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SURVIVAL OF THERMOTOLERANT CAMPYLOBACTERS IN WATER

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GONDROSEN, BJØRN: *Survival of thermotolerant campylobacters in water.* Acta vet. scand. 1986, 27, 1—10. — Three bacterial strains, classified as Campylobacter jejuni biotype 1, Campylobacter coli, and NARTC (nalidixic-acid-resistant thermophilic campylobacters), were tested for survival in water specimens kept at 4, 12, and 20°C. Five different water milieus were compared: sterile physiological saline, chlorinated tap water, dechlorinated tap water, polluted river water, and sterile filtered river water. With few exceptions, all organisms survived better at 4°C than at 12 or 20°C, regardless of the water milieu. Briefest survival was detected at 20°C; no viable campylobacters could be demonstrated after more than 2 days at this temperature. Of the 5 water milieus tested, the highest mean survival time for all strains was obtained with dechlorinated tap water. In this medium, all 3 strains remained viable for 15 days at 4°C, 10 days at 12°C, and 2 days at 20°C. Briefest survival was obtained in chlorinated tap water. Even residual amounts of Cl_2 drastically reduced the survival of all strains tested. Only small variations in viability were detected for 2 of the strains tested after sterile filtration of a water source with a dense bacterial population. The results are discussed in relation to waterborne outbreaks of campylobacteriosis.

chlorination; Campylobacter jejuni; Campylobacter coli; temperature.

The bacterial group referred to as thermotolerant campylobacters (synonyms: Campylobacter fetus subsp. jejuni, Campylobacter jejuni/Campylobacter coli) is now recognized as comprising important human pathogens of world-wide distribution (*Skirrow* 1977, *Butzler & Skirrow* 1979, *Skirrow* 1982, *Prescott & Munroe* 1982, *Kist* 1983, *Blaser et al.* 1984). Food products of animal origin are probably the most important source of infection (*Blaser et al.* 1984, *Doyle* 1984, *Rosef et al.* 1984), though many epidemiological aspects remain to be clarified. The bacteria concerned have been frequently isolated from the faeces or

intestinal contents of a wide range of warmblooded domestic and wild animal species (*Prescott & Munroe* 1982, *Kapperud & Rosef* 1983, *Resef et al.* 1983, *Blaser et al.* 1984). This circumstance provides an opportunity for dissemination of campylobacters to drinking water, through faecal contamination of catchment areas and subsequent drainage into water reservoirs. It has been claimed that drinking raw water involves a greater risk of contracting campylobacteriosis than drinking raw milk or eating undercooked chicken (*Hopkins et al.* 1984). Indeed, several large outbreaks, involving hundreds of people, have been reported in which surface water was implicated as the most probable source of infection (*Tiehan & Vogt* 1978, *Mentzing* 1981, *Taylor et al.* 1982, *Vogt et al.* 1982, *Palmer et al.* 1983, *Penner et al.* 1983, *Taylor et al.* 1983). However, the causative organism has rarely been isolated from water during such outbreaks. In most cases, bacteriological examination of a suspected water reservoir is not initiated until several days after the outbreak has been recognized, and the question arises whether the infective organism is still detectable. One factor which strongly influences the recovery of campylobacters is their viability in different water milieus. The present study was conducted to ascertain whether temperature, chlorination, or competing microorganisms have any effect on the survival of thermotolerant campylobacters in water.

MATERIALS AND METHODS

Bacterial strains

Relevant properties of the 3 Campylobacter strains studied are listed in Table 1. The strains were classified as C. jejuni biotype 1, C. coli, and NARTC (nalidixic-acid-resistant thermophilic campylobacters), respectively, according to the scheme of *Skirrow & Benjamin* (1980). The serotype affiliation was determined on the basis of heat-stable antigens, which were identified by means of the passive haemagglutination technique as described by *Lauwers & Penner* (1984) (courtesy of Dr. S. Lauwers, Infectious Disease Unit, Free University of Brussels). Information on the source of isolation was supplied by the donors. All strains were stored at —70°C in heat-inactivated horse serum with 17 % glycerol. Prior to experiments, the strains were thawed and used within the fourth subculture.

Table 1. Campylobacter strains studied.

