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The incidence and significance of salmonella carriage by gulls (*Larus* spp.) in Scotland

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SUMMARY

Salmonella carriage in 5888 gulls sampled by cloacal lavage was found to be 7·8%. Marked geographical and seasonal differences in carriage rates were found. These differences appeared to be associated with human population density and seasonal differences in the reported incidence of human salmonellosis. The maximum duration of salmonella excretion in 17 laboratory-maintained gulls was 4 days and the number of salmonellae excreted was never more than 170 per gram of faeces. On the basis of this study it is suggested that gulls are not important factors in the aetiology of human salmonellosis.

INTRODUCTION

Intestinal carriage of salmonellae by wild birds has been recognized for many years (Shirlaw & Iyer, 1937), and although the percentage carrier rate detected in most species has been low – less than 5% in most cases (Faddoul, Fellows & Baird, 1966; Wilson & MacDonald, 1967) – one group of birds (the *Larus* gulls) regularly has been shown to have relatively high carrier rates of 7–31% (Edel *et al.* 1976, 1978; Fenlon, 1981, 1983). In addition, other infectious agents such as *Taenia saginata* ova and campylobacters have been recovered from seagull faecal material (Crewe, 1967; Skirrow & Benjamin, 1980; Fricker, Girdwood & Munro, 1983*a*).

The population of gulls in Scotland has increased dramatically during most of this century and is now believed to be several millions. In particular, the population of the herring gull (*Larus argentatus*) has been increasing at a rate of 13% per annum, resulting in a doubling of the population every six years (Chabrzyk & Coulson, 1976). These increases in the gull population have resulted in the utilization of new feeding and nesting sites, often in close proximity to man and his domestic animals and have accentuated concern over the role of gulls in the transmission and dissemination of pathogenic agents (Monaghan, 1983). These

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changes in gull ecology suggest that such problems as these birds may create are likely to increase.

Pollution of water-storage reservoirs by roosting gulls has presented a major problem to some Water Authorities (Fennell, James & Morris, 1974; Benton *et al.* 1983). In addition, gulls have been implicated in the passive transmission of salmonellae to domestic animals and 13 such incidents were reported to the Communicable Diseases (Scotland) Unit between 1978 and 1980 (CDS, 1981). Furthermore two discrete incidents of bovine salmonellosis possibly transmitted by seagulls have been described (Williams *et al.* 1977; Johnston *et al.* 1981).

Previous studies of salmonella carriage in gulls have relied upon isolation by culture of faecal deposits or cloacal swabs (Williams, Richards & Lewis, 1976). Collection of faecal deposits from contaminated areas such as refuse tips and sewage-treatment plants could result in contamination of the faeces, and conversely if faeces are not collected soon after deposition a combination of desiccation, exposure to ultraviolet light and low pH (due to the presence of uric acid in the faecal material) may reduce the number of recoverable salmonellae. In addition, since individual gulls may leave several faecal deposits, the prevalence of salmonella carriage cannot be determined reliably. The isolation of salmonellae by culture of cloacal swabs allows the results to be related to individual gulls of known species, sex and age, although the efficiency of this method of collection of material in relation to intestinal carriage is not known.

The techniques used for the culture of environmental samples for the recovery of salmonellae markedly affect the results (Vassiliadis *et al.* 1978; Harvey & Price, 1982; Harvey, Price & Xirouchaki, 1979; Vassiliadis, 1983; Fricker, 1984*a*, *b*) and thus efficient techniques for the culture of seagull faecal material are required. Previous reports on avian carriage of salmonellae have described a variety of isolation techniques (Faddoul, Fellows & Baird, 1966; Plant, 1978; Sharma, Sethi & Singh, 1980; Fenlon, 1981; Butterfield *et al.* 1983) and therefore comparison of results is complicated by the differences in the efficiencies of the different protocols. We have reported previously on the differences in efficiency of enrichment broths (Fricker, Girdwood & Munro, 1983*b*) and solid media (Fricker & Girdwood, 1984) in recovering salmonellae from seagull faecal material. The optimum protocols recommended in these papers have been adopted for the work reported here.

