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The occurrence and significance to animal health of salmonellas in sewage and sewage sludges

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SUMMARY

A total of 882 samples of settled sewage, sewage sludges and final effluents from eight sewage treatment plants were examined for the presence of salmonellas. Of these samples 68 % were positive, isolations being made most frequently from settled sewage (85 %), raw sludge (87 %) and anaerobically digested sludge (96 %). Fewer isolations were made from final effluent (24 %) and processed sludges (58 %). Samples usually contained less than 200 salmonellas/100 ml and arguments are presented that such concentrations should not lead to disease in animals if suitable grazing restrictions are followed.

INTRODUCTION

Water authorities dispose of sewage sludge, either raw or following processing, on agricultural land which may be grazed by cattle. The sludge is a valuable source of nutrients and humus but may contain potentially pathogenic bacteria (Wray, 1975). Salmonellas are probably the most important group of organisms involved but a potential hazard may also be presented by the possible presence of *Brucella abortus*, *Bacillus anthracis*, enteropathogenic strains of *Escherichia coli*, pathogenic mycobacteria and pathogenic leptospires.

This communication describes studies designed to demonstrate the degree to which salmonellas occur in sewage sludges which have been variously treated or have received no treatment. Studies on the presence of other potentially pathogenic bacteria will be the subject of a future publication. Initially samples of sewage and sludge from seven sewage-treatment plants within the Thames Water Authority were examined. Samples from an eighth plant were included during the early stages of the work.

MATERIALS AND METHODS

Sewage-treatment plants

Samples from eight sewage-treatment plants, chosen to be representative of those in operation in the Thames Water Authority (T.W.A.) area, were examined between October 1976 and September 1977. A brief description of the plants is

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given in Table 1. Samples of settled sewage, raw sludge and final effluent were received from all eight plants. Additional samples were examined as shown in Table 2. Samples were taken at intervals of approximately 14 days by staff of the T.W.A. and transported to the Institute for Research on Animal Diseases (IRAD) on the day of collection.

Table 1. *Sewage treatment at plants sampled*

Site	Flow ($m^3 d^{-1}$)	Secondary treatment	Tertiary treatment
A	32600	Activated sludge (diffused air)	None
B	4500	Percolating filters (partial recirculation)	None
C	8600	Percolating filters (alternating double filtration)	Lagoon
D	17700	Percolating filters (single pass)	Land irrigation
E	2400	Percolating filters (partial recirculation)	None
F	510000	Activated sludge (diffused air)	None
G	56200	Activated sludge (diffused air)	None
H	35200	Percolating filters (partial recirculation)	Rapid gravity sand filters

Isolation of salmonellas

Salmonellas were isolated by direct enrichment of 10 ml volumes of sample in two 100 ml volumes of Rappaport broth (Rappaport, Konforti & Navon, 1956) and two 100 ml volumes of selenite brilliant green broth (SBG, Difco Laboratories). In addition salmonellas were isolated by pre-enrichment of 10 ml of sewage in 100 ml of buffered peptone water (Peptone (Difco B118) 10 g, sodium chloride 5 g, disodium hydrogen orthophosphate 12 H_2O 9 g, potassium dihydrogen orthophosphate 1·5 g, distilled water 1 l, pH 7·2) for 24 h at 37 °C followed by enrichment of 10 ml of the buffered peptone water in Rappaport broth, SBG, and Muller–Kauffmann tetrathionate broth (Oxoid CM 343). The Rappaport broths were incubated at 37 °C and the SBG and Muller–Kauffmann tetrathionate broths at 43 °C. After 24 and 48 h incubation all enrichment broths were inoculated on modified brilliant green agar (Oxoid CM 329) with the addition of sulphadiazine (120 mg/l; B.D.H.). Plates were incubated at 37 °C and examined after 24 and 48 h. Non-lactose and non-sucrose fermenting bacteria resembling salmonellas in colony morphology were identified biochemically according to the method of Edwards & Ewing (1962) and serologically according to the method of Kauffmann (1972).

In addition to the above procedure 10 ml of each sample from treatment plant E was enriched in two 100 ml volumes of brilliant green MacConkey broth (Smith, 1959). One broth was incubated at 37 °C and the other at 43 °C. After 24 and 48 h incubation each broth was inoculated on modified brilliant green agar and salmonellas identified as previously described.