Strain	Species	Biotype	Serotype	Source of isolation	Donor
CCUG 6824[a]	C. jejuni	1	LAU 1	Faeces, human enteritis	*E. Falsen*
CCUG 8320[a]	C. coli		LAU 8/11	Pig	*E. Falsen*
C 219	NARTC[b]		LAU 14	Gull, cloacal swab	*G. Kapperud*

[a] Culture Collection, University of Göteborg, Sweden.
[b] Did not produce H_2S by the method of *Skirrow & Benjamin* (1980). This strain, however, produced H_2S when the method described by *Lior* (1983) was used.

Water milieus tested

The survival of the Campylobacter strains in 5 different milieus was studied:

A. Sterile, physiological saline (pH 6.5).
B. Chlorinated tap water (pH 7.4) with ≤ 0.06 mg Cl_2/l. Viable count: 5 colony forming units (CFU)/ ml. Coliform count: 0 CFU/100 ml. Chemical oxygen demand ($K_2Cr_2O_7$): 8 mg O_2/l.
C. Dechlorinated tap water (pH 7.4). Dechlorination was achieved by addition of sodium thiosulphate to medium B.
D. Polluted river water (pH 6.7) obtained from the Akerselva river in the city of Oslo. Viable count: 6.800 CFU/ml, Coliform count: $>$ 30.000 CFU/100 ml. Faecal coliform count: 25.000 CFU/100 ml. Faecal streptococci: 2.500 CFU/100 ml. Turbidity: 4.7 Nephelometric Turbidity Units (NTU).
E. Sterile filtered river water (pH 6.8) obtained by filtration of medium D through a 0.45 µm and a 0.22 µm membrane filter (HAWG 047S3 and GSWP 047S0; Millipore Corporation, Bedford, Massachusetts, USA). Chemical oxygen demand: 8.0 mg $KMnO_4$/l. Turbidity: 0.6 NTU.

To ensure that only the test strains were present during the experiments, medium B and D were checked for thermotolerant campylobacters prior to inoculation.

Inoculation

Strains were prepared for inoculaion by cultivation on blood agar plates at 42°C for 48 h under microaerobic conditions. An appropriate microaerobic atmosphere was achieved in anaerobic jars without catalysts, using gas generating sachets (BR 38; Oxoid Ltd., Basingstoke, Hampshire, England). The bacteria

were transferred to tubes containing 10 ml nutrient broth (CM 67, Oxoid) which were incubated under microaerobic and static conditions as described above. The bacterial density was then adjusted to approx. 10^7 CFU/ml, and 0.9 ml of this suspension were added to 900 ml of each of the 5 water milieus to be tested, giving a final concentration of approx. 10^4 CFU/ml. The 900 ml suspensions were shaken thoroughly and subdivided into three 500 ml Duran bottles (no. 21801443, Schott, Mainz, West Germany), each containing 300 ml of bacterial suspension. These bottles were incubated at 4, 12, and 20°C, respectively, under aerobic and static conditions with no exposure to light.

Withdrawal of samples

At daily intervals, the bottles were shaken, and 10 ml samples were withdrawn. The samples were double-filtered through a 0.7 μm and a 0.22 μm membrane filter (HCWG 047S3 and GSWP 04700; Millipore). Both filters had a diameter of 47 mm. The double filtration was carried out as a one-step procedure, using a prefilter attachment (SM 16807, Sartorius GmbH, Göttingen, West Germany), combined with a vacumfilter holder (SM 16201, Sartorius). After filtration of each sample, 10—20 ml sterile physiological saline were processed through the filter system to ensure that any campylobacters remaining on the walls of the filter holder were trapped by the filters. All apparatus was disinfected between each sample.

Determination of survival

Filters were cut into halves which were placed on the surface of 2 dissimilar selective agar media: colistin-amphotericin-keflin (CAK) agar (*Rosef et al.* 1983) and Skirrow's agar (*Skirrow* 1977). The plates were incubated at 42°C in a microaerobic atmosphere, as specified above, and the filters were checked for growth of campylobacters after 24 and 48 h. The criteria for identification of Campylobacter spp. have been presented previously (*Rosef et al.* 1983). For determination of endpoints, filters were placed in tubes containing 10 ml of Campylobacter enrichment broth (*Rosef* 1981). The endpoint was defined as the time (no. of days) when campylobacters could no longer be detected. The enrichments were incubated at 42°C for 24 and 48 h under microaerobic conditions, and 0.1 ml was subsequently plated out onto CAK and Skirrow's agar. Plates were incubated and read as before.

Table 2. Survival of 3 Campylobacter strains in 5 different water milieus kept at 4, 12, and 20°C.

