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Prevalence and Survival of *Campylobacter jejuni* in Unpasteurized Milk

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Campylobacter jejuni was isolated from 1 of 108 (0.9%) milk samples obtained from the bulk tanks of nine grade A dairy farms and from 50 of 78 (64%) cows producing grade A milk. Survival of eight *Campylobacter* strains in unpasteurized milk (4°C) varied greatly: the most tolerant strain showed a <2-$\log_{10}$ decrease in viable cells after 14 days, and the most sensitive strain showed a >6-$\log_{10}$ decrease after 7 days. One strain was still recoverable 21 days after the inoculation of milk. Inactivation of the different strains corresponded with an increase in milk aerobic plate count and a decrease in milk pH; however, no absolute correlation could be made between the rates of change of these parameters and the rates of campylobacter inactivation. When held at 4°C, *C. jejuni* was most stable in brucella broth, died most rapidly in unpasteurized milk, and was inactivated at an intermediate rate in sterile milk. Our results indicate the presence and possible persistence of *C. jejuni* in raw grade A milk and reaffirm the need for pasteurization of milk.

Campylobacter jejuni is now recognized as a leading cause of acute bacterial gastroenteritis in humans and has been implicated as the causative agent of several food-associated outbreaks of disease (4, 6). Several such outbreaks have been associated with the consumption of unpasteurized milk (6, 12, 17, 19, 25, 26). The bovine intestinal tract is a known reservoir of *C. jejuni* (15, 18, 24); hence, feces may contaminate milk with the organism. In addition, Lander and Gill (13) have shown that *C. jejuni* may establish infection and multiply within the bovine udder, subsequently resulting in the production of *Campylobacter*-contaminated mastitic milk. Hence, the bovine udder may also serve as a source of *C. jejuni* in milk.

Blaser et al. (3) have reported that *C. jejuni* may survive in sterile milk initially containing $>10^7$ cells per ml for up to 22 days at 4°C and for no more than 3 days at 25°C. Similar observations were made by Christopher et al. (5), who reported an approximately 4-$\log_{10}$ decrease in cells in sterile skim milk after 14 days at 1°C and a 5- to 6-$\log_{10}$ decrease after 2.5 days at 20°C. Recently, Barrell (1) reported the survival profiles of different strains of *C. jejuni* in unpasteurized milk held at 4 or 21°C. He observed that four of seven strains were recoverable after 48 h at refrigeration temperature and that only two of seven strains were recoverable after 48 h at 21°C. One strain was still detected after 5 days at refrigeration temperature. Unfortunately, these were short-term studies, lasting only 2 to 5 days, and the sensitivity of the recovery procedure was rather limited, the minimum level of sensitivity being $<10^3$ to 10^4 cells per ml. Thus, only a 2- to 4-$\log_{10}$ reduction of cells per ml could be monitored.

The purpose of this study was to assess the incidence of *C. jejuni* in unpasteurized milk produced by grade A dairy herds and in cows that produce grade A milk and to determine the long-term survival profiles of a number of *Campylobacter* strains in unpasteurized milk stored at refrigeration temperature, relative to milk aerobic plate count (APC) and pH.

MATERIALS AND METHODS

Screening unpasteurized milk for *C. jejuni*. Raw milk obtained from nine grade A dairy herds that supply the University of Wisconsin-Madison milk processing facility was screened for the presence of *C. jejuni*. Samples were taken from the bulk tank at each farm when milk was picked up by the hauler and were maintained at 4°C until analyzed, usually within 6 h after sampling. Samples of milk (25 ml) from each herd were assayed by an enrichment procedure (8) (100 ml of enrichment medium per sample) on 12 occasions over a 3-month period (October through December 1981).

