Jackdaws and magpies as vectors of milkborne human campylobacter infection

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SUMMARY

In 1990 we reported that milk bottles pecked by jackdaws and magpies were a probable source of human campylobacter infection. During April to June 1990 an extended study of campylobacter infections was carried out in the Gateshead area. Prior to the study a health education programme was undertaken in an attempt to reduce human infection. Fifty-nine cases of human infection were recorded and 52 were interviewed. Thirty were entered into a case control study which demonstrated a very strong association between consumption of pecked milk and human campylobacter infection ($\chi^2 = 12.6$, P < 0.0004). It was estimated that between 500 and 1000 jackdaws (Corvus monedula) were present in the area where milk bottles were pecked and 63 isolates of campylobacter were made from the bill and cloaca. Target bottles were put out in the early mornings and campylobacters were isolated from 12 of 123 pecked bottles. Typing of the campylobacters revealed a wide distribution of strains amongst birds, pecked milk and human infections. The health education programme had only limited success.

INTRODUCTION

There were over 34552 laboratory reports of faecal isolates of campylobacter in England and Wales reported to the PHLS Communicable Diseases Surveillance Centre in 1990 (provisional figures). This represents an annual incidence of about 80 per 100000. Almost all cases are sporadic and unexplained although outbreaks due to raw milk [1] and unchlorinated water [2] have occurred. In outbreaks associated with raw milk campylobacter has not been isolated from milk.

Gateshead has a population of 206 900 residents, the majority of whom live in urban areas. One hundred and sixty-eight cases of campylobacter enteritis were recorded in Gateshead in 1989 (81 per 100 000). Just under a quarter of the population (46 110) live in a rural area which is to the west of the River Derwent. Surveillance of campylobacter enteritis has been routinely carried out since 1981

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and during April, May and June 1989 revealed that of the 62 cases of illness reported in the district as a whole 46 had occurred in this western area. Many of the cases lived in newly developed housing adjacent to open countryside. In 1989 evidence was obtained from food histories collected by environmental health officers that milk consumed by patients had been pecked by jackdaws (Corvus monedula) and magpies (Pica pica). In addition Campylobacter jejuni was isolated from bottles of milk that had been pecked [3].

In view of the 1989 information, during March 1990 a health education campaign advised members of the public not to consume milk from bottles that had been pecked by birds. This was achieved with the help of local media and the delivery of local information leaflets by the local dairy.

Preliminary results from South Wales have provided further evidence of a link between human campylobacter infection and drinking milk from pecked bottles [4]. We now report in full the results of our investigation which was carried out during the months of April to June 1990.

METHODS

A primary case was defined as a person with laboratory-confirmed campylobacter infection with onset between 1 April and 30 June 1990 living in Gateshead (Fig. 1). Each case who appeared to have acquired his or her infection whilst resident in Gateshead was asked to nominate a neighbourhood control of similar age, sex and occupation. The purpose of the investigation was explained to both cases and controls. In accordance with previous local practice the majority of interviews were conducted at home by one environmental health officer (K.R.). When this was not possible interviews were conducted by telephone.

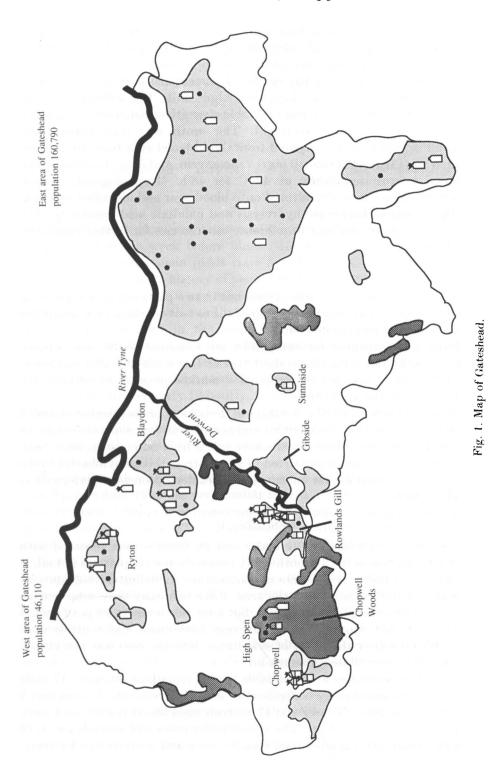
Both cases and controls were interviewed using a standardized food questionnaire which included details of residence, occupation, recent travel abroad, duration and nature of symptoms. Occupations were classified into the socioeconomic group used by the Office of Population Censuses and Surveys. Food histories included details of handling and consumption of raw or undercooked animal products, untreated water, handling of human or animal faecal material and particulars about 18 specified food items including cooked chicken in the week prior to the onset of symptoms. Information was also collected about diarrhoeal symptoms in other members of the household. If the control reported diarrhoea in the week before or during the illness of the matched case another control was found.

