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Campylobacters in man and the environment in Hull and East Yorkshire

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SUMMARY

Campylobacter organisms isolated from water samples taken weekly from ponds and land-drains in the City of Hull were compared with isolates from humans. Of 314 campylobacter organisms isolated from patients, 237 (75·5%) of the strains were identified as typical *Campylobacter jejuni*, whilst of 125 identified strains isolated from the water samples, 85 (68%) resembled *C. jejuni* in most respects but were hippurate hydrolysis negative by the Hwang and Ederer method. The ponds and land drains in the city were therefore not a source of campylobacteriosis in the people living near these water courses. The atypical *C. jejuni* strains isolated from the environment may be mistaken for the *C. jejuni* strains which cause human infection. It is therefore essential that such strains are fully identified before attributing human and animal infections to their ingestion.

INTRODUCTION

Since Skirrow (1977) first described simple methods for isolating *Campylobacter jejuni*, campylobacter enteritis has been recognized as a major cause of acute enteritis. The mode of spread in sporadic cases has not yet been fully elucidated, although raw and undercooked poultry are likely sources and outbreaks have commonly been attributed to contaminated milk. Large outbreaks have also been described where polluted water was the source.

The nurses from the Infectious Diseases Section of the Hull Health Authority visit all notified cases of suspected food-poisoning within the city boundary. They observed that campylobacter cases appeared to cluster in areas near to the many water courses found within the city. The detection of cases in the areas close to the water courses studied could have occurred by chance because of a large *Shigella sonnei* dysentery outbreak in those areas and because there was a heightened public awareness of gastroenteritis following the publicity surrounding that outbreak.

Not withstanding that possibility a survey was undertaken by the Hull Environmental Health Department to sample these waters to see if there was any similarity in the campylobacter organisms present in the water and in the human cases.

METHODS

Environmental surveys

Samples of 200 ml of water were taken weekly, excluding public holidays, for 1 year, and at less regular intervals during the preceeding 2 years, from sites within the city boundary by Environmental Health Officers of the Hull Environmental Health Department. Samples were not taken during periods of exceptionally bad weather and at times of staff shortage. The sites included lakes and ponds at:

Costello playing fields, a circular, concrete-lined children's boating pond without resident wildfowl. Large flocks of duck and Blackhead gulls congregate there in the autumn.

East Park, the largest recreational lake in the city, approximately 1 km in length used for rowing and motor-boating, angling and is a wildfowl sanctuary. The samples were taken in the motor boating area which attracts ducks, geese and swans in large numbers for the plentiful supply of food provided by visitors.

Pickering Park, similar to East Park lake but smaller.

Pearson Park, a small pond lined with willow and other trees whose leaves fall into the water in the autumn. There is a large wildfowl population for its small size.

Hymers School, similar to Pearson Park but the wildfowl population is more variable. Gulls use the surrounding playing field for roosting during the winter. Water run-off from the playing field enters the pond.

From land drains, which cross the agricultural land of the Holderness plain and drain into the River Hull or Humber:

Barmston Drain at Hall Road,

Holderness Drain at Preston Road,

and small land drains on the city boundary at:

Hopewell Road and Wold Road.

None of these waters was known to receive sewage or sewage effluent, although the land drains receive rain-water run-off from agricultural land and may receive farm effluent.

River Hull at Sutton Road Bridge in the tidal section approximately 1 km south of a sewage outfall.

Three surveys of the River Hull both above and below the River abstraction plant at Tophill Low, near Driffield, East Yorkshire were undertaken by laboratory staff. At each site 1 l of water was taken. These were examined for the presence of campylobacter and salmonella.

Bacteriological methods

Faecal samples. Faecal samples from cases of food poisoning, family contacts and pets were plated directly onto Preston medium (Bolton & Robertson, 1982). The plates were incubated in a microaerobic atmosphere for 48 h at 42 °C.

All faecal samples were also examined for the shigella and salmonella groups and, where indicated, *Staphylococcus aureus*, *Clostridium perfringens*, the vibrio/aeromonas group and for parasitic ova and cysts.

Environmental samples. In the laboratory 100 ml of the samples were filtered

through a 0·45 μm membrane which was placed face up on Preston medium. After overnight incubation at 42 °C in a microaerobic atmosphere the membranes were removed and the plates again incubated for a further 48 h.