Screening dairy cows for *C. jejuni*. The University of Wisconsin-Madison dairy herd was twice sampled during 2 successive weeks in November 1981. Sampling was accomplished by soaking a sterile swab in 0.1% peptone, inserting it 1 to 2 in. (ca. 2.5 to 5 cm) into the rectum, rotating it for approximately 10 s, and placing it in 3 ml of 0.1% peptone (2 to 4°C). Swab samples (maintained at 2 to 4°C for no longer than 1 h)

were mixed with a Vortex blender for approximately 15 s, and 0.1 ml was streaked onto each campy BAP agar plate (4), which was supplemented with 50 mg of cycloheximide per ml. The addition of cycloheximide was necessary to suppress the growth of molds which developed on unsupplemented selective media during preliminary studies. The cycloheximide effectively suppressed the growth of molds without restricting the recovery of *C. jejuni*. Plates were incubated for 48 h at 42°C in 5% O_2–10% CO_2–85% N_2. Wet mounts of representative colonies characteristic of *C. jejuni* were prepared and examined microscopically. Colonies showing characteristic darting, corkscrew-shaped movements were confirmed to be *C. jejuni* by biochemical and growth characteristics, using the procedures of Smibert (22), Holdeman et al. (11), and Skirrow and Benjamin (20, 21).

Survival of *Campylobacter* strains in milk. Fresh raw milk was obtained from the University of Wisconsin-Madison herd, stored at 4°C, and used within 18 h after being drawn. Portions (25 ml) of the milk to be inoculated were screened for *C. jejuni* by selective enrichment (8); none were positive. Seven strains of *C. jejuni* and one nalidixic acid-resistant thermophilic *Campylobacter* (NARTC) sp. were used for these studies. The strains of *C. jejuni* included the following: FRI-CF3, FRI-CF6, and FRI-CF8, which were of human origin; FRI-CF33P, which was of porcine origin; FRI-CF74C, which was of avian origin; and FRI-CF145B and FRI-CF147B, which was of bovine origin. The NARTC strain, FRI-CF31P, was of porcine origin. Each strain was grown to late-log phase in brucella broth supplemented with 0.3% sodium succinate and 0.01% cysteine-hydrochloride as described previously (7). Each strain was studied individually by inoculation of 1 ml (ca. 10^9 colony-forming units [CFU]) into 99 ml of unpasteurized milk. Milk was held in the dark at 4°C and sampled at 1- to 2-day intervals for 14 days; one strain was also tested 21 days after inoculation. At each sampling, milk was assayed for *Campylobacter* strains, APC, and pH. Surviving campylobacters were enumerated by serially diluting (1:10) milk in 0.1% peptone and surface plating 0.1-ml portions onto campy-BAP agar. Plates were incubated for 48 h at 42°C, and colonies typical of *C. jejuni* were counted. Wet mounts of representative colonies were prepared and examined microscopically for cells having darting, corkscrew-shaped movements typical of *C. jejuni*. Selected colonies were confirmed to be *C. jejuni* or NARTC by the methods referred to above. APC were determined by procedures described in *Standard Methods for the Examination of Dairy Products* (16).

In conjunction with studies of survival in raw milk, three strains (FRI-CF3, FRI-CF6, and FRI-CF74C) were also tested for the ability to survive in sterile milk (heated at 121°C for 15 min) and brucella broth, which were inoculated and assayed in the same manner as was the unpasteurized milk.

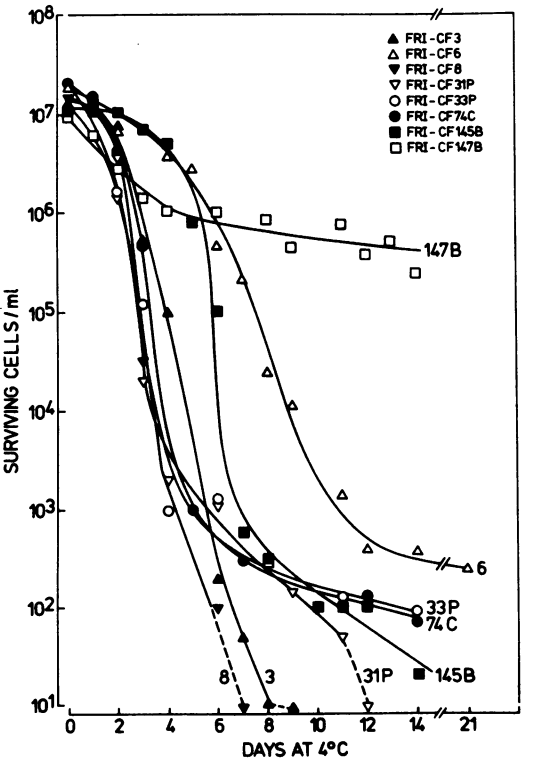

FIG. 1. Survival of *C. jejuni* and NARTC in unpasteurized milk held at 4°C. — — —, No campylobacters were detected at the <10-CFU/ml level (minimum level of sensitivity) in the final sampling.

