195

Seagulls (*Larus* spp.) as vectors of salmonellae: an investigation into the range of serotypes and numbers of salmonellae in gull faeces

By D. R. FENLON

Bacteriology Division, North of Scotland College of Agriculture, 581 King Street, Aberdeen AB9 1UD

SUMMARY

Of 1242 samples of seagulls faeces examined, 12·9% were found to contain salmonellae. The number of positive samples was significantly higher (17–21%) near sewage outfalls. Twenty-seven serotypes were isolated, including a new serotype named *Salmonella grampian*. The range and frequency of serotypes carried by gulls was similar to those in the human population, suggesting sewage as a possible source of gull infection.

The number of salmonellae found in positive samples was low ($0·18–191 \text{ g}^{-1}$ faeces). This was similar to the numbers found in sewage, $10–80 \text{ l}^{-1}$, suggesting gulls may only carry infected material without infecting themselves. Antibiotic resistance in the isolates was low, only 21 showing resistance to the antibiotics tested, although most of these were determined by resistance transfer plasmids.

INTRODUCTION

For some time birds have been considered as vectors of salmonellosis. However, studies have shown that most species present little hazard, with carrier rates of less than 1% (Wilson & MacDonald, 1967; Plant, 1977), although higher rates can occur during epidemics (Macdonald & Cornelius, 1969). One group of apparently healthy birds, the gulls (*Larus* spp.) have shown carrier rates consistently higher than other species. For example, in a variety of studies salmonellae have been isolated from faeces at rates varying from 7 to 31% (Fennell, James & Morris, 1974; Williams, Richards & Lewis, 1976).

Gull species, particularly the herring gull (*Larus argentatus*), the black-headed gull (*Larus ridibundus*), and the less frequent more solitary great black-backed gull (*Larus marinus*) readily become adapted to feeding on urban waste (Hickling, 1967), and all show a tendency to scavenge for food at rubbish tips and sewage outfalls (Vernon, 1970, 1972). After feeding the birds roost in nearby fields (Vernon, 1970), where they rest, preen and defaecate. These fields are usually well grazed, thus providing for the birds' instinctive requirements for good all-round vision, enabling them to see approaching predators and be seen by other gulls (Hickling, 1967). These pastures can therefore be contaminated by faecal organisms and provide a possible source of infection to grazing livestock (Williams *et al.* 1977).
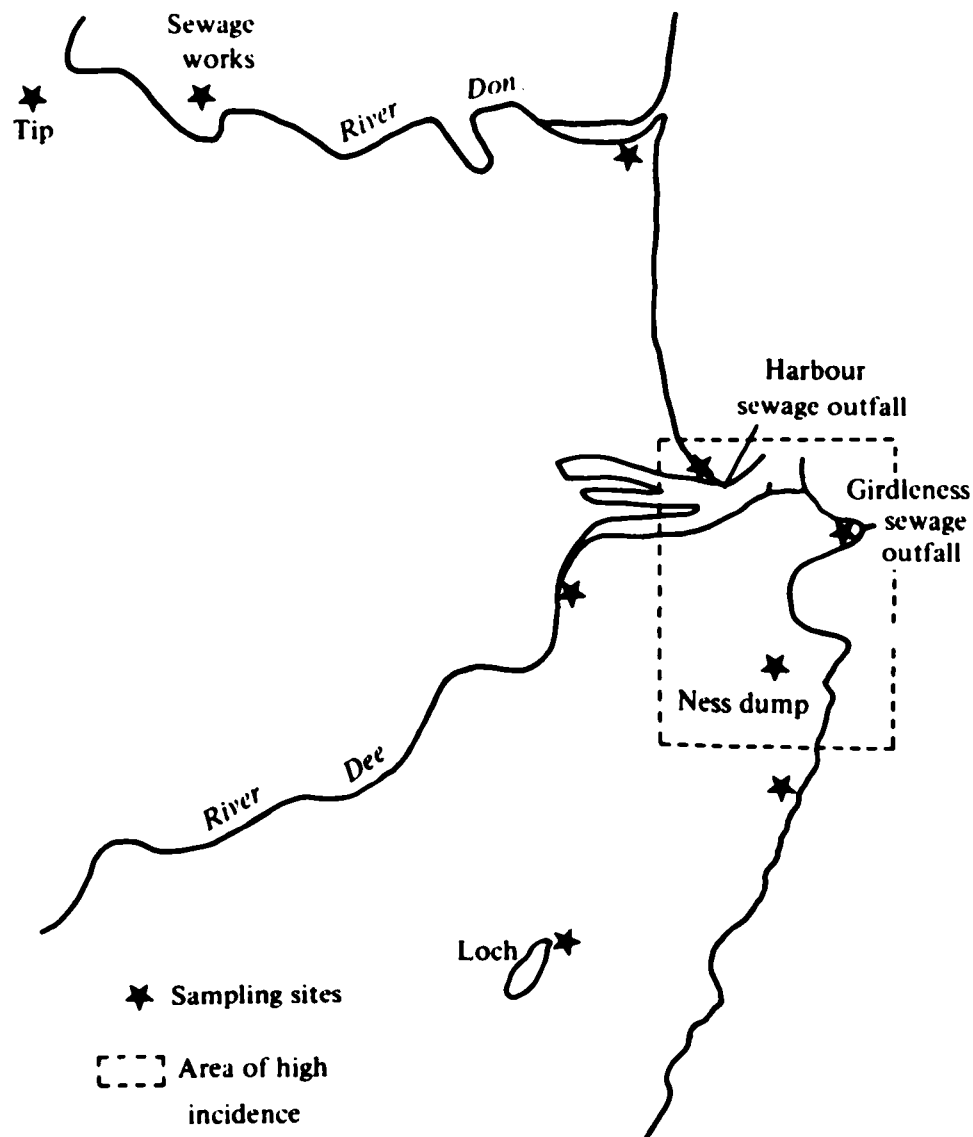
Fig. 1. Map of survey area showing sampling sites.