Typical colonies were identified by methods described by Skirrow (Skirrow & Benjamin, 1980*a*) including Gram film, catalase production, sensitivity to triphenyltetrazolium chloride (TTC) compared with a *C. jejuni* control, hippurate hydrolysis, sensitivity to nalidixic acid and resistance to cephalothin. All strains were sent to Manchester Public Health Laboratory for serotyping by the Penner method (Penner & Hennessy, 1980).

Most Probable Number (MPN) counts of presumptive coliforms and *Escherichia coli* type 1 were performed using methods described in a report by the PHLS Water Sub-Committee (1958). Equal volumes of water were added to 5 tubes containing 10 ml, 5 of 1 ml and 5 of 0·1 ml double strength MacConkey Broth. These were incubated at 37 °C for 18 h and tubes showing the presence of gas were subcultured into MacConkey broth and Tryptone water. These were incubated at 44 °C for 18 h and tubes showing the production of gas and indole were considered to be *Escherichia coli* type 1. An MPN was calculated for presumptive coliforms and *Escherichia coli* by reference to tables (de Mann, 1975).

RESULTS

A total of 314 campylobacter organisms isolated from patients between 1 January and 30 June 1983 were identified. Two hundred and thirty-seven (75·5%) of the strains isolated were identified as typical *C. jejuni* (hippurate positive, TTC sensitive compared with a *C. jejuni* control). Twelve (3·8%) were identified as *C. coli* (hippurate negative, TTC resistant) and 33 (10·5%) resembled the atypical *C. jejuni* strains being hippurate negative, TTC sensitive. Other aberrant *C. jejuni* strains which were hippurate sensitive, TTC resistant were isolated from 32 (10·2%). In the same period, 24 strains were isolated from dog faeces and 1 from a cat. Eighteen (72%) were typical hippurate positive, TTC sensitive *C. jejuni*, 2 (8%) were aberrant TTC resistant *C. jejuni* and 5 resembled the atypical environmental *C. jejuni* strains.

Campylobacter organisms were isolated from all of the ponds, lakes, land drains and river sites on more than one occasion. Campylobacter organisms were isolated from 98/211 (46·4%) of pond samples and 78/175 (44·6%) of drain samples. Eighty-five (68%) of 125 strains identified resembled *C. jejuni* in most respects (flat effuse colonies, TTC sensitive relative to *C. coli*, nalidixic acid sensitive, cephalothin resistant) but were hippurate hydrolysis negative by the Hwang and Ederer method. Eighteen (14·4%) strains were identified as typical *C. jejuni*, 2 (1·6%) as aberrant TTC resistant *C. jejuni*, 8 (6·4%) as *C. laridis*, (nalidixic acid resistant thermophilic campylobacter) and 12 (9·6%) as *C. coli*, hippurate negative, TTC resistant.

The distribution of serotypes identified in strains isolated from the environment and from humans was determined by the Penner system. A greater proportion (58 strains, 52%) of the 112 environmental strains, were not typable by the Penner serotyping scheme compared with 63 (21·6%) of the 292 human isolates. Many of the serological reactions were common to both human and environmental strains,

