Acta vet. scand. 1981, *22*, 578—588.

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THE EFFECT OF NaCl ON CAMPYLOBACTER JEJUNI/COLI

By

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HÄNNINEN, MARJA-LIISA: *The effect of NaCl on Campylobacter jejuni/coli.* Acta vet. scand. 1981, *22*, 578—588. — The growth of 2 strains of Campylobacter jejuni/coli was investigated in 0—2.0 % NaCl in Brucella broth at 35° C and 30° C. Both strains tolerated more NaCl in the growth medium at 35° C than at 30° C. 2 % NaCl was bacteriocidic at both temperatures. The strains also grew in the medium without added NaCl. At 35° C, low concentrations of NaCl stimulated the growth of strain 5616, but not the growth of strain B33. At 30° C, strain 5616 grew in NaCl concentrations up to 1.0 % and strain B33 in 0 % and at the control concentration (0.5 % NaCl).

The survival of 22 C. jejuni/coli strains in 2.0 % NaCl at 4° C and 35° C was also investigated. Human strains showed significantly greater tolerance to 2.0 % NaCl at both temperatures than did the strains isolated from animals. These findings suggest that the salting of food can be effective in preventing the growth or survival of C. jejuni/coli.

C. jejuni/coli; NaCl; growth; survival.

Campylobacter jejuni/coli has been implicated as a cause of human gastroenteritis. Although the epidemiology of human gastroenteritis is still partly unresolved, some epidemics have proven to be water- or food-borne (*Butzler & Skirrow* 1979). C. jejuni/coli bacteria have been isolated from unprocessed foods, e.g. red meat (*Butzler & Skirrow, Stern* 1981), poultry meat (*Grant et al.* 1980), and coastal sea water samples (*Pearson et al.* 1977).

Salt-tolerance has been used as a criterion in differentiating catalase-positive Campylobacter species; C. faecalis can usually grow in 3.5 % NaCl, but C. fetus subsp. fetus, subsp. jejuni (C. jejuni/coli), and subsp. intestinalis cannot (*Smibert* 1974). It has also been shown by some authors (*Fletcher & Plastridge* 1964, *Lawson et al.* 1975, *Hänninen* to be publ.) that there are differen-

ces between C. jejuni/coli strains in salt-tolerance, if NaCl concentrations 2.0 % or less in the cultivation medium are used.

Salt is the most widely used of food preservatives. The microbiological effect of NaCl as a growth inhibitor for microbial cells in food probably depends on the osmotic withdrawal of water, and will reflect the water activity of the system. The effect of salt is often similar to that of drying (*Scott* 1957). The lethal action of NaCl, like that of other food preservatives, is known to be reduced at low temperatures (*Ingram & Kitchell* 1967). There are also several reports which demonstrate an effect of sodium chloride in rising the minimum temperature for growth (*Ingram & MacKey* 1976).

Because C. jejuni/coli has a relatively low salt-tolerance, the salt concentrations used in foods can be effective in preventing the growth or survival of Campylobacter cells. C. jejuni/coli does not grow at the temperatures commonly used for storage of perishable foods, since its minimum growth temperature is about 30° C (*Smibert*). The survival of non-growing cells of C. jejuni/coli at low NaCl concentrations is not known.

The purpose of this study was to investigate the growth of C. jejuni/coli at different low NaCl concentrations. The tolerance of C. jejuni/coli strains to 2.0 % NaCl at growth and refrigeration temperatures was also investigated.

MATERIALS AND METHODS

Bacterial strains

The bacterial strains used were isolated from human gastroenteritis (5616, 2107, 12650, 11299, 6407, 7535, 9000; isolated at the Department of Bacteriology and Immunology, University of Helsinki, by T. Kosunen. Strain NCTC 11168 was obtained from the Center for Disease Control, Atlanta, Georgia, USA). Bovine (N104, N120, N149, N191), ovine (L5B, L53, L69), chicken (B33, B42, B86, B103) and swine (S12, S14, S17) strains were isolated from intestinal contents or rectal swabs. The sources and methods of isolation are described elsewhere (*Hänninen & Raevuori* 1981). After isolation the strains were stored frozen at —20° C.