RESULTS

Incidence of *C. jejuni* in unpasteurized milk. *C. jejuni* was isolated from 1 of 108 (0.9%) milk samples obtained from bulk tanks of nine grade A dairy farms. The level of sensitivity of the recovery procedure was approximately 0.1 campylobacter per ml of raw milk (8).

Incidence of *C. jejuni* in a dairy herd. Of 78 cows screened, 38 (49%) were positive for *C. jejuni* at the first sampling, and 45 of 77 (58%) were positive at the second sampling. Of 77 cows, 19 (25%) were positive for *C. jejuni* at one sampling but not at the other, and of 78 cows, 50 (64%) were positive at one sampling but not at the other.

Survival of *Campylobacter* strains in unpasteurized milk. Survival of the eight *Campylobacter* strains in refrigerated unpasteurized milk varied greatly (Fig. 1). For instance, strain FRI-CF147B, a bovine isolate, showed a <2-$\log_{10}$ decrease in viable cells after 14 days, whereas strain FRI-CF8, a human isolate, showed a >6-$\log_{10}$ decrease in viable cells to a nondetectable level after 7 days. Interestingly, one strain, FRI-CF6, was still recoverable at the 250-CFU/ml level at 21 days after inoculation.

Inactivation of the strains corresponded with an increase in milk APC and a decrease in milk pH (Table 1). The initial milk APC ranged from 2.8×10^3 to 1.1×10^5 CFU/ml and reached 1.6×10^7 to 5.7×10^8 CFU/ml by day 14. The initial

TABLE 1. Inactivation of *C. jejuni* and NARTC at 4°C in unpasteurized milk, relative to milk APC and pH

Strain	Day	Campylobacters/ml	APC (CFU/ml)	pH
FRI-CF3	0	1.3×10^7	3.5×10^3	6.6
	3	5.2×10^5	1.9×10^4	6.5
	6	2.0×10^2	7.1×10^6	6.4
	9	<10	1.6×10^7	6.3
FRI-CF6	0	1.9×10^7	1.1×10^5	6.7
	6	4.7×10^5	3.6×10^7	6.4
	9	1.1×10^4	7.0×10^8	6.3
	12	4.0×10^2	3.7×10^8	6.3
	14	3.9×10^2	2.8×10^8	6.2
	21	2.5×10^2	8.1×10^8	5.9
FRI-CF8	0	1.4×10^7	1.7×10^4	6.7
	3	3.2×10^4	2.2×10^5	6.4
	6	1.0×10^2	6.4×10^5	6.4
	9	<10	5.5×10^7	6.3
FRI-CF31P	0	1.2×10^7	2.3×10^3	6.7
	3	2.0×10^4	5.9×10^5	6.6
	6	1.1×10^3	1.8×10^7	6.5
	9	1.4×10^2	3.6×10^8	6.4
	12	<10	1.7×10^8	6.3
FRI-CF33P	0	2.0×10^7	2.8×10^3	6.7
	3	1.2×10^5	5.9×10^5	6.6
	6	1.3×10^3	1.8×10^7	6.5
	9	2.3×10^2	3.6×10^8	6.4
	12	9.0×10^1	1.7×10^8	6.3
	14	9.0×10^1	5.7×10^8	6.0
FRI-CF74C	0	2.1×10^7	8.6×10^4	6.6
	3	4.7×10^5	5.2×10^5	6.5
	5	1.0×10^3	1.5×10^7	6.5
	8	6.0×10^2	7.2×10^7	6.4
	12	1.3×10^2	7.5×10^8	6.2
	14	7.0×10^1	1.0×10^9	6.1
FRI-CF145B	0	2.9×10^7	1.5×10^3	6.6
	3	7.0×10^6	1.7×10^3	6.5
	6	1.0×10^5	2.0×10^5	6.3
	9	1.0×10^2	3.9×10^6	6.3
	12	1.0×10^2	1.6×10^8	6.3
	14	2.0×10^1	7.5×10^8	ND[a]
FRI-CF147B	0	9.4×10^6	2.3×10^3	6.7
	3	1.4×10^6	5.9×10^5	6.6
	6	1.0×10^6	1.8×10^7	6.5
	9	4.4×10^5	3.6×10^8	6.4
	12	3.8×10^5	1.7×10^8	6.3
	14	2.4×10^5	5.8×10^8	6.0

[a] ND, Not determined.

pH of the milk was 6.6 to 6.7, which is within the normal range of freshly drawn milk, and declined to 6.0 to 6.2 by day 14.