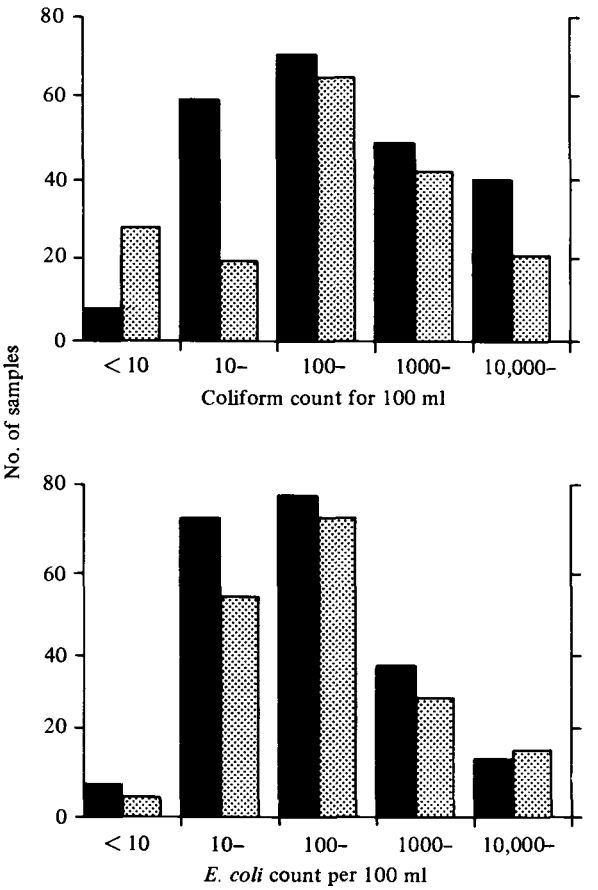

Fig. 1. Distribution of presumptive coliform and *E. coli* counts for campylobacter positive ▨ and negative ■ waters.

Table 1. *Seasonal variation in the isolations of campylobacter organisms from environmental samples*

	Ponds			Drains		
	Samples positive	%	Total	Samples positive	%	Total
Jan.	19	(95)	20	14	(88)	16
Feb.	4	(40)	10	3	(43)	7
Mar.	7	(44)	16	5	(71)	7
Apr.	1	(11)	9	3	(38)	8
May	0	(0)	29	0	(0)	25
June	24	(48)	50	15	(38)	40
July	15	(50)	30	7	(29)	24
Aug.	15	(50)	30	9	(38)	24
Sep.	23	(47)	49	22	(55)	40
Oct.	27	(90)	30	23	(96)	24
Nov.	13	(45)	29	15	(54)	28
Dec.	9	(47)	19	10	(63)	16

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although in the latter group the individual numbers were small. The antigens common to both included 1, 2, 3, 4, 8, 11, 16, 18, 19, 24, 31, 44, 46 and 55. The commonest antigen group amongst the human isolates included the 4/13/16/50 complex in 19 (31·2%) strains; this complex was found on only nine (8%) of environmental strains. No other antigen complex represented over 10% of human or environmental isolates.

The distribution of presumptive coliform and *E. coli* counts for samples from which campylobacter organisms were and were not isolated are shown in Fig. 1. Whilst small differences in the distributions were noted, there was no consistent relationship between the numbers of coliforms present and the presence or absence of campylobacter organisms.

The seasonal variation in recovery of campylobacter from environmental sources is shown in Table 1.

DISCUSSION

During 1981/2 it was noted that cases of campylobacter enteritis were particularly common in people living close to the park lakes and land drains within the city of Hull. The city with a population of 350000 people, lies on the north bank of the River Humber estuary and is divided into East and West Hull by the River Hull. Most of the modern city of Kingston-upon-Hull is built on the boulder clay of the Holderness Plain and lies close to sea-level. The existence of salt and fresh water marshes, which were drained to provide agricultural land and later, building land, is recorded in street names (Markham, 1987) such as 'Ings Road, Saltshouse Road, Tweendykes Road and Inglemire Lane' (Igel, Old English = Leech). As the city grew radially from the old town a number of parks with boating lakes were created within the suburbs. Large open land drains cross the city; the largest, Holderness Drain, passes through the large estates built between the wars in the eastern part of the city.

Some areas of Hull with the highest incidence of campylobacteriosis have a large uncontrolled dog population and it was possible that the dogs were being infected by drinking the water from these ponds and drains. Infection could then be spread to families by direct contact as described by Khan (1983). The large land drains and lakes are sometimes used illicitly for swimming by children who could similarly become infected and introduce it into their households. If environmental waters are, directly or indirectly, a source of human campylobacteriosis then it would be expected that the same biotypes and serotypes of campylobacter organisms would be present in human and dog faeces and environmental samples. This study was designed to investigate the distribution of campylobacter organisms in the environment and compare them with human isolates.