Strain	Viability[a] (days) in				
	Sterile physiological saline	Chlorinated tap water	Dechlorinated tap water	Polluted river water	Sterile filtered river water
4°C					
C. jejuni (CCUG 6824)	21	4	15	15	15
C. coli (CCUG 8320)	4	0	15	12	8
NARTC (C 219)	4	1	15	10	9
12°C					
C. jejuni (CCUG 6824)	15	3	10	12	12
C. coli (CCUG 8320)	5	0	10	11	9
NARTC (C 219)	5	0	10	6	4
20°C					
C. jejuni (CCUG 6824)	2	2	2	2	2
C. coli (CCUG 8320)	1	0	2	2	0
NARTC (C 219)	1	0	2	0	0

[a] The endpoint was defined as the time (no. of days) when campylobacters could no longer be detected.

RESULTS

Of the 3 Campylobacter strains tested, CCUG 6824 (C. jejuni) showed the longest survival time at all 3 temperatures and in all 5 water milieus compared (Table 2). The mean survival times across all water milieus were: (i) CCUG 6824 (C. jejuni): 14.0 days (4°C), 10.4 days (12°C), and 2.0 days (20°C), (ii) CCUG 8320 (C. coli): 7.8 days (4°C), 7.0 days (12°C), and 1.0 day (20°C), (iii) C 219 (NARTC): 7.8 days (4°C), 5.0 days (12°), and 0.6 day (20°C). The highest viability detected in this study was recorded for strain CCUG 6824 in physiological saline kept at 4°C. This strain remained viable for 21 days, whereas strains CCUG 8320 and C 219 both survived for only 4 days under these conditions.

Of the 3 temperatures investigated, the longest mean survival time for all strains and media was recorded at 4°C (9.9 days), followed by 12°C (7.5 days), and 20°C (1.1 days). With few exceptions, all organisms survived better at 4°C than at 12 or 20°C, regardless of the test medium. Briefest survival was detected at 20°C; no viable campylobacters could be demonstrated after more than 2 days in any of the media.

Among the 5 water milieus investigated, the longest mean survival time for all strains was obtained with dechlorinated tap water, regardless of the temperature. In this medium, all 3 strains remained viable for 15 days at 4°C, 10 days at 12°C, and 2 days at 20°C. Briefest survival was obtained in chlorinated tap water (Table 2). Residual amounts of chlorine (≤ 0.06 mg Cl_2/l) drastically reduced the survival of all strains tested. CCUG 6824 seemed to be more resistant to chlorine than the other 2 strains, though the difference in survival time were insignificant.

The viability detected in polluted river water was 10—15 days at 4°C, 6—12 days at 12°C, and 0—2 days at 20°C. Sterile filtration lead to decreased survival for strain CCUG 8320 and C219, whereas CCUG 6824 seemed to be unaffected.

DISCUSSION

Valuable information on the survival of pathogenic microorganisms in water may be provided by laboratory studies. There are, however, important limitations associated with such models, and the data obtained should therefore be interpreted with caution. Firstly, the survival times detected are of course relative to the isolation procedure employed. Methods based on 100 % sterility never have sharp endpoints and a difference of a few days is not rare, even among tests with the same environmental conditions and initial densities. Secondly, the survival of wild-type strains may be significantly different from laboratory isolates which have been subjected to repeated subculture and artificial storage conditions. Thirdly, bacteria living in natural ecosystems are exposed to a set of variable physiochemical conditions and to a network of interactions within the microbial community, all of which may be vastly different from laboratory settings. In the present work, an attempt was made to establish laboratory conditions that would approximate those in the field.

The Campylobacter strains tested showed highest viability at 4°C. Incubations at 20°C caused a marked reduction in survival compared with 4 and 12°C. The difference between 4 and 12°C was less pronounced. In accordance with this finding, *Blaser et al.* (1980) reported that 2 strains of C. fetus subsp. jejuni placed in specimens of autoclaved stream water survived from 1—4 weeks at 4°C, whereas organisms in samples kept at 25°C died within 4 days. A similar temperature-dependent viability has been observed for thermotolerant campylobacters in other bio-

logical milieus, including foods (*Blaser et al.* 1980, *Doyle & Roman* 1981, *Svedhem et al.* 1981, *Blankenship & Craven* 1982). Due to the climatic conditions prevailing in Norway, the temperature of most drinking water reservoirs is close to 4°C for several months a year. Hence, untreated drinking water may be a more effective vehicle for thermotolerant campylobacters in Norway and other northern countries than in the tropical region, when there has been a point source of contamination (*Blaser et al.* 1980). An extensive indigenous reservoir of thermotolerant campylobacters has been detected among Norwegian wild and domestic animals (*Kapperud & Rosef* 1983, *Rosef et al.* 1983), and contamination of surface waters may consequently occur. Therefore, more investigations should be made to determine the significance of drinking water as a potential source of human Campylobacter infections in this country.

