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An evaluation of strontium chloride, Rappaport and strontium selenite enrichment for the isolation of salmonellas from man, animals, meat products and abattoir effluents

BY J. B. IVESON AND E. M. MACKAY-SCOLLAY
Salmonella Diagnostic and Reference Laboratory,
State Health Laboratory Service, Box F312, Perth, Western Australia

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SUMMARY

Strontium chloride enrichment broth was found to be comparable to Rappaport broth for the recovery of a wide range of *Salmonella* serotypes from man, animals, meat products and effluents. With the exception of cloacal samples from reptiles, both procedures were superior to selenite F.

The performance of strontium chloride M and selenite F enrichment was improved when effluent samples were incubated at 43° C.

Strontium chloride M and Rappaport enrichment were superior to selenite F for the isolation of *Arizona* species from reptiles.

Strontium chloride B, strontium selenite and Rappaport broths were found suitable for the isolation of multiple *Salmonella* serotypes from sea water contaminated with abattoir effluents. The strontium chloride B and strontium selenite enrichment media were superior to Rappaport broth when samples were incubated at 43° C.

Modified bismuth sulphite agar was found superior to Salmonella–Shigella agar as a solid subculture medium.

The investigation of a food poisoning outbreak due to *Salmonella typhimurium* phage type 21 is reported.

The significance of the choice of sampling and isolation techniques in salmonellosis in man and animals is discussed.

INTRODUCTION

Meat products have often been implicated as a vehicle of salmonella infection, furthermore it has been demonstrated that there is a close relationship between the types of *Salmonella* isolated from abattoirs and meat processing centres, and from human cases in the same area (Galton, Smith, McElrath & Hardy, 1954; McDonagh & Smith, 1958; Harvey & Phillips, 1961). Assessments of the frequency of salmonella infection have shown wide variation, and Schothorst & Kampelmacher (1967) and Vassiliadis, Trichopoulos, Papadakis & Politi (1970) have attributed differences to slaughterhouse practice, meat processing procedures, and methods of laboratory examination.

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The selenite F enrichment medium of Leifson (1936) and the tetrathionate broth of Muller (1923) are widely used for the isolation of salmonellas. Important modifications include the tetrathionate broth of Kauffmann (1930, 1935) and the selenite broth of Hobbs & Allison (1945).

The inhibitory action of chlorides on the growth of certain Enterobacteriaceae was first investigated by Eisenburg (1918) and Hotchkiss (1923). Subsequently, lithium chloride was used by Gray (1931), magnesium chloride by Rappaport, Konforti & Navon (1956), and strontium chloride by Iveson & Mackay-Scollay (1969) and Iveson (1971), as selective ingredients in salmonella isolation media.

In trials of Rappaport broth, Collard & Unwin (1958), Iveson, Kovacs & Laurie (1964), Hooper & Jenkins (1965) and Iveson & Kovacs (1967) demonstrated improved salmonella recoveries over both selenite and tetrathionate media. The medium was also found suitable for the isolation of the selenite and tetrathionate sensitive *S. cholerasuis* from pigs by Iveson & Mackay-Scollay (1969), Riley (1970) and Beh (1971). On the other hand, Harvey & Price (1968) found Rappaport broth less suitable for the isolation of *S. dublin* and *S. pullorum*. The medium was also not suited to incubation at 43° C., a temperature effective in improving the performance of selenite F broth (Harvey & Thomson, 1953).

In comparative trials of two new enrichment media containing strontium ion, Iveson & Mackay-Scollay (1969) observed that in tests with sewage and human and swine faeces, strontium chloride M medium compared favourably with Rappaport broth in the isolation of salmonellas and, except for the isolation of *S. typhi*, was superior to selenite F. They also found that strontium selenite broth was superior to Rappaport, strontium chloride M, tetrathionate and selenite F for the isolation of *S. typhi*.

Similar results were obtained by Chau & Huang (1971), who recommended the combination of strontium selenite and Rappaport broths for the isolation of *Salmonella*. However, the methods quoted were unsuitable for the isolation of *Edwardsiella tarda*, and Iveson (1971) introduced strontium chloride B broth for the improved isolation of *Edwardsiella* as well as *Salmonella* and *Arizona* species. The new medium was also well suited to the elevated temperature technique.

In the present study an evaluation of the relative performance of strontium chloride M, strontium chloride B, strontium selenite, Rappaport and selenite F enrichment media was undertaken during a survey of salmonella infection in animals, reptiles and poultry, in a study of abattoir effluents and in an investigation of a food-poisoning outbreak. The methods of examination and results obtained are presented in this report.

