J. Hyg., Camb. (1985), **94** 301–307
Printed in Great Britain

Salmonellae in sewage sludge and abattoir effluent in South-east Scotland

By K. A. LINKLATER, MARGARET M. GRAHAM

East of Scotland College of Agriculture, Veterinary Investigation Centre, Greycook, St. Boswells, Roxburghshire TD6 0EU

AND J. C. M. SHARP

Communicable Diseases (Scotland) Unit, Ruchill Hospital, Glasgow G20 9NB

(*Received* 10 *December* 1984; *accepted* 28 *December* 1984)

SUMMARY

A survey into the prevalence of salmonella organisms in sewage in the Borders Region of South-east Scotland is described. A total of 317 isolates representing 34 different serotypes were made, of which only 5 serotypes appeared in animals, supporting the view that the spreading of sewage sludge on to pastureland presents little risk to livestock provided the recommended guidelines are followed. Nevertheless, *Salmonella typhimurium* phage type 12, identified in sewage, was also recovered from animals in incidents on 11 farms, including 4 which had received sludge from this source. A further 48 isolates (13 serotypes) were obtained from the parallel monitoring of abattoir effluents, indicating that the background level of salmonella infection in the animal population appears to be low in comparison to that in humans.

INTRODUCTION

It has long been recognized that many serotypes of salmonella can be present in human sewage, and many conventional methods of sewage treatment have little effect on their numbers (Wray, 1975).

As one of the main methods of disposal of sewage is by spreading on agricultural land, this obviously presents a hazard to grazing animals. However, guidelines for the application of sewage sludge to pasture land have been laid down, and a minimum of three weeks between application and grazing has been recommended (DOE/NWC Guidelines, 1981 and the Scottish Agricultural Colleges, 1981).

In South-east Scotland most of the sewage is spread as sludge on agricultural land. Therefore it was considered appropriate to monitor the serotypes of salmonella present in the sludge and to see if there was any association with the serotypes identified as being responsible for animal and human disease in the area.

MATERIALS AND METHODS

Sampling

(*a*) One litre samples of sludge were collected weekly from each of eight sewage works in different towns designated A to H in the Borders Region for a three-month

period (June to August 1982). Subsequently samples were collected every two weeks for another nine months (September 1982 to May 1983). Samples were taken at the point where the tankers were being filled for spreading.

(*b*) At the same time as the sewage sludge was being monitored, Moore's swabs were placed in drains in the abattoirs in towns A, C and D. In all of these they were placed in the drains from the lairage and the slaughterhall, and also from the offal house in C.

(*c*) For the second part of the investigation during August and September 1983, attention was focused on the sewage plant in town A. Moore's swabs were placed in the effluent which flowed out into a river and in the five incoming drains, i.e. from the abattoir and four different areas of the town designated B, S, T and W. Swabs were left in place for one week and the investigation was carried out for an eight-week period. Each time the swabs were collected a sample of sludge was taken as previously described.

Isolation techniques

(*a*) *Sludge*. The sludge was homogenized to release salmonellae from clumps of other enteric bacteria and 15 ml transferred to 100 ml phosphate-buffered peptone water (Oxoid CM 509) as recommended by Edel & Kampelmacher (1973). After incubation for 18–24 h at 37 °C, 10 ml were transferred to 100 ml Rappaport broth and incubated for a further 18–24 h at 43 °C. Forty-eight hours' incubation gave no better isolation. Subculture was made on two brilliant green agar (BGA) plates (Oxoid CM329) as recommended by Edel & Kampelmacher (1969), which allows better salmonella colonial differentiation than the use of a single plate. The method depends on transferring the inoculum from the first plate to the second without intermediate sterilization of the loop. This allows dilution of contaminating organisms and gives good separation of colonies. Incubation was for 18–24 h at 37 °C.

Five colonies resembling *Salmonella* sp. were picked off and characterized biochemically and serologically. Subcultures of all isolates were sent to the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow for confirmation of serotype and subsequently for phage-typing where relevant to the Division of Enteric Pathogens, Colindale, London.

(*b*) *Moore's swabs*. These were placed in 100 ml double-strength selenite broth and incubated for 18–24 h at 37 °C. Sub-cultures were again made on BGA plates.

However, in investigation (*c*) into the sewage plant in town A, the Moore's swabs taken from the incoming drains were almost totally unrecognizable, being partly eaten by rats and covered in large amounts of extraneous material. The contamination was such that we were unable to get reasonable colonial separation. In this investigation, therefore, the swabs were treated in the same way as the sludge.

