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A Research Note

Occurrence and Survival of *Campylobacter jejuni* in Milk and Turkey[1]

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ABSTRACT

An enrichment procedure was used to determine the presence of *Campylobacter jejuni* in milk and ground turkey. This procedure consisted of subculturing a sample in antibiotic-supplemented brucella broth incubated at 37°C for 24 h, transferred to fresh broth, incubated microaerophilically at 42°C for 8 h and plated on agar medium selective for *C. jejuni* for detection. *C. jejuni* was suspected in 9 of 50 samples of raw milk, but was not confirmed. The organism was not recovered from rectal swabs of cows. Storage of whole milk and ground turkey inoculated with *C. jejuni* at 4, 37 and 42°C resulted in decreases in *C. jejuni* counts in milk at 4 and 42°C; and increases in counts in ground turkey at 37 and 42°C. No survivors were detected when suspensions of the organism were exposed to 10 ppm chlorine for 30 s or three common commercial sanitizers used according to manufacturers' specifications.

Campylobacter jejuni has recently been implicated as one of the leading causes of gastrointestinal illness. As early as 1946, this organism was suspected to be a foodborne pathogen, but it was not until 1973 that *C. jejuni* was shown to be commonly associated with gastroenteritis (*4*).

Earlier studies failed to show the organism as an important enteropathogen because *C. jejuni* is difficult to isolate in the laboratory as a result of its fastidious requirements. The development of highly selective culture media for the isolation of *C. jejuni* has greatly simplified the study of this organism. The true incidence of foodborne infections caused by *C. jejuni* remains undetermined because of incomplete reporting of outbreaks, difficulty in culturing and identification of the organism in suspect foods (*16*).

The epidemiology of human enteric infections due to *C. jejuni* is not well understood. Studies to date have shown certain warm-blooded animals, such as fowl, cattle, swine and sheep, to be reservoirs of the organism (*1,8,10*). Meat, eggs, raw milk and water have been suspected as sources of the organism in some outbreaks (*2,9,12,15-18*).

This report provides information on an enrichment procedure for isolation of *C. jejuni* from food samples and the effect of storage conditions and sanitizers on survival of the organism.

MATERIALS AND METHODS

Cultures

C. jejuni cultures were obtained from the Oregon State University Student Health Services from victims of campylobacter gastroenteritis outbreaks. Stock cultures were maintained on Skirrow selective agar plates (*13*), transferred every 48 h, and incubated at 42°C in jars containing a gas mixture of 5% O_2:10% CO_2:85% N_2. Reserve stock cultures were maintained on Skirrow agar slants under normal atmosphere at 4°C. Maximum storage of reserve stock cultures was one month.

Samples

Raw milk and rectal swab samples were taken from 50 cows randomly selected from the Oregon State University dairy herd. The milk was withdrawn from sanitized weighing jars attached to the milking apparatus. Rectal swabs were obtained using sterile cotton swabs and placed in screw cap tubes containing 10 ml of brucella broth (Difco) supplemented with Skirrow antibiotics (*13*) and 0.16% agar. Samples were packed in ice, transported to the laboratory, stored between 0-4°C and analyzed within 4 h.

Each rectal swab was streaked onto prepared Skirrow agar plates and incubated microaerophilically at 42°C for 48 h. The swabs were returned to the transport media, incubated microaerobically at 42°C for 24 h, again cross-streaked onto agar plates and incubated microaerophilically at 42°C for 48 h. Also, 100 ml of each milk sample were enriched in flasks containing 100 ml of brucella broth with antibiotics as previously described, shaken, and incubated microaerophilically at 37°C for 24 h. After incubation, 10 ml were transferred to five replicate tubes containing 10 ml of brucella broth with antibiotics and incubated microaerophilically at 42°C for 8 h. After the second incubation, appropriate dilutions were made to facilitate isolation of the organism and were spread onto selective agar plates which were incubated at 42°C for 48 h.

The identity of suspect *C. jejuni* colonies were confirmed by Gram stain reaction, motility test, morphology examination and biochemical tests (*2*).

Survival studies

Sterile whole milk was inoculated with 6.0×10^5 colony forming units (CFU)/ml of *C. jejuni* and stored at 4 and 42°C for 72 h. At regular intervals, appropriate dilutions (0.1% peptone) were plated on Skirrow agar plates and enumerated for *C. jejuni*. Samples inoculated with 1.2×10^2 and 1.7×10^4 CFU/ml were stored at 4°C. At regular intervals, appropri-

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ate dilutions were made and enumerated until less than 10 viable cells per ml were obtained.