MATERIALS AND METHODS

The investigation comprised three complementary studies. The first, Study I, involved the testing of 940 specimens from cattle, pigs, sheep, poultry, rats, mice, guinea-pigs, dogs, cats, snakes, lizards and ticks. Strontium chloride M, Rappaport and selenite F enrichment media were used to examine samples of faeces, glands, intestinal contents and viscera. Study II consisted of the investigation of a

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salmonella food-poisoning outbreak in humans. The same enrichment media were used to examine a total of 1541 samples which comprised human faeces, raw and cooked meats and the effluents from meat-processing centres and abattoirs. Study III was directed particularly to the isolation of multiple *Salmonella* serotypes from 18 samples of sea water heavily contaminated with abattoir effluent using strontium chloride B, strontium selenite and Rappaport enrichment broth media, incubated at 37 and 43° C.

Study I

With the exception of cloacal swabs from reptiles, which were placed direct into 2 ml. of Sachs (1939) transport medium, all samples were homogenized in equal volumes of the transport solution and inoculated on SS agar (Oxoid) and modified bismuth sulphite agar (Iveson, 1971). Approximately 0·5 ml. volumes of each sample were added to 10 ml. volumes of strontium chloride M, Rappaport and selenite F enrichment media respectively and incubated at 37° C. Subcultures were made from enrichment media to one SS agar and one modified BS agar plate after 18–24 hr incubation.

Study II

Faeces and effluent samples were collected as described by Iveson & Mackay-Scollay (1969). The surface swabs of meat and meat-processing equipment were collected on folded gauze swabs held with sterile forceps and immediately placed into 100 ml. of $\frac{1}{4}$ strength Ringer's solution. After mixing, 15 ml. volumes were added to 150 ml. volumes of the three enrichment media, strontium chloride M, Rappaport and selenite F broths which were incubated at 37° C. In addition, selected meat processing and abattoir effluents were also inoculated into strontium chloride M and selenite F enrichment media and incubated at 43° C. Meat and effluent samples were subcultured to SS and modified BS agar at 24 and 48 hr incubation. Faeces samples were similarly subcultured but only after 24 hr incubation.

Study III

Samples of sea water heavily contaminated with effluent were collected at nine sampling points close to the shoreline, and not farther than half a mile from the abattoir effluent outflow. Moore swabs were immersed for approximately 6 hr., lifted and replaced by swabs left overnight at the sampling points.

Samples were transported to the laboratory in 100 ml. $\frac{1}{4}$ strength Ringer's solution, the contents mixed and 15 ml. inoculated into 150 ml. quantities of strontium chloride B, Rappaport and strontium selenite enrichment media, and incubated at 37 and 43° C. The remaining fluid was drained from the container and the swab (which contained approximately 15 ml. retained fluid) was covered with 150 ml. strontium chloride B broth and incubated at 43° C. Each sample was subcultured to a plate of SS and one of modified BS agar after 24 hr. incubation, and to a BS agar plate only at 48 hr. In addition, 14 of the samples were subcultured to BS agar after 72 hr. incubation.

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Media

Strontium chloride M enrichment broth

Bacto tryptone (Difco)	0·5 g.
Sodium chloride	0·8 g.
Potassium dihydrogen phosphate	0·1 g.
60 % strontium chloride (B.D.H.)	6·0 ml.
0·4 % malachite green (Merck)	1·0 ml.
Distilled water	100 ml.

The medium was distributed in 10 ml. or 150 ml. volumes as required, and sterilized by steaming for 30 min. The final concentration of strontium chloride was 3·4 % and the pH was 5·0–5·5.

Strontium selenite enrichment broth

Bacto tryptone (Difco)	0·5 g.
Sodium chloride	0·8 g.
Strontium hydrogen selenite (Ajax)	0·2 g.
Potassium dihydrogen phosphate	0·1 g.
Distilled water	100 ml.

The broth was adjusted to pH 6·8, distributed in 150 ml. amounts and steamed for 30 min. The strontium hydrogen selenite salt, supplied by Ajax Chemicals Ltd. of Australia, replaced the salt previously prepared in our laboratories (Iveson & Mackay-Scollay, 1969).

Other enrichment broth media

The strontium chloride B, Rappaport and selenite F media were prepared as reported by Iveson (1971).

An average of three suspect colonies were selected from each of the plating media and examined biochemically and serologically.

Supplementary sero-recovery technique.

In addition to routine colony identification, the BS agar plates from the 24 hr. subculture routine in Study III were examined for the presence of further *Salmonella* serotypes by a modification of the serological recovery method of Harvey & Price (1967). Swabs of positive BS plates were inoculated to Craigie tubes which contained the homologous flagellar phase 1 and phase 2 antisera of the serotypes previously identified from the same plates. After overnight incubation, growth from Craigie tubes was subcultured to BS agar and colonies examined in the usual way.