In an attempt to elucidate the role of gulls in human and animal infection this communication presents our findings from a two-year study of salmonella carriage in gulls. Data are presented for gulls captured in different localities, at different times of year and for different species. In addition, data on the duration of salmonella carriage in captive birds and on the prevalence of salmonellae in seagull eggs and laboratory-reared chicks are reported. Information on the effects of age and sex of gulls, together with their feeding ecology, on the rate of salmonella carriage will be reported elsewhere (Monaghan *et al.* 1985).

MATERIALS AND METHODS

Gull sampling

During the period February 1982 to February 1984, 5324 herring gulls, 360 lesser black-backed gulls (*Larus fuscus*) and 204 black-headed gulls (*Larus ridibundus*)

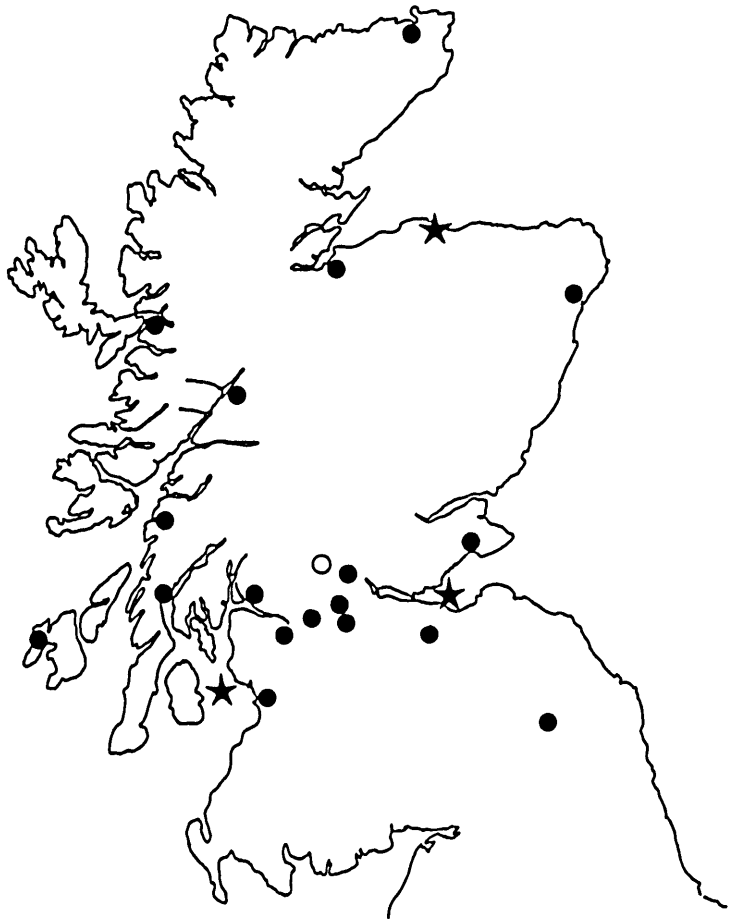

Fig. 1. The areas from which gulls were sampled for salmonella carriage. ●, Sites where gulls were captured by cannon-netting. ★, Breeding colonies from which gulls culled in control measures were obtained. ○, Breeding colony from which eggs were obtained.

were captured by cannon netting in a variety of localities throughout Scotland (Fig. 1). The herring and lesser black-backed gulls were caught at refuse tips; 59% of the black-headed gulls were caught at tips and the remainder in fields.

