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The significance of wild birds (*Larus* sp.) in the epidemiology of campylobacter infections in humans

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INTRODUCTION

During much of the present century, the population of herring gulls in Britain has increased rapidly (Cramp, Bourne & Saunders, 1973; Chabrzyk & Coulson, 1976; Monaghan & Coulson, 1977). Accompanying this change was an increased utilization by these birds of human waste as a food supply, particularly sewage and refuse emanating from our towns and cities (Monaghan, 1983; Horton *et al.* 1983). This, coupled with their habits of roosting on agricultural land and water storage reservoirs and of breeding on inhabited buildings, has given rise to concern over the role of these birds in the spread of disease to man and domestic animals (e.g. Fenlon, 1981; Reilly *et al.* 1981; Butterfield *et al.* 1983; Monaghan *et al.* 1985; Girdwood *et al.* 1986).

It has been known for some time that many birds can and do carry *Campylobacter* spp. in their gut (e.g. Smibert, 1969; Hussong *et al.* 1979; Skirrow & Benjamin, 1980b; Leuchtefeld *et al.* 1980; Fenlon *et al.* 1982; Fricker & Metcalfe, 1984). Gulls in particular appear to be associated with a nalidixic acid resistant type of *Campylobacter* spp. termed *C. laridis* (Skirrow & Benjamin, 1980b; Benjamin *et al.* 1983). Human illness arising from *C. laridis* is rare (Karmali & Skirrow, 1984).

Gulls also carry *C. coli* and *C. jejuni*, which are more commonly associated with human gastroenteritis. However, until the recent development of serogrouping schemes for *Campylobacter* spp., it has not been possible to assess the degree of similarity between strains of these *Campylobacter* species carried by gulls and those occurring in the human population. The purpose of this study was to examine the extent of campylobacter carriage by herring gulls and, using the Penner serogrouping scheme (Penner & Hennessy, 1980), to assess the degree of similarity between serotypes found in gulls and those infecting humans in the same geographical area.

METHODS

Gull samples

Samples of faeces were obtained by cloacal swabbing from 942 herring gulls *Larus argentatus* caught by canon-netting at refuse tips in 1982 and 1984–5 in the

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Fig. 1. The locations at which gulls were sampled. 1, Thurso (tip); 2, Aberdeen (tip); 3, Moodiesburn (tip); 4, Bishopbriggs (tip); 5, Horse Island (breeding colony); 6, Bowmore (tip).

localities shown in Fig. 1. Small numbers of lesser black-backed gulls (*Larus fuscus*) were also caught and these have been considered separately (see Whelan, 1986). Gulls were caught in Islay, Inner Hebrides (October–November), the Thurso area (October) and the Glasgow area (September–October) in 1982, and in Aberdeen (June) and the Glasgow area (November–December, January–February and April) in 1984–5. These birds were colour-ringed and released after sampling.

Cloacal swabs were also taken from 33 freshly dead, breeding herring gulls culled by the Royal Society for the Protection of Birds in control measures at a breeding colony in the Firth of Clyde in 1985.

Gulls were aged using plumage characteristics (see Grant, 1981 for details) into the following three categories: first years, intermediates (2–4 years) and adults (5 years or older). A proportion of the birds was sexed using the combined head and bill length (Coulson *et al.* 1983).

The cloacal swabs were immediately submerged in a Preston enrichment broth (Bolton & Robertson, 1982); all samples were examined for the presence of *Campylobacter* spp. using the method outlined below for human samples. Penner serogrouping was carried out on the *C. jejuni* and *C. coli* isolates from the 378 birds examined between November 1984 and August 1985.

Human samples

A total of 650 confirmed *Campylobacter* spp. isolates from infected humans was obtained between July 1984 and June 1985 from Aberdeen City Hospital, Monklands District General Hospital, Falkirk Royal Infirmary, Dumfries Royal Infirmary and Stobhill General Hospital, Glasgow. A Preston agar plate (Bolton & Robertson, 1982) was inoculated with each isolate and incubated in a microaerophilic environment in a Gaspak jar for 48 h at 42 °C. A single colony was randomly selected from the Preston plate and incubated in a Preston enrichment broth for 48 h at 42 °C. A sample from the broth was then plated for confluent growth onto four blood agar plates containing FBP supplement (Goossens *et al.* 1984), incubated as above, and subjected to hippurate hydrolysis and nalidixic acid susceptibility tests, using the methods of Skirrow & Benjamin (1983). The purity of the cultures was checked by a Gram stain and 10 μl of the culture was then placed in each of two FBP glycerol vials and retained for subsequent serogrouping (see below). Vials were stored at −70 °C. The method for dealing with the gull samples was essentially similar and follows that outlined in Fricker *et al.* (1983).