Chlorination is widely used for disinfection of drinking water. *Wang et al.* (1983) demonstrated that 1.25 mg of hypochlorite per litre killed 3 Campylobacter strains within 1 min, at an inoculum size of 10^3—10^4 CFU/ml. This observation indicates that standard chlorination procedures are probably sufficient to prevent the spread of campylobacters along aqueducts and pipelines. All waterborne outbreaks of campylobacteriosis of which the author is aware have involved consumption of unchlorinated or inadequately chlorinated water (*Tiehan & Vogt* 1978, *Mentzing* 1981, *Taylor et al.* 1982, *Vogt et al.* 1982, *Palmer et al.* 1983, *Penner et al.* 1983, *Taylor et al.* 1983). After chlorination, the concentration of Cl_2 falls rapidly. The present results indicate that even residual amounts of Cl_2 ($\leq$0.06 mg/l) in tap water drastically reduce survival. Nevertheless, one of the strains tested (CCUG 6824) survived for 2—4 days depending on the temperature (Table 2). This may reflect a further reduction of Cl_2 concentration after the experiments was started, since no efforts were made to maintain a constant Cl_2 level. In any case, the possibility of contamination by leakage into pipes or aqueducts after the final chlorination remains as a potential hazard. This is especially pertinant with regard to water sources with a higher pH and a higher content of organic matter than the tap water examined in this study. Both these factors are known to counteract the effect of chlorination. In this context, the low infective dose of thermotolerant campylobacters (*Blaser et al.* 1980, *Robinson* 1981) is naturally of concern.

Interspecific competition and other negative interactions with indigenous microorganisms are important factors limiting the survival of pathogens in natural aquatic ecosystems. In this study, however, only small variations in viability were detected after elimination of competing microorganisms by sterile filtration of river water with a dense bacterial population. It should be mentioned, though, that sterile filtration may also influence viability by removal of particulate organic matter. The survival of faecal indicator bacteria in water has been shown to decline with increasing eutrophication (*Østensvik* 1981). No marked differences were observed between the viability of thermotolerant campylobacters in dechlorinated tap water (oligotrophic) as compared to polluted river water (more eutrophic) (Table 2). However, interpretation of the results is complicated by the presence of small amounts of chemical pollutants in the river water, and by the different pH of the 2 water sources compared.

The conclusions listed above were drawn from the study of only 3 Campylobacter-isolates. Examination of greater numbers of strains is necessary to justify definite conclusions. The 3 strains included in this study exhibited different viability. More work is needed to ascertain whether these differences reflect inherent dissimilarities between C. jejuni, C. coli, and NARTC, or merely indicate individual variations among the strains concerned.

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SAMMENDRAG

Overlevingsevnen til termotolerante Campylobacter i vann.

Tre stammer klassifisert som C. jejuni biotype 1, C. coli og NARTC, med henholdsvis serotype LAU 1, LAU 8/11 og LAU 14, ble testet for overlevingsevne i 5 vanntyper ved 4, 12 og 20°C. Vanntypene var som følger: sterilt fysiologisk saltvann, klorholdig springvann, avklorert springvann, forurenset elvevann og sterilfiltrert elvevann. Lengst overlevelse blant de testede stammene ble funnet for C. jejuni-stammen i fysiologisk saltvann ved 4°C (21 døgn). Den lengste gjennomsnittlig overlevingstid, uansett vanntype og stamme, ble oppnådd ved 4°C (9,9 døgn), fulgt av 12°C (7,5 døgn) og 20°C (1,1 døgn). Som overlevingsmedium viste avklorert drikkevann seg best egnet, uansett stamme og temperatur, men selv små rester av klor ($\leq$ 0,06 mg Cl_2/l) i drikkevannet reduserte overlevingsevnen drastisk. Sterilfiltrering ga noe kortere overlevingsevne for 2 av de 3 testede Campylobacter-stammene. Resultatene blir satt i relasjon til vannbårne utbrudd av campylobacteriose.

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