Both cases and controls gave details of doorstep milk delivery, the occurrence of milk bottles being pecked by birds and whether such milk had been consumed. Unmatched analysis was performed using 'Epi info' software version 5 (Centres for Disease Control). χ^2 and Fisher's exact tests were used.

Environmental studies

Milk

In 1989 photographic evidence was obtained of a jackdaw removing tops and drinking from milk bottles in the early morning in High Spen, an area adjacent to Rowlands Gill. Verbal reports were also received of magpies displaying the same



behaviour. It was therefore decided to put milk bottles outside houses in these areas in a way that reflected the normal pattern of milk delivery to the households as regards timing, numbers and composition of whole (silver top), skimmed (blue/white top), and semi-skimmed (red/white top) milk.

Between 18 March and 1 July 157 bottles of pasteurized milk were submitted for examination, the majority of which were whole milk. Ten millilitres of well-mixed milk was transferred into 10 ml of double-strength enrichment broth which was then incubated at 42 °C overnight. The broth was then plated onto campylobacter selective media prepared from Oxoid blood agar base No. 2, lysed horse blood, 5 mg/l trimethoprim. 10 mg/l vancomycin, and 5 mg/l colistin which was incubated microaerophilically at 42 °C for 48 h. Morphologically typical campylobacter colonies were subcultured onto blood agar and identified by Gram stain, oxidase reaction, hippurate hydrolysis and nalidixic acid sensitivity. The isolates were lyophilized and sent to reference laboratories for further characterization and typing.

Birds

The investigation of jackdaws was carried out in two parts: an investigation of approximate numbers and an investigation of the extent to which they and other corvids were carrying campylobacter.

When birds were captured for campylobacter examination 38 were ringed. Following this jackdaws standing on short turf at Rowlands Gill were examined through a telescope to measure the proportion marked in order to estimate the probable numbers in the area [5].

Thirty-five birds (26 jackdaws. 5 magpies. 2 rooks (Corvus frugilegus) and 2 carrion crows (Corvus corone) were netted and sampled on four separate occasions between mid April and late June. Swabs were taken from outer beak, inner beak and cloacae of each bird and transported in single-strength campylobacter broth to the laboratory. Campylobacter culture and characterization were performed as for the milk samples.

RESULTS

Fifty-nine Gateshead residents, 31 males and 28 females were recorded with campylobacter infection in the 3-month study period. Seven people were not interviewed: 2 were on holiday and 5 were uncontactable, including 3 residents who had acquired their infection outside the area. Fifty-two cases were interviewed. Fifteen were unable to nominate a control, but a further seven cases put forward controls who were not contactable. On average both cases and controls were interviewed 10 days after the onset of symptoms, but one case was not visited until 6 weeks after their illness commenced.