In the north of Scotland there is a very large gull population with ready access to agricultural land, which is confined principally to coastal areas. Untraceable cases of salmonellosis in cattle and sheep, especially isolated incidents involving 'exotic' serotypes, in an area with very little importation of livestock, have thrown suspicion on gulls as the vectors (Johnston, Maclachlan & Hopkins, 1979; CDS Salmonellosis Annual Summary of Isolations, 1975, 1976, 1977, 1978).

The purpose of the work reported here, conducted in the Aberdeen area over a twelve-month period commencing January 1979, was to determine the carrier rate, serotypes, numbers and probable sources of salmonella in gulls, and their role in the cycle of infection in the environment.

MATERIALS AND METHODS

Flocks of gulls at the sampling sites shown in the map of the survey area (Fig. 1) were disturbed and fresh individual faecal samples collected using sterile 28 ml wide-necked universal containers and sterile cotton swabs. The isolation procedure is shown in Fig. 2.

Positive isolates were confirmed using the API 20E Microtube system (API Laboratory Products Ltd, Farnborough, Hampshire) before sending to the

Faecal sample

Pre-enrichment 20 ml nutrient broth 24 h, 37 °C

2 ml

20 ml Muller Kauffman
tetrathionate broth
(Oxoid)

20 ml Selenite
broth
(Oxoid)

24 + 48 h
43 °C

Brilliant green agar
(Difco)