RESULTS

(*a*) *Sewage sludge*. A total of 228 isolations of salmonellae were made covering 32 serotypes (Table 1). Most isolates were made from the largest town (A), otherwise there did not seem to be any correlation between the size of population and the number of isolates.

Table 1. *Salmonella serotypes isolated from sewage sludge from eight towns in the Borders Region of Scotland – June 1982 to May 1983*

Town...	A	B	C	D	E	F	G	H	Totals
Population (thousands)...	16·70	4·17	5·76	13·31	1·68	0·61	1·62	0·81	44·66
S. montevideo	4	—	12	2	1	2	5	—	26
S. virchow	3	2	4	1	5	4	—	5	24
S. newport	—	—	—	—	13	8	—	—	21
S. panama	6	1	3	5	—	2	—	2	19
S. anatum	1	1	—	12	—	—	—	—	14
S. typhimurium (other)*	1	3	2	2	1	1	—	1	11
S. java†	9	2	—	—	—	—	—	—	11
S. heidelberg	1	2	2	—	—	—	6	—	11
S. derby	5	1	1	—	—	1	—	2	10
S. stanley	1	1	1	1	1	2	—	—	7
S. london	6	—	1	—	—	—	—	—	7
S. infantis	3	1	—	1	—	—	1	—	6
S. livingstone	1	—	—	—	1	4	—	—	6
S. typhimurium 10	—	5	—	—	—	—	1	—	6
S. agona	—	2	—	1	1	—	1	—	5
S. saint-paul	—	—	3	—	1	1	—	—	5
S. typhimurium 12	2	1	—	—	1	—	—	—	4
S. indiana	—	1	1	—	1	—	—	1	4
S. brandenburg	—	—	—	—	—	—	1	1	2
S. enteritidis‡	—	—	—	1	—	—	—	1	2
S. eimsbuettel	1	1	—	—	—	—	—	—	2
S. ohio	1	1	—	—	—	—	—	—	2
Other serotypes§	9	3	4	2	—	—	5	—	23
Totals	54	28	34	28	26	25	20	13	228

* *S. typhimurium* – other phage types 8, 35, 49, 49a, 193, 204a and untyped.
† *S. java* – phage types 1 var 3, 1 var. 9, Dundee.
‡ *S. enteritidis* – phage types 8, untyped.
§ Other serotypes – *S. give* (3), *S. senftenberg* (3), *S. kedougou* (2), *S. bareilly* (2), *S. hadar* (2), *S. bredeney*, *S. takoradi*, *S. schwarzengrund*, *S. stanleyville*, *S. chester*, *S. tennessee*, *S. mbandaka*.

Table 2. *Salmonella serotypes isolated from effluent fron abattoirs in three towns in the Borders Region of Scotland – June 1982 to May 1983*

Town	A	C	D	Totals
S. montevideo	9	12	3	24
S. typhimurium 12	—	2	3	5
S. virchow	1	—	1	2
S. arizonae	1	—	1	2
S. ohio	—	—	2	2
S. panama	2	—	—	2
S. derby	—	2	—	2
S. typhimurium 41	—	—	1	1
S. kedougou	—	1	—	1
S. infantis	—	1	—	1
S. uganda	—	1	—	1
S. stanley	1	—	—	1
S. agona	—	—	1	1
– rough strain	1	—	—	1
Totals	15	18	13	46

https://doi.org/10.1017/S0022172400061520 Published online by Cambridge University Press

Table 3. *Salmonella serotypes isolated from incoming drains, effluent and sludge from sewage works in town 'A' on a weekly basis in August and September* 1983

