Growth and Survival of *Campylobacter fetus* subsp. *jejuni* as a Function of Temperature and pH

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**ABSTRACT**

The objective of this study was to determine what effect temperature and pH have on the ability of *Campylobacter fetus* subsp. *jejuni* to grow and survive. None of three strains of *C. fetus* subsp. *jejuni* could grow at 30 C and below or at 47 C and above. The optimum temperature for growth was in the range of 42 to 45 C. Only one of three strains, FRI-CF8, could grow at pH 4.9 and none could grow at pH 4.7. The optimum pH for growth was in the range of 6.5 to 7.5; however, all strains grew well at pH 5.5 to 8.0. Rate of cell death at pH 3.0 to 4.5 was temperature-dependent. At comparable pH, cells of *C. fetus* subsp. *jejuni* died most rapidly at 42 C, less rapidly at 25 C and at the slowest rate at 4 C. For example, at pH 4.5, a 3 log₁₀ decrease of cells occurred within 8 h when incubated at 42 C but took 4 days when incubated at 4 C. At 25 C and pH 4.5, cells were inactivated at an intermediate rate. Rates of thermal inactivation of five strains of *C. fetus* subsp. *jejuni* were determined at 48, 50, 53, and 55 C in a skim milk heating menstruum. At 48 C, D-values ranged from 7.2 to 12.8 min while at 55 C they ranged from 0.74 to 1.00 min. The times and temperatures used to pasteurize milk should be sufficient to free milk of even unusually large numbers of viable cells of *C. fetus* subsp. *jejuni.

*Campylobacter fetus* subsp. *jejuni* has recently been recognized as a prominent cause of acute bacterial gastroenteritis in humans (3). Its rate of isolation is comparable to and in many studies exceeds the rate at which *Salmonella* is isolated from diarrheal stools of hospitalized patients.

Both food and water have been implicated as vehicles responsible for transmitting campylobacters to susceptible individuals (1, 3, 5, 11-13, 21, 22). Foods derived from animals are of particular concern. Many species of animals, both wild and domestic, harbor *C. fetus* subsp. *jejuni* as part of their normal intestinal flora (7). Examples include poultry (4, 16), swine (10), cattle (7), sheep (15) and waterfowl (9). Since *C. fetus* subsp. *jejuni* may be present on meat (2, 5, 13, 18) or in milk (1, 7, 11, 12, 14, 21) obtained from such animals, it is important to know how these organisms will respond to conditions that may be present when foods are prepared and/or stored.

Temperature and pH are two factors commonly employed by food processors to control or inactivate pathogens and undesirable microorganisms in foods. The purpose of this study was to determine what effect temperature and pH have on the ability of *C. fetus* subsp. *jejuni* to proliferate and/or survive in a medium supportive of its growth. Such information may be useful in identifying conditions and treatments that would render foods safