Survival of Campylobacter fetus subsp. jejuni in Cheddar and Cottage Cheese

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(Received for publication February 8, 1982)

ABSTRACT

Campylobacter fetus subsp. jejuni inoculated into Cheddar cheese milk at concentrations ranging from \(10^2\)-\(10^8\) cells per ml was not detectable in the curd after 30-60 d of curing. When milk for cottage cheese manufacture was inoculated with \(10^5\) to \(10^7\) cells of C. fetus subsp. jejuni, the organism was not detectable in the whey or curd after cooking for 30 min at 55°C.

Campylobacter fetus subsp. jejuni is now recognized as a potential human pathogen that can cause gastroenteritis in humans through consumption of contaminated water or food. Consumption of raw milk has been associated with several outbreaks of Campylobacter gastroenteritis (1,5-7). Recently, Christopher et al. (2) reported that C. fetus subsp. jejuni at a concentration of \(10^6\) to \(10^7\) viable cells per ml failed to survive heating in skim milk at 60°C for 1 min. Calculated \(D_{50C}\) values for their test strains of C. fetus subsp. jejuni ranged from 1.3 to 4.5 min. To our knowledge, no case of Campylobacter enteritis has been associated with the consumption of commercially pasteurized milk. According to Christopher et al. (2), several strains of C. fetus subsp. jejuni survived in sterile skim milk at commercial refrigeration temperatures for at least 2 weeks, survival time dependent upon strain and level of contamination. Survival in skim milk was temperature dependent, at 1 or 10°C it was at least 11-12 d, at 20-40°C, it was 30-54 h.

Some cheeses are often manufactured from raw milk. In this country, cheese prepared from raw milk is stored for 60 to 17°C or above before consumption. The present paper provides information on the survival of C. fetus subsp. jejuni in Cheddar and cottage cheese prepared from milk inoculated with this species.

MATERIALS AND METHODS

ABSTRACT

CULTURES

The following strains of C. fetus subsp. jejuni were used: 6 (C. E. Park, Microbiology Research Division, Health and Welfare Canada, Ottawa), 18177 (B. D. Firehammer, Department of Veterinary Science, Montana State University, Bozeman, MT), C 56 (S. M. Harvey, Food and Drug Administration, Los Angeles, CA) and 29428 (American Type Culture Collection, Rockville, MD). Test strains were maintained in culture maintenance broth (CMB) consisting of brucella broth with 0.15% agar and 0.05% filter-sterilized sodium pyruvate. Cultures were transferred weekly. Inocula for the cheese milk consisted of cultures grown in CMB for 48 h at 42°C in 5% \(O_2\):10% \(CO_2\):85% \(N_2\). The amount of inoculum was calculated to yield approx. \(10^5\)-\(10^6\) organisms per ml for Cheddar cheese manufacture; for cottage cheese one level of inoculum, \(10^2\)-\(10^3\) cells per ml of milk, was used.

Media

Brucella-FBP-AM (antimicrobial agents) broth consisted of brucella broth supplemented with 0.15% agar, 0.05% FeSO_4 \(\cdot\) \(H_2\)O, 0.05% sodium metabisulfite, 0.05% sodium pyruvate, vancomycin (10 mg/L), trimethoprim (5 mg/L), polymyxin B sulfate (250 IU/L), amphotericin B (2 mg/L) and cephalothin (15 mg/L). The brucella broth and agar were mixed, brought to a boil and autoclaved at 121°C for 15 min. The supplements were filter-sterilized and added to the medium after it had cooled to 50°C. Brucella-campylobacter agar consisted of brucella broth supplemented with 1.75% agar, 5% defibrinated horse blood, vancomycin (10 mg/L), trimethoprim (5 mg/L), polymyxin B sulfate (250 IU/L), amphotericin B (2 mg/L) and cephalothin (15 mg/L). The brucella broth and agar were mixed, brought to a boil and then autoclaved at 121°C for 15 min. After cooling to 50°C, the blood and filter-sterilized supplements were added to the medium. After pouring, plates were allowed to dry overnight. Brucella broth diluent consisted of brucella broth supplemented with 0.05% FeSO_4 \(\cdot\) \(H_2\)O, 0.05% sodium metabisulfite and 0.05% sodium pyruvate.