Ground turkey (25 g) was blended 2 min in a Waring Blendor with 225 ml of sterile phosphate buffer. *C. jejuni*, 1.4×10^9 CFU/ml, was inoculated into flasks of the turkey homogenate (1:10 dilution) and stored at 4, 37 and 42°C for 48 h. After the holding period, portions (0.1 ml) were spread onto selective agar plates and incubated microaerophilically at 42°C for 48 h. Additionally, a sample containing a lower inoculum (4.0×10^3 CFU/ml), was held at 4 and 42°C for 72 h. Appropriate dilutions were made and enumerated every 12 h.

Germicidal tests

Stock solutions of three commercially available sanitizers (Sanidu and Iodip, Wesmar Co., Seattle, WA, and Dyne, WestAgro Chemical Co., Shawnee Mission, KS) were prepared according to the manufacturers' directions and serially diluted fivefold with sterile phosphate buffer to achieve the predetermined concentration of sanitizer (*4*). A 1-ml inoculum (1.0×10^4 CFU/ml) of *C. jejuni* was added to 9 ml of sanitizer, shaken and allowed to react at 42.5°C, 30 s and 2 min, for Sanidu and Dyne, and at 22°C, 30 s, for Iodip.

After the reaction time, the solutions were diluted twofold with sterile phosphate buffer, spread onto selective agar plates, incubated microaerophilically at 42°C for 48 h and colonies were enumerated.

Two stock solutions of chlorinated water, i.e., 5555 and 1111 ppm, were prepared from Master-X Bleach which contained 5.25% sodium hypochlorite. The stock solution was diluted fivefold which sterile phosphate buffer. A 1-ml inoculum of *C. jejuni* (1.0×10^5 CFU/ml) was added to 9 ml of a predetermined concentration of chlorinated buffer, shaken and allowed to react for 30 s and 2 min. After the reaction time, the solution was diluted twofold with sterile phosphate buffer and 0.1 ml was spread onto selective agar plates. The plates were incubated microaerophilically at 42°C for 48 h and colonies were enumerated.

RESULTS AND DISCUSSION

The milk sampled from the Oregon State University dairy herd was of good microbial quality, i.e., 46 of 50 samples had standard plate counts of less than 20,000 CFU/ml, the Oregon standard for grade A raw milk (*11*). Thirty-nine of 50 samples had *E. coli* counts of < 10 MPN/ml.

There was no positive confirmation of *C. jejuni* in the raw milk samples, however 9 of the 50 samples included suspect colonies (based on typical motility). Gram staining of these suspect smears revealed mixture cultures. Subculturing of the mixed cultures resulted in pure cultures, but none of the colonies was confirmed as *C. jejuni*. All rectal swab samples were negative for *C. jejuni*.

One of three raw milk samples purchased from local supermarkets contained an organism with typical cultural, microscopic and biochemical characteristics of *C. jejuni*. However, the culture was mixed and upon further subculturing the suspect *C. jejuni* was lost. The same sample, when enriched, did not reveal any suspect *C. jejuni*. The enrichment treatment used had an incubation period of less than 8 h. Possibly if a longer incubation period had been used, *C. jejuni* would have been isolated.

Effect of temperature on survival of C. jejuni *in milk and poultry*

Holding *C. jejuni* in whole milk at 4 and 42°C resulted in a decrease in counts (Fig. 1). Survival was best at 4°C with less than a 1-$\log_{10}$ decrease after 60 h regardless of level of inoculum (data not shown). At 42°C, counts were

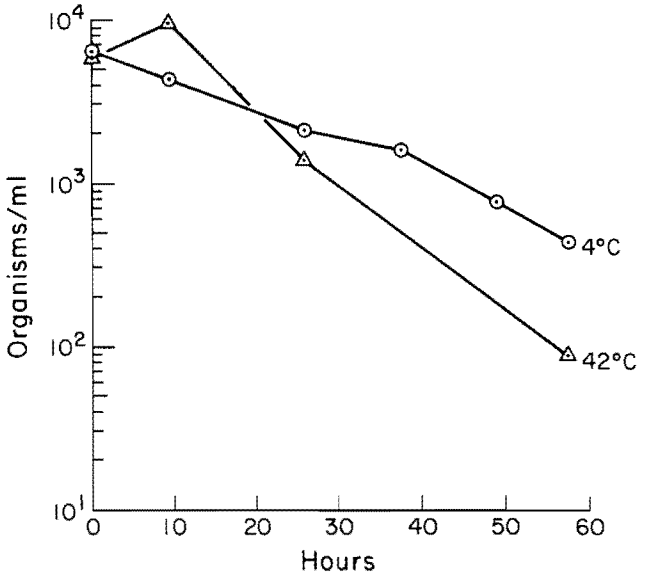

Figure 1. *Survival of* C. jejuni *in pasteurized, homogenized whole milk held at 4 and 42°C.*

reduced 2-$\log_{10}$ within 69 h of storage. Christopher et al. (*6*) observed a similar response for different strains of *C. jejuni* in skimmilk. Robinson et al. (*12*) reported *C. jejuni* survived 164 d in milk kept at 4°C, the level of inoculum, however, was not stated.