Faecal samples were obtained from individual birds by cloacal lavage (Fricker, 1983) and in some cases (847) by both cloacal lavage and cloacal swab. A further 401 herring gulls and 503 lesser black-backed gulls were obtained from culls carried out by the Royal Society for the Protection of Birds and the Ministry of Agriculture, Fisheries and Food during control measures (Fig. 1). The entire gut (excluding the gall-bladder) was removed from these birds for microbiological examination within 12 h of collection.

Culture procedures

Cloacal lavage samples and cloacal swabs were placed in 20 ml of buffered peptone water (BPW) and transported back to the laboratory. For the examination of dead birds the gut was cut into pieces approximately 3 cm in length and placed

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into 100 ml of BPW. In an attempt to prevent cross-contamination between carcasses five sets of dissecting scissors were used in rotation. The sets which were not in use were stored in Clearsol 2%. Sterile disposable gloves were used and changed after processing batches of five gulls. The operators' gloved hands were rinsed in Clearsol 2% after processing each bird. All pre-enrichment cultures were incubated aerobically at 37 °C for 24 h. Investigations to determine optimum isolation techniques for salmonellae from gull faecal material were carried out early in the study and the results of these experiments have been reported elsewhere (Fricker, Girdwood & Munro, 1983*b*; Fricker & Girdwood, 1984). The technique which was adopted was: pre-enrichment in BPW, enrichment in Rappaport–Vassiliadis (RV) medium (inoculation ratio 1:100) and plating at 24 and 48 h on brilliant green agar (Oxoid CM 329) containing sulphamandelate supplement (Watson & Walker, 1978) and Hynes's deoxycholate citrate agar (Oxoid CM 227). Plates were examined after 24 h incubation at 37 °C and up to four presumptive salmonella colonies indentified by standard biochemical and serological procedures.

Investigation into the efficiency of cloacal swabs and cloacal lavage in detecting salmonella carriage in gulls

Cloacal swabs were taken from 456 of the dead gulls and placed into 20 ml of BPW. The entire gut was then removed from each bird and cultured as described above. The results of culture of the swab and entire gut were then compared for each bird.

A total of 847 gulls captured in the field were sampled by cloacal swabbing followed by cloacal lavage and the samples obtained cultured separately. Cloacal lavage was performed by inserting a plastic Pasteur pipette containing about 2 ml of BPW into the cloaca, discharging the pipette and aspirating the cloacal contents.

Enumeration of salmonellae in gull faecal material and assessment of the duration of excretion

During the period of the study 84 herring gulls caught at refuse tips were taken into captivity and held individually in specially designed cages for periods up to 3 weeks. The gulls were fed daily on tinned pilchards and clean drinking water. Faecal deposits were collected from the bases of the cages twice daily. In order to prevent perpetuation of infection of the gulls by their ingestion of their own faecal material the sides of the cages were scrubbed daily and fresh cage bases were inserted daily. Used cage bases were scrubbed and steam-cleaned. Birds were transferred to totally steam-cleaned cages every 3 days. The deposits from each bird at each collection time were pooled and a weighed representative sample was suspended in BPW to give a final volume of 10 ml. Five 1:10 and five 1:100 dilutions were prepared in 10 ml of BPW and the number of salmonellae present per gram of faeces was calculated using a 15-tube most probable number (MPN) procedure based on the isolation protocol detailed above.

Examination of herring gull eggs and laboratory-reared chicks for the presence of salmonellae

One hundred and thirty-four seagull eggs were collected from a breeding colony at Flanders Moss in Central Scotland (Fig. 1) and cultured for salmonellae. The surface of the eggs was sterilized by immersion in alcohol followed by ignition of the surface spirit. The eggs were then broken into 100 ml of BPW and cultured in the same way as outlined above. In addition, a further 72 eggs were hatched in an incubator, the entire intestinal contents of the resultant chicks being removed at periods ranging from 6 h to 7 days and cultured in the same way as for the eggs.