Enumeration of salmonellas

The concentration of salmonellas in samples taken between October 1976 and March 1977 was estimated by a 'spread plate' technique in which 0·1 ml volumes

Table 2. *Sludge treatment at plants sampled*

Site	Processed sludge sample	Type of sludge treated	Retention/storage time	Chemical conditioner used
A	Mesophilic anaerobically digested	Primary	34 days	—
	Consolidated digested	Mesophilic anaerobically digested	40 days	—
	Consolidated activated	Surplus activated	24 hours	—
	Vacuum filter cake	Consolidated digested	—	Aluminium chlorohydrate
B	Cold aerobically digested	Cosettled primary and humus	100 days	—
	Drying bed cake	Cold digested	Variable > 1 month	
	Belt filter press cake	Cosettled primary and humus	—	Polyelectrolyte (Zeetag 94)
C	Centrifuge cake	Cosettled primary and humus	—	Polyelectrolyte (Zeetag 92)
D	Drying bed cake	Cosettled primary and humus	Variable > 1 month	—
	Centrifuge cake	Cosettled primary and humus	—	Polyelectrolyte Zeetag 92)
E	Filter press cake	Cosettled primary and humus	—	Lime and copperas
F	Mesophilic anaerobically digested	Cosettled primary and surplus activated	23 days	—
	Lagooned	Mesophilic anaerobically digested	< 2 years	—
G	Mesophilic anaerobically digested	Cosettled primary and surplus activated	28 days	—
	Lagooned	Mesophilic anaerobically digested	> 2 years	—
H	Mesophilic anaerobically digested	Cosettled primary and humus	50 days	—
	Filter press cake	Cosettled primary and humus	—	Polyelectrolyte (Zeetag 94)
	Stockpiled cake	Filter press cake	Variable	Polyelectrolyte (Zeetag 94)

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of undiluted sewage or 1/10 and higher dilutions in 0·85 % saline were spread over the surface of modified brilliant green agar; the plates were incubated at 37 °C for 24 h and colonies agglutinating in *Salmonella* polyvalent 'O' serum (Wellcome Laboratories) were counted.

The concentration of salmonellas in all samples from treatment plant H and in samples from plants A, B, C and D taken between April 1977 and September 1977 was estimated by the 'Most Probable Number' technique (Taras *et al.* 1971). Samples of final effluent were inoculated into buffered peptone water and then enriched in Muller–Kauffmann tetrathionate broth. All other samples were enriched in SBG. The technique used was a three dilution test with three tubes per dilution each containing 10, 1 and 0·1 ml of sample respectively.

RESULTS

Isolation of salmonellas

Salmonellas were isolated from 597 (68 %) of 882 samples examined (Table 3). Isolations were made most frequently from settled sewage (85 % of samples positive), raw sludge (87 % of samples positive) and anaerobically digested sludge (96 % of samples positive). Fewer isolations were made from final effluent (24 % of samples positive) and processed sludges (58 % of samples positive). There was considerable variation between isolations from processed sludges depending upon the type of treatment employed.

The value of the various enrichment techniques used in the isolation of salmonellas is shown in Table 4. The most successful technique used was enrichment in SBG. However, the success of a technique depended upon the sample examined. Thus, although SBG was most successful for settled sewage, raw sludge, anaerobically digested sludge and other processed sludges, Muller–Kauffmann tetrathionate broth after pre-enrichment in buffered peptone water was the best method for isolation from samples of final effluent.

Isolations were not made from the brilliant green MacConkey broth used for samples from plant D.

Effect of treatment on the Salmonella *content of sewage*

The effect of treatment of sewage on the *Salmonella* population is summarized in Table 5 which illustrates the percentage of samples of each sludge shown to contain salmonellas. The percentage of isolations ranged from 0 % (filter press cake, plant E) to 100 % (filter press cake, plant H).

The effect of treatment on the actual number of salmonellas is illustrated in Table 6. The figures are those obtained by the 'Most Probable Number' (MPN) technique. None of the samples contained sufficient salmonellas (greater than 10^4/100 ml) to be countable by the spread plate technique. The highest concentration of salmonellas was found in raw sludge (mean MPN/100 ml > 194·0), anaerobically digested sludge (mean MPN/100 ml > 171·4) and consolidated activated sludge (mean MPN/100 ml 114·0). The concentration of salmonellas was significantly reduced by most of the sludge-processing treatments. Similarly fewer

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Table 3. *Isolation of salmonellas from eight sewage-treatment plants*