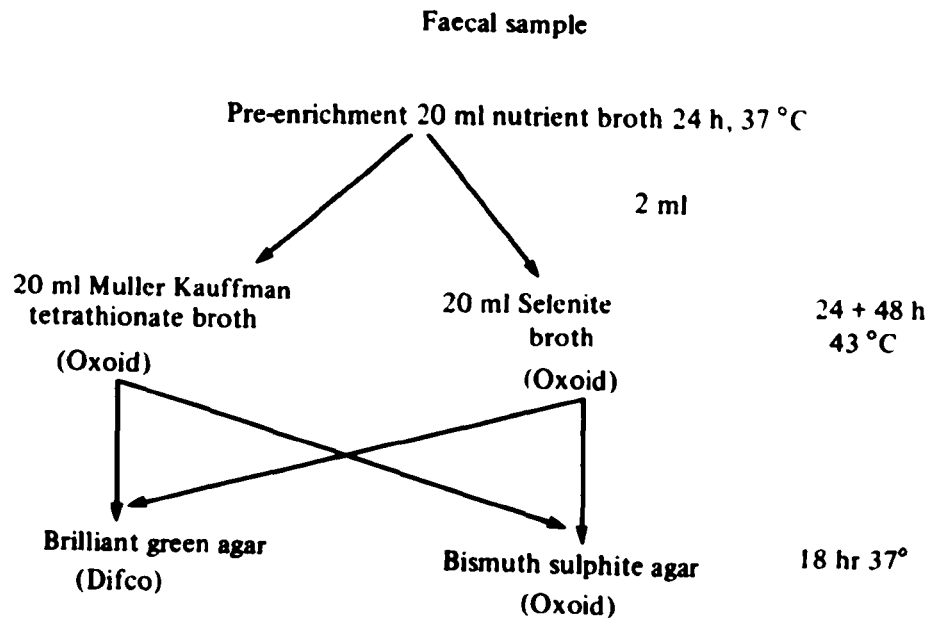Bismuth sulphite agar
(Oxoid)

18 hr 37°

Fig. 2. Procedure for isolation of salmonellae from gull faeces. Typical colonies were confirmed by slide agglutination test with polyvalent H antiserum (Wellcome Reagents Ltd, Beckenham, England).

Salmonella Reference Laboratory, Stobhill General Hospital, Glasgow for sero-typing.

The number of salmonellae per g gull faeces was estimated using a five-tube Most Probable Number technique (*Standard Methods*, 1967) on suspensions of individual droppings from sites known to have a high incidence of carriers. Dilutions made were then treated as in Fig. 2 except that Muller Kauffman was the sole enrichment medium. Sewage was examined as a possible source of infection and counts to determine the numbers of salmonellae present were made in the same way.

Antibiotic sensitivity of the isolates was assessed using Multodisk 3866E (Oxoid). Isolates were tested for resistance to sulfamethoxazole/trimethoprim 25 μg; chloramphenicol 10 μg; streptomycin 10 μg; tetracycline 10 μg; neomycin 10 μg; ampicillin 10 μg; and furazolidone 15 μg on iso-sensitest agar (Oxoid).

R factor transfer was carried out as described by Linton, Howe & Osborne (1975) using as recipients nalidixic-acid-resistant mutants of *S. agona*, *S. brandenberg* and *S. muenchen*, which were sensitive to the test antibiotics. These were prepared by exposure of a shallow suspension of the organisms, in 1/4 strength Ringers', to a source of u.v. irradiation at 254 nm (Camag Universal u.v. Lampen type TL900/U, Muttenz, Schweiz) at a distance of 10 cm for 30 s. A 10^{-1} dilution of the irradiated suspension in nutrient broth was incubated at 37° for 2 h to enable recovery, then 0·1 ml was spread on an iso-sensitest agar plate containing 20 μg ml^{-1} nalidixic acid (NX) and incubated for 18 h at 37°. Colonies were then picked off and plated on iso-sensitest agar containing 40 μg NX. These cultures were used as recipients in the resistance transfer tests. After incubation of the donor/recipient mixture 0·1 ml was spread on iso-sensitest agar supplemented with 40 μg ml^{-1} NX, on which, when dry, a 3866E Multodisk was placed. Transmitted resistance was shown by colonies in the zones of clearing around the tips of the multodisk. These were picked off and confirmed for transmitted resistance.