Examination of refuse

Fifty-seven samples of materials (food scraps which gulls typically eat) found on the refuse tips were collected and placed into sterile containers. Approximately 10 g of refuse was placed into 100 ml BPW and incubated at 37 °C for 24 h. These cultures were then treated in exactly the same way as the seagull faecal material. In addition, 36 surface-water samples of 50 ml volume were processed in the same manner.

RESULTS

Efficiency of swabbing/lavage

Of the 456 gulls examined by culture of a cloacal swab and the entire gut, 71 were shown to be carrying salmonellae by examination of the entire gut. Only 44 (62 %) were positive by cloacal swabbing. All positive samples detected by cloacal swabbing were detected by culture of the entire gut. The efficiency of cloacal lavage against cloacal swabbing was compared in 847 gulls. Salmonellae were yielded in 91 samples by at least one method. Ninety were positive by lavage and 69 (77 %) by swabbing.

Isolation of salmonellae from gulls

Of the 5888 live gulls examined, 459 (7·8 %) were found to be carrying salmonellae at the time of sampling. Table 1 shows the number of live gulls of each species examined during the study and the proportion found to be carrying salmonellae. The overall percentage of lesser black-backed gulls carrying salmonellae was higher than either herring or black-headed gulls (Table 1). However, when the salmonella carriage rate in gulls of different species obtained by the same method and from the same site at the same time of year were compared, herring gulls had a significantly higher incidence of salmonella than lesser black-backed gulls (Table 2).

Seasonal variations in carriage rates were investigated by examining the incidence of salmonellae in herring gulls caught in the same area (the Clyde Estuary area) during each month of the year for which a sufficient sample was available (Table 3). Carriage rates were found to be highest during the autumn period between August and November, and low during the winter months (Table 3).

To examine geographical variation in salmonella carriage by gulls it was necessary to consider birds caught in different areas at the same time of year. Table

Table 1. *Number of live individuals of each gull species examined and the proportion found to be carrying salmonellae*

Gull species	No. examined	No. positive	Positive (%)
Herring gull	5324	410	7·7
Lesser black-backed gull	360	41	11·4
Black-headed gull	204	8	3·9
Total	5888	459	7·8

Table 2. *The proportion of herring and lesser black-backed gulls examined in the same area, and by the same isolation method, which were found to be carrying salmonellae (the difference between the two is significant:* ($\chi^2 = 7{\cdot}8$, 1 D.F., $P < 0{\cdot}01$))

Gull species	No. examined	No. positive	Positive (%)
Herring gull	163	62	38·0
Lesser black-backed gull	464	121	26·1

These data are based on gulls culled at Horse Island in the Firth of Clyde (Fig. 1) during late May.

Table 3. *Number of herring gulls examined from refuse tips in the Clyde area during each month of the year, showing the proportion found to be carrying salmonellae*

Month	No. examined	No. positive	Positive (%)
January	301	9	3·0
February	260	10	3·9
March	552	48	8·7
April	319	31	7·9
May	243	20	8·2
August	102	26	25·5
September	310	49	15·8
October	319	42	13·2
November	148	44	29·7
December	405	35	8·6

A total of only 26 birds was sampled during the months of June and July: three were found to be carrying salmonellae.

Table 4. *Number of herring gulls caught by cannon-netting in different areas between April and July which carried salmonellae*

Location	Capture site	No. examined	No. positive	Positive (%)
N.E. Scotland	Aberdeen	177	9	5·1
Highland Region	Fort William and Inverness	235	3	1·3
Clyde area	Bishopbriggs and Helensburgh	536	48	9·0

$\chi^2 = 16{\cdot}81$, 2 D.F., $P < 0{\cdot}001$.

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Table 5. *The proportion of herring gulls carrying salmonellae in different localities in Scotland (see Fig. 1) in different months of the year*

(The localities marked * denote sites from which culled birds were examined. At the other sites, the gulls were cannon-netted at refuse tips. For details of herring gulls examined in the Clyde area, see Tables 2 and 3.)