Plant sample	Settled sewage	Final effluent	Raw sludge	Digested sludge (anaerobic)	Digested sludge (aerobic)	Processed sludge	Total
A	22/24 (92%)	5/24 (21%)	20/24 (83%)	22/24 (92%)	—	Vacuum filter cake 12/15 (80%)	119/159 (75%)
						Consolidated digested 16/24 (67%)	
						Consolidated activated 22/24 (92%)	
B	19/24 (83%)	4/23 (17%)	18/24 (75%)	—	9/24 (38%)	Drying bed cake 5/15 (33%)	68/113 (60%)
						Belt filter press cake 13/14 (93%)	
C	18/23 (78%)	5/23 (22%)	20/22 (91%)	—	—	Centrifuge cake 17/22 (77%)	60/90 (67%)
D	15/23 (65%)	3/23 (13%)	18/23 (78%)	—	—	Drying bed cake 16/23 (69%)	59/101 (58%)
						Centrifuge cake 7/9 (78%)	
E	16/23 (69%)	6/23 (26%)	21/23 (91%)	—	—	Filter press cake 0/17 (0%)	43/86 (50%)
F	22/22 (100%)	9/22 (41%)	22/22 (100%)	22/22 (100%)	—	Lagooned sludge 5/41 (12%)	80/129 (62%)
G	21/22 (95%)	4/22 (18%)	20/22 (91%)	21/22 (95%)	—	Lagooned sludge 9/20 (45%)	75/108 (69%)
H	16/16 (100%)	7/16 (44%)	14/16 (87%)	16/16 (100%)	—	Filter press cake 16/16 (100%)	79/96 (82%)
						Stockpiled filter press cake 10/16 (62%)	
Total	149/177 (89%)	43/176 (24%)	153/176 (87%)	81/84 (96%)	9/24 (38%)	148/256 (58%)	583/882 (66%)

organisms were present in final effluent (mean MPN/100 ml 1·9) than in settled sewage (mean MPN/100 ml 20·7).

Individual figures for the five sewage treatment plants examined by the MPN technique are shown in Table 7. Variations in counts between individual samples from each plant are shown in Figs. 1–10.

The efficiency of the five plants in reducing the number of salmonellas present in settled sewage is shown in Table 8. The plants ranged from 71·8% efficient to 100% efficient. There was no correlation between total flow and efficiency. There

Table 4. *Comparison of methods for the isolation of salmonellas from sewage*

	Pre-enrichment			Enrichment	
	MK	RAPP	SBG	RAPP	SBG
Settled sewage	65	38	34	50	75
Final effluent	74	46	28	37	42
Raw sludge	56	28	55	35	79
Anaerobically digested sludge	78	43	63	83	87
Processed sludges	53	32	47	53	63
Total of all samples	61	34	46	50	71

MK = Muller–Kauffmann tetrathionate broth; RAPP = Rappaport broth; SBG = selenite brilliant green broth.

The figures represent the number of occasions (expressed as a percentage) on which any particular method was successful in isolating *Salmonella* from a sample shown to be positive by any of the methods.

Table 5. *Effect of treatment on the* Salmonella *content of sewage*

	Qualitative data	
Sample	Plant	Samples positive (%)
Settled sewage	All plants	85
Final effluent	All plants	24
Raw sludge	All plants	87
Mesophilic digested sludge (anaerobic)	A, F, G, H	96
Mesophilic digested sludge (aerobic)	B	38
Filter press cake	H	100
Belt filter press cake	B	93
Consolidated activated sludge	A	92
Vacuum filter cake	A	80
Centrifuge cake	D	78
Centrifuge cake	C	77
Drying bed cake	D	69
Consolidated digested sludge	A	67
Stockpiled filter press cake	H	62
Lagooned sludge (< 2 years old)	G	45
Drying bed cake	B	33
Lagooned sludge (< 2 years old)	F	25
Lagooned sludge (> 2 years old)	F	4
Filter press cake (lime + coperas)	E	0

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appeared to be a correlation between efficiency and type of process although only one plant using an activated sludge process was examined.

The efficiency of the digestion process in removing salmonellas from raw sludge is shown in Table 9.

The figures shown in Tables 8 and 9 were produced from an analysis of the mean figures presented in Table 7.

Identification of Salmonella *serotypes*

A total of 2973 isolates were identified as members of the genus *Salmonella*. Three hundred and seventy-three (12%) were assigned to serotype as shown in Table 10.

Table 6. *Effect of treatment on the* Salmonella *content of sewage*

	Quantitative data	
Sample	Plant	Mean MPN/100 ml
Settled sewage	A, B, C, D, H	20·7
Final effluent	A, B, C, D, H	1·9
Raw sludge	A, B, C, D, H	> 194·0
Mesophilic digested sludge (anaerobic)	A, H	> 171·4
Consolidated activated sludge	A	114·0
Filter press cake	H	56·2
Drying bed cake	B	46·5
Centrifuge cake	C	22·4
Centrifuge cake	D	16·9
Belt filter press cake	B	14·1
Stockpiled filter press cake	H	9·5
Consolidated digested sludge	A	3·9
Vacuum filter cake	A	1·7
Mesophilic digested sludge (aerobic)	B	0·3
Drying bed cake	D	0·0

DISCUSSION

Salmonellas were isolated from 68% of all samples examined. It is perhaps surprising that, despite the extensive enrichment procedures used, almost a third of the samples were shown to be free from salmonellas. There was however a considerable variation between treatment plants in the number of positive samples (plant E, 50%, plant H, 82%). This did not appear to relate to the total flow of sewage through the plant or the size of population served by the plant but rather to differences in treatment at each plant.