Table 1. *Salmonellae isolated from gulls compared with human and veterinary (cattle and sheep) serotypes isolated during* 1978

Serotype	Gull survey (Number of isolations)	Ranking order of serotypes		
		Gulls	Humans, 1978	Cattle and sheep, 1978
S. agona*	25	1	2	8
S. anatum*	3	—	12	11
S. braenderup*	1	—	—	—
S. brandenberg	3	—	—	—
S. bredeney	2	—	—	—
S. chester*	2	—	—	—
S. cubana	2	—	—	—
S. derby*	6	10	—	—
S. give	2	—	—	11
S. goldcoast	1	—	—	—
S. grampian†	3	—	—	—
S. hadar*	10	4	3	—
S. heidelberg*	4	11	4	3
S. infantis*	10	4	7	6
S. kedougou	1	—	—	—
S. lille	1	—	—	—
S. london	1	—	—	—
S. muenchen*	10	94	6	—
S. panama*	20	2	9	—
S. saint paul*	9	8	11	—
S. san diego	4	11	—	—
S. tennessee	2	—	—	—
S. thompson	8	8	12	6
S. typhimurium*	16	3	1	1
S. virchow*	10	7	8	—
S. worthington	3	—	—	11
S. 13, 23; gt,-	1	—	—	—

* Isolated from humans in Grampian area, 1979.

† New serotype found during survey antigenic structure 6, 7; r, lw.

RESULTS

A total of 1242 individual faecal samples were tested and 160 (12·9%) were positive for salmonellae. A list of the serotypes and their frequency of isolation is shown in Table 1. Also shown are the serotypes found most frequently in humans, cattle and sheep in Scotland during 1978 (Communicable Diseases Scotland, 1978). Within the area of study there was a wide variation in the carriage of salmonellae, which appears to be related to the probable food source available to the gulls (Table 2). The results of counts on raw and treated sewage and sea water near a large untreated sewage outfall are shown in Table 3.

Individual gull faeces samples were examined to determine the number of salmonellae per g; 14 were positive and the populations ranged from 0·18 to 191 salmonellae g⁻¹ (mean 39·7, s.d. 52·7).

Most isolates were sensitive to all antibiotics, but 21 were resistant to one or more of the following: chloramphenicol, streptomycin, neomycin, tetracycline or

Table 2. *Distribution of gulls carrying salmonellae in relation to nearest feeding site*

Sampling site	Percentage carrying salmonellae (no. of samples tested)	Probable food source
Girdleness and Ness dump area	21·1 (431)	Dumps, sewage outfall
River Don	6·5 (299)	Agriculture, sewage works
River Dee	8·3 (234)	Fish processing and urban litter
Tip	2·1 (95)	Domestic rubbish only
Harbour	16·7 (48)	Sewage outfall

Table 3. *Numbers of salmonellae and serotypes isolated from sewage*

	Salmonellae (no. l^{-1})	Serotypes
Raw sewage	80	*S. agona, brandenberg, bredeney*
Settled sewage	80	*S. virchow, thompson*
Treated sewage	20	*S. agona*
Sea water at outfall	10–50	*S. agona, virchow*

Table 4. *Antibiotic resistance and transmissible R factors in salmonella serotypes isolated from gulls*

Serotype	Antibiotic resistance	R factors
S. typhimurium	C, S, T, N, A	C, S, T, S, N, A
S. typhimurium	S, T, N	S, T
S. typhimurium	S, T	S, T
S. typhimurium (× 2)	S	S
S. typhimurium	S	—
S. typhimurium	T	T
S. derby (× 5)	T	T
S. give (× 2)	S, T, N	S, T, N
S. panama (× 2)	T	T
S. agona	T	T
S. agona	T	—
S. chester	S, T	S, T
S. hadar	S	S
S. virchow	S, T	—

Key: C, Chloramphenicol; S, Streptomycin; T, Tetracycline; N, Neomycin; A, Ampicillin.

ampicillin. The results of the antibiotic sensitivity tests of those serotypes showing resistance are shown in Table 4, together with those showing transmissible resistance.

DISCUSSION

The evidence for the transmission of salmonellae by gulls to farm livestock is now very strong (Johnstone *et al.* 1979; Williams *et al.* 1977). The major questions arising are how the serotypes involved in livestock infections compare with those

infecting gulls, and whether the levels of salmonellae carried can provide a sufficiently high infective dose for farm animals.