Serogrouping

The serogrouping method used in this study was passive haemagglutination as described by Penner & Hennessy (1980). All Penner strains were obtained from Dr J. L. Penner via Dr D. M. Jones, Public Health Laboratory, Manchester and the Culture Collection of the University of Goteborg.

Antisera were prepared by standard methods using New Zealand White rabbits. The decision on which antisera to use for this study was based on the work of Jones *et al.* (1984). In all, 29 sera were used (made to Penner types 1–11, 13, 15, 16, 18, 19, 20, 23, 24, 27, 30, 31, 35, 37, 42, 44, 45, 48, 55). Serotyping was carried out in two stages; firstly by testing the isolates with pools of antisera and subsequently with individual antisera from the pool which yielded a positive result.

Full details of all procedures used can be found in Whelan (1986).

RESULTS

Isolation of campylobacter from gulls

The proportion of herring gulls caught at refuse tips which carried *Campylobacter* spp. varied between 30 and 97%. Overall, 64% of the birds examined were found to be excreting *Campylobacter* spp., 55% of which was *C. laridis* and the remaining 45% *C. jejuni* and *C. coli* in a ratio of approximately 2:1. Campylobacter carriage was examined in relation to the age of the birds; 70% of first year herring gulls, 69% of intermediates and 60% of adult herring gulls were found to be carrying *Campylobacter* spp. There was no significant difference in carriage rates in relation to age in any of the 1982 samples of gulls; in the 1984–5 samples, a significant difference was found in only one of the samples (November–December) in which the number of young gulls examined was very small (only seven first years). Accordingly, we did not consider there to be any evidence of a consistent difference in carriage rates of herring gulls in relation to age, and the age classes were

Table 1. *Comparison of the proportion of herring gulls with* Campylobacter *species in three different locations caught at refuse tips at the same time of year in 1982*

	No. with *Campylobacter* spp.	No. without *Campylobacter* spp.
Bishopbriggs	131 (66%)	69 (34%)
Bowmore	97 (53%)	86 (47%)
Thurso	98 (54%)	83 (46%)

Comparing all three areas: $\chi^2 = 7{\cdot}58$, D.F. = 2, $P < 0{\cdot}025$.

Table 2. *Comparison of the proportion of herring gulls with* C. laridis *and with* C. jejuni/coli *caught at refuse tips at the same time of year in 1982 in three different locations*

	No. with *C. laridis*	No. without *C. laridis*	No. with *C. jejuni. coli*	No. without *C. jejuni/coli*
Bishopbriggs	58 (29%)	142 (71%)	73 (37%)	127 (63%)
Bowmore	61 (33%)	122 (67%)	36 (20%)	147 (80%)
Thurso	48 (27%)	133 (73%)	50 (28%)	131 (72%)

Comparing all 3 areas: *laridis* $\chi^2 = 2{\cdot}08$, D.F. = 2, NS. *jejuni/coli* $\chi^2 = 13{\cdot}41$, D.F. = 2, $P < 0{\cdot}005$.

Table 3. *The proportion of herring gulls caught at refuse tips in two areas of Scotland during summer 1985 which carried different* Campylobacter *species*

	No. examined	No. with *C. laridis*	No. with *C. jejuni/coli*
N.E. Scotland	97	47 (48%)	18 (19%)
Glasgow Area	50	21 (42%)	22 (44%)

(*laridis* $\chi^2 = 0{\cdot}32$, D.F. = 1, NS) (*jejuni/coli* $\chi^2 = 123$, D.F. = 1, $P < 0{\cdot}001$)

Table 4. *Comparison of the proportion of herring gulls feeding at the same refuse tip positive for* Campylobacter *species over three time periods*

	No. with *Campylobacter* spp.	No. without *Campylobacter* spp.
Nov./Dec.	60 (58%)	43 (42%)
Jan./Feb.	77 (79%)	20 (21%)
April	30 (97%)	1 (3%)

Comparing all three time periods: $\chi^2 = 21{\cdot}40$, D.F. = 2, $P < 0{\cdot}001$

combined in subsequent analyses. Data on the sex of the sampled gulls was available for the birds examined in 1984–5; no significant difference was found between males and females in *Campylobacter* spp. carriage rates in any of these samples.

