Occurrence of *Campylobacter* spp. in water in Northern Ireland: implications for public health

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**SUMMARY**

The occurrence of *Campylobacter* spp was examined in a variety of waters in Northern Ireland. Conventional cultural techniques were employed with 768 water specimens, including drinking waters (tap, spring, bore hole and bottled) and recreational waters (swimming pool, lough, river and sea). Positive waters included 1/11 (9.1%) drinking water from untreated well water, as well as 5/12 (41.7%) untreated surface waters from loughs and 7/8 (87.5%) untreated river waters.

Overall, untreated surface waters may represent a source of contamination with *Campylobacter* spp. in Northern Ireland, where they have a recreational involvement or are used as a drinking source by man or agricultural livestock. Therefore waterborne campylobacteriosis should be considered in patients presenting with acute enteritis and a history of participation in water sports/activities. As faecal coliform organisms have been previously shown to be poor markers of water quality, especially for *Campylobacter* spp, new criteria should be established to assess the risk of this infection and to evaluate and monitor the quality of water used for recreational purposes.

**INTRODUCTION**

The past three decades have seen the rise of *Campylobacter* enteritis in man from virtual obscurity to notoriety, with present isolation rates superseding those of other enteric pathogens such as *Salmonella* spp. and *Shigella* spp. in most developed countries. Unlike the salmonellae and other enteric pathogens, the majority (c.99%) of clinical reports concerning *Campylobacter* are sporadic and *Campylobacter* enteritis outbreaks are rare. The lack of well-developed typing schemes has hindered the epidemiological investigations seeking natural reservoirs of the organism and modes of transmission from these sources to man. Only about 15% of clinical isolates are identified to species level thus making epidemiological investigations extremely difficult to perform.

Although campylobacters are not completely new to applied bacteriology, they have evaded traditional techniques used for the isolation of pure cultures, apart from single isolations that were free from competing organisms. However it was not until 1957, when King¹ reported on infection in man caused by a closely “related vibrio”, that more awareness was given to the disease potential of *Campylobacter* spp. King observed two distinctly different types of vibrio organisms being isolated from blood cultures of infected patients. The first were typical of the organisms designated as *Vibrio fetus*, but the second group were distinctly different in that they had a much higher optimal growth temperature (42°C) and all were isolated from patients with gastrointestinal disease. She concluded that these “related vibrios” were the causative agents of the gastroenteritis, and such organisms were a more common cause of gastroenteritis than was recognised at that time.

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King concluded that the relative absence of microaerophilic vibrios from stools was due to the organisms being fastidious in nature and slow-growing.

A major breakthrough occurred when Dekeyser et al. developed a procedure to selectively isolate microaerophilic vibrios from stool specimens. The isolation technique involved filtering suspensions of stools through 0.65 μm membrane filters and inoculating the filtrate on to selective agar. Since King first described human infection with the "related vibrios" only 14 cases were reported until the discovery of a selective medium for the isolation of enteropathogenic Campylobacter spp. However it was not until 1977, when Skirrow described a more direct technique for the isolation of campylobacters from stool samples from individuals with diarrhoea that the true importance of Campylobacter as a causative agent for acute human gastroenteritis was fully revealed. Skirrow employed selective antibiotics in the media formulation in order to eliminate competing flora and to promote the isolation of campylobacters. From this work it was established that campylobacters were indeed a very frequent, if not the most frequent, cause of diarrhoea, particularly amongst paediatric patients.

Since the development of more sophisticated isolation techniques, the true disease potential of these organisms has become apparent, and today campylobacteriosis is regarded as a zoonosis which is capable of being transmitted to man by a wide range of domestic animals. At present the laboratory isolation of these organisms has become routine from clinical as well as from environmental specimens, and although relatively complicated to perform routine isolation has been carried out with success for the past 20 years or so. Until recently, Campylobacter has been known mainly as an human pathogen generally of zoonotic origins; however there have been several reports of waterborne acquisition of this organism. Therefore, it was the aim of this study to examine the occurrence of Campylobacter spp in waters in Northern Ireland and to assess subsequently their importance to public health.