Comparison of inactivation rates in unpasteurized milk, sterile milk, and brucella broth. The three strains studied responded similarly in the same milieu, survival being greatest in brucella broth and lowest in unpasteurized milk. After 14 days at 4°C, very little death occurred in brucella broth; however, campylobacters were less hardy in milk (Fig. 2). Death rates in sterile milk were lower than but parallel to death rates in unpasteurized milk. Little change occurred in the pH of sterile milk or brucella broth during the 14-day study.

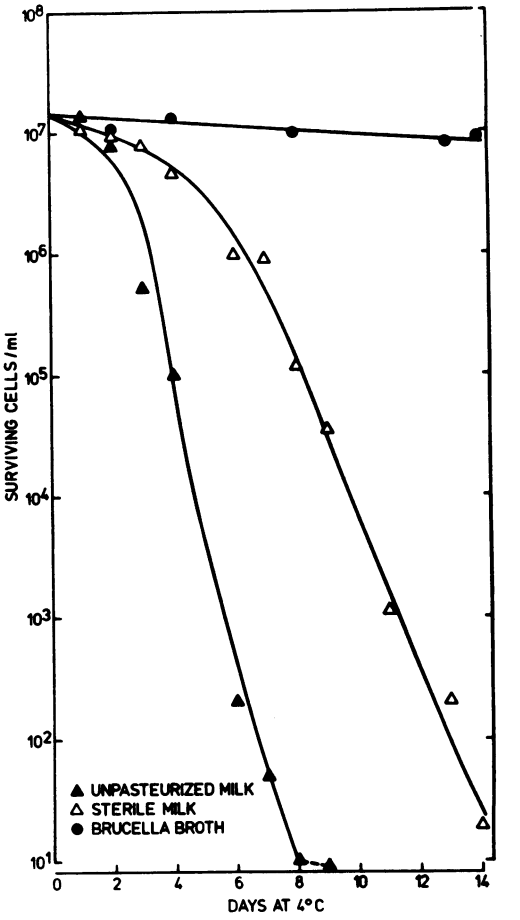

FIG. 2. Survival of *C. jejuni* FRI-CF3 in unpasteurized milk, sterile milk, and brucella broth at 4°C.

DISCUSSION

Although *C. jejuni* was found in only 1 of 108 samples of fresh raw milk, its presence in the intestinal tracts of 64% of the cows in one grade A herd suggests that milk handled in an unsanitary manner may easily be contaminated with the organism. Our finding of *C. jejuni* in 49 and 58% of the same herd sampled on two occasions is similar to the finding of Luechtefeld and Wang (15), who isolated the organism from the cecal contents of 43% of 130 slaughter cattle. Others who have studied the incidence of *C. jejuni* in feces or cecal contents of cattle have reported

isolation rates of 0% of 31 cattle (23), 2.5% of 202 cattle (18), and 19% of 90 cattle (24). One can only speculate as to why such variability exists. Herd management practices, type of housing, and type and quality of feed and drinking water may influence the existence and spread of *C. jejuni* in cattle. Methods used to obtain and transport samples and isolate the organism may also be important factors.

One strain of *C. jejuni*, bovine isolate FRI-CF147B, survived exceptionally well in unpasteurized milk at 4°C. A <2-$\log_{10}$ reduction in cells occurred after 14 days, indicating that under the appropriate conditions, large numbers of campylobacters may survive in raw milk for several days. Strains from several sources, including poultry and swine, were evaluated because, as shown by Lior et al. (14), *C. jejuni* strains of the same serogroup can be isolated from a variety of different animals. Hence, similar strains of the same serogroup may be isolated from both poultry and cattle. Furthermore, since cattle may be exposed to swine and poultry and their excrement, it is possible that campylobacters from these animals may be ingested and colonize cattle.