As may have been expected a high proportion of isolations were made from settled sewage and raw sludge and salmonellas were present in the highest concentration in raw sludge. The salmonellas present in settled sewage were significantly reduced following either passage through percolating filters or the activated sludge process. Percolating filters appeared to be more efficient in this respect but since only one activated sludge plant was examined further work is required. The efficiency of plants in removing salmonellas ranged from 72% (plant A) to 100% (plants C and D) although the 100% efficiency does not imply that all salmonellas

were removed but merely that they could not be detected by the methods used on the volumes of sample examined. It would appear from the data obtained from plant A that a large number of the organisms removed from settled sewage by the activated sludge process are concentrated in the surplus sludge where they are present even after gravity consolidation for approximately 24 h (mean MPN/100 ml 114·0).

Table 7. *'Most Probable Number' of salmonellas in samples of sewage from five sewage-treatment plants*

	Range/100 ml	Mean/100 ml
Plant A		
Settled sewage	0–210	22·0
Final effluent	0–43	6·2
Raw sludge	0– > 2400	> 273·0
Digested sludge (anaerobic)	0– > 2400	> 104·0
Consolidated digested sludge	0–43	3·9
Consolidated activated sludge	0–460	114·0
Vacuum filter cake	0–9	1·7
Plant B		
Settled sewage	0–93	16·9
Final effluent	0–7	1·6
Raw sludge	0–460	93·8
Digested sludge (aerobic)	0–3	0·3
Drying bed cake	0–93	46·5
Belt filter press cake	0–35	14·1
Plant C		
Settled sewage	0–24	3·1
Final effluent	0	0·0
Raw sludge	0– > 2400	> 255·7
Centrifuge cake	0–93	22·4
Plant D		
Settled sewage	0–23	5·3
Final effluent	0	0·0
Raw sludge	0–460	90·2
Drying bed cake	0	0·0
Centrifuge cake	0–44	16·9
Plant H		
Settled sewage	0–93	22·5
Final effluent	0–15	1·7
Raw sludge	9– > 2400	> 218·9
Digested sludge (anaerobic)	0–1100	219·5
Filter press cake	0–460	56·2
Stockpiled filter press cake	0–93	28·4

Salmonellas were also removed from raw sludge by the various sludge treatment processes examined. Mesophilic anaerobic digestion appeared to be the least efficient in this respect. In fact the concentration in raw sludge (mean MPN/100 ml > 194·0) may not have been reduced by the anaerobic digestion process (anaerobically digested sludge mean MPN/100 ml > 171·4). A slightly greater proportion of samples of anaerobically digested sludge were shown to contain salmonellas by the enrichment techniques used. In one treatment plant (plant H) the mean MPN of *Salmonella*/100 ml was similar in digested sludge and raw sludge. The possibility

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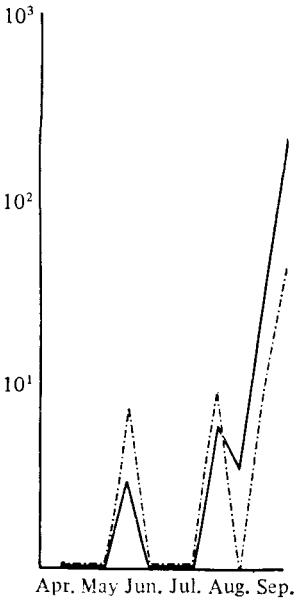

Fig. 2

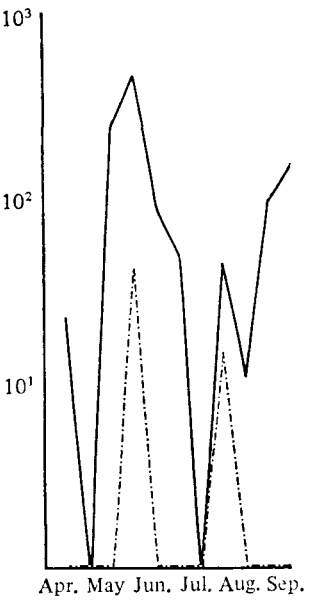

Fig. 2

Fig. 1. MPN counts (salmonellas/100 ml) plant A. ——, Settled sewage; —·—, final sludge.

Fig. 2. MPN counts (salmonellas/100 ml) plant A.——, Raw sludge; —·—, digested sludge.