**MATERIALS AND METHODS**

**Collection and processing of water specimens**

All samples (1000 ml) were collected by Environmental Health Officers from the 26 local council authorities within Northern Ireland in sterile plastic disposable containers. The samples were collected as part of a routine programme from various domestic and commercial premises (Table 1). In addition, samples were collected from various leisure facilities, such as public leisure centres and hotel leisure complexes. Surface waters from lakes, reservoirs, springs and wells were also analysed. All samples were transported and maintained at 4°C prior to analysis and were processed within 24 hours following collection.

**Conventional culture of Campylobacter spp**

Water specimens (400 ml) were initially filtered through a sterile polycarbonate membrane filter (43 mm diameter; pore size 0.20 μm) (Whatman Ltd., England) employing a sterile Millipore Water Filtration system (Millipore Inc., USA). Filters containing filtrate were removed and placed in Nutrient Broth no. 2 (Oxoid Ltd., England) supplemented with Preston Selective agents (Oxoid SR 117, Oxoid Ltd., England) and placed in universal containers (approximately 20 ml) with the minimum headspace volume allowed. Broths were incubated at 37°C for 24 hours followed by a further incubation at 42°C for 24 hours prior to streaking on to Preston Selective agar (Oxoid Ltd., England). All presumptive colonies were further characterised using phenotyping methods, as previously described. The type strain C. jejuni NCTC 11351 was employed as a positive control for both extraction and characterization and sterile distilled water was employed as a suitable negative control.

**Molecular confirmation of Campylobacter by 23S rRNA & flaA/flaB PCR**

All isolates were further confirmed by PCR employing both the flagellin (flaA/flaB) gene and the 23S rRNA gene, as previously described.

**Penner serology**

Campylobacter isolates were serotyped according to the method of Penner and Hennessey, as previously described.

**ENUMERATION OF COLIFORM ORGANISMS FROM WATER SPECIMENS**

Water specimens (100 ml) were initially filtered through a sterile polycarbonate membrane filter (43 mm diameter; pore size 0.45 μm) (Type HA, Whatman Ltd., England) employing a sterile Millipore Water Filtration system (Millipore Inc., USA). Filters containing filtrate were removed and placed in a sterile petri dish containing a petri pad soaked in Lauryl Sulphate Broth (Oxoid

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TABLE

Occurrence of Campylobacter spp. in waters in Northern Ireland

| Water Type          | No. of specimens examined | No. positive | Species       | Penner serotype | Source          | Coliform count (cfu/100 ml) |
|---------------------|---------------------------|--------------|---------------|-----------------|-----------------|----------------------------|
| (i) Drinking waters |                           |              |               |                 |                 |                            |
| Tap                 | 367                       | 0            | –             |                 |                 |                            |
| Spring              | 13                        | 0            | –             |                 |                 |                            |
| Bore hole           | 9                         | 0            | –             |                 |                 |                            |
| Well                | 11                        | 1(9.1%)      | C. jejuni-ND  | ND              | Private Dwelling| ND                        |
| Bottled             | 2                         | 0            | –             |                 |                 |                            |
| (ii) Recreational waters |                     |              |               |                 |                 |                            |
| Swimming pool       | 345                       | 0            | –             |                 |                 |                            |
| Lough               | 12                        | 5 (41.7%)    | C. coli NT    | Lough Neagh     | 1.1 x 10^3      |
|                     |                           |              | C. coli NT    | Lough Neagh     | 1.4 x 10^3      |
|                     |                           |              | C. coli NT    | Lough Neagh     | 9.1 x 10^3      |
|                     |                           |              | C. coli NT    | Lough Neagh     | 5.4 x 10^3      |
|                     |                           |              | C. coli 11    | Lough Neagh     | 1.6 x 10^4      |
| River               | 8                         | 7 (87.5%)    | C. coli NT    | River Lagan     | 3.5 x 10^4      |
|                     |                           |              | C. coli 11    | River Lagan     | 9.1 x 10^4      |
|                     |                           |              | C. coli NT    | River Lagan     | >10^4           |
|                     |                           |              | C. coli NT    | Annesborough river | 1.1 x 10^4 |
|                     |                           |              | UPTC t        | River Lagan     | 3.5 x 10^4      |
|                     |                           |              | C. jejuni NT  | River Lagan     | 3.4 x 10^4      |
| Sea                 | 1                         | 0            | –             |                 |                 |                            |