Location	Month	No. examined	No. carrying salmonellae	Gulls carrying salmonellae
Castletown, Caithness	Oct.	180	2	1·1
Kinloss, Morayshire*	June	196	6	3·1
Aberdeen	May	57	0	0·0
	July	120	9	7·5
	Dec.	179	4	2·2
Kircaldy	Jan.	124	0	0·0
	June	57	0	0·0
	Sept.	112	10	8·9
	Dec.	175	13	7·4
Inchmickery, Firth of Forth*	May	42	0	0·0
Penicuick, Midlothian	Feb.	192	5	2·6
Duns, Berwickshire	Feb.	160	2	1·3
Stirling	Mar.	121	15	12·4
	April	7	0	—
	July	11	3	—
Bowmore, Islay	June	70	4	5·7
	Oct.	107	4	3·7
	Nov.	76	0	0·0
Oban	Oct.	58	3	5·2
Fort William	June	114	1	0·9
	Dec.	18	1	—
Inverness	May	121	2	1·7
	Dec.	233	15	6·5
Plockton, Kyle of Lochalsh	June	47	0	0·0

Table 6. *Number of culled herring gulls in two different areas which carried salmonellae*

Location	Site	No. examined	No. positive	Positive (%)
N.E. Scotland	Kinloss	196	6	3·1
Clyde Area	Horse Island	163	62	38·0

$\chi^2 = 68{\cdot}64$, 1 D.F., $P < 0{\cdot}001$.

(Since only 42 birds were obtained from culls in the Forth area these have not been included. No salmonellae were isolated from these birds).

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Table 7. *The salmonella serotypes isolated from gulls during this study*

Salmonella serotype	Herring	Lesser	Black-headed	Total
S. agona	6	5	0	11
S. anatum	5	1	0	6
S. bareilly	1	1	0	2
S. braenderup	0	1	0	1
S. brandenburg	1	0	0	1
S. bredeney	29	20	0	49
S. derby	1	0	0	1
S. enteritidis	0	1	0	1
S. hvittingfoss	1	0	0	1
S. haardt	7	0	0	7
S. hadar	10	6	1	17
S. heidelberg	4	0	0	4
S. indiana	5	6	0	11
S. infantis	12	5	0	17
S. java	1	0	0	1
S. kedougou	0	1	0	1
S. livingstone	4	0	0	4
S. mbandaka	12	2	0	14
S. montevideo	10	3	0	13
S. muenchen	2	0	0	2
S. newport	13	2	1	16
S. panama	1	2	0	3
S. reading	0	1	0	1
S. saint-paul	4	1	0	5
S. schwarzengrund	1	0	0	1
S. stanley	11	4	0	15
S. thompson	0	1	0	1
S. typhimurium	124	51	5	180
S. virchow	215	56	1	272
S. virginia	1	0	0	1
4, 12: d	1	0	0	1
6, 7: r	1	0	0	1

Table 8. *The 'top ten' most frequently isolated salmonella serotypes obtained from gulls during this study compared with their ranking and frequency of isolation from humans, cattle and sheep*

	Seagull	Human	Cattle	Sheep
S. virchow	1 (271)	3 (571)	3 (78)	—
S. typhimurium	2 (175)	1 (2487)	1 (1002)	2 (83)
S. bredeney	3 (49)	9 (64)	—	—
S. infantis	4 (17)	—	—	—
S. hadar	5 (16)	—	—	—
S. meunchen	6 (15)	—	—	—
S. stanley	6 (15)	—	6 (9)	—
S. mbandaka	8 (14)	—	—	—
S. montevideo	9 (13)	—	6 (9)	—
S. agona	10 (11)	8 (65)	4 (28)	—

Figures in parentheses in the seagull column indicate the number of isolations made in this study and in the other columns indicate the numbers of isolates reported by the Communicable Diseases (Scotland) Unit during the same period (1982, 1983).