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Table 1. *The relative efficiency of direct culture and selenite F, Rappaport and strontium chloride M enrichment in the isolation of* Salmonella *from domestic animals, rodents and reptiles*

Samples		Tests	Culture method							
			D	S	R	C	S+R	S+C	R+C	S+C+R
Livestock	tissues	348	20*	38	50	50	51	51	56	57
	faeces	371	17	52	68	78	82	90	90	97
	rumen	10	2	4	4	7	5	7	7	7
Domestic pets faeces		32	0	3	11	13	12	14	14	14
Rodents faeces		83	5	11	28	27	28	28	31	31
Reptiles faeces		82	28	47	58	59	64	62	67	68
Reptiles ticks		14	0	3	2	4	4	4	5	5
Totals		940	72	158	221	238	246	256	270	279
Efficiency (%)		.	25·8	56·6	79·2	85·3	88·2	91·8	96·8	100

* The figures show the number of positive cultures.
D = direct culture; S = selenite F; R = Rappaport; C = strontium chloride M.

RESULTS

A total of 864 isolations which comprised 828 *Salmonella*, 35 *Arizona* and 1 *Edwardsiella* strain were isolated from 631 positive samples. *Salmonella typhimurium* was most frequently isolated and was recovered from 248 (39 %) of the positive samples. A total of 51 *Salmonella* and 14 *Arizona* serotypes were identified. The distribution of the *Salmonella* serotypes is shown in Table 5.

In tests on animals (Study I) a total of 340 strains comprising 306 *Salmonella*, 33 *Arizona* and 1 *Edwardsiella* strain, were isolated from 279 positive specimens. Of the positive samples 72 (26 %) were obtained by direct culture and 279 (100 %) by enrichment culture. The enrichment media recovery totals were strontium chloride M 238 (85 %), Rappaport 221 (79 %) and selenite F 158 (57 %). The relative efficiency of direct culture and enrichment methods is shown in Table 1. In tests on cattle, sheep and pig samples there was no significant difference in the performance of strontium chloride M and Rappaport broths, and both procedures were superior to selenite F.

Similar results were obtained in tests on rodent, dog, cat and snake samples. Two possible exceptions were noted in the lizard and poultry test series, where selenite F was comparable to Rappaport medium in performance and only slightly inferior to strontium chloride M broth.

In the course of the animal investigations, 45 *Salmonella*, 13 *Arizona* and *Edwardsiella tarda* serotype recoveries were made.

Figures in Table 2 show that in the recovery of Arizonas, strontium chloride M and Rappaport enrichment methods were each superior to either direct culture or selenite F methods. In fact, from the 18 reptiles where *Arizona* species were recovered exclusively only six were positive by a combination of direct culture and selenite enrichment, compared with 15 recoveries through Rappaport and 13 through strontium chloride M enrichment broths. Conversely, in the 35 reptiles

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Table 2. *Relative efficiency of strontium chloride M, Rappaport and selenite F enrichment cultures and direct culture in the isolation of* Salmonella, Arizona *and* Edwardsiella *species from 54 reptiles*

	Salmonella		*Arizona*		*Edwardsiella*	
Culture method	No. positive	Efficiency (%)	No. positive	Efficiency (%)	No. positive	Efficiency (%)
Strontium chloride M	32	91·4	13	72·2	0	.
Rappaport	29	82·9	15	83·3	0	.
Selenite F	32	91·4	6	33·3	0	.
Direct culture	17	48·6	6	33·3	1	.
Totals	35	100	18	100	1	.

Table 3. *The relative efficiency of direct culture and selenite F, Rappaport and strontium chloride M enrichment in the isolation of* Salmonella *from human faeces, meats, meat factories and abattoirs*

Samples		Tests	Culture method							
			D	S	R	C	S+R	S+C	R+C	S+C+R
Human faeces		1111	88	142	155	161	164	167	168	172
Meats		177	.	27	34	29	39	41	39	42
Effluents	Meat factory	63	.	9	12	14	20	17	22	24
	Abattoir	52	.	18	45	45	45	46	52	52
Swabs	Meat	62	.	7	14	13	16	15	18	19
	Equipment	58	.	5	10	13	15	15	19	19
Totals		1523	.	208	270	275	299	301	318	328
Efficiency, %		.	.	63·4	82·3	83·8	91·2	91·8	97·0	100

Notes as in Table 1.

which yielded *Salmonella* exclusively, the performance of the three enrichment methods were comparable though direct culture was again greatly inferior. However, the single *Edwardsiella* isolation was made exclusively from direct culture.