Notes: ND, not determined; §NT, non-typable; *cfu/100 ml, colony forming units/100 ml water; UPTC t, urease-positive thermophilic Campylobacter

MM615, Oxoid Ltd., England) and the inverted pads were incubated at 30°C for 4 hours, followed by further incubation at 37°C for 14 hours. Coliform organisms were confirmed by gas production in Brilliant Green Bile Broth (Oxoid CM31, Oxoid Ltd, England) and oxidase activity [oxidase negative]. Colony counts were expressed as colony forming units per 100 ml water examined [cfu/100 ml].

RESULTS

Viable Campylobacter spp. were only isolated from untreated well, lake and river waters, as detailed in Table. None of the chlorinated drinking water specimens were positive for this organism. The present study showed that all chlorinated swimming-pool waters were also free from viable campylobacters. Ninety seven percent of unchlorinated drinking-water specimens were negative, with one unchlorinated well-water supply yielding C. jejuni biotype 6102. In contrast to this, approximately 57% of untreated surface waters from loughs and rivers were positive for campylobacters. Of these, 83% were C. coli, 8.5% C. jejuni and the remainder urease-positive thermophilic Campylobacter (UPTC), as confirmed by both phenotypic and molecular (flaA/flaB PCR) analyses. The majority of campylobacters isolated from surface waters were untypable using the Penner serology scheme, with the exception of two isolates, which were Penner serotype 11. Coliforms were enumerated for Campylobacter-positive water specimens, as a marker of faecal contamination, and yielded mean counts of 3.72 x 10^3 cfu/100 ml and 1.86 x 10^4 cfu/100 ml for loughs and rivers respectively, indicating a moderate level of faecal pollution in these sources.

DISCUSSION

In Northern Ireland, Campylobacter spp. is the most common cause of acute bacterial gastroenteritis, exceeding other common faecal pathogens including Salmonella, Shigella, pathogenic E. coli (VTec, EPEC, EHEC) and protozoa including Cryptosporidium parvum,
Campylobacter in water

Annual laboratory reports of campylobacters isolated from faeces over the period 1990-1999.

with present annual laboratory reports of approximately 800-900 cases. The trend for this pathogen is demonstrating an annual rise, even in the present food safety climate. However, Northern Ireland infection rates for this pathogen are substantially lower than the rest of the UK, whereas the rates for England/Wales and Scotland are relatively similar. With the exception of the late Spring peak periods in 1997 and 1999, the rates of infection in Northern Ireland per 100,000 population has remained in the range 6-14, whereas the rate in England/Wales and Scotland has ranged from 15-40 persons per 100,000 population. It has been postulated that this difference may be attributed to (i) a colder and wetter climate, curtailing summer picnics and barbeques, (ii) a higher rate of consumption of red meat than white meat, (iii) a social liking for “well-done” foods and (iv) a virtual absence of consumption of unpasteurised milk by the general public.

Campylobacters still remain the most common cause of acute bacterial food-poisoning in Northern Ireland, first exceeding gastrointestinal-related Salmonella isolation rates in 1991 and continuing to show an annual rise of approximately 21%, as shown in Figure 1. Although the majority of diagnostic clinical laboratories locally do not characterise isolates to the species level, studies at the Northern Ireland Public Health Laboratory have demonstrated that approximately 90% of local infections relating to this genus are caused by C. jejuni, followed by 8% C. coli with the remaining 2% made up of C. lari, C. upsaliensis and other unusual species. Furthermore, as no local laboratories are carrying out non-selective differential filtration isolation, it is difficult to predict the prevalence and clinical significance of antibiotic-sensitive species in the Northern Ireland population.