Geographical variation in *Campylobacter* spp. carriage rates in gulls feeding at refuse tips was examined in both the 1982 samples and the 1984–5 samples for gulls caught at the same time of year, autumn 1982 (i.e. September–November) and summer 1985 (June). For the 1982 samples, there was a significant difference between sites (Table 1), with the highest carriage rate being found in gulls in the Glasgow area (Bishopbriggs). When these data were examined further in relation

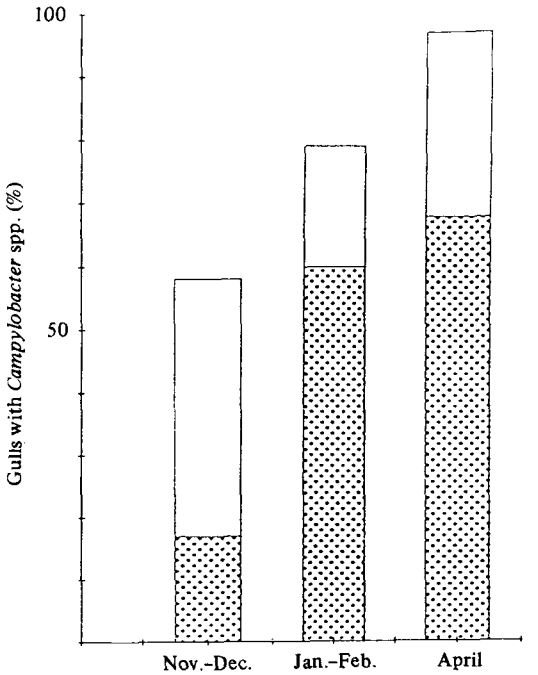

Fig. 2. The proportion of gulls caught at Moodiesburn tip in the Glasgow area during three time periods which carried *Campylobacter* species. The data have been broken down into those which carried *C. laridis* (▨) and *C. jejuni/coli* (□).

to the species of *Campylobacter* isolated from the gulls, there was no significant difference in the proportion of gulls in the three areas which were found to carry *C. laridis* (Table 2); however, there was a highly significant difference in the proportion carrying *C. jejuni* and *C. coli*, which was highest in the gulls caught at tips in the Glasgow area (Table 2). A similar pattern was found for the 1985 samples (Table 3).

Since the carriage rates of gulls feeding at the same tip in the Glasgow area were available for three time periods in 1984–5, seasonal variation in carriage rates was examined, and a highly significant difference was found (Table 4); carriage rates were highest in April. This difference related to both *C. laridis* ($\chi^2 = 46{\cdot}43$, D.F. = 2, $P < 0{\cdot}001$, Fig. 2) and *C. jejuni* and *coli* ($\chi^2 = 11{\cdot}21$, D.F. = 2, $P < 0{\cdot}005$, Fig. 2). The majority of isolates from herring gulls in the Glasgow area in November–December were of *C. jejuni* and *C. coli*; in January–February the picture was reversed, with a large increase in the proportion of birds carrying *C. laridis* and a decline in the proportion with *C. jejuni* and *C. coli* (Fig. 2).

Overall, 24% of the serotypes of *C. jejuni* and *C. coli* isolated from herring gulls were typeable with the Penner antisera used, and 17 different serotypes were identified; these are given in Table 5. The most common serotypes were Penner 30, 42 and 24, which together accounted for 46% of the typed isolates.

Isolation of campylobacter from humans

Most of the 650 *Campylobacter* spp. isolates from humans were *C. jejuni*, with *C. coli* accounting for only 3·3%; 75·7% (492) were typeable with the 29 antisera

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Table 5. *Serotypes of* C. jejuni *and* C. coli *isolated from herring gulls*

Date	Site	Serotype (number of isolates)
29. xi . 84	Moodiesburn	P30 (2)
		P18 (2)
		P 3
		P 4
		P37
		P42
13. xii . 84	Moodiesburn	P30 (2)
		P15
		P16
		P42
21. ii . 85	Moodiesburn	P31
		P44
17. iv . 85	Moodiesburn	P24 (2)
		P44/7
24. v . 85	Horse Island	P24
24. vi . 85	Aberdeen	P 1
		P 8
		P24
13. viii. 85	Bishopbriggs	P42 (2)
		P 2
		P 3/13

Table 6. *Overall frequency distribution of Penner serotypes of 650 human campylobacter isolates received from various sources in Scotland* (*see text for details*)