Media

In the survival studies at 2 % NaCl, 1.5 % NaCl was added to dehydrated Brucella broth (Difco), filled with distilled water

in a volumetric flask and autoclaved at 121° C for 15 min. In the growth studies the Brucella broth was prepared in the laboratory: pancreatic digest of casein (Difco) (10 g), peptic digest of animal tissues (Difco) (10 g), yeast extract (Difco) (2 g) and sodium bisulphite (Merck) (0.1 g) were added to 1000 ml of distilled water. The final pH of the medium was 7.0±0.1. NaCl was added in the appropriate concentrations to achieve final concentrations of 0.5 % (concentration in the normal Brucella broth), 1 %, 1.5 %, 1.75 % and 2.0 %. The media were autoclaved at 121° C for 15 min. The volumes of the media were carefully tested after autoclaving to ensure that the salt concentrations had not increased during autoclaving. Only fresh media were used in the experiments.

In the determination of the Campylobacter count, Brucella agar with 7 % defibrinated bovine blood was used. All the media were incubated microaerophilically (85 % N_2, 15 % CO_2 and 5 % O_2). The dilution fluid used was 0.1 % peptone water.

Survival and growth studies

The inoculum for both the survival and the growth studies was prepared by adding a loopful of blood agar culture (2 days at 35° C) to Brucella broth and incubating this microaerophilically for 40—44 h at 35° C.

In the survival studies, 1 ml of the Brucella broth culture of a C. jejuni/coli strain was inoculated into 50 ml of Brucella broth with 2 % NaCl in 100 ml glass bottles. The Campylobacter count was made immediately after inoculation and after storage for 48 h at 4° C or 35° C by serial tenfold dilutions in 0.1 % peptone water and plating of 0.1 ml on Brucella blood agar plates. All the experiments were duplicated.

In the growth studies, 1 ml of the 40—44 h Brucella broth culture of a C. jejuni/coli strain was inoculated into 100 ml of freshly prepared Brucella broth in a 200 ml glass bottle containing the desired levels of sodium chloride. The inoculated Brucella broth was divided into test tubes, 5 ml per tube, and incubated microaerophilically at 30° C and 35° C. After incubation for various time periods, 2 tubes were taken for determination of the Campylobacter count as described above for survival studies.

In the statistical analysis of the results, Student's t-test was used.

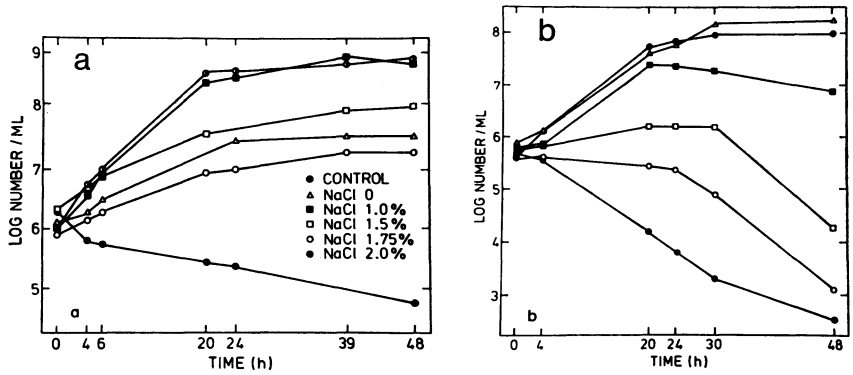

Figure 1. Growth and survival of Campylobacter jejuni/coli strains 5616 (a) and B33 (b) at 35° C in Brucella broth with 0 to 2 % NaCl.

RESULTS

The results of growth studies of C. jejuni/coli strains 5616 and B33 in NaCl concentrations varying from 0 % to 2.0 % are presented in Figs. 1a, 1b, 2a, and 2b. The growth of both strains is greatly influenced by the incubation temperature. Both strains grew more slowly and tolerated less NaCl at 30° C than at 35° C.