Of the eighteen serotypes isolated from human cases in the Grampian area (as reported to the CDS unit Glasgow by Aberdeen City Hospital, personal communication), thirteen (72 %) were also found in gulls' faeces during the same period. Also the types in gulls were similar in range and frequency to those isolated from human cases in Scotland the previous year. McCoy (1979) stated that serotypes in the human population tend to be found in sewage sludges. The interpretation may be that the gulls' habit of feeding at sewage outfalls results in similar serotypes infecting both the human and gull populations. The serotypes involved in incidents where gulls are suspected of being vectors of salmonellosis (S. agona, S. panama, S. typhimurium, S. heidelberg, S. brandenberg and S. give) fall mainly into the same category. The ability of gulls to provide the infective dose required by cattle or sheep to acquire salmonellosis is much more difficult to explain.

The birds seen at the sampling sites showed no obvious signs of disease, although Barnes (1979) reported that poultry chicks can harbour 10^8 S. typhimurium g^{-1} of caecal contents with no clinical symptoms. However, none of the birds sampled carried numbers near those levels. The range found (0·18–191 salmonellae g^{-1} faeces) compares more nearly with the levels found in sewage (10–80 salmonellae l^{-1}), suggesting that gulls are indeed only vectors transferring the organism from one place to another.

The absence of the high numbers associated with the true sub-clinical carrier state in the gulls sampled may be explained by the rapid progression of food through the bird gut, together with the growing evidence (Barnes, 1979) that the caecal contents of the healthy adult bird can prevent infection by salmonellae.

The numbers of salmonellae required to infect healthy cattle and sheep appear high. Taylor & Burrows (1971), using an avirulent S. dublin, showed that 4 of 6 calves excreted the organism when feeding on grass containing 10^4–10^5 salmonellae g^{-1}, but none was infected by doses of half this level. Studies using S. infantis (Browne, Gross & Smith, 1977) to infect sheep showed that whereas a dose of 2×10^{11} gave overt clinical symptoms, 2×10^{10} organisms produced symptoms in only 2 of 6 sheep and, perhaps significantly, these two had only recently been moved to their pens and therefore were subject to more stress. Spence & Westwood (1978) successfully infected sheep with doses of 10^9 S. agona having first starved them for 24 h. The authors stated that in their experience the stress of modern husbandry largely determines the infective dose and the severity of the disease.

The number of salmonellae carried by gulls is very low compared to the infective doses shown experimentally to be required to cause disease in the healthy animal. The effect of even a large roosting flock of gulls on a pasture, for example, 500 birds each excreting 5 g faeces containing 200 salmonellae g^{-1}, would be small, 5×10^5 organisms in total.

Low infective doses have been reported. Brownlie & Grau (1967) have shown that starvation of cattle for 2–3 days enhances the ability of salmonellae to colonize the rumen, whereas when normal feeding was uninterrupted added salmonellae were rapidly eliminated. Smith (1977) working with S. lomita reported that

8×10^3 organisms given to sheep starved for 48 h colonized the gut and were excreted at levels of $10^4–10^6$ g^{-1} of faeces. A second animal given a dose of 4.4×10^2 *S. typhimurium* after 48 h starvation developed clinical disease and died 6 days later with 10^9 salmonellae g^{-1} faeces.

This suggests that stress or starvation must play an important role if the infection of farm livestock by gulls is to take place. The higher frequency of salmonellae in gulls near untreated sewage outfalls, and the similarity in the serotypes involved, suggest sewage as a probable source, other food sources, for example domestic (bin) rubbish being of little danger. Therefore pastures frequented by gulls which feed at sites where sewage is accessible may be contaminated by low levels of salmonellae. These could cause infection in a susceptible animal, which once infected is more likely to be an infective agent for other animals and gulls, because of its higher excretion rate.

The level of antibiotic resistance in the salmonellae isolated from gulls was lower than that found in isolates from farm animals (Sojka, Wray & Hudson, 1977). Gulls are therefore unlikely to be a major source of infection with multiple resistant strains, but the ability to receive and transmit resistant plasmids is an ever-present danger.