Serotype	Number	%	Serotype	Number	%
1	43	6·6	20	1	0·2
2	95	14·6	23	18	2·8
3	11	1·7	24	8	1·2
4*	78	12·0	27	5	0·8
5	15	2·3	30	6	0·9
6	21	3·2	31	33	5·2
7	—	—	35	1	0·2
8	15	2·3	37	8	1·2
9	14	2·2	42	5	0·8
10	14	2·2	44	6	0·9
11	20	3·1	45	1	0·2
13	27	4·1	48	—	—
15	4	0·6	55	6	0·9
16	12	1·8	5/31	3	0·4
18	10	1·5	NT†	158	24·3
19	12	1·8			

* Includes 4/16.

† NT, not typable.

used. The most common serotypes were Penner 2, 4, 1 and 31 which together accounted for 38·3% of the isolates examined and 50·6% of those which were typeable. There was no significant difference in the proportion of typeable strains from the different hospitals or in the serotypes of the isolates. The overall frequency distribution of the serotypes of the human isolates is given in Table 6.

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DISCUSSION

In contrast to the situation with salmonella carriage, which is higher in female than in male herring gulls (Monaghan *et al.* 1985), no sex differences in carriage rates of *Campylobacter* spp. were found in gulls feeding at refuse tips, nor did carriage rates vary with the age of the birds involved. However, carriage rates of *Campylobacter* spp. were found to be highest in gulls feeding at refuse tips near areas of high human population density, particularly with respect to *C. jejuni* and *C. coli* which commonly cause gastroenteritis in humans. This difference, which has also been found with respect to salmonella carriage (Girdwood *et al.* 1986), may be due to an increased probability of the birds ingesting contaminated material at tips near urban areas, or an increased contamination of their roosting sites with sewage products.

Of the three time periods examined, gull carriage rates of *C. jejuni* and *C. coli* were found to be highest in the November–December period. *Campylobacter* infection in humans is generally highest in summer (Skirrow, 1987), and it is possible that subsequently high levels of environmental contamination with campylobacter in autumn is reflected in the gull population. However, an alternative, and perhaps more likely, explanation of the peak in carriage rates in gulls is that this relates to the higher winter prevelance of campylobacter in natural waters, which has been attributed to their longer survival at cooler temperatures (Blaser *et al.* 1980; Reisinger, 1985; Bolton *et al.* 1987; Carter *et al.* 1987). The drinking of contaminated water at winter roosting sites (to which the gulls themselves will contribute campylobacter) may increase carriage rates. It is more difficult to explain the high percentage of gulls excreting *Campylobacter* spp. in April, and the reversal in the ratios of *C. jejuni* and *C. coli* to *C. laridis* in January and February in comparison with the November to December figures. We do not have, at present, sufficient information on the aetiology of *C. laridis* infection in wild birds, but its increased presence in gulls could be connected with their heavy use of refuse tips during periods of hard weather. This possibility is supported by the fact that gulls feeding at refuse tips have an increased prevalance of *C. laridis* carriage compared with those examined at an island breeding colony at the same time of year (Whelan, 1986). The latter make more use of traditional marine food sources. However, this aspect requires further investigation.

The considerable seasonal and geographical variation found make it difficult to give an overall figure for the rate of *Campylobacter* spp. carriage by herring gulls; carriage rates varying between 30% and 97% were found in this study.

Only 3·3% of the human isolates examined were *C. coli*. This is in agreement with the findings of Skirrow & Benjamin (1982) who found the frequency of *C. coli* in Britain to be between 3 and 5%. The predominance of Penner serotypes 2, 4 and 1 in the human isolates examined in this study has also been reported elsewhere both in Britain (Jones *et al.* 1984) and North America (Karmali *et al.* 1983; Penner *et al.* 1983). All of the Penner strains isolated from the gulls were present in the human population. However, the serotypes most commonly present in the gulls were not those most common in humans and only a comparatively small proportion (24%) of the gull isolates of *C. jejuni* and *C. coli* serotyped with the panel of 29 Penner antisera used, in contrast to 76% of the human isolates.

This may be due to differential survival of serotypes in the environment or in the digestive tract of the birds. It does suggest that gulls are unlikely to be a direct source of infection to humans, though, as with the Salmonellae, large numbers of birds roosting on potable water supplies may be a potential health hazard (Monaghan *et al.* 1986). However, due to their increased use of sewage and refuse as a food source, gulls do represent a good indicator of the extent of contamination of the environment with human waste products.

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