Case control questionnaires were available for the remaining 30 cases. 17 male and 13 female. Four cases and 4 controls were under 15 years old. 9 cases and 7 controls aged 16 35 years. 13 cases and 17 controls aged 36–55 years with 4 cases and 2 controls over 55 years of age. The age range for cases and controls was 1–76 years, and the mean difference between ages for cases and controls was 4·4 years. s.d. 3·7. Twenty-three cases and 19 controls (Table 1) reported doorstep milk delivery and there were no significant trends in the odds ratio for this factor |odds

Table 1. Doorstep delivery of milk and pecked bottles

	No. (%		
	Cases $(n = 30)$	Controls $(n = 30)$	Odds ratio (95% confidence interval)
Doorstep milk delivery	23 (77%)	19 (63%)	$1.90 \ (0.54-6.83) \ P = 0.4$
Consumption of fresh cold milk	25 (83%)	28 (93%)	$0.36 \ (0.04-2.38) \ P = 0.4$
Pecked bottles in week before illness	15 (50%)	5 (17%)	5.00 (1.32 - 19.92) P = 0.01
Drinking milk from pecked bottles in week before	12 (40%)	0 (0%)	Not calculable $P = 0.0004$
illness			

ratio 1.9 (0.5–6.8)]. However, there was a significant association between bottles being pecked in the week before illness, 15 of the cases and 5 of the controls reporting this phenonomen [odds ratio 5.0 (1.3–19.9)]. Twelve of 30 cases with matched controls gave a history of pecked milk consumption compared with none of the controls [odds ratio 0 (not calculable)]. Three of 12 cases witnessed jackdaws and magpies attacking milk, and many more commented on the large numbers of jackdaws and magpies in the area. There was no correlation between the consumption of cold milk and the development of campylobacter enteritis. None of the other factors investigated were found to be significant.

In 1 of the 6 cases admitted to hospital who was treated with 200 mg ciprofloxacin intravenously 12-hourly, to which there was a good clinical response, resistance developed. *C. jejuni* Lior non-typable, Penner serogroup 35 was isolated which was initially sensitive (minimum inhibitory concentration 0·125 mg/l) but was subsequently found to be resistant to ciprofloxacin (minimum inhibitory concentration 8 mg/l).

The total number of cases of campylobacter enteritis recorded in the district from April to June 1990 was very similar to that in 1989 (59 as against 62). In 1990 dates of onset of illness were more evenly distributed throughout the same period, with 15 cases in April, 22 in May and 22 in June. In 1989 only 2 cases of campylobacter enteritis had dates of onset in April and there was a sharp increase in May (37) with 23 cases in June. The total number of faeces submitted to the laboratory in Gateshead was similar (944 v. 1012) in both periods with the highest percentage of isolates in relation to specimens received occurring in May. In 1990 there was a reduction in the number of cases in the western sector (31 v. 46). In the western area the number of cases increased (28 v. 16). The reduced incidence in the west could be attributed to the health education programme, but it was apparent that a considerable number of people interviewed were unaware of the publicity campaign. Some people stated that it was not always possible to guarantee that milk was covered, particularly when extra milk was delivered at weekends. Few of those interviewed could give details of delivery time in relation to milk collection from the doorstep.

Information was collected about doorstep delivery, the occurrence of pecked bottles and consumption of pecked milk in 52 of the 59 cases. Of 31 cases which occurred in the rural western part of the district doorstep milk delivery was

Table 2. Consumption of pecked milk in relation to area of residence and date of onset of illness

Area	Number of cases	Number of households	Onset date
Sunniside	1	1	1 April
Rowlands Gill/Blaydon	9	7	29 April to 29 May
Ryton	1	1	29 May
Chopwell	3	2	9 June to 16 June

Table 3. Serotypes of campylobacter derived from birds, milk and human sources

Penner type	Birds	Milk	Human	Human, where pecked milk consumed
1	0	0	11	3
$\overset{\bullet}{2}$	ő	0	6	ő
5	1	0	Ö	ŏ
6	$\frac{1}{2}$	ő	ŏ	ŏ
11	õ	0	1	ŏ
15	$\overset{\circ}{2}$	0	î	ŏ
19	2	ő	1	ŏ
21	1	ŏ	Ô	ŏ
23	0	ő	1	1
24	ŏ	4	1	Ô
27	$\ddot{3}$	0	Ô	Ö
31	Ö	ŏ	ĺ	ő
35	1	ŏ	1	ŏ
39	1	ĭ	Ô	ŏ
45	$\overline{2}$	0	$\overset{\circ}{2}$	1
53	ō	ŏ	1	Õ
55	1	5	1	Õ
9, 37	4	ō	$\overline{3}$	1
Other multiply reacting types	0	Ô	9	1 (15, 46)
Non-typable	8	4	9	1 (C. laridis)
Not available	35	ō	9	6
Total	63	14	59	14