The following *Arizona* serotypes were recovered from reptiles; the figures in parentheses indicate the frequency of isolations: *Arizona* 5:29–21 (1); 5:29–30 (1); 15:24–31 (1); 16:23–25 (9); 16:23–37 (3); 16:27–25 (1); 26:24–25 (1); 26:33–31 (4); 28:23–25 (1); 28:32–28 (2); 29:29–25 (2); 30:26–21 (2); 1, 33:23–21 (4). *Arizona* 1, 33:23–21 was also recovered from ticks taken from one reptile.

In the food-poisoning investigations, Study II, a total of 419 isolations comprising 417 *Salmonella* and 2 *Arizona* strains were isolated from 328 positive samples. The enrichment media recovery totals were, strontium chloride M, 275 (84 %); Rappaport, 270 (82 %); and selenite F, 208 (63 %) positive samples. The relative performance of the isolation methods used is shown in Table 3.

In tests on human faeces, strontium chloride M and Rappaport enrichment were slightly superior to selenite F enrichment when the broths were incubated at 37° C. Similar results were obtained in tests on meat products, meat swabs and meat

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Table 4. *Relative efficiency of selenite F and strontium chloride M enrichment at 37° and 43° C. and Rappaport enrichment at 37° C. in the isolation of* Salmonella *from 30 meat factory and 39 abattoir effluent samples*

Effluent	Culture method and isolation										
	37° C.							43 °C			
	S	C	R	SC	SR	RC	SCR	S	C	SC	All methods
Meat factory	9	14	14	18	20	22	24	12	19	21	30
Abbattoir	15	34	35	35	35	39	39	28	39	39	39
Totals	24	48	49	53	55	61	63	40	58	60	69
Efficiency (%)	34·8	69·9	71·0	76·0	79·7	88·4	91·3	58·0	84·1	87·0	100·0

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Table 5. Salmonella *serotypes isolated from all sources in Studies I, II and III*

Salmonella serotype	Source and isolations								Totals
	Human faeces	Live-stock	Abat-toir	Meat pro-cessing	Kangaroo meat	Domestic pets	Rodents	Reptiles	
abony	.	3	.	.	.	.	.	.	3
adelaide	3	31	24	3	3	7	.	1	72
alsterdorf	.	.	.	.	.	.	.	1	1
anatum	.	2	8	5	5	3	.	.	23
bahrenfeld	.	2	3	.	.	.	.	.	5
birkenhead	.	1	.	.	.	.	.	.	1
boeker	.	.	.	.	.	.	.	1	1
bootle	.	.	.	.	.	.	.	1	1
bovismorbificans	.	5	10	2	.	.	.	.	17
bredeney	.	4	12	.	.	.	.	.	16
bukavu	.	.	1	.	.	.	.	.	1
bullbay	.	.	.	.	.	.	.	4	4
champaign	.	1	.	.	.	.	.	1	2
charity	.	.	.	.	1	.	.	1	2
chester	1	48	11	1	2	1	1	5	70
cholerae-suis	.	11	.	.	.	.	.	.	11
derby	.	18	61	1	.	.	.	.	80
eastbourne	.	.	1	.	.	.	.	.	1
eimsbuettel	.	.	2	.	.	.	.	.	2
emmastad	.	.	.	.	.	.	.	1	1
enteritidis	.	.	.	.	.	.	5	1	6
give	.	4	10	1	.	1	.	4	20
havana	4	.	18	.	.	.	.	.	22
houten	.	.	.	.	2	.	.	. (1)	3
hvtttingfoss	.	.	1	.	.	2	.	1	4
litchfield	.	2	.	.	.	.	.	.	2
livingstone	.	.	14	.	.	.	.	.	14
kibusi	.	.	.	.	.	.	.	2	2
kisarawe	.	.	.	.	.	.	.	3	3

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Table 5 (*cont.*)

Salmonella serotype	Source and isolations: Human faeces	Live-stock	Abat-toir	Meat pro-cessing	Kangaroo meat	Domestic pets	Rodents	Reptiles	Totals
muenchen	5	5	4	4	19	.	22	2	61
nashua	.	.	.	.	.	.	.	14	14
new-brunswick	.	1	.	.	.	.	.	.	1
newington	.	1	6	7	1	1	.	1	17
onderstepoort	.	4	.	.	2	.	.	.	6
oranienburg	.	3	5	.	.	.	.	.	8
orientalis	.	.	.	.	.	.	.	2 (1)	3
orion	1	6	2	2	5	.	.	.	16
ohlstedt	.	.	.	.	.	.	.	2 (1)	3
potsdam	.	.	1	.	1	1	.	.	3
pullorum	.	6	.	.	.	.	.	.	6
rubislaw	3	.	.	.	.	.	.	.	3
saint-paul	.	7	.	.	.	2	2	5	16
senftenberg	.	.	7	2	.	.	1	.	10
sydney	.	.	.	.	.	.	.	. (1)	1
tennessee	.	1	4	3	.	.	.	.	8
typhimurium	154	19	18	47	2	.	7	1	248
urbana	.	2	.	.	.	.	.	.	2
wandsbek	.	.	3	.	.	.	.	4	7
waycross	.	.	.	.	2	.	.	.	2
welikada	.	.	.	.	2	.	.	.	2
42 : z : –	.	.	.	.	.	.	.	1	1
Totals	171	187	226	78	47	18	38	59 (4)	828

Figures in parentheses indicate isolations from ticks on reptiles.

