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Occurrence of 'thermophilic' campylobacters in sewage and their removal by treatment processes

By S. M. ARIMI,* C. R. FRICKER and R. W. A. PARK

Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ, UK

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SUMMARY

Removal of thermophilic campylobacters from sewage at three different stages of treatment at a trickling filter sewage works has been assessed. Samples of incoming sewage, primary sedimentation effluent and final effluent were taken daily from 06.00 h to 20.00 h for 5 consecutive days and the numbers of campylobacters determined by using a most probable number method. Each sample was cultured using 2 h pre-enrichment followed by enrichment in Preston broth for 48 h and detection by plating. Over 78% of the incoming campylobacters were removed after primary sedimentation and < 0·1% remained in the final effluent. *Campylobacter jejuni* biotype I and biotype II constituted 81·5% and 15·9% respectively of the 232 isolates tested. Serotypes common in sewage were common in human faeces. It appears that the trickling filter sewage works removes most of the campylobacters entering the sewage works, but large numbers, estimated to be approximately 10^{10}, are released into the environment daily from a local sewage works.

INTRODUCTION

In recent years campylobacters have become recognized as an important cause of bacterial enteritis in human beings in many parts of the world (Steele & McDermott, 1978; Butzler & Skirrow, 1979; Blaser *et al.* 1980; Richardson, Koornhof & Bokkenheuser, 1981; Skirrow, 1982; Riley & Finch, 1985). Enteritis is commonly associated with *Campylobacter jejuni* and, to a lesser extent, *C. coli*. However, recent reports indicate that *C. laridis* (Simor & Wilcox, 1987), *C. fetus* subsp. *fetus* (Devlin & McIntyre, 1983; Harvey & Greenwood, 1983; Klein *et al.* 1986), *C. cinaedi* and *C. fennelliae* (Totten *et al.* 1985) may also be involved in enteritis. *C. jejuni* has been isolated from a wide range of animal species (Luechtefeld, Cambre & Wang, 1981; Rosef *et al.* 1983; Fricker & Metcalfe, 1984; Waterman, Park & Bramley, 1984) and water (Knill, Suckling & Pearson, 1982; Bolton, Coates & Hutchinson, 1985). Unpasteurized cows' milk (Hutchinson *et al.*

Correspondence should be addressed to Dr R. W. A. Park, Microbiology Department, Reading University, London Road, Reading RG1 5AQ.

* Present address: Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya.

1985, Robinson *et al.* 1979) and improperly prepared poultry (Brouwer *et al.* 1979; Skirrow, 1982) are thought to be the major sources of human infections with *C. jejuni*; contaminated water has also been implicated (Mentzing, 1981; Palmer *et al.* 1983). Water may be involved in the infection of human beings either by the consumption of water from a contaminated supply or by accidental ingestion during recreational pursuits. Campylobacters may reach domestic water supplies and recreational waters by a number of routes, for example by faecal contamination from wild birds or animals or drainage from agricultural land grazed by, or treated with faecal waste from, animals or human beings carrying these organisms. Little is known about the role of sewage treatment plants in the contamination of the environment with campylobacters but Bolton, Coates & Hutchinson (1985) reported that *C. jejuni* isolated from river water downstream from sewage effluent discharge sites were frequently of the same serotypes as those isolated from human faeces.

Sewage contains human, animal and industrial wastes; many microorganisms pathogenic for man and animals may be present (Jones & Watkins, 1985). The removal of various bacteria during sewage treatment was reviewed by Pike (1975). Other workers have reported a 90% or complete removal of salmonellas during sewage treatment (Kampelmacher & Van Noorle Jansen, 1970; Yaziz & Lloyd, 1979). Sewage is potentially an important vehicle for the spread of campylobacters in the environment and thus a possible source of infection for man and animals, but very little is known about these bacteria in sewage. We thought that their known microaerophilic nature might make them particularly sensitive to treatment and so we assessed the incidence of 'thermophilic' campylobacters in sewage and their removal by treatment processes.