Enumeration of C. fetus subsp. jejuni

Milk (10 ml), whey (25 ml), curd (25 g) or cheese (25 g) was placed in brucella-FBP-AM broth to give a 1:10 dilution. The samples were macerated in a Stomacher 400 for 1.5 min and then placed in sterile 600-ml glass jars with screw-top lids. One ml of the initial dilution (0.25 ml on each of 4 plates) and 0.1-ml volumes of appropriate decimal dilutions (brucella broth diluent) were streaked over the surface of brucella-campylobacter agar plates with a sterile bent glass rod. In addition, 1-ml volumes of the same dilutions were added to a series of tubes with brucella-FBP-AM broth (9 ml) in a 3-tube MPN series. Jars and tubes were held first at 4°C for 12 h and then at 42°C for 48 h in 5% \(O_2\):10% \(CO_2\):85% \(N_2\). A loopful of the contents of each jar and MPN tube was then streaked onto brucella-campylobacter agar plates. Plates were incubated for 48 h at 42°C in 5% \(O_2\):10% \(CO_2\):85% \(N_2\). Suspect colonies (smooth, convex, translucent, colorless to cream-colored, pin-point to 1-2 mm in diameter or often spreading colonies) of C. fetus subsp. jejuni were examined by the following tests: gram reaction (+ or +); morphology (vibrio form); oxidase reaction (+); catalase reaction (+); motility (+); \(H_2S\) (+); growth in 1% glucose (+); growth at 42°C (+); growth at 30.5°C (-); sensitivity to nalidixic acid (sensitive), triphenyltetrazolium chloride (sensitive) and cephalothin (resistant); and hippurate hydrolysis (+). Concentrations of...
<table>
<thead>
<tr>
<th>Sample</th>
<th>Strains of C. fetus subsp. jejuni used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Milk, just after inoculation with Campylobacter</td>
<td>$4.6 \times 10^5 b$</td>
</tr>
<tr>
<td></td>
<td>$8.9 \times 10^3 c$</td>
</tr>
<tr>
<td>Milk, just before adding rennet</td>
<td>$2.4 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>$2.5 \times 10^4$</td>
</tr>
<tr>
<td>Whey, at time of draining</td>
<td>$2.3 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>$1.2 \times 10^3$</td>
</tr>
<tr>
<td>Curd, at time of draining whey</td>
<td>$4.6 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>$2.8 \times 10^6$</td>
</tr>
<tr>
<td>Curd, after salting, before pressing</td>
<td>$&gt;2.4 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>$2.7 \times 10^6$</td>
</tr>
<tr>
<td>Cheese, after 15 d curing</td>
<td>$&lt; 0.3$</td>
</tr>
<tr>
<td></td>
<td>$&lt; 1$</td>
</tr>
<tr>
<td>Cheese, after 30 d curing</td>
<td>$&lt; 0.3$</td>
</tr>
<tr>
<td></td>
<td>$&lt; 1$</td>
</tr>
<tr>
<td>Cheese, after 60 d curing</td>
<td>$&lt; 0.3$</td>
</tr>
<tr>
<td></td>
<td>$&lt; 1$</td>
</tr>
</tbody>
</table>

*Count per ml (milk, whey) or g (curd, cheese).  
\(^b\)MPN.  
\(^c\)Plate count on brucella-campylobacter agar.  
\(^d\)Agar streak from contents of jar containing 25 g of cheese and 225 ml of brucella-FBP-AM broth was positive for *C. fetus* subsp. *jejuni* after incubation for 48 h at 42°C.
Microaerophilic environment

Stock cultures, inocula, MPN tubes and plating media were incubated in an A.N.E.E. (National Appliance Company) controlled environment system obtained by filling and evacuating the incubator twice with N₂ and then filling it with a gas mixture consisting of 5% O₂:10% CO₂:85% N₂.

Cheddar cheese manufacture

Raw milk from the Texas A&M University dairy processing plant was pasteurized at 72°C for 16 s and cooled to 4°C. Pasteurized milk (15 L) was placed in a plexiglass vat (30 x 60 x 15 cm), warmed to 30°C at which time C. fetus subsp. jejuni and lactic starter (0.7% by volume, Hansen DVM culture) were added. Annato color (1 oz per 1000 lb milk) was added to the milk 30 min after inoculation. Rennet (3.5 oz per 1000 lb milk) diluted with 20 volumes of cold, sterile, distilled water was added to the milk 45 min after inoculation. Coagulation of the milk occurred within 45 min after addition of rennet. The coagulum was cut with 0.25-in. knives and left undisturbed for 15 min. At cutting, the titratable acidity of the whey was 0.10-0.11%. Dielectric heating was used to increase the temperature of the curd and whey from 30 to 38.8°C within 30 min. This temperature was maintained for an additional 60 min with the curd being agitated continuously. At the conclusion of cooking, the titratable acidity of the whey was 0.12-0.13%. Whey was drained and the curd collected in two packs. Cheddaring was conducted by turning the curd every 15 min until the titratable acidity increased to 0.40-0.45%. During cheddaring the temperature of the curd decreased from 38.3°C to 33.8°C. The curd was cut into strips approx. 0.63-in. wide and 2-3 in. long. Salt (2.5 lb per 1000 lb milk) was added in three portions with a 10-min interval between each addition. The salted curd was placed in hoops made from 1-lb coffee cans and pressed overnight at 31±1°C. The pressed cheese was dried for 4 h, waxed and then stored at 4°C. Samples of milk, whey or curd were tested for Campylobacter jejuni subsp. jejuni at various stages during manufacture; cheese was sampled after 15, 30, and 60 d of storage.