REFERENCES

BARNES, E. M. (1979). The intestinal microflora of poultry and game birds during life and after storage. *Journal of Applied Bacteriology* **46**, 407–19.

BROWNE, D. D., GROSS, J. G. & SMITH, A. F. G. (1977). Experimental infection of sheep with *Salmonella infantis*. *British Veterinary Journal* **133**, 435–41.

BROWNLIE, L. E. & GRAU, F. H. (1967). Effect of food intake on growth and survival of salmonellas and *Escherichia coli* in the bovine rumen. *Journal of General Microbiology* **46**, 125–4.

COMMUNICABLE DISEASES SCOTLAND. Salmonellosis summary of isolations 1975, 1976, 1977 and 1978. CDS Unit, Ruchill Hospital, Glasgow.

FENNELL, H., JAMES, D. B. & MORRIS, J. (1974). Pollution of a storage reservoir by roosting gulls. *Water Treatment and Examination* **23**, 1, 5–20.

HICKLING, R. A. O. (1967). The inland wintering of gulls in England, 1963. *Bird Study* **14**, 104–13.

JOHNSTON, W. S., MACLACHLAN, G. K. & HOPKINS, G. F. (1979). The possible involvement of seagulls (*Larus* sp.) in the transmission of salmonella in dairy cattle. *Veterinary Record* **105**, 526–7.

LINTON, A. H., HOWE, K. & OSBORNE, A. D. (1975). The effect of feeding tetracycline, nitrovin and quindoxin on the drug resistance of coli-aerogenes bacteria from calves and pigs. *Journal of Applied Bacteriology* **38**, 255–75.

MACDONALD, J. W. & CORNELIUS, L. W. (1969). Salmonellosis in wild birds. *British Birds* **62**, 28–30.

McCOY, J. (1979). The risks to public health from pathogens in sewage sludges. In *Proceedings of the WRC Conference on Utilization of Sewage Sludge on Land*, pp. 191–7. Stevenage: WRC.

PLANT, C. W. (1977). Salmonellosis in wild birds feeding at sewage treatment works. *Journal of Hygiene (Cambridge)* **81**, 43–8.

SMITH, M. G. (1977). Transfer of R factors from *Escherichia coli* to salmonellas in the rumen of sheep. *Journal of Medical Microbiology* **10**, 29–35.

SOJKA, W. J., WRAY, C. & HUDSON, E. B. (1977). A survey of drug resistance in salmonellae isolated from animals in England and Wales during 1973 and 1974. *British Veterinary Journal* **133**, 292–311.

SPENCE, J. B. & WESTWOOD, A. (1978). *Salmonella agona* infection in sheep. *Veterinary Record* **102**, 332–6.

Standard Methods for the Examination of Water and Waste Water, 12th ed. (1967). New York: American Public Health Association Inc.

TAYLOR, R. J. & BURROWS, M. R. (1971). The survival of *Escherichia coli* and *Salmonella dublin* in slurry on pasture and the infectivity of *S. dublin* for grazing calves. *British Veterinary Journal* **127**, 536–42.

VERNON, J. D. R. (1970). Feeding habits and food of the black-headed and common gulls. 1. Feeding habits. *Bird Study* **17**, 287–96.

VERNON, J. D. R. (1972). Feeding habits and food of the black-headed and common gulls. 2. Food. *Bird Study* **19**, 173–86.

WILLIAMS, B. M., RICHARDS, D. W. & LEWIS, J. (1976). Salmonella infection in the herring gull. *Veterinary Record* **98**, 51.

WILLIAMS, B. M., RICHARD, D. W., STEPHENS, D. P. & GRIFFITH, T. (1977). The transmission of *Salmonella livingstone* to cattle by the herring gull (*Larus argentatus*). *Veterinary Record* **100**, 45.

WILSON, J. E. & MACDONALD, J. W. (1967). Salmonella infection in wild birds. *British Veterinary Journal* **123**, 212–18.