Week number	Drain ex abattoir	Drain B	Drain S	Drain T	Drain W	Effluent	Sludge
1	Negative	Negative	Negative	*S. indiana* *S. montevideo* *S. virchow*	*S. bredeney*	*S. typhimurium* 12 *S. indiana* *S. java* 1 var. 3	*S. indiana* *S. typhimurium* 12
2	Negative	*S. typhimurium* 195 *S. eimsbuettel* *S. saint-paul* *S. virchow* *S. heidelberg*	*S. infantis*	*S. virchow* *S. concord* *S. java* 1 var. 3	*S. agona* *S. virchow*	*S. kedougou* *S. eimsbuettel* *S. brandenburg*	*S. concord* *S. typhimurium* 110 *S. brandenburg*
3	*S. indiana* *S. typhimurium* 124	*S. worthington*	*S. brandenburg* *S. panama* *S. eimsbuettel*	*S. concord* *S. virchow* *S. java*	*S. virchow*	*S. panama* Group D	*S. concord* *S. panama*
4	Negative	*S. meleagridis*	*S. salford* *S. eimsbuettel*	*S. java* 1 var. 3 *S. virchow*	*S. virchow* *S. bareilly* *S. minnesota*	*S. salford*	*S. panama*
5	Negative	*S. infantis*	*S. muenchen* *S. salford*	*S. eimsbuettel* *S. java* *S. panama*	Negative	*S. livingstone*	*S. muenchen* *S. indiana* Group C2 *S. java*
6	Negative	*S. virchow*	*S. newport* *S. typhimurium* *S. bredeney* *S. blockley*	*S. java*	*S. indiana*	*S. kedougou* *S. heidelberg*	Rough strain
7	Negative	*S. typhimurium* *S. worthington* *S. eimsbuettel*	*S. derby* *S. infantis*	No sample	*S. kottbus* *S. indiana*	*S. java* *S. kedougou* *S. concord*	*S. kedougou* *S. derby* *S. eimsbuettel*
8	Negative	*S. kapemba*	*S. kapemba*	*S. java*	No sample	*S. kapemba* *S. mbandaka*	*S. typhimurium* *S. kapemba*

S. montevideo was the most common serotype found, but this was mainly due to the large number of isolates from town C. Similarly the high rating for *S. newport* was entirely due to its having been regularly isolated from two sites only (towns E and F). On the other hand *S. virchow*, the second most common serotype, was recovered at least once from all but one town, while *S. typhimurium* was recovered at least once from every town.

There was no seasonal pattern to the isolations, with the total number of serotypes being recovered on each sampling occasion varying from 6 to 13. Neither was there any seasonal trend in individual serotypes, with the most common ones varying from 1 to 3 at each sampling.

(*b*) *Abattoir drains*. The serotypes identified and the frequency with which they were recovered from drains in towns A, C and D are shown in Table 2. *S. montevideo* accounted for fully 50% of the isolations and was especially common in abattoirs A and C. Most other serotypes, including *S. typhimurium*, were sporadic.

(*c*) *Sewage works in town A*. A total of 25 serotypes were present in the incoming drains, 13 (plus an unidentified group D) in the effluent and 11 (plus a rough strain and an unidentified group C2) in the sludge, with 8 being common to all three categories (Table 3). The most striking feature was the low incidence of isolates in the abattoir drains compared with those from other parts of the town. *S. java* was regularly recovered from drain T but not any other drains, suggesting an ongoing localized source of the infection within the town. *S. virchow* was the most commonly isolated from the inlets, but was completely absent from effluent and sludge. On the other hand *S. kedougou* was recovered from effluent and sludge on four occasions, but not from any of the Moore's swabs.

DISCUSSION

The large number of salmonella serotypes found in the sewage sludge agrees with the findings of other workers and confirms the potential hazard to animals from applying this to pasture land. This emphasizes the need to observe the guidelines on the length of time to elapse before grazing treated pastures laid down by the DOE/NWC (1981) and the Scottish Agriculture Colleges, (1981). A period of 3 weeks has been recommended for all livestock for treated sludge and up to 6 months for untreated sludge, but the longer period is mainly for tapeworm eggs rather than for pathogenic bacteria. In the case of cattle producing milk which is not to be pasteurized the 'lay off' period is recommended by the DOE/NWC to be 5 weeks.

In the Borders Region there was little evidence of spread of salmonellae to animals following the application of sludge. During the period under review, of 51 incidents of salmonellosis in animals investigated by the St Boswells Veterinary Investigation Centre, 38 were due to *S. typhimurium*, 7 to *S. montevideo*, 3 to *S. dublin* and 1 each to *S. heidelberg*, *S. hadar* and *S. virchow*. Therefore of the 34 serotypes recovered in the sewage sludge in the Region (Tables 1 and 3) only 5, *S. typhimurium*, *S. montevideo*, *S. heidelberg*, *S. hadar* and *S. virchow* also appeared in the animals. This tends to support the view that the procedure of spreading sludge on land presents little risk to the animals of salmonellosis (Reilly *et al.* 1981) provided the guidelines are followed.

However, *S. typhimurium* phage type 12 was confirmed in 11 animal incidents,

4 of which occurred on neighbouring farms near town A. As the same phage type was recovered from sludge from town A which was being spread on pasture on these farms, it is highly likely that this was the source of their infections.