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Table 9. *Salmonella serotypes isolated from 11 of 57 refuse-tip debris samples and 19 of 36 surface-water samples, showing whether or not the same serotype was isolated from gulls captured on the same day as the samples were collected*

Salmonella serotype	Surface debris	Surface water	Isolated from gulls
S. agona	2	1	No
S. anatum	1	3	No
S. derby	3	0	No
S. newport	1	7	No
S. panama	0	2	No
S. stanley	0	5	No
S. typhimurium	4	0	Yes (2)
S. virchow	0	1	Yes (1)
Total	11	19	(3)

Figures in parentheses show the number of occasions when the same salmonella serotype was isolated from refuse-tip debris or surface-water and from gulls captured on the same site on the same day.

4 shows the salmonella carriage rates of herring gulls caught in three different regions of Scotland between April and July. Insufficient samples were taken from the other 13 sites at this time of year to permit meaningful comparison. Full details of carriage rates found at the other sites are given in Table 5. There are considerable differences between these areas, with the carriage rate in the Clyde area being markedly higher than that found elsewhere. This high carriage rate in the Clyde area is corroborated by comparing the incidence of salmonellae in gulls culled in this area in May with the incidence in gulls from north-east Scotland at the same time (Table 6). The carriage rate in Clyde birds was found to be more than twelve times that recorded in north-east Scotland.

A total of 32 salmonella serotypes was isolated from gulls during the study. *Salmonella virchow* and *S. typhimurium* were the commonest serotypes encountered, and together accounted for over 70 % of the total salmonella isolates. Table 7 lists the serotypes obtained and Table 8 lists the ten most frequently isolated serotypes in gulls and compares this with their frequency of isolation from humans, cattle and sheep during the same period.

Culture of gull eggs and laboratory-reared chicks

No salmonellae were isolated from any of the eggs or chick guts examined.

Enumeration of salmonellae in gull faecal material and duration of excretion

Of the 84 herring gulls maintained in captivity for a minimum of 3 weeks, 17 (20·2 %) were found to be excreting salmonellae. The maximum duration of excretion was 4 days (2 birds) and the mode was 3 days (8 birds). The maximum number of salmonellae found in the positive faecal samples was 170 organisms/g and the mean number was 22.

Examination of refuse tips

Fifty-seven refuse-tip and 36 surface-water samples were examined for the presence of salmonellae. A total of 763 gulls was sampled at the same time as samples were taken from refuse tips. The results are shown in Table 9. Eleven refuse-tip samples and 19 surface-water samples yielded salmonellae but on only three occasions was a salmonella of the same serotype isolated from the gulls and refuse samples (debris or water) on the same day.

DISCUSSION

The overall incidence of salmonella carriage in gulls was found to be 7·8% and herring gulls were found to be more commonly associated with salmonella carriage than either of the other two species examined. The carriage rate in black-headed gulls was considerably lower than that of the larger gulls and presumably reflects differences in their feeding ecologies. Black-headed gulls in Scotland do not feed regularly at refuse tips and more than 40% of these examined in this study were caught in fields. Higher rates of salmonella carriage have been found in black-headed gulls feeding around sewage works. If cloacal swabbing had been used to obtain faecal material the detection rate would have been considerably reduced as this method of specimen collection was shown to detect only 62% of carriers. Use of cloacal lavage increased the number of carriers detected by about 25% and therefore this method of specimen collection will detect approximately 80% of carriers.

The differences in salmonella carriage rates in gulls captured in different localities in this study are probably a reflexion of the degree of environmental contamination in the areas of capture. The higher carriage rates are found in areas associated with relatively higher human population densities. These areas will also be associated with more refuse tips and a greater amount of sewage. Even when the differences in sampling techniques are taken into account the carriage rates of birds culled at breeding colonies are higher than those found at refuse tips. This presumably reflects the wider range of foraging specialists found at colonies and is discussed further in Monaghan *et al.* (1985).