MATERIALS AND METHODS

Enumeration of campylobacters in sewage

Samples of incoming sewage, primary sedimentation tank effluent and final effluent were collected from a local trickling filter sewage works at hourly intervals from 06.00 h to 20.00 h for 5 consecutive days. The samples were cultured within 1 h of collection using a pre-enrichment technique. This involved inoculation into non-selective broth (NSB) followed by incubation microaerobically at 42 °C for 2 h prior to addition of antimicrobials. NSB consisted of nutrient broth No. 2 (Oxoid CM 67), 5% saponin-lysed horse blood, and FBP supplement. FBP supplement (George *et al.* 1978) was added to give 0·05% (w/v) final concentration of each ingredient. Antimicrobials added were rifampicin, trimethoprim, cycloheximide, and polymyxin B sulphate (Sigma) to give a final concentration of, respectively, 10 μg ml^{-1}, 10 μg ml^{-1}, 100 μg ml^{-1}, 5 i.u. ml^{-1}. On the basis of experience from preliminary studies to assess the effect of duration of incubation and incubation atmospheres on enumeration of campylobacters, the volume of sample used per bottle was: crude sewage, 50 μl contained in 1·0 ml NSB; primary sedimentation effluent, 500 μl added to 0·5 ml NSB; final effluent, deposit from 10 ml (centrifuged at 20000 ***g*** for 10 min) suspended in 1·0 ml NSB, and 1/10 and 1/100 of these volumes. Each dilution was inoculated into 10 × 5 ml broths. Broths were incubated with the caps applied loosely in a microaerobic atmosphere

produced by evacuating an anaerobic jar without a catalyst to 500 mmHg below atmospheric pressure and refilling with a mixture of H_2 (95%) and CO_2 (5%). After incubation for 2 h, addition of antimicrobials, and further incubation for 48 h, the enrichment cultures were plated on freshly prepared Preston agar (Fricker, 1985). These plates were examined for the growth of campylobacters after microaerobic incubation for 48 h at 42 °C. The MPN was determined for each sample as described by Bolton *et al.* (1982), having recorded as ' + ' any bottle that yielded campylobacters on plates.

Speciation and serotyping of the campylobacter isolates

Usually only a single colony from each of the samples in which campylobacters were isolated was checked for purity and tested for the ability to hydrolyse sodium hippurate and to produce H_2S in FBP broth according to the method of Skirrow & Benjamin (1982). Serotyping of 95 isolates was undertaken based on soluble heat-stable antigens using a modification of the Penner passive haemagglutination technique (Penner & Hennessy, 1980). Details of the technique have been described elsewhere (Fricker, Alemohammad & Park, 1987). A total of 25 Penner antisera which included serotypes most common in both man and animals as used These were Penner 1–11, 15, 16, 18, 19, 20, 23, 24, 27, 30, 31, 35, 37, 44 and 55.

RESULTS

Numbers of campylobacters in sewage and their removal by treatment

Campylobacters were detected in all but 6 of the 225 incoming sewage, primary sedimentation effluent and final effluent samples used for enumeration of the organisms. The mean numbers (MPN) and ranges at the three stages of treatment are shown in Table 1. The numbers fluctuated hourly, but they showed an increasing trend from 06.00 h when sampling was started by reaching peak numbers of $1{\cdot}8 \times 10^5$, $3{\cdot}9 \times 10^4$ and $1{\cdot}7 \times 10^2$ per litre at 12.00, 14.00 and 19.00 h in the incoming sewage, primary sedimentation effluent and final effluent respectively before declining. The peak hours suggested sewage retention time of 2 h from crude influent to primary sedimentation effluent and 7 h from crude influent to final effluent. These times corresponded with the mean flow times of the sewage through the works as recorded by the management.

Campylobacters were present in large numbers in the incoming sewage and were sequentially reduced in numbers during settlement and/or the trickling filter treatment processes by 99·9% in the final effluent. The sequential reduction in numbers is shown in Table 2 using, as an example, 5-day means of campylobacter numbers at peak hours.