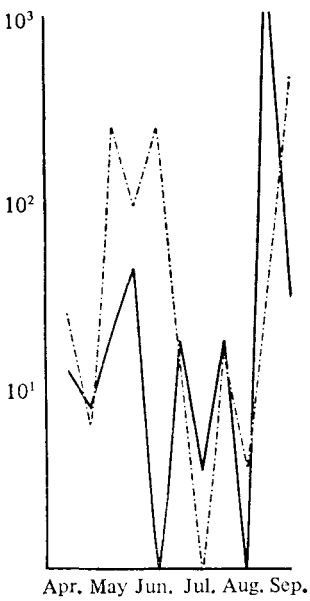

Fig. 3

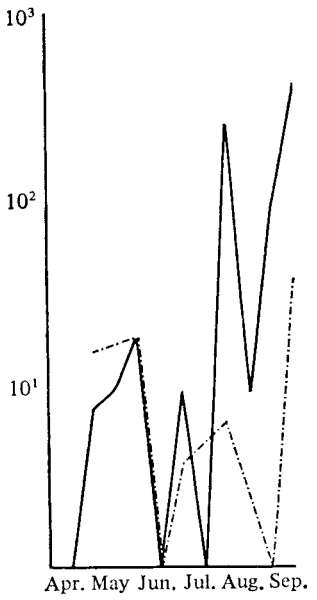

Fig. 4

Fig. 3. MPN counts (salmonellas/100 ml) plant A.——, Consolidated activated sludge; —·—·— consolidated digested sludge.

Fig. 4. MPN counts (salmonellas/100 ml) plant B. ——, Raw sludge; —·—, belt filter press cake.

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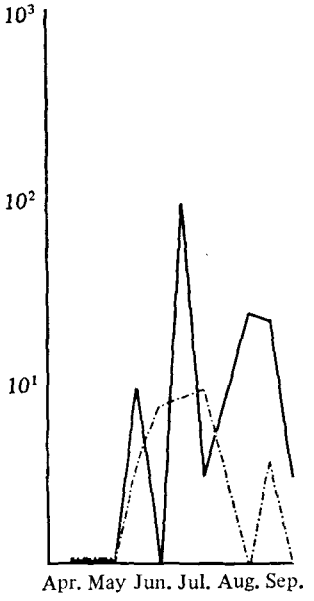

Fig. 5

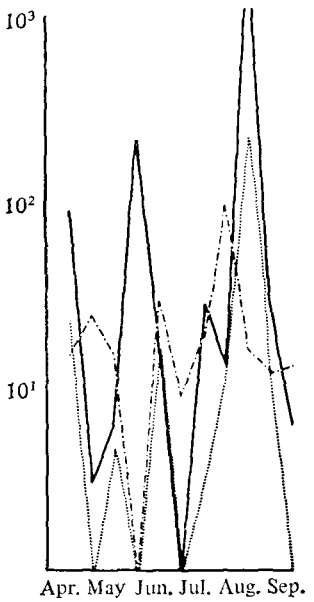

Fig. 6

Fig. 5. MPN counts (salmonellas/100 ml) plant B. ——, Settled sewage; —·—, final effluent.

Fig. 6. MPN counts (salmonellas/100 ml) plant C. ——, Raw sludge; —·—, centrifuge cake; ..., settled sewage.

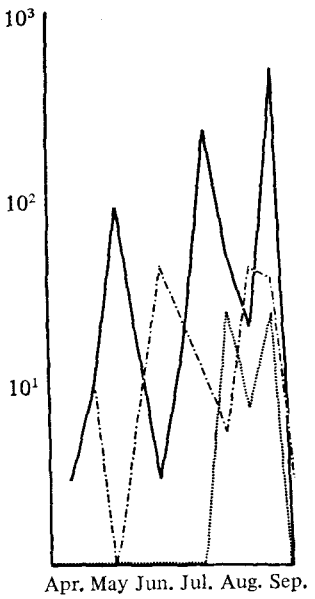

Fig. 7

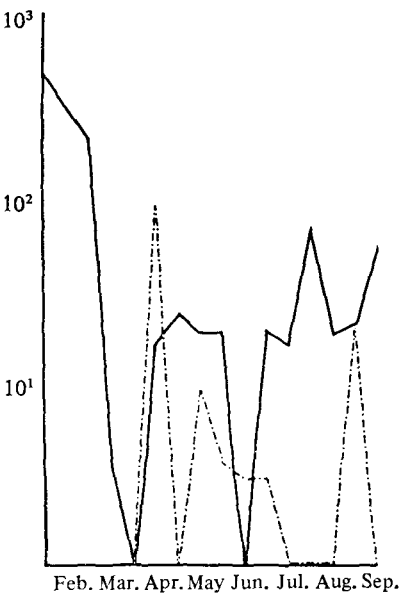

Fig. 8

Fig. 7. MPN counts (salmonellas/100 ml) plant D.——, Raw sludge; —·—, centrifuge cake; ..., settled sewage.