When ground turkey homogenate was inoculated with *C. jejuni*, counts decreased during storage at 4°C and increased when stored at 37 and 42°C (Fig. 2). Others have reported similar results. Blankenship and Craven (*3*) found that survival of *C. jejuni* inoculated onto the surface of

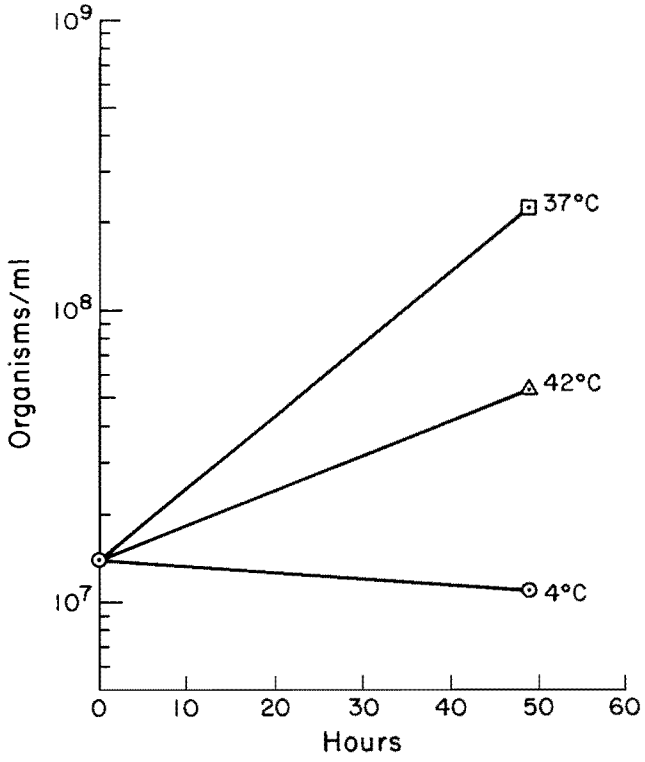

Figure 2. *Survival of* C. jejuni *in ground turkey homogenate held at 4, 37 and 42°C.*

chicken meat varied with temperature during 17 d of incubation. The organism grew for 4 d followed by minimal die-off at 4°C. Grant et al. (*8*) found that *C. jejuni* survived 96 h in five of six chicken intestines kept at 4°C. Results of this study show *C. jejuni* is capable of surviving storage temperatures commonly associated with perishable foods. For some foods, if the product is subjected to higher temperatures, the microbial population can readily increase. The public health significance of this is unknown but it is advisable to avoid cross-contamination with foods that may be contaminated with *C. jejuni*.

C. jejuni was inactivated by commercial sanitizers commonly used in the food industry that were tested in this study. Sanidu is a chlorine sanitizer that is normally used at 100 ppm with a 2-min contact time on dairy equipment surfaces, but inactivated *C. jejuni* to below detectable numbers with a 30-s contact time. Dyne is an iodophor and was effective at 10 ppm for 30 s. Iodophors are effective against most microorganisms at 25 ppm with a 2-min contact time on all dairy equipment surfaces (*5*). Iodip is normally used at full strength, 100 ppm, to dip the cow's teats in before milking. Iodip was effective even at 100 ppb for 30 s. The breakpoint for chlorinated water being effective against *C. jejuni* was 5 ppm. *C. jejuni* survived exposure to 5 ppm chlorine for up to 2 min; however, at 10 ppm, the organism did not survive a 30-s contact time. The level of residual chlorine in municipal water supplies and chlorinated washes in food processing plants should be set high enough to adequately kill *C. jejuni*.

C. jejuni may represent a significant public health hazard in certain raw or improperly processed foods. The survival of *C. jejuni* at 4°C shows that refrigeration is not an adequate means of controlling *C. jejuni*. Doyle and Roman (*7*) reported the D-value of *C. jejuni* in skimmilk at 50°C was 3.5 to 5.4 min and at 55°C it was 0.75 to 1.0 min. Christopher et al. (*6*) reported $D_{50^\circ C}$ values for *C. jejuni* in skim milk from 1.3 to 4.5 min. Present standards for pasteurization are 30 min at 62.8°C or 16 s at 71.7°C. Properly pasteurized milk should, therefore, be completely free from *C. jejuni*. Meats properly cooked and not subjected to cross-contamination from raw food and held at proper temepratures also should not be a health hazard.

Properly sanitized dairy equipment should pose no health hazard. Contaminated water could be a health hazard if residual levels of chlorine are not adequate for destruction of *C. jejuni*.

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