At 35° C the cells of strain 5616 were able to grow in salt concentrations 0 to 1.75 %. At 2 % NaCl the number of cells decreased during the 48 h incubation. The growth in 1.0 % NaCl was comparable with the control growth. At 1.5 %, 1.75 % and 0 % NaCl the log numbers of cells obtained during the growth

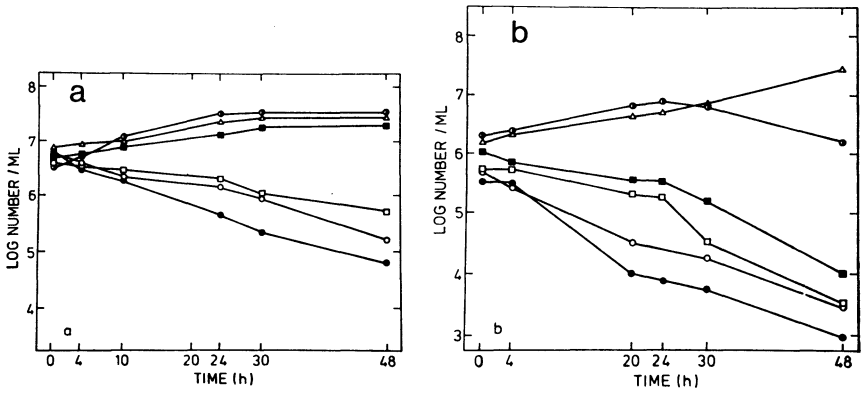

Figure 2. Growth and survival of Campylobacter jejuni/coli strains 5616 (a) and B33 (b) at 30° C in Brucella broth with 0 to 2 % NaCl. Symbols: see Fig. 1 a.

period were lower than those of the control. The final number of cells decreased when the salt concentration increased from 1.5 % to 1.75 %. At 35° C low concentrations of NaCl stimulated the growth of strain 5616. Strain B33 grew at 35° C in NaCl concentrations 0 % to 1.5 %, although the number of cells increased onyl slightly in 1.5 % NaCl. After about 30 h incubation the number of cells in 1.5 % NaCl decreased. NaCl concentrations of 1.75 % and 2.0 % were bacteriocidic.

At 30° C, strain 5616 grew only in NaCl concentrations 0 % to 1.5 %, NaCl concentrations 1.75 % to 2.0 % were bacteriocidic. The log numbers of cells obtained during incubation were almost the same in NaCl concentrations 0 % to 1.0 %. For strain B33,

Table 1. Differences between the log numbers/ml of cells of C. jejuni/coli before and after storage at 35° C or 4° C in Brucella broth with 2 % NaCl for 48 h.

Strain	Mean differences[1]	
	35° C	4° C
5616	1.40	0.57
2107	1,30	0
12658	0.90	0.69
6407	0	ND[2]
NCTC 11168	0.75	0.25
7535	2.12	ND
9000	ND	0.1
11299	1.20	0.86
B86	3.88	1.26
B42	3.38	0.61
B103	3.67	0.61
B33	1.84	0.30
N191	3.12	1.14
N120	4.24	1.04
N149	1.30	1.26
N104	2.81	0.1
S14	1.58	0.89
S17	2.35	0.68
S12	3.12	0.67
L58	2.35	ND
L53	1.86	1.66
L69	2.30	1.26

[1] the log numbers of cells/ml were determined before and after incubation of cells at 35° C or 4° C for 48 h in duplicated bottles of Brucella broth with 2 % NaCl.

[2] ND = not determined.

1 % NaCl was bacteriocidic. The final number of cells in 0 % NaCl was even higher than that of the control.

The results of the survival experiments of C. jejuni/coli strains at 4° C and at 35° C in Brucella broth with 2.0 % NaCl are presented in Table 1. As can be observed, the killing effect of 2.0 % NaCl was greater at 35° C than at 4° C for all the strains studied. The inoculation levels were adjusted to 10^6—10^7 cells per ml of the Brucella broth with 2.0 % NaCl. The mean Campylobacter count decreased by 1.02 logs ($s = 0.72$) for human strains and 2.6 logs ($s = 0.85$) for animal strains at 35° C. At 4° C the mean cell numbers decreased 0.42 logs ($s = 0.37$) and 0.87 logs ($s = 0.45$), respectively. The mean difference of log Campylobacter counts before and after incubation in 2.0 % NaCl both at 35° C and 4° C were significantly greater ($P < 0.05$) for the animal strains than for the human strains. The human strains thus exhibited better NaCl tolerance at both temperatures.