Fig. 8. MPN counts (salmonellas/100 ml) plant H. ——, Filter press cake; —·—, stockpiled filter press cake.

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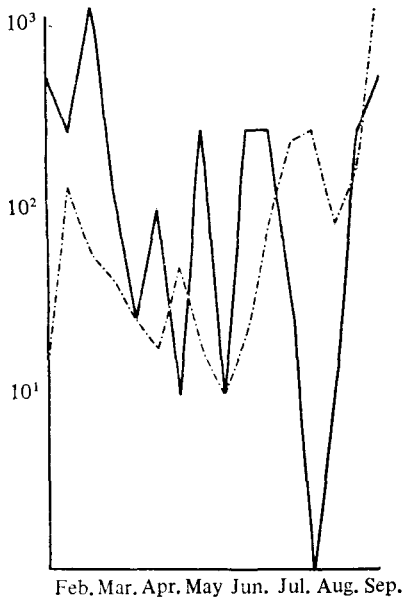

Fig. 9

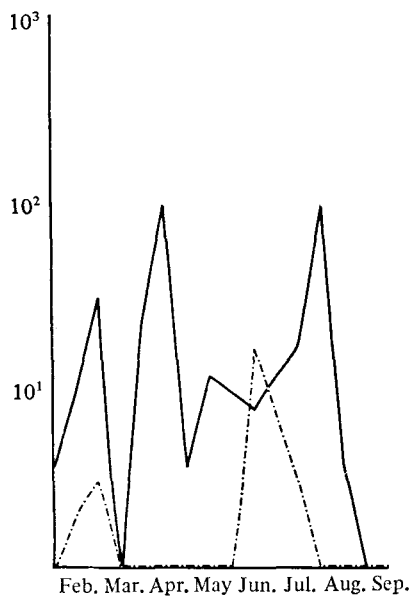

Fig. 10

Fig. 9. MPN counts (salmonellas/100 ml) plant H. ——, Settled sewage; —·—, final effluent.

Fig. 10. MPN counts (salmonellas/100 ml) plant H. ——, Digested sludge; —·—; raw sludge.

Table 8. *Efficiency of five sewage-treatment plants in removing salmonellas from settled sewage*

Treatment plant	Treatment	Reduction (%)
A	Activated sludge (diffused air)	71·8
B	Percolating filters (partial recirculation)	90·8
C	Percolating filters (alternating double filtration) followed by lagooning	100
D	Percolating filters (single pass)	100
H	Percolating filters (partial recirculation) followed by rapid gravity sand filters	92·5

Table 9. *Efficiency of three sewage-treatment plants in removing salmonellas from raw sludge by digestion*

Treatment plant	Raw sludge (MPN)	Treatment	Post-treatment (MPN)	Reduction (%)
A	> 273·0	Mesophilic anaerobic digestion	> 104·0	61·9 (minimum)
	> 273·0	Mesophilic anaerobic digestion and consolidation	3·9	98·9 (minimum)
H	> 218·9	Mesophilic anaerobic digestion	219·5	cannot be calculated
B	93·8	Mesophilic aerobic digestion	0·3	99·7

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that salmonellas may multiply during the digestion process obviously merits further investigation.

The mesophilic aerobic digestion process used at plant B appears to be far more successful than anaerobic digestion. There was however, a far higher isolation rate of salmonellas from such sludge (67 % of samples positive) during the first 6 months of the survey than the final 6 months (8 % of samples positive) when the digester was only infrequently fed with raw sludge. A similar variation was found when

Table 10. *Serotypes of* Salmonella *isolated from eight sewage treatment plants*