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Table 6. *Distribution and relative frequency of* Salmonella *serotypes isolated by five procedures from* 18 *samples of sea water contaminated with abattoir effluent*

Method	Temperature °C	Incubation (hr.)	Plating medium	Colony tests No.	Colony tests Pos.	*livingstone*	*derby*	*bredeney*	*typhimurium*	*give*	*havana*	*senftenberg*	*adelaide*	*bovismorbificans*	*bahrenfeld*	*wandsbek*	*anatum*	*bukavu*	*chester*	*hvittingfoss*	*muenchen*	*newington*	Isolations	Isolations (subtotal)	Isolations (method)	Positive samples	Positive samples (subtotal)	Positive samples (method)
I. Strontium selenite	37	24	SS	40	3	+	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	3			2		
		24	BS	47	23	+	+	+	.	+	+	.	+	.	.	.	.	.	.	.	.	.	15	20		11	13	
		48	BS	21	10	.	+	.	+	+	+	.	+	.	.	+	.	.	.	.	.	.	8		41	7		16
	43	24	SS	40	22	+	+	+	+	.	+	.	.	.	.	.	.	.	.	.	.	.	14			13		
		24	BS	42	35	+	+	+	+	+	+	.	+	.	.	.	.	.	.	.	.	.	23	35		16	16	
		48	BS	30	25	+	+	+	+	+	+	.	+	.	+	.	.	.	+	.	.	.	20			13		
II. Rappaport	37	24	SS	27	19	+	.	+	+	+	.	+	.	.	.	.	.	+	.	.	.	.	12			10		
		24	BS	41	40	+	.	+	+	+	+	+	.	+	.	.	+	+	.	.	.	.	20	29		14	14	
		48	BS	30	29	+	+	.	+	+	+	+	.	.	+	.	+	+	.	.	.	.	17		42	11		15
	43	24	SS	30	21	+	+	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	15			11		
		24	BS	34	32	+	+	+	+	+	+	+	.	+	.	.	.	.	.	.	.	.	18	29		12	13	
		48	BS	31	29	+	+	+	+	.	+	+	.	.	.	.	+	.	.	.	.	.	17			12		
III. Strontium chloride B	37	24	SS	39	11	+	+	+	.	.	.	+	.	.	+	.	.	.	.	.	.	.	8			7		
		24	BS	40	33	+	+	+	+	+	.	.	+	+	.	.	+	.	.	.	.	.	19	35		14	16	
		48	BS	29	20	+	+	.	+	+	+	.	+	.	.	+	.	.	.	.	.	.	17		60	12		16
	43	24	SS	33	27	+	+	+	+	+	+	+	.	.	+	+	.	.	.	.	.	.	20			14		
		24	BS	44	41	+	+	+	+	+	+	+	+	+	+	.	.	.	.	+	.	.	24	47		15	15	
		48	BS	36	35	+	+	+	+	+	+	+	.	.	+	+	.	.	.	.	.	+	24			15		
IV. Strontium chloride B with swab	43	24	SS	41	10	+	+	.	+	.	.	.	.	+	.	+	.	.	.	.	.	.	8			7		
		24	BS	50	48	+	+	+	+	+	+	+	+	+	.	+	.	.	.	.	.	.	35	49		17	17	
		48	BS	37	33	+	+	+	+	+	+	+	+	+	.	.	.	.	.	.	.	.	24			16		
Serum recovery technique	37	24	BS	77	72	+	+	+	+	+	+	+	.	+	.	.	.	+	.	.	.	.	23	57				
	43	24	BS	127	126	+	+	+	+	+	+	+	.	+	+	.	.	.	+	.	+	.	48					
Totals				966	744	15	13	11	9	7	9	4	5	9	3	3	2	1	3	1	1	1		97			18	

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factory effluents. Differences in the performance of enrichment media were most evident with samples of abattoir effluent, where Rappaport and strontium chloride M broths were each considerably better than selenite F enrichment. However, the performance of selenite F was considerably improved and that of strontium chloride M broth further improved when effluent samples were incubated at 43° C. (Table 4). Rappaport broth was not incubated at the higher temperatures.