documented in 22 (74%) households. In contrast doorstep delivery was recorded in only 13 (46%) instances in the rest of the district. Pecked milk bottles were reported on 15 occasions west of the River Derwent, but on only two occasions in the area east of the river (see Fig. 1). Two of the 15 cases which had dates of onset of illness in April 1990 had consumed pecked milk. During the study period 12 cases reporting consumption of pecked milk occurred between 10 May and 16 June in the west of the district (Table 2).

In 3 of 5 households where more than one case occurred there was a history of consuming milk from pecked bottles. These cases either had identical dates of onset or occurred within 48 h of each other. Of the remaining households, in one two people had probably acquired their infection abroad, and in the other two young children acquired infection with *C. jejuni* with onset dates 8 weeks apart. *C. jejuni* was isolated from faeces in all cases except for one in which the organism

Table 4. Biotypes of Campylobacter sp. derived from human, milk and bird sources

No. of isolations	Human	Milk	Bird
C. jejuni	58	9	25
$C.\ coli$			2
$C.\ laridis$	1	2	3
$Campylobacter \ { m sp}$	-	3	33

Table 5. Milk bottle survey results and occurrence of cases associated with the consumption of pecked milk by week

Week commencing	No. of bottles submitted	No. pecked	No. positive	No. of cases reporting consumption of pecked milk within 7 days
18 March	5	3	0	1
25 March	6	4	0	0
1 April	4	4	0	1
8 April	0	0	0	0
15 April	2	2	0	0
22 April	3	3	0	0
29 April	2	2	0	1
6 May	3	2	0	4
13 May	14	4	3	3
20 May	28	21	8	0
27 May	16	13	0	2
3 June	23	18	1	2
10 June	24	18	0	1
17 June	12	9	0	0
24 June	20	15	0	0
1 July	5	5	0	0
Total	157	123	12	15

was identified as C. laridis. Results of the serotypes of Campylobacter sp. derived from birds, milk and human sources are shown in Table 3.

Milk

Of the 157 bottles of milk sampled all 34 unpecked milk bottles were negative whereas 12 (9 whole milk and 3 skimmed) of 123 pecked bottles yielded either C. jejuni, C. laridis or other campylobacters (Table 4). From two bottles more than one serotype of campylobacter was isolated. Eleven of the 12 bottles from which Campylobacter sp. were isolated were collected between 14 and 24 May (Table 5). Eight of the bottles from which organisms were isolated were from two dwellings where four cases of campylobacter enteritis occurred.

Birds

Marker-recapture studies estimated that there were between 500 and 1000 jackdaws in the area. During the breeding season 112 nests were found in chimney stacks in Rowlands Gill. One hundred additional pairs breeding in the derelict Gibside Hall or nearby were often observed flying to Rowlands Gill, 2 km away, to feed.

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Table 6. Campylobacter isolated from corvids

No.	campylobacter	isolated
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Bird isolates	${f No.}$ sampled	No. totally negative	Cloaca	Inner bill	Outer bill	Total
Jackdaw	26	1	24	17	7	48
Magpie	5	0	6	2	1	9
Rook	2	1	1	0	0	1
Carrion crow	2	0	2	2	1	5

Thirty-five corvids were sampled and a total of 63 strains of various *Campylobacter* sp. were isolated from 33 of them. More than one strain was isolated from some birds. Thirty-three of the isolates were from cloacal swabs, 21 from the internal bill and 9 from the external bill (Table 6). Typing results on 28 organisms showed a wide variety (Table 3).

DISCUSSION

Our findings support those of workers in Bridgend who carried out a study in May 1990 which demonstrated that there is a strong association between campylobacter infection and doorstep delivery of milk bottles; a history of milk bottle attack by birds and consumption of milk from attacked bottles during the week before illness [4]. In addition we were also able to obtain evidence of microbiological contamination of pecked milk bottles.

