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The pathogenicity of environmental campylobacters – a human volunteer experiment

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SUMMARY

Three human volunteer experiments were performed in which river water expected to contain campylobacter organisms was ingested. Despite the ingestion of over 44000 organisms in one experiment, the subject did not suffer any symptoms, nor were campylobacter organisms excreted, nor was an antibody response to the ingested strains detected. The campylobacter organisms ingested resembled *Campylobacter jejuni* on colonial and microscopic morphology but were hippurate negative, and were distinct from *C. coli*. These environmental campylobacter strains appear to be non-pathogenic, however they may be mistaken for *C. jejuni* or *C. coli* if they are not fully identified.

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.

Early in 1983, an unusually high incidence of campylobacter infection in humans was noted in Hull after a period of heavy rain. This observation prompted a series of investigations into the relationship between environmental strains of campylobacter, including those found in the River Hull near to the abstraction plant for the water supply to East Hull, and human infections. Surveys of water courses within the Hull city boundary and of the River Hull had shown that atypical strains of *C. jejuni* were commonly present in these waters (Mawer, 1988). These in turn led to a human volunteer experiment to determine the potential pathogenicity of the strains which enter the water abstraction plant on the River Hull.

METHODS

Bacteriological methods

Faecal samples. Human faecal samples were plated directly onto Preston medium (Bolton & Robertson, 1982) and enriched by inoculation into Preston medium broth (Bolton & Robertson, 1982). The plates were incubated in a

microaerobic atmosphere for 48 h at 42 °C. The enrichment broths after 24 h incubation at 42 °C were subcultured onto Preston medium and were similarly incubated for 48 h.

Environmental samples. In the laboratory the samples were filtered through a 0·45 μm membrane which was placed face up onto 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 and sensitivity to nalidixic acid. All strains were sent to Manchester Public Health Laboratory for serotyping by the Penner method (Penner & Hennessy, 1980).

Serological tests. The serum samples were tested for the presence of complement fixing (CF), bactericidal and enzyme linked immunosorbent assay (ELISA) detectable antibodies to the ingested strains by Dr D. M. Jones of Manchester Public Health Laboratory (Jones, Robinson & Eldridge, 1981). The CF and ELISA tests use a crude antigen which detects antibody to almost all *C. jejuni* and *C. coli* strains. The bactericidal tests used the 13 isolates from the ingested water to detect strain-specific antibodies.

Human volunteer experiments

Three experiments were performed in which river water, expected to contain campylobacter organisms, was ingested. The ingested waters were taken from two sites upstream of the water intake of the river abstraction plant at Tophill Low on the River Hull. At both sites the water, originating from chalk springs, was clear with only a small amount of suspended matter. Previous culture results had shown that campylobacter organisms were normally present and it was expected that there would be between 100 and 500 organisms in the ingested water.

The volunteer for all three experiments, the author, was 37 years of age and was healthy without a known history of campylobacter infection. The volunteer was fully aware of the potential risks of infection and of ingesting untreated river water.

In the first experiment on 19 January 1983 (day 0) a site close to the inlet of a trout farm was chosen. Three samples were taken, one of 1 l and two of 200 ml; from one of the latter samples, 100 ml was ingested on an empty stomach, immediately after it had been taken. The remainder was returned to the laboratory where it was filtered in 1·0, 10 and 100 ml amounts. The 1 l sample was also filtered and cultured similarly for the isolation of campylobacters. The membranes were cultured in the manner described above.

Faecal samples were taken for 3 days before the experiment as control samples and on days 1, 2, 4, 5, 6, 7 and 8 afterwards. These were cultured as described above. A control sample of blood was taken on day 0 and post exposure samples on day 12 and day 28. These were sent to Manchester Public Health Laboratory for serological examination together with the strains isolated from the water.

The second experiment on 18 February 1983 was a duplicate of the first but no

campylobacters were isolated from 100 ml of the duplicate sample of ingested water. The experiment was abandoned.

A different site, a tributary of the River Hull at Bell Mills near Driffield, was chosen for the third experiment. This water was expected to contain more campylobacter organisms because of the presence of more water-fowl than on the river near the trout farm. On 14 July 1983 (day 0), 800 ml of river water was collected, of which half was ingested. The remaining water was filtered in the laboratory in 3×10 ml, $3 \times 1{\cdot}0$ ml, $3 \times 0{\cdot}1$ ml, and $3 \times 0{\cdot}01$ ml amounts as described above and the most probable number (MPN) of campylobacter organisms calculated from the numbers possible by reference to tables (McCoy, 1962). Any remaining water was also filtered and cultured similarly. Colonies showing the typical campylobacter morphology were taken from each plate and identified as described above. Subcultures from 13 colonies were sent to Manchester Public Health Laboratory for serotyping and to Dr M. B. Skirrow for confirmation of the biotyping results.

Six faecal samples were collected before the experiment as control samples and on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 11 and 12, and serum samples were collected on days 0, 7, 15 and 20. The faecal samples were examined as described above. All of the water taken to the laboratory was used for the campylobacter isolation and none remained for presumptive coliform and *Escherichia coli* counts.