The *Salmonella* serotypes recovered from human faeces, meats and meat-processing centres during the food-poisoning investigations are listed in Table 5. *Arizona* 26:26–25 was also recovered from human faeces and *Arizona* 16:23–25 was isolated from a sample of kangaroo meat.

The results obtained in the third investigation, Study III, are set out in Tables 6–8. One hundred and five *Salmonella* isolations, comprising 19 serotypes, were made from 18 positive samples of sea water contaminated with abattoir effluent. At a temperature of 37° C. (Table 6) strontium chloride B enrichment was slightly better than either strontium selenite or Rappaport in the total number both of isolations and of individual serotype recoveries. However, the total of positive *samples* achieved by each method was fully comparable.

The performance of strontium chloride B and strontium selenite was enhanced when the enrichment media were incubated at 43° C. But Rappaport was more suited to the lower temperature of incubation. The distribution and relative frequency of *Salmonella* serotypes, isolated by the four enrichment methods subcultured at 24 and 48 hr. and by the supplementary sero-recovery technique, are shown in Table 6.

If a single enrichment broth, a single incubation temperature and a single plating medium are to be used, strontium chloride B medium when added to the swab sample and incubated at 43° C. with subculture after 24 hr. incubation to the modified BS agar, was the best single method consistent with economy of materials; 17 (94 %) positive samples, 10 (59 %) serotypes, and 35 (33 %) isolations were recorded; furthermore, only 50 (5·2 %) colonies were examined and of these 48 (96 %) were positive. There was little difference in the isolations and positive sample totals when strontium chloride B medium was used to examine either the fluid or swab sample by the elevated temperature technique. However, three more serotypes were isolated from the fluid sample than from the swab.

The *Salmonella* isolations, serotypes and positive samples recovered from enrichment media at 24, 48 and 72 hr. and without the use of the supplementary sero-recovery technique are detailed in Table 7. The combined 24 hr. subculture procedures recovered a total of 78 (80 %) isolations, 14 (74 %) serotypes and 18 (100 %) positive samples, compared with 64 (66%) isolations, 15 (79%) serotypes and 18 (100%) positive samples at 48 hr. The recoveries from the 14 samples subcultured to BS agar at 72 hr. were 38 isolations, 16 serotypes and 14 positive samples. The 48 hr. subculture routine increased the 24 hr. isolation total by 11 and the serotype numbers by 2, whereas the 72 hr. subculture increased the isolations by a further 8, and three additional serotypes *S. muenchen*, *S. oranienburg*, and *S. potsdam* were isolated. *S. muenchen* was also recovered by the 24 hr supplementary sero-recovery method (Table 6).

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Table 7. Salmonella *isolations, serotypes and positive samples from* 18 *effluent samples examined by four enrichment methods, incubated at* 37 *and* 43° C. *and subcultured to* SS *agar and modified* BS *agar after* 24, 48 *and* 72 *hr.*

Subculture after (hr.)	Plating media	No. of *Salmonella* isolations at (° C.)			*Salmonella* serotypes at (° C.)			Positive samples at (° C.)		
		37	43	37/43	37	43	37/43	37	43	37/43
24	SS	19	39	46	9	10	11	13	17	17
24	BS	38	57	68	11	12	14	16	17	18
24	SS/BS	45	65	78	12	12	14	16	17	18
48	BS	32	55	64	11	14	15	15	17	18
24/48	SS/BS	52	80	89	13	15	16	16	17	18
*72	BS	19	29	38	11	13	16	11	13	14
Totals		56	85	97	15	17	19	16	17	18

* Results from 14 samples only.

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Table 8. *Distribution and relative frequency of* Salmonella *serotypes in 909 colonies on subculture media related to time and temperature of incubation of enrichment culture*

		No. of positive colonies after								
		24 hr. at				48 hr. at		72 hr.* at		
		37° C. on		43° C. on		37 °C on	43 °C on	37 °C on	43 °C on	No. of
Serotype	Pos. cols.	SS	BS	SS	BS	BS	BS	BS	BS	isolations
livingstone	209	15	42	39	53	12	24	8	16	15
derby	78	1	7	8	19	8	27	3	5	13
bredeney	61	3	5	5	15	.	18	.	14	10
typhimurium	66	5	11	8	15	14	11	2	.	10
bovismorbificans	36	.	8	2	15	.	7	1	3	8
havana	61	2	6	11	14	5	8	6	9	8
give	61	2	6	2	11	8	10	14	8	7
adelaide	18	.	3	.	4	2	2	1	6	6
senftenberg	21	2	2	1	4	4	4	2	2	4
bahrenfeld	11	1	.	1	3	1	3	.	2	3
wandsbek	8	.	.	3	1	2	2	.	.	3
anatum	10	.	5	.	.	2	3	.	.	2
newington	11	.	.	.	.	.	1	5	5	2
bukavu	6	2	1	.	.	1	.	2	1	1
chester	3	.	.	.	.	.	2	.	1	1
hvittingfoss	2	.	.	.	2	.	.	.	.	1
muenchen	1	.	.	.	.	.	.	.	1	1
oranienburg	2	.	.	.	.	.	.	.	2	1
potsdam	1	.	.	.	.	.	.	1	.	1
Totals	666	33	96	80	156	59	122	45	75	97
Colony tests	909	106	128	144	170	80	134	62	85	.
% positive	73·3	31·1	75·0	55·6	91·8	73·8	91·0	72·6	88·2	.