Biotypes and serotypes of the isolates

Of a total of 232 isolates, 226 (97·4%) were recognized as *C. jejuni*, 189 (81·5%) being *C. jejuni* biotype I. The remaining six isolates were hippurate negative and did not produce hydrogen sulphide in FBP broth. They were therefore presumptively classified as *C. coli*. Serotyping of 95 of the *C. jejuni* strains was done using 25 Penner antisera and 68% were typable. In addition 73 *C. jejuni* strains isolated from diarrhoeic human faeces obtained from Royal Berkshire

Table 1. *Number of campylobacters (MPN) in incoming crude sewage, primary sedimentation effluent and final effluent (minimum, maximum and mean of 5 days) at Reading sewage treatment works, sampled from 16–20 September 1985*

Sampling time	Incoming sewage flow rate (l/sec)			Incoming sewage			Campylobacters/l in primary sedimentation effluent			Final effluent		
	Min	Max	Mean	Min × 10^4	Max × 10^4	Mean × 10^4	Min × 10^4	Max × 10^4	Mean × 10^4	Min	Max	Mean
06.00	22·2	372·2	160·0	1·0	4·4	2·1	0·4	2·4	1·3	22	92	64
07.00	66·7	388·9	225·6	< 1·0	2·0	1·8*	< 0·2	3·2	1·7*	10	69	41
08.00	127·8	527·8	357·8	2·0	5·1	2·7	0·2	2·4	1·0	10	69	35
09.00	422·2	911·1	624·4	1·0	10·0	4·0	0·4	1·0	0·8	22	69	43
10.00	572·2	900·0	728·9	1·0	13·8	7·7	0·7	2·4	1·4	10	92	48
11.00	483·3	855·6	668·9	2·0	7·2	4·9	0·4	4·6	2·2	22	36	30
12.00	616·7	800·0	684·3	5·4	46·0	18·0	0·7	4·6	1·8	51	96	64
13.00	494·4	588·9	545·6	2·0	24·0	11·0	2·4	4·6	3·3	22	230	103
14.00	511·1	783·3	628·3	< 1·0	2·2	1·8*	2·4	4·6	3·9	10	> 230	96*
15.00	433·3	738·9	493·3	2·0	10·0	4·2	1·8	> 4·6	3·2*	36	230	109
16.00	350·0	650·0	526·7	< 1·0	2·2	1·8*	1·4	> 4·6	1·9*	36	230	109
17.00	355·6	666·7	473·3	2·0	7·2	5·3	1·8	2·4	2·3	22	> 230	130*
18.00	333·3	750·0	554·4	1·0	4·4	2·3	1·4	3·2	2·0	10	230	150
19.00	311·1	488·9	363·3	< 1·0	2·0	1·7†	0·7	3·2	1·9	51	230	167
20.00	283·3	338·9	312·0	< 1·0	2·2	2·1*	0·2	2·4	1·3	22	160	76

MPN, Most probable number; min, minimum; max, maximum; *, based on four counts; †, based on three counts.

Table 2. *Removal of campylobacters by sewage treatment processes*

(Figures used are 5-day means of numbers at peak hours.)

Stage of treatment	Time of sampling	MPN/l	Reduction in campylobacter numbers from last stage	Reduction in campylobacter numbers from intake
Incoming sewage	12.00	$1{\cdot}8 \times 10^5$	—	—
Primary sedimentation effluent	14.00	$3{\cdot}9 \times 10^4$	78·3%	—
Final effluent	19.00	$1{\cdot}7 \times 10^2$	5 h 99·6%	7 h 99·9%

MPN/l, Most probable number/l, mean of five daily samples at peak hours.