RESULTS

In the first human volunteer experiment, it was estimated that between 10 and 100 hippurate negative, TTC-sensitive campylobacter organisms were ingested. Growth was present on the membranes through which 10 ml and 100 ml of river water had been filtered but not on the 1 ml membrane. The strains from the ingested water were non-typable by the Penner serotyping system. No symptoms were experienced by the volunteer, no campylobacter organisms were isolated from the faecal samples and no antibody was detected by CF or ELISA IgG and IgM tests in the three serum samples.

The second experiment failed because the ingested water did not contain campylobacter organisms and was abandoned.

The water ingested in the third experiment contained more campylobacter organisms than expected. Every portion cultured contained hippurate negative, TTC sensitive strains which gave an MPN of greater than 11000 organisms per 100 ml. Thus the volunteer had ingested over 44000 campylobacter organisms. Of 13 isolates from the ingested water that were serotyped, 1 belonged to type 4, 3 to type 28 (1 a weak reactor), 2 to type 39 (1 weak) and 7 were not typable. Dr Skirrow confirmed that the 20 strains examined by him 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. No typical *C. jejuni* or *C. coli* were isolated from any of the water samples. None remained for conventional tests of water purity.

Although over 44000 campylobacter organisms were ingested, no symptoms were experienced by the volunteer and no campylobacter organisms were isolated from any of his faecal samples. No antibody to any of the strains ingested was

detected by campylobacter bactericidal test, CFT or by ELISA for IgG and IgM in any of the four serum samples.

DISCUSSION

The ingestion of contaminated water as the cause of outbreaks of campylobacter enteritis has been described on a number of occasions (Editorial, 1983). Evidence of pollution was a prominent feature of the episode described. In one episode a group of scouts were believed to have been infected when they drank from a stream in which there was a sheep carcase and another group of scouts were infected by drinking from a polluted river (PHLS Communicable Disease Surveillance Centre, unpublished). In other episodes there was evidence of pollution of piped water supplies (Mentzing, 1982). The source of infection of sporadic cases in the community remains uncertain.

A river abstraction scheme, commissioned in 1959, takes water from the non-tidal part of the River Hull, 15 km to the north near Driffield. The water is treated by rapid gravity sand filtration and super-chlorination before being pumped to the part of the city which it supplies, east of the River Hull. Although it seemed very unlikely that *C. jejuni* could survive the treatment process because of its susceptibility to chlorine, at the time it was considered this was worthy of further investigation. When the River Hull is swollen by heavy rain much of the increased flow is caused by water run-off from agricultural land through which the tributaries flow. Particulate matter may then be carried further downstream by the increased flow, increasing the bacterial load of the water (McCoy, 1971).

The human volunteer studies were designed to assess the pathogenicity of environmental campylobacters. For these experiments the volunteer ingested raw waters which were expected to contain between 100 and 1000 naturally occurring campylobacter organisms. A site upstream of the Tophill Low River Hull abstraction plant was chosen close to the water intake of a trout farm. As trout are very sensitive indicators of the presence of chemical and pollutants in the water, this appeared to be a good source of water for the experiment. There were no sewage outfalls into the river so that the risk of transmission of other agents to the volunteer was considered to be negligible.

In the first experiment between 10 and 100 hippurate-negative non-typable campylobacter organisms were ingested with no ill-effect. For the third experiment another site was chosen where the water was obviously not as clear and the river had a substantial wild-fowl population. On that occasion it is estimated that over 44000 campylobacter organisms were ingested. With an inoculum of *C. jejuni* similar to that of the environmental campylobacter strains ingested in these experiments, comparison with the results of Black (1983) suggests that there should have been a greater than 50% chance of infection and a detectable antibody response. However, in Black's experiments *C. jejuni* was ingested in milk which might have protected the inoculum from the action of stomach acid.

In these experiments the volunteer suffered no symptoms, did not excrete campylobacter organisms and did not show any serological response to the strains ingested. This suggests that environmental campylobacter organisms like these are not pathogenic even in large numbers. Robinson (1981) showed that 500

organisms of a pure culture of *C. jejuni* added to milk produced symptoms and a serological response. Black (1983) in a series of human volunteer experiments showed that the probability of infection and symptoms increased with an increasing dose of *C. jejuni* and confirmed that as few as 800 organisms could produce both infection and symptoms. The water from the River Hull was, therefore, unlikely to have been a source of sporadic campylobacteriosis in Hull.

This investigation highlights the importance of biochemical and serological typing of campylobacter organisms isolated from water. The environmental campylobacter described here are indistinguishable on colonial or microscopic morphology from *C. jejuni*. The strains, resembling some of the environmental strains studied in the '1001' isolate survey (Skirrow & Benjamin, 1980*b*), were hippurate-negative by the Hwang and Ederer method but otherwise more closely resembled *C. jejuni* than *C. coli* (Skirrow, personal communication). Some apparently hippurate-negative strains are in fact positive when tested by more sensitive methods (Morris *et al.* 1985). If the strains described are normal aquatic organisms and are not pathogenic, it will be essential for all strains isolated from water to be fully identified before their presence can be used as an indicator of water quality or in the case of water-borne disease be implicated as the source of infection.

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