* Results from 14 samples only.

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The multi-serotype serum recovery method performed on the 24 hr positive BS agar plates increased the 24 hr BS isolation total from 68 to 83 and the serotype total from 14 to 16. The combined enrichment media isolation total was increased from 97 to 105. However, 42 isolations detected by the routine 24 hr. isolation procedures were again recovered by the serum method, and additional serotypes were subsequently detected in the 48 and 72 hr. subculture.

In the 24 hr. enrichment subculture routine, where both SS and BS agar media were used (Table 7) the modified BS agar was considerably better as a plating medium; for example, after enrichment at 37° C. only 19 *Salmonella* isolations, 9 serotypes and 13 positive samples were recovered from SS agar, compared with 38 isolations, 11 serotypes and 16 positive samples from BS agar. Salmonella isolations from SS plating media were increased from 19 to 39 when enrichment media were incubated at 43° C. Isolations from BS agar were increased from 38 to 57, when the higher enrichment temperature was used.

The 24 hr. isolation total from enrichment media plated out on modified BS agar was 68 isolations, 14 serotypes and 18 positive samples, compared with 46, 11 and 17 respectively from SS. agar. Furthermore, 252 (85 %) colonies were positive, compared with 113 (45 %) positive colonies from SS. However, the SS plating routine increased the BS agar isolation total from 68 to 78. The distribution and relative frequency of *Salmonella* serotypes in the 659 colonies selected from BS agar and in the 250 colonies examined from SS plating medium are detailed in Table 8.

DISCUSSION

In a report on salmonellosis in Australia, Atkinson (1964) commented that the epidemiology of the disease was not well understood, few outbreaks had been closely studied and individual cases seldom followed up. Nevertheless, *Salmonella typhimurium*, *S. derby*, *S. anatum* and *S. adelaide* were noted as widespread pathogens, passing between man, animals and the environment with considerable efficiency. *S. typhimurium* was involved more than any other serotype, but an outbreak of *S. derby* infection was reported by Rubbo (1948); and Lee & Mackerras (1955) reported a severe outbreak of gastro-enteritis due to *S. bovismorbificans*.

In the present investigations *S. typhimurium* was directly implicated in the food-poisoning outbreak and this serotype, as well as *S. derby*, *S. anatum*, *S. adelaide* and *S. bovismorbificans*, was frequently detected in samples from domestic animals, abattoirs and meat-processing effluents. *S. typhimurium* was rarely found in kangaroo meat, domestic pets and reptiles. *S. muenchen* was widely distributed, particularly in kangaroo meat and rodents. *S. rubislaw* was recovered exclusively from human specimens during the food-poisoning investigations, but this serotype has been isolated from cattle and rodents in Australia (Atkinson, 1964) and from lizards (Lee & Mackerras, 1955; Iveson, Mackay-Scollay & Bamford, 1969).

S. typhimurium was isolated from 86 of the 97 patients (172 isolations) diagnosed during the outbreak. The source of infection was traced to a meat smallgoods factory, and samples of corned beef, ham, ham salami, polony, saveloys, frankfurts,

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and swabs from shelves, benches, floor, tables, mincer, meat pack sealing machine, delivery truck, refrigerated store and sewage, also yielded *S. typhimurium*. Salmonella strains from meat products, eight food handlers, and from human cases in different suburbs were identified as *S. typhimurium* phage type 21.

With the exception of *S. rubislaw*, the serotypes recovered from patients and contacts during the food-poisoning investigations were all recovered from livestock, abattoir effluents and meat processing centres. The isolation of *S. chester* and *S. havana* were of particular interest as both serotypes were subsequently associated with outbreaks of human infection. The *S. havana* outbreak was traced to infected food handlers and meat products. The serotype was also recovered frequently from livestock at the abattoir supplying the meat centre. The prevalence of a serotype in an abattoir followed subsequently by human infection has been reported by Harvey & Price (1970).