DISCUSSION

As a rule relatively low concentrations of salt will stimulate the growth of micro-organisms, while higher concentrations inhibit them (*Ingram & Kitchell* 1967). In this study, NaCl concentrations up to 1 % are shown to stimulate the growth of the C. jejuni/coli strains investigated if the incubation temperature used is near the optimum growth temperature. At higher salt concentrations, there are differences in salt tolerance between the strains investigated. Salt concentrations over 1.5 % inhibited growth or were bacteriocidic. The salt-tolerance of C. jejuni/coli is relatively low compared to that of other enteric Gram-negative bacteria. Salmonellae are able to grow at 7—8 % NaCl in the cultivation medium, if the growth is tested at 37° C (*Matches & Liston* 1972), and Y. enterocolitica is reported to grow in 5 % NaCl at 25° C (*Stern et al.* 1980).

The tolerance to 3.5 % NaCl has been used as one criterion in distinguishing C. fetus from C. bubulus and C. fecalis, which usually grow in 3.5 % NaCl (*Smibert* 1974). In this study no strains grew at 2 % NaCl, although *Fletcher & Plastridge* (1964) reported the growth of certain human strains at this concentration. The cultivation and incubation conditions which they used were not the same as in the present study.

The strains investigated tolerated more NaCl in the growth medium nearer the optimum growth temperature of 42° C (*Smi-*

bert 1974) than at 30° C. *Matches & Liston* reported that Salmonella likewise tolerated more salt near the optimum growth temperature than at lower growth temperatures. The range of temperatures and salt concentrations in which Salmonella can grow in much larger than those for Campylobacter. In this study it is shown that the range of salt concentrations at which C. jejuni/coli can grow is very narrow and that a small change in the salt concentration has a marked effect on the growth.

The best growth at both temperatures investigated was in salt concentrations under 1 %. This study indicates that C. jejuni/coli can grow at very low NaCl concentrations. At a temperature near the minimum growth temperature, the medium without added salt probably even stimulated the growth of some particularly salt-sensitive strains. Whether other subspecies of C. fetus can grow without added salt is not known, but both C. fecalis and many vibrios (it is to be noted that campylobacters were formerly classified in the same genus as vibrios) need salt for growth (*Smibert, Shewan & Veron* 1974). The growth in the present Brucella broth, without added salt, can probably be used as a criterion in distinguishing C. fecalis from C. jejuni/coli.

The functioning of sodium chloride in the stimulation or inhibition of bacterial growth is not fully understood. One of its functions in growth inhibition is its ability to decrease the water activity (a_w) in the growth medium. However, a growth medium also contains other solutes which influence the a_w (*Scott* 1957). The theoretical a_w value of NaCl concentrations 0.9 % and 1.7 % in water have been reported to be 0.995 and 0.99, respectively (*Evans & Niven Jr.* 1960). The minimum a_w for growth of C. jejuni/coli is not known. It is known, however, that bacteria can usually grow at the above range of a_w. The a_w of most moist fresh foods is above 0.99. It seems unlikely that the relatively low salt concentrations used in this study would alter the a_w of Brucella broth so as to be unfavourable for growth of Campylobacter. The limiting a_w for the growth of many Gram-negative enteric bacteria is reported to be 0.945—0.95 (*Lee & Riemann* 1971).