The variations in the prevalence of salmonella carriage in gulls at different times of year broadly reflect the variations seen in the number of reported human cases (CDS, unpublished). We feel that when there is a low infection rate in humans the amount of environmental contamination with salmonellae will be similarly low and therefore the opportunity for gulls to feed on salmonella-contaminated food sources is reduced.

We have shown that gulls carry a wide range of salmonella serotypes, some of which are commonly found in infected humans and domestic animals. The two commonest serotypes isolated from gulls were *S. virchow* and *S. typhimurium* and these two serotypes were also among the three commonest serotypes reported in human and cattle infections during the same period, although in humans and cattle *S. typhimurium* is commoner than *S. virchow*. Similar associations have been reported by others (Fenlon, 1981; Butterfield *et al.* 1983) who suggested that gulls

ingested salmonellae whilst feeding on sewage. In a study of the salmonella serotypes found at a sewage-treatment plant and in gulls feeding at the same site it was demonstrated that similar serotypes were present in both types of sample. Furthermore the appearance of *S. takoradi* (a serotype which is uncommon in Scotland) in the sewage was closely followed by the same serotype being found in the gull faeces (Fricker, 1984*c*). Many gulls feed extensively at refuse tips which are frequently contaminated with salmonellae (Durrant & Beatson, 1981) and refuse may therefore act as a source of salmonella infection for gulls. Preliminary studies were carried out on the role of refuse tips as a source of salmonella for gulls. Refuse material was examined on several occasions. Although salmonellae were often isolated, no specific association was found between the serotypes present on the tip and in gulls feeding at the same site. The ecology of tips is complex and this together with the selective feeding habits of scavenging birds is worthy of further study.

Laboratory studies on the duration and number of salmonellae excreted demonstrated that the gulls did not excrete salmonellae for long periods (fewer than 5 days). Furthermore the numbers of salmonellae excreted in these naturally infected birds were low, (fewer than 200/g). Experimental infections of gulls with salmonellae have shown that the numbers excreted are directly proportional to the number ingested (Fricker *et al.*, in preparation) and that the duration of excretion was normally about 4 days. Examination of seagull eggs and laboratory-reared chicks did not reveal any salmonella isolates and therefore it appears that vertical transmission of salmonellae from mother to chick via egg is uncommon.

Because of the above factors it appears unlikely that gulls could infect humans directly, or harbour salmonellae for a long time. Any role which they may have in the cycle of salmonella infections would presumably be in the contamination of pastureland, with the subsequent infection of farm animals, and both Johnston, Maclennan & Hopkins (1979) and Williams *et al.* (1977) suggested that gulls have been the source of salmonella infections in cattle. Whilst experimental infections of farm animals have demonstrated that large numbers of salmonellae are normally required to produce clinical symptoms in healthy animals (Brown, Ross & Smith, 1976, 1977; Hall & Jones, 1978), it has been suggested that low doses of salmonella can cause infections in stressed animals (Josland, 1953; Dennis & Armstrong, 1965; Tannock *et al.* 1971; Spence & Westwood, 1978). Although gulls excrete low numbers of salmonellae, large numbers of gulls can collect on pastureland and water supplies, and when infections of farm animals occur without the source of the organism being found, the possibility of contamination of pastureland, water or feedstuffs by gulls should not be ignored.

In addition, if potable water supplies used for roosting are untreated before distribution, this is a potential source of infection to man, especially if the water is used in the preparation of food and the organisms have the opportunity to multiply.

We conclude that whilst a substantial proportion of gulls carry salmonellae, and gulls have considerable powers of dispersal, the low numbers and short duration of excretion suggest that they do not present a major public health hazard, although they may occasionally be involved in the infection of stressed farm

animals. It seems likely therefore that salmonella carriage in gulls is more usually an indicator of environmental contamination rather than an important causative factor in animal or human infections.

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