Campylobacter organisms were isolated from all of the sites sampled. Neither the *E. coli* nor the presumptive coliform counts gave an indication of the presence of campylobacters in the water (Fig. 1). In general, campylobacter organisms were isolated more frequently from the ponds and drains when the presumptive coliform and *E. coli* counts were higher. As the waters studied did not receive human sewage as far as is known, the source of the *E. coli* in these waters is uncertain. Atypical *C. jejuni* were isolated on some occasions from water which contained fewer than 20 coliforms per 100 ml, the lowest limit of detection of the

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MPN range used in this study. There was thus no consistent relationship between the indicators of faecal pollution and the presence of atypical *C. jejuni*. The double strength MacConkey broth used in this study, in our experience, gives satisfactory results for this type of water. It was not possible in this study to determine whether these atypical *C. jejuni* strains were of avian, mammalian or invertebrate origin. Whilst it is possible that these organisms form part of a complex ecosystem in environmental waters, an avian, waterfowl or small mammal source appears to be the most likely.

There was a marked seasonal variation in the frequency of isolation of campylobacter organisms from these waters (Table 1). The reason for this remains unclear. The highest frequency occurred during the coldest and wettest parts of the year. At those times water run off from land would be at its greatest and there may have been prolonged survival of the organisms despite the cold conditions. Wildfowl and gulls migrating from the harsher conditions in Northern Europe, congregate on these waters and the adjacent land which appeared to be the most likely source. Campylobacter organisms then disappeared during the late spring. The reappearance of campylobacter organisms occurred in the late summer, when many of the waters became covered in phytoplankton, 'blanket weed', which prevents sunlight from penetrating beneath the surface of the water.

The campylobacter organisms isolated from these waters were culturally and morphologically identical to the strains isolated from the human cases but only 14·4 % were hippurate positive, compared with 75·5 % of the human strains, which were identified as *C. jejuni*. These results indicate that the environmental waters in the city were unlikely to be associated with human cases and the proximity of cases to these waters was almost certainly fortuitous. Similarly, the isolations from dogs were mainly of *C. jejuni* and few resembled the atypical strains found in the environment. It is therefore more likely that humans and dogs are infected from a similar source but not from environmental water.

The technique used for the isolation of campylobacter organisms from water was based on their presence or absence in 100 ml. The effuse nature of the campylobacter colony made any attempt at a direct quantification impossible and the strains identified would probably represent the predominant organism present. If other strains were also present in smaller numbers, they would not be detected. The isolation technique for the water samples used in these studies, which required passage of campylobacter organisms through a 0·45 μm membrane, might also have selected out the atypical strains but have attracted little comment in other environmental surveys (Pearson, 1982, Jones *et al.* 1984). Jones *et al.* (1984) did not include TTC in their identification scheme and recorded that *C. coli* was commonly found in environmental samples. Steele & McDermott in 1984 noted that aero-intolerant, hippurate negative campylobacter organisms were isolated from human faeces by using 0·45 μm membranes in a similar manner but not on media containing antibiotics. Unfortunately they did not fully identify the strains and it is therefore not possible to determine whether they were atypical *C. jejuni*.

Considerable difficulties were experienced with these organisms in the laboratory when trying to apply biochemical tests. The environmental strains were particularly aero-intolerant and often failed to survive subculture. We were

unable to subdivide the human strains by the H_2S test (Skirrow, 1980*b*) which, in our hands, proved to be unreliable. Many of the strains, particularly the environmental ones, sent by post to Manchester Public Health Laboratory failed to survive.

This investigation does highlight the importance of biochemical and serological typing of campylobacter organisms isolated from water that is thought to have caused an outbreak. The environmental campylobacter organisms described here are indistinguishable on colonial and microscopic morphology from *C. jejuni*. In order to define the atypical *C. jejuni* strains more fully and to determine their source, further studies will be required. However, the presence in the environment of probably non-pathogenic campylobacter organisms which may easily be wrongly identified as *C. jejuni* should be taken into consideration when trying to determine the sources of human and animal campylobacteriosis. The existence of these atypical *C. jejuni* strains will also need to be considered if legislative bodies include campylobacter species in microbiological standards for recreational and other waters.

A preliminary analysis of the data collected prior to the main study period from January to June 1983 indicated that a human volunteer experiment would be required to determine the pathogenicity of atypical *C. jejuni* found in fresh water. That study (Mawer, 1988) suggested that the atypical *C. jejuni* isolated from the environment were not pathogenic to humans.

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