Table 3. *Serotypes of* C. jejuni *isolates from diarrhoeic human faeces (73) and sewage (95) typed by PHA using 25 Penner antisera*

Serotype	Human isolates No.	Human isolates %	Sewage isolates No.	Sewage isolates %
1	2	2·7	8	8·4
2	9	12·3	1	1·1
3	0	0	1	1·1
4, 16	15	20·5	37	38·9
6	5	6·8	4	4·2
8	3	4·1	2	2·2
9	1	1·4	0	0
11	1	1·4	0	0
15	1	1·4	0	0
19	5	6·8	3	3·2
23	0	0	1	1·1
24	0	0	1	1·1
27	0	0	1	1·1
31	4	5·5	3	3·2
37	0	0	1	1·1
44	2	2·7	1	1·1
55	1	1·4	1	1·1
Untypable	24	32·9	30	31·6
Total	73	100	95	100

Hospital, Reading, were serotyped using the same antisera. Serotypes common in the sewage were also found to be common in human faeces (Table 3).

DISCUSSION

In order to increase the chances of recovering campylobacters from food, faecal or environmental samples, selective enrichment, and where necessary pre-enrichment before plating, is now considered an essential requirement (Chan & MacKenzie, 1982; Doyle & Roman, 1982; Fricker, Girdwood & Munro, 1983; Fricker, 1984; Ribeiro & Price, 1984; Rogol *et al.* 1985; Humphrey, 1986*a*, *b*). Using such procedures with carefully chosen amounts of sample, time of addition of antibiotics and duration of enrichment we have been able to study the survival of campylobacters in sewage treatment works. All but 6 of the 225 samples used

for enumeration of campylobacters yielded positive plates after enrichment, a clear indication that campylobacters remain present in sewage throughout the treatment process. Large numbers were detected in the incoming sewage and were substantially reduced in the final effluent (99·9%; Tables 1 and 2). Thus the trickling filter process is effective in removing a large proportion of campylobacters from sewage. The activated sludge process has been reported to be more efficient than the trickling filter process in removing salmonellas (Yaziz & Lloyd, 1979) and could presumably be more efficient in removing campylobacters, particularly in view of their oxygen sensitivity. The reason for the marked reduction in numbers of detectable campylobacters in the fluid component by the sewage treatment was not determined. There are several probabilities. They may be killed, stressed or injured (Ray & Johnson, 1984; Humphrey & Cruickshank, 1985) or converted to viable but non-culturable phase (Rollins & Colwell, 1986), resulting in failure to recover them by the isolation techniques used; or concentrated and removed in the sludge. Bearing in mind the disposal practices for sewage sludge on arable land and pastures (Carrington, 1981; Jones & Watkins, 1985), it would seem that further studies should be made. For example more information is needed on the survival time of campylobacters in sludge applied to land and the hazards that survivors might pose to man and animals. Since the infective dose of *C. jejuni* can be as low as 500 organisms (Robinson, 1981), we can only speculate on the possibility that human beings might acquire campylobacter infection from raw crops obtained from agricultural land treated with sewage sludge.

The serotypes of *C. jejuni* common in sewage are also common in human faeces, a finding consistent with human faeces being an important contributor of these organisms. In a survey of campylobacters in a river system subject to sewage effluent discharge, Bolton, Coates & Hutchinson (1985) concluded that sewage is an important source of *C. jejuni* in river water and that biotypes and serotypes common in human faeces were also common in river water especially at sampling points downstream of sewage effluent discharge sites. However, the campylobacters strains could also have originated at least partly from other sources (e.g. a poultry farm, slaughterhouse). Apart from direct contact with sewage itself (Sumathipala & Morrison, 1983), or the possibility of acquiring infection as a result of sewage sludge disposal on land, it is also possible that human beings and animals may acquire campylobacter infections from river waters receiving sewage effluent discharge. Although the percentage of campylobacters remaining in the final effluent is small, the numbers are substantial. We estimate that approximately 10^{10} campylobacters are released from Reading sewage works into a nearby river daily. Clearly, sewage works effluent is an important source of campylobacters entering the environment and hence a potential source for infecting man and animals.

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