Cottage cheese manufacture

Pasteurized (72°C for 16 s) skim milk (15 L) was placed in the plexiglass vat, warmed to 32°C after which C. fetus subsp. jejuni, lactic starter (5% by vol, Hansen Dri-Vac) and rennet (1 ml per 1000 lb milk) were added. The temperature of the milk was maintained at 32°C. The coagulum was cut with 0.25-in. knives when the pH was 4.75. Dielectric heating was used to increase the temperature of the whey and curd to 55°C at a rate of 2°C per 5 min. The temperature was maintained at 55°C for 30 min to permit development of proper curd firmness. The pH at the end of the cooking process was 4.5. Curd and whey were gently agitated throughout the cooking process. Cooked curd was washed three times. The first two washes were with tap water and the final wash was with chilled water (4°C). Before each wash, one-half of the fluid volume in the vat was drained from the curd. Wash water was added to replace the volume of liquid drained from the curd and the contents of the vat were agitated gently. At the conclusion of the third wash all the liquid was drained from the curd. Washed curd was then stored in a refrigerator at 4°C. Samples of milk, whey or curd were tested for C. fetus subsp. jejuni at various stages during manufacture.

RESULTS AND DISCUSSION

When Cheddar cheese milk was inoculated with either low (10²-10⁶ cells per ml) or high (10⁴-10⁶ cells per ml) concentrations of C. fetus subsp. jejuni, all curd samples (at draining of whey and after salting, before pressing) were positive for C. fetus subsp. jejuni (Table 1). Concentrations of this organism in the curd from the milks with a high inoculum ranged from 10⁶-10⁷ per g, those in curds prepared from milks with a low inoculum ranged from 10²-10⁶ per g of curd. C. fetus subsp. jejuni was not detected in cheese samples cured for 30 or 60 d. However, in three instances (Cultures 6, 18177, C56) C. fetus subsp. jejuni was present, although in low concentrations, in cheese held for 15 d. Although the precise mechanisms, for the rapid destruction of C. fetus subsp. jejuni in Cheddar cheese curd during ripening have not been studied, it is most likely that a low pH and sodium and the presence of salt are involved. Substantial decreases in viable cells of C. fetus subsp. jejuni in brucella broth at pH 5.0 were reported by Doyle and Roman (4) at 4 and 25°C and by Christopher et al. (2) at 37°C. C. fetus subsp. jejuni added to milk did not survive the manufacture into cottage cheese (Table 2). The absence of C. fetus subsp. jejuni in cottage cheese curd and whey after cooking (30 min at 55°C) is not surprising.

Table 2. Count of Campylobacter fetus subsp. jejuni in milk, whey and curd during manufacture of cottage cheese.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Strain of C. fetus subsp. jejuni used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, just after inoculation with Campylobacter sp.</td>
<td>10⁷</td>
</tr>
<tr>
<td>Coagulum just before cutting</td>
<td>10⁶</td>
</tr>
<tr>
<td>Whey, at end of cooking</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Curd, at end of cooking</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Curd, after final wash</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Count per ml (milk, whey) or g (curd).

Plate count on brucella-campylobacter agar.

Christopher et al. (2) reported that this organism, at a level of 10²-10⁶ per ml of skim milk, did not survive heating at 55°C for 5 min. According to Doyle and Roman (4), D₅₅°C values for five strains of this organism ranged from 0.74 to 1.00 min. However, the organisms did survive in the low pH environment until the curd was cut. Procedures for the isolation of C. fetus subsp. jejuni from foods are relatively new. Preliminary experiments with a limited number of strains in foods such as milk, eggs and turkey meat showed that fewer than 10 viable cells per g can consistently be detected by the enrichment-plating procedure. Improvements in sensitivity of detection as compared with previously reported results (3) may have resulted from the addition of FeSO₄·7H₂O and sodium metabisulfite to the enrichment broth. When reporting the presence or absence of C. fetus subsp. jejuni, it should be recognized that the cells in this study may have been subjected at one time or another to conditions of stress (refrigeration temperature, heating, chemicals such as NaCl and metabolic products from the microorganisms). To our knowledge, no published information exists on the detection of potentially injured cells of Campylobacter jejuni in milk, whey, curd and cheese are reported both as MPN per g or ml and as the plate count of confirmed colonies on brucella-campylobacter agar.
**C. fetus subsp. jejuni.** Additional experiments with raw milk containing various levels of this organism are needed to determine whether the survival of **C. fetus subsp. jejuni** in curd, whey and cheese from raw milk is similar to that reported for these products using pasteurized milk. In summary, under the present conditions of recovery, **C. fetus subsp. jejuni** inoculated in Cheddar cheese milk could not be detected in the curd after 30-60 d of curing. No **C. fetus subsp. jejuni** was detected in cottage cheese whey or curd after cooking.

**ACKNOWLEDGMENTS**

Journal paper No. 17423 of the Texas Agricultural Experiment Station, College Station, Texas.

**REFERENCES**


   technique than with those obtained by the double swab technique. This finding was substantiated by the calculated values of the coefficient of variation, standard deviation, correlation coefficient and coefficient of determination. It was also found that the excision technique value could be predicted most accurately with the modified agar sausage technique. According to this study, the modified agar sausage technique can be recommended for monitoring microbiological counts on carcass surfaces because of its simplicity, speed and accuracy.

   **ACKNOWLEDGMENT**

   The authors wish to thank Dr. C. Z. Roux for assistance with the statistical analyses.

   **REFERENCES**