With the exception of *S. pullorum*, *S. houten* and *S. waycross*, the 39 *Salmonella* serotypes recovered from livestock, domestic pets, rodents, abattoirs, meats and meat processing centres have all been isolated from cases of human infection in Western Australia. A total of 18 (72 %) serotypes recovered from reptiles, have also been isolated from man. Seven serotypes, *S. boecker*, *S. bootle*, *S. bullbay*, *S. kibusi*, *S. kisarawe*, *S. sydney* and *S.* 42: z: –, have been recovered exclusively from reptiles. The public health significance of salmonella carriers in livestock and birds has been reported by Hobbs (1961).

The detection of a single salmonella serotype in a contaminated sample, whilst important from a diagnostic public health viewpoint, does not necessarily provide accurate epidemiological data, particularly in the examination of foodstuffs and environmental samples. The problems of assessing the diagnostic and epidemiological significance of multiple serotype infections has been reported by Winkle & Rohde (1958), and, more recently, Harvey & Price (1967) have drawn attention to the technical problems associated with the isolation of multiple serotypes from heavily contaminated materials, and have commented further that the choice of technique depends on the time available for the examination and the epidemiological importance of achieving comprehensive results. A wide choice of isolation techniques are available and, in comparative studies, the results obtained by different laboratories have shown wide variation (Edel & Kampelmacher, 1968, 1969). The importance of the sampling and salmonella isolation procedure in relation to the final result has also been stressed by Van Schothorst & Kampelmacher (1967). They reported that isolations in their laboratory varied from 28 to 57 % according to the method of examination.

In the present study in tests on domestic animals, rodents and reptiles, the inclusion of strontium chloride M and Rappaport broth, along with the direct culture combined with selenite F routine, increased salmonella isolations from 159 to 234 (47 %), and 159 to 219 (38 %) respectively. The combined increase was 159 to 280 (76 %). Reduced selenite performance was most evident with faeces inocula from pigs, cats, dogs and rats, where *Proteus* species were frequently encountered. The problems of *Proteus* overgrowth with selenite F and tetrathionate media has been reported by Hobbs & Allison (1945), Smith & Buxton (1951),

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Cruickshank & Smith (1949), and Galton, Scatterday & Hardy (1952). In the animal studies both Rappaport and strontium chloride M were effective in suppressing the growth of *Proteus* species, and salmonellas were often isolated by both procedures when the corresponding selenite F subculture showed a heavy growth of *Proteus* or, occasionally, *Pseudomonas* species. Using laboratory strains, Anderson & Kennedy (1965), and Vassiliadis (1968), also showed that Rappaport broth was more efficient than selenite F or tetrathionate media in suppressing *Proteus* species, and at the same time allowing satisfactory growth of *S. choleraesuis*.

However, in contrast to the results of the present investigation (cf. Table 2), the latter author found selenite F broth superior to Rappaport enrichment medium in a growth test series in which *Arizona* strains were used.

In tests on human faeces Chau & Huang (1971) reported that strontium selenite broth was superior to selenite F, both in the inhibition of *Proteus*, and in the isolation of *S. typhi*. Strontium chloride M and Rappaport broth were found suitable for the isolation of *S. choleraesuis*. The latter methods were also effective in the recovery of the selenite-sensitive serotype from pig glands in the present investigation.

The strontium chloride M and Rappaport media were slightly better than selenite F in tests on human faeces, meat products and effluent samples examined in the food-poisoning investigations reported here. However, when effluent samples were incubated at 43° C., the performance of selenite F was improved considerably, although it was still inferior to enrichment in strontium chloride M.

Salmonella organisms were detected in each of the 70 abattoir effluent samples examined, whether diluted with sea water (Study III) or not, and a total of 226 isolations, which comprised 24 serotypes, were achieved. In the final study in which strontium selenite, strontium chloride B and Rappaport enrichment were combined with the serum recovery method, 105 salmonella isolations were obtained from 18 sea-water samples contaminated with abattoir effluent. One sample yielded 10 *Salmonella* serotypes, and a total of 19 serotypes were identified in specimens collected over a 24 hr period.

The value of a comprehensive testing programme in achieving both diagnostic and epidemiological data, together with the work load involved, has been amply demonstrated in the present investigation. It has also been shown, particularly in tests on abattoir effluents reported in Table 6, that the new strontium chloride and strontium selenite enrichment media are fully capable of achieving comprehensive results when used singly or in combination. The choice of a single enrichment broth, suitable plating medium, and number of colonies selected for identification, will be governed by both epidemiological requirements and the resources of the laboratory. The new media have shown that they can perform efficiently when incubated at 43° C., and provide a sound basis for the application of the supplementary serum recovery method to the single 24 hr. modified BS agar subculture routine for the isolation of multiple serotypes.

Throughout the investigations, the modified bismuth sulphite agar plating medium was greatly superior to SS agar and provided ready differentiation of *Salmonella* and *Arizona* species within 24 hr. incubation.

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