Case control studies must be carefully designed in order to avoid bias [5]. In our study potential sources of bias were the selection methods used for cases and controls; the low response rate, interviewer and recall bias. It was impractical to obtain controls by any other method and detailed replies were obtained from those available for interview who fulfilled the selection criteria. It was not possible for interviews to be conducted blind and therefore both cases and controls were questioned thoroughly about birds thus minimizing recall bias.

In 1990 many more cases of illness were found in April and one case where pecked milk was drunk was recorded in late March (Table 5) before the study began. Perhaps this change in the pattern of illness can be attributed to the abnormally warm spring which may have led to the birds starting breeding earlier and hence pecking milk bottles earlier in the season.

Workers in Oslo found the highest rate of avian campylobacter carriage to occur in crows [7]. We were able to verify a high rate of campylobacter carriage by corvids in the vicinity where human cases of campylobacter infection occurred that were associated with pecked milk consumption. Of the 59 cases of campylobacter infection occurring in the study period 14 have been shown to be associated with drinking milk from bottles pecked by birds. It was not possible to match the serotype of a patient isolate with that from contaminated milk because the suspect milk connected with an individual was not available for examination. However the wide variety of serotypes from the bird isolates is mirrored by several serotypes obtained from the milk (Table 3). Similarly the serotypes of patients strains were very varied. Moreover different serotypes and species of campylobacter were isolated from individual birds and milk bottles.

C. jejuni was isolated from all human cases except one during the course of the study. In the one case where C. laridis was isolated a history of drinking milk from a pecked bottle was given. C. laridis is a recognized bird-associated species [8] and has previously been isolated from a resident with enteritis in the affected area (S. J. Hudson, personal communication). It is common practice in routine diagnostic laboratories to use only one selective media which may be inadequate to isolate some Campylobacter species [9]. Further the identification of campylobacters is often rudimentary and the hippurate test may give doubtful results [10]. Thus the variety of campylobacters currently causing diarrhoea may be underestimated. The emergence of fluoroquinolone resistance in campylobacters [11] means that it can no longer be assumed that a nalidixic acid-resistant thermophilic isolate is not C. jejuni and further highlights the need for accurate identification.

Milk was not sampled quantitatively as preliminary studies had indicated that the number of organisms present was very low. Under normal household or laboratory conditions C. jejuni does not multiply in milk [12]. Park and Stankiewicz [13] have performed working indicating that a variety of culture media and incubation periods are desirable to maximize the isolation of campylobacter from milk. We may have missed or underestimated the number of organisms present as only one culture medium and one incubation temperature were used. It has been reported that as few as 500 C. jejuni can cause infection [14] and thus even minor contamination by bacteria simply washed off beaks may be sufficient to constitute a health hazard in the pecked milk.

Corvids are known to be scavengers. Videographic evidence was obtained during the course of the study of magpies tearing tops off milk bottles, and as has been reported elsewhere [4] they have been seen tearing rubbish bags open. When examined corvids' beaks were found to be covered with organic material. They were seen feeding on sewage and dead birds were retrieved from sewage treatment tanks. An increased isolation rate of campylobacter in sewage has been attributed to zoonotic infections which may also peak in May [15]. Campylobacter are widely distributed in nature [16] and there are many natural hosts, but the routes of transmission between these hosts are not known. Further work is needed to ascertain whether there is seasonal variation in corvids' rate of campylobacter carriage and feeding habits.

We have demonstrated that campylobacter enteritis is a cause of considerable morbidity in our area. In 1986 Sockett and Pearson [17] estimated the total cost of a single case of human campylobacter infection to be £587. Allowing for inflation, in Gateshead costs of over £100000 would have been incurred in 1989. Any steps that will reduce the incidence must be worthwhile.

Although there was publicity prior to our investigation advising the public not to drink milk that had been pecked by birds, contaminated milk still accounted for 14 (24%) of 59 cases of campylobacter enteritis in the 3-month study period. The health education campaign only had a limited effect as cases associated with pecked milk still continued to occur. It is perhaps unreasonable to expect people to discard all pecked bottles, especially if they live in areas where such pecking is widespread. An alternative solution would be to find alternative packaging for delivered milk that is less attractive to corvids.

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