Not only is sewage sludge potentially dangerous but also effluent can be a source of infection. For example in town A the effluent flowed into a river. Downstream from the sewage works sheep which drank from the river succumbed to *S. typhimurium* phage type 12 which had been recovered from the effluent. This was exacerbated by a period of drought which had the dual effects of decreasing the flow of water in the river and increasing the thirst of the sheep.

Of the 34 serotypes recovered from sewage only 10 were also isolated directly from man in the Borders Region during the study period, namely *S. enteritidis* phage type 8 (48 isolations), *S. typhimurium* types 10, 12 and 110 (17), *S. saint-paul* (8), *S. stanley* (4), *S. heidelberg* (3), *S. indiana* (2), *S. montevideo* (2), *S. virchow* (2) and single isolates of *S. newport* and *S. bredeney*. Only *S. virchow* and *S. stanley*, which were among the top six human serotypes recorded in Scotland, were identified from persons resident in towns A and D, respectively, coincidental with the sewage isolates. Such findings, however, represent only a small proportion of human infection prevalent in the community. Conversely, of the other 24 serotypes isolated from sludge but not from man, only 2 namely *S. minnesota* and *S. takoradi*, were not reported from any other source in Scotland, with the former never previously have been recorded at any time. Surprisingly *S. enteritidis*, a common serotype in man, was recovered on only two occasions from sewage throughout the survey.

S. montevideo was by far the most common serotype present in the three abattoirs monitored (Table 2). This is especially reflected in town C, which also had the highest recovery rate of *S. montevideo* in the sewage sludge (Table 1). This serotype has caused outbreaks of disease in animals throughout much of eastern Scotland during the past 15 years (Sharp *et al.* 1983) and has become an important cause of abortion in sheep (Linklater, 1983). In spring 1982 it was associated with especially heavy losses in flocks in South-east Scotland (Miller & Linklater, 1985) and it is likely that the high recovery rate from the abattoirs is a direct reflection of this.

Otherwise the range of serotypes coming from the abattoirs into the sewage appears to be low, and the intensive study in sewage works A confirmed this. The background of salmonella infection in the animal population therefore appears to be low in comparison to that in humans, as reflected in isolates from sewage especially in the range of serotypes identified. The value of monitoring sewage as an index of human infection is again demonstrated.

The co-operation of the staff of the Borders Regional Council Water and Drainage Services who arranged for the collection of the sewage samples, and of colleagues in the Animal Health Division of the Department of Agriculture for Scotland who helped with the monitoring of the abattoir effluent is gratefully acknowledged. We are also grateful to Mr F. Jones, North West Water Authority, Warrington and Mr C. Weaver, Ministry of Agriculture, Veterinary Investigation Centre, Thirsk for helpful advice on techniques for the isolation of salmonellae from sewage sludge. Typing was expeditiously carried out by Mrs J. Hall.

REFERENCES

DOE/NWC (1981). Standing Committee on the Disposal of Sewage Sludge, 1981. *Report of the Sub-committee on the Disposal of Sewage Sludge to Land*. Standing Technical Committee Report No. 20, National Water Council, London.

EDEL, W. & KAMPELMACHER, E. H. (1969). Salmonella isolation in nine European laboratories using a standardised technique. *Bulletin of World Health Organisation* **41**, 297–306.

EDEL, W. & KAMPELMACHER, E. H. (1973). The comparative studies of the isolation of sublethally injured salmonellae in nine European laboratories. *Bulletin of World Health Organisation* **48**, 167–174.

LINKLATER, K. A. (1983). Abortion in sheep associated with *Salmonella montevideo* infection. *Veterinary Record* **112**, 372–374.

MILLER, J. K. & LINKLATER, K. A. (1985). Clinical and epidemiological features of a series of outbreaks of ovine abortion due to *Salmonella montevideo* in one season. *Veterinary Record*. In preparation.

REILLY, W. J., FORBES, G. I., PATERSON, G. M. & SHARP, J. C. M. (1981). Human and animal salmonellosis in Scotland associated with environmental contamination. *Veterinary Record* **108**, 553–555.

SCOTTISH AGRICULTURAL COLLEGES (1981). *Disposal of Sewage Sludge on Agricultural Land*. Publication No. 76, March 1981.

SHARP, J. C. M., REILLY, W. J., LINKLATER, K. A., INGLIS, D. M., JOHNSTON, W. S. & MILLER, J. K. (1983). *Salmonella montevideo* infection in sheep and cattle in Scotland 1970–81. *Journal of Hygiene* **90**, 225–232.

WRAY, C. (1975). Survival and spread of pathogenic bacteria of veterinary importance within the environment. *The Veterinary Bulletin* **45**, 543–550.