	Plant								
	A	B	C	D	E	F	G	H	
agona	6	3	2	2	—	3	—	2	18
azteca/fyris	1	—	—	—	—	—	—	—	1
anatum	2	3	1	2	5	5	1	—	19
bardo/feruch	—	—	—	—	1	—	—	—	1
braenderup	—	1	—	1	—	—	—	—	2
brandenburg	1	—	—	—	—	—	—	—	1
bredeney	—	—	—	1	—	—	—	2	3
derby	3	1	—	1	—	2	—	—	7
drypool	2	—	—	—	—	1	2	—	5
dublin	—	—	—	—	—	1	—	—	1
enteritidis	1	—	—	—	1	—	—	2	4
give	8	2	—	1	—	7	5	—	23
haardt	—	—	—	—	—	1	—	—	1
hadar	—	3	1	—	—	8	4	3	19
halmstad	—	1	—	—	—	—	—	—	1
heidelberg	1	3	—	1	3	3	5	1	17
indiana	—	—	—	—	—	2	—	—	2
infantis	1	—	—	—	—	—	—	—	1
java	1	—	—	—	—	—	—	—	1
kaapstad	1	—	—	—	—	1	2	—	4
kedougou	—	—	—	—	—	—	—	1	1
kentucky	—	—	—	—	—	1	—	—	1
kingston	—	—	2	—	—	1	2	1	6
kinshasa	1	—	—	1	—	1	—	—	3
lovelace	—	—	—	—	—	—	—	2	2
montevideo	—	—	—	—	1	—	—	—	—
muenchen	—	1	—	—	—	2	—	—	3
newhaw/newington	4	—	—	—	2	—	1	—	7
newport	2	1	1	2	3	5	10	1	25
new rochelle	—	—	—	—	—	—	—	1	1
oranienburg	14	10	3	6	8	20	14	3	78
panama	1	—	—	—	—	1	—	1	3
paratyphi B	12	1	—	—	—	1	1	4	19
raus	1	—	1	1	—	—	—	—	3
reading	1	—	—	—	—	—	1	—	2
remo	—	—	—	—	1	1	—	—	2
saintpaul	5	5	1	—	—	2	3	—	16
senftenberg	6	2	—	—	2	10	—	—	20
stormont	—	—	—	—	—	1	1	—	2
thompson	—	1	—	—	—	—	—	—	1
typhimurium	11	8	5	4	3	7	7	1	46
	86	46	17	23	30	87	59	25	373

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samples of drying bed cake from plant D were examined; 100 % of samples positive during the first 8 months compared with 0 % for the final 4. This variation is probably also explained by the drying bed not receiving fresh sludge during the final 6 months.

Of the other sludge processing methods examined the most efficient was lime and copperas conditioning followed by dewatering (plant E, all samples negative). This process raised the pH of the sludge above 10·0, a level at which salmonellas in sludge are instantly killed (Jones, 1977, unpublished observations). Storage of sludge in lagoons was also an efficient method of reducing the *Salmonella* concentration and the reduction was directly related to time (plant F–storage for more than 2 years, 4 % of samples positive). It is not necessary to discuss in detail the success of all the treatment methods individually. Reference to Tables 4 and 5 will show that all treatments of raw sludge other than anaerobic digestion led to a reduction in salmonellas of at least 70 %.

It is not really possible to speculate on the origin of the large number of *Salmonella* serotypes isolated since all types may possibly on occasion be isolated from both man and other animals. It was however interesting that the sewage plant receiving the highest proportion of farm animal excreta in crude sewage (plant D) had the lowest isolation rate (65 %) of salmonellas from settled sewage. Only one isolation of the host-adapted serotype for cattle, *S. dublin*, was made and this was from a treatment plant (plant F) which received sewage from a predominantly urban area. The most likely source of the isolate was thus an abattoir or butcher's shop. If animal excreta was contributing a large proportion of the serotypes to the sewage a larger number of isolations of *S. dublin* might have been expected. Only a small proportion of the isolates of *Salmonella* were serotyped and it is therefore difficult to speculate on the frequency of isolation of various serotypes. *S. oranienburg* was the most frequently isolated serotype and *S. paratyphi B* was isolated from plant A with greater frequency than from other plants.

The figures arrived at using the MPN technique are the most accurate that can be achieved at present. However, they should be treated with some caution. The MPN technique may provide an under estimate of the numbers actually present when compared with spread plate counts (Jones, 1974 – unpublished observations). In addition it is possible to speculate that enrichment techniques may be more efficient at isolating salmonellas from one sample than another. This may relate to the identity and concentration of other bacteria in the samples with which the salmonellas must compete during the incubation of the enrichment broths.

Of the enrichment broths used SBG without pre-enrichment produced the largest number of isolations. It was also superior to the other methods for all samples with the exception of final effluent for which pre-enrichment followed by enrichment in Muller–Kauffmann tetrathionate broth gave the best results. For this reason this method was used for MPN counts on final effluent during the last 6 months of the survey. SBG was used for all other samples. Table 4 shows the value of using more than one isolation technique and indicates that the more extensive the examination the greater the chance of success.

While the numbers of salmonellas present in samples were similar to those

reported for cattle slurries (Jones & Matthews, 1975) and pig slurries (Jones *et al.* 1976) the percentage of samples positive greatly exceeded those in cattle slurries (11 %) and pig slurries (19 %). Table 3 shows that 87 % of raw, 83 % of digested and 58 % of processed sludges contained salmonellas. This finding may be interpreted to mean that whilst 1/10 pastures receiving cattle slurry and 2/10 pastures receiving pig slurries presented potential hazards to animal health, 9/10 receiving crude sludge presented the same hazard.