Although there was wide variation in the ability of the strains to survive in unpasteurized milk, direct comparisons among the strains cannot be made because the milk used for these studies, although obtained from the same herd, was obtained on different days. Hence, the composition of the microbial population and other constituents of the milk that may influence the ability of the organism to survive may vary. However, the same lot of milk was used to assess the survival characteristics of strains FRI-CF147B and FRI-CF31P, making a direct comparison of these strains possible. Strain FRI-CF147B was clearly more tolerant of the antimicrobial factors in refrigerated raw milk than was strain FRI-CF31P.

In all instances, as the *Campylobacter* population decreased in unpasteurized milk, the APC increased and the pH decreased. However, no absolute correlation could be made between the rate of change in milk APC or pH and the rate of campylobacter inactivation. Likely differences in the bacterial composition of the APC may partially explain this lack of correlation: the populations of bacteria producing metabolites toxic to campylobacters may vary in number and type among different lots of milk. Furthermore, as indicated above, there are likely to be differences among strains in tolerance to adverse conditions.

Although the pH was shown to decline as campylobacters were inactivated, it is unlikely that the decline was the sole factor influencing the inactivation rate. We have shown previously that FRI-CF8, the strain that died most rapidly at 4°C in unpasteurized milk, shows an approximately 2.5-$\log_{10}$ reduction after 14 days in brucella broth adjusted to pH 5.0 and held at 4°C, whereas a >6-$\log_{10}$ reduction occurs after 7 days in raw milk, which ultimately reaches pH 6.3 (7).

Inactivation of *Campylobacter* strains in unpasteurized milk paralleled but was greater than inactivation of strains in sterile milk, suggesting that the psychrotrophic microflora of raw milk may have produced metabolites toxic to *C. jejuni*. Alternatively, unlike sterile milk, raw milk contains lactoperoxidase, which, in combination with H_2O_2 and SCN^-, produces metabolites that are bactericidal to many gram-negative bacteria. The activity of the lactoperoxidase system may at least partially explain why the *Campylobacter* strains were inactivated to a greater degree in raw milk than in sterile milk. Perhaps the H_2O_2 produced by bacteria growing in raw milk increases the generation of lactoperoxidase-mediated metabolites toxic to *Campylobacter* strains. *C. jejuni* was more readily inactivated in sterile milk (pH 6.6 to 6.7) than in brucella broth (pH 6.9). Since the pH values of both media were comparable and since there was little or no change in the pH of either medium during storage, it is unlikely that pH was a major factor in the inactivation of the organism in sterile milk. Possibly milk possesses a heat-stable factor that is bactericidal to *C. jejuni*, or, more likely, brucella broth possesses properties that are protective of campylobacters. We have found that brucella broth is more protective of campylobacters during drying than is sterile skim milk (8a). We speculated that the bisulfite in brucella broth acts as an antioxidant and suppresses the generation of toxic derivatives formed by oxygen. *C. jejuni* is believed to be particularly sensitive to such metabolites (11). Hence, bisulfite may be a factor that gives brucella broth its protective advantage.

The presence and possible persistence of *C. jejuni* in raw grade A milk reaffirms the need for pasteurization. We have reported D_{55} (time at 55°C required to inactivate 90% of the population) values of 1.0 min or less for inactivation of several strains of *C. jejuni* in skim milk (7). Hence, the vat process of holding milk at 62.7°C for 30 min should be sufficient to free milk of unusually large numbers of *Campylobacter* strains. Results reported by Gill et al. (9) confirm that the high-temperature, short-time process (71.7°C, 15 s), which is commonly used to pasteurize milk in the United States, is sufficient to inactivate 2.3×10^6 campylobacters per ml. It is unlikely that contaminated milk would contain more than 10^6 to 10^7 campylobacters per ml: mastitic milk from *Campylobacter*-infected ud-

ders has been found to contain $\leq 10^5$ campylobacters per ml (13), and fecal material entering milk at the farm would likely be diluted sufficiently to reduce the number of campylobacters to $<10^7$ CFU/ml. Hence, properly pasteurized milk should be free of viable *C. jejuni*.

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