The stimulating effect of low concentration of NaCl on the growth of bacteria may be related to Na^+. Na^+ is known to have at least two functions in the metabolism of halophilic bacteria (*Gow et al.* 1981). It is required specifically for the transport of a number of solutes into the cells; it functions also less speci-

fically, as an osmotic agent, in preventing the loss of intracellular solutes from the cells (*MacLeod et al.* 1978). Non-halophilic bacteria possess an intracellular tonicity equivalent to that produced by about 0.85—0.9 % NaCl. The other ingredients in the present Brucella broth without added NaCl probably compensated the need of NaCl for intracellular tonicity. The Na^+ concentrations in Brucella broth without added NaCl was found to be 40 mmol/l and that in normal Brucella broth 124 mmol/l. The Na^+ concentration of 40 mmol/l is probably enough for the metabolic functions of Na^+ in Campylobacter cells. Since Campylobacter cells are highly salt sensitive, some of the functions of Na^+ in the metabolism of cells are probably quite different from those of halophilic bacteria.

This study showed a significant difference in salt-tolerance between strains isolated from humans and animals. *Fletcher & Plastridge* (1964) have similarly reported that human isolates of V. fetus tolerated more salt than avian isolates. *Stern et al.* (1980) investigated environmental and clinical strains of Y. enterocolitica and found significant differences in salt and pH-tolerance; the clinical strains were more salt and pH tolerant than the environmental strains. Whether the difference in salt-tolerance observed in the present study is a general phenomenon or is limited only to the strains investigated remains a question for further studies.

Blaser et al. (1980) recognized that Campylobacter survived better at 4° C than at 25° C or 37° C in water, milk, faecal material, human bile and urine. The present survival studies showed that Campylobacter will survive better at 4° C than at 35° C in a salt concentration (2 %) at which they usually do not grow. The same phenomenon is recognized for certain other enteric Gram-negative pathogens. For example Salmonella is rapidly destroyed in curing brines when kept at room temperature, but survives for weeks at 5° C (*Shipp* 1957). E. coli behaves similarly (*Ingram & Kitchell* 1967). The survival of Campylobacter at NaCl concentrations higher than 2 % was not investigated, but in studies by *Shipp* and *Matches & Liston* (1972) there was virtually no difference in the survival of Salmonella at a low temperature when higher or lower salt concentrations were used.

It is known that bacterial cells in the exponential phase of growth rapidly loose viability when cooled ('cold shock') (*Sherman & Albus* 1923, *Straka & Stokes* 1959). In the present sur-

vival studies the cells from the stationary phase of growth were transferred to a supporting 2 % NaCl medium, which then was gradually cooled to 4° C. Microbial cells, if stored under conditions in which they cannot grow, usually die gradually. The number of Campylobacter cells will remain unchanged for at least 2 days in Brucella broth (own unpublished results) or in other non-inhibitory media (*Blaser et al.* 1980).

From the practical point of view, the application of the present findings to the survival and growth of Campylobacter in foods with a low salt content is important. If such foods are stored at refrigeration temperatures, Campylobacter will survive but will not grow until the perishable foods are spoiled. Also low salt content in foods will diminish the survival of Campylobacter cells. Perishable foods with a low salt content are not usually stored at temperatures permitting the growth of Campylobacter.

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SAMMANFATTNING

Effekten av NaCl på Campylobacter jejuni/coli.

Växten av 2 stammar av C. jejuni/coli (5616, B33) i Brucella brot med 0 till 2 % NaCl under 48 h undersöktes. Båda bakteriestammarna visade en bättre salt tolerans i 35° C än i 30° C. 2 % NaCl var bakteriocid i de båda använda temperaturerna. Bakteriestammarna växte också i näringsubstratet utan NaCl tillsats. Låga konsentrationer av

salt stimulerade växten av stammen 5616 i 35° C, men inte växten av stammen B33. I inkubationstemperaturen 30° C växte stammen 5616 i salt konsentrationer upp till 1.0 % och stammen B33 i medium utan NaCl tillsats ocr i medium med 0.5 % NaCl.

Inverkan av 2 % NaCl på överlevandet av C. jejuni/coli stammar i 4° C och 35° C undersöktes också. De stammar som isolerats från människor hade signifikant bättre salt tolerans än stammarna isolerade från husdjur. På grund av resultaten av denna undersökning kan man anta, att saltningen är effektiv för att förhindra av växten eller överlevandet av C. jejuni/coli i livsmedel.

(Received October 31, 1981).

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