It is difficult to predict the ease with which animals grazing pasture dressed with sewage sludge might become infected, since the dose of *Salmonella* required to initiate an infection under natural conditions is not known and may vary from serotype to serotype. The dose of *S. dublin* required to produce an infection is high but may offer a guide to the infectious dose of other serotypes. Hall, Jones & Aitken (1978) failed to initiate infection in adult cattle with a dose of 10^{10} organisms administered directly into the rumen via a canula. However, many factors as yet not fully understood, such as concurrent infection with the liver fluke, *Fasciola hepatica*, may lower the infectious dose (Aitken *et al.* 1976).

The numbers of salmonellas present in all samples were 10^8–10^9 times lower than the probable infectious dose of *S. dublin* for adult cattle and would presumably be reduced even further following spreading on pasture. The survival of salmonellas in soil is influenced by many factors (Rudolfs, Falk & Ragotzkie, 1950) and is therefore difficult to predict. Survival of various serotypes is reported as less than 30 days (Melick, 1917) to more than 1 year (Delage, 1961). Survival after application of infected animal slurry is equally variable but salmonellas may still be isolated from such soil after 20 weeks (Findlay, 1971). Survival on pasture depends upon the length of grass. Taylor & Burrows (1971) recovered *S. dublin* from the upper levels of grass for up to 10 days, from the lower levels for up to 19 days and from the soil for up to 12 weeks. However in all the trials quoted above the numbers of salmonellas used to infect soil or grass were 10^4–10^6 times higher than those normally found in sewage sludge. Survival times after application of infected sewage sludge would presumably, therefore, be shorter.

No reliable information is available on the infectivity of pasture spread with sewage sludge to grazing cattle. However, experimental exposure of calves to pasture contaminated with *S. dublin* in cattle slurry suggests that grazing animals are not easily infected from this source. Taylor (1973) failed to infect calves allowed to graze pasture 7 days after spreading with slurry containing 10^5 *S. dublin*/ml. However, outbreaks of salmonellosis in cattle associated with contaminated cattle slurry have been reported (Jack & Hepper, 1969; Rasch & Richter, 1956). Similarly in Switzerland seasonal increases in the incidence of isolation of salmonellas from cattle have been associated with the spreading of large quantities of sewage sludge (Hess & Breer, 1975). In Holland, regular disposal to land of sewage sludge on a dairy farm was thought to have raised the percentage of cattle infected with salmonellas significantly above the national average (Strauch, 1977).

In an experiment designed to measure the infectivity to cattle of salmonellas in raw sludge, Hall & Jones (1978) included raw sludge containing up to 10^4 naturally occurring salmonellas/100 ml. in the diet of four grown calves at the rate of

1 l/animal/day for 28 days. Salmonellas were not isolated from the faeces of the animals or from their tissues at *post-mortem* examination. It is thus probable that the number of salmonellas normally contained in sewage sludge will not cause infection in grazing animals, particularly if pasture is not grazed for a suitable period after spreading. Four weeks has been recommended for animal slurries (Jones, 1976) and a similar period would probably be acceptable for sewage as far as the risk of salmonellosis is concerned. This is particularly true for most processed sludges in which the number of salmonellas is reduced. Most danger is presented by raw sludge, anaerobically digested sludge and, on the evidence of the present data, consolidated activated sludge. These should still not present a greater hazard than farm animal slurries if grazing restrictions are observed. On the basis of the figures produced here there appears to be little logic in differentiating between raw and anaerobically digested sludge, both of which contain similar numbers of salmonellas. However, results of further investigations of the anaerobic digestion process should be considered before a firm conclusion on this matter is reached.

The principal restraint on sewage sludges is based on the introduction of salmonellas exotic to the premises while animal slurries are usually only contaminated with salmonellas already present in the animals. The exception exists where pig slurry, which is often contaminated with salmonellas (Jones *et al.* 1976) is spread on pasture to be grazed by cattle.

The above comments relate only to the possible risk of transmission of salmonellosis while a final decision on the health hazard of sewage sludge may depend as much on the presence of infective stages of helminths, particularly *Cysticercus bovis*, in the sludge. Similarly the role of potentially toxic or hazardous metals has not been considered in this paper.

Recommendations or codes of practice can only consist of procedures to reduce the risk of infection to an acceptably low level. Such a risk cannot be completely eliminated unless the pathogen itself is eliminated, by for example lime treatment, since it is not possible to guarantee that a small number of organisms may not on occasion cause infection.

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