The natural habitat of most Campylobacter spp. is the intestine of warm-blooded animals,
particularly poultry. Although the enteropathogenic campylobacters have been shown to cause disease in a wide variety of animals and man, certain species have been shown to have a preferred niche. *C. jejuni* have been shown to be most prevalent in poultry, whilst pigs appear to be the preferred niche of *C. coli*. Hence the ecological niche occupied by the *Campylobacter* spp may be of significant importance in relation to the epidemiology of the disease. Likewise, contamination of both recreational and drinking water with faeces from human and animal positive shedders represents a significant risk to the safety of such waters.

In the present study, it was reassuring to note the absence of campylobacters from a large sample population of chlorinated drinking water as well as from swimming pool water, indicating the maintenance of effective disinfection and quality control procedures. Swimming pool-associated outbreaks of human campylobacteriosis have not been reported in the literature indicating that this is not an established mode of transmission of this pathogen to man, unlike numerous outbreaks of cryptosporidiosis, which have been transmitted in this manner.10

All environmental samples were collected over a two month period in the winter time, when it would have been more likely to detect positive specimens by culture than in the summer time, as the rate of isolation has been previously shown to decline due increased ambient temperature, but also increased hours of daylight.11 One untreated drinking water from a well in a private residence was positive for *C. jejuni* indicating the importance of proper control and disinfection through chlorination as an effective means of rendering water potable. In England and Wales there have been at least six outbreaks of human campylobacteriosis associated with the consumption of water from private supplies,12 as well as an outbreak from unchlorinated well water in Canada due to contamination with meltwater.13 Consequently, individuals consuming unchlorinated water from private supplies, including wells, bore holes and springs, should have their supply monitored microbiologically at regular intervals to maintain a safe and potable supply and to discuss any irregularities with the Environmental Health Officer from their local council authority.

The most significant risk of human infection is from the accidental ingestion of contaminated untreated surface waters during recreational use, even in non-outbreak settings. A variety of such recreational activities including rowing, canoeing, water skiing, jet skiing, wind-surfing, angling and boating are popular at many sites (River Lagan and Lough Neagh). These are subject to contamination by campylobacters from point sources, including sewage releases, water sheds, run-off from agricultural and residential areas, faecal contamination from wildlife, as well as floods. In addition, positive waters may serve as a source of infection for domestic livestock as well as household pets.

In this study, three species of *Campylobacter* were isolated, i.e. *C. coli*, *C. jejuni* and the UPTC group. Both *C. coli* and *C. jejuni* are well-established human pathogens, whilst the latter group of campylobacters are atypical to other defined species within this genus, as these organisms have well marked urease activity, similar to their close phylogenetic neighbour, *Helicobacter pylori*.14 As this group of organisms is not found in domestic animals or man, this suggests that these organisms may be endogenous to the aquatic environment, probably through introduction by water fowl and migratory birds.

Little is known about the survival and transmission of *Campylobacter* spp. in the environment or how domestic and wild animals which serve as a natural source become infected. Several epidemiological reports have implicated water as the source of campylobacters, although no *Campylobacter* spp. could be isolated from the suspected water. This has introduced the concept of the viable but non-culturable cell. Rollins and Colwell15 reported on a viable but non-culturable *C. jejuni*, which changed form from a culturable spiral to an non-culturable coccoidal structure. These workers attempted to explain the dormant state of the *Campylobacter* spp. and the inability to culture these cells employing conventional techniques. McKay16 reported on the significance of the viable but non-culturable form of *C. jejuni* and concluded that reliable detection methods must be available in order to allow detection of this form of the pathogen, as non-culturability cannot be equated with non-viability. Consequently the existence of a viable but non-culturable form of campylobacters has serious implications for both epidemiology and methods for detection, especially in the detection of enteropathogenic campylobacters in quality control situations in water microbiology. As a
result of such work, the “pseudosenescent” state was proposed, where bacteria lose the ability to multiply as a result of certain stresses, but remain completely functional as individuals – the so-called “viable but non-cultur able” state. *C. jejuni* have been shown to have this viable but non-culturable form.\(^1^5\) *C. jejuni* was shown to be viable in water samples by non-conventional culturing techniques, but could not be detected by conventional culture methods. These workers concluded that the inability to culture these cells was due a number of factors, including (i). survival of the organism in a viable but non-culturable state, (ii). persistence in a biofilm and adherence to surfaces – non-culturable by conventional methods, (iii). cells may be present in numbers below the threshold necessary to establish growth on laboratory media.

Consequently future studies should concentrate on the adoption of molecular detection systems employing molecular markers of viability such as detection of mRNA through RT-PCR, to account for physiological adaptation with this genus.

In conclusion, untreated surface waters may represent a source of contamination with campylobacters in Northern Ireland, where they have a recreational involvement or are used as a drinking source by either man or agricultural livestock. Therefore consideration of waterborne campylobacteriosis should be given to patients presenting with acute enteritis and a history of participation in water sports/activities. As faecal coliform organisms have been previously shown to be poor markers of water quality, especially for *Campylobacter* spp, new criteria should be established to assess the risk from campylobacter and to evaluate and monitor the quality of water used for recreational purposes.

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**REFERENCES**

1. King E. Human infections with *Vibrio fetus* and a closely related *Vibrio*. *J Infect. Dis.* 1957; 101: 119-28.
2. Dekeyser P, Gossuin-Detrain M, Butzler J P, Sternon J. Acute enteritis due to related vibrio: first positive stool cultures. *J Infect Dis* 1972; 125: 390-2.
3. Skirrow M B. Epidemiology of *Campylobacter* enteritis. *Int J Food Microbiol* 1991; 12: 9-16.
4. Munroe D L, Prescott, JF, Penner, J L. *Campylobacter jejuni* and *Campylobacter coli* serotypes isolated from chickens, cattle and pigs. *J Clin Microbiol* 1983; 18: 877-81.
5. Bolton FJ, Wareing D R A, Skirrow M B, Hutchinson D N. Identification and biotyping of Campylobacters. In: Identification methods in applied and environmental microbiology (R. G. Board, D Jones, F A Skinner, eds.), pp. 151-161. Blackwell Scientific Publications, London 1992.
6. Nacharnkin I, Bohachick K, Patton, C M. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J Clin Microbiol* 1993; 31: 1531-6.
7. Wegmüller B, Lüthy J, Candrian U. Direct polymerase chain reaction detection of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and dairy products. *Appl Environ Microbiol* 1993; 59: 2161-5.
8. Penner J L, Hennessy J N. Passive haemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J Clin Microbiol* 1980; 12: 732-7.
9. Anon. Review of Communicable Diseases 1999. Communicable Disease Surveillance Centre (Northern Ireland). pp 7-12.
10. Stafford R, Neville G, Towner C, McCall B. A community outbreak of *Cryptosporidium* infection associated with a swimming pool complex. *Commun Dis Intell* 2000; 24: 236-9.
11. Obiri-Danso K, Paul N, Jones K. The effects of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni*, *Camp. coli*, *Camp. lari* and urease-positive thermophilic campylobacters (UPTC) in surface waters. *J Appl Microbiol* 2001; 90: 256-67.
12. Furtado C, Adak G K, Stuart J M, Wall P G, Evans H S, Casemore D P. Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992-5. *Epidemiol Infect* 1998; 121: 109-19.
13. Millson M, Bokhout M, Carlson J, Spielberg L, Aldis R, Borczyk A, Lior H. An outbreak of *Campylobacter jejuni* gastroenteritis linked to meltwater contamination of a municipal well. Can J Public Health 1991; 82: 27-31.
14. Owen R J, Costas M, Sloss L, Bolton F J. Numerical analysis of electrophoretic protein patterns of *Campylobacter lari* dis and allied thermophilic campylobacters from the natural environment. *J Appl Bacteriol* 1988; 65: 69-78.
15. Rollins D M, Colwell R R. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Appl Environ Microbiol* 1986; 52: 531-8.
16. McKay A M. Viable but non-culturable forms of potentially pathogenic bacteria in water. *Lett Appl Microbiol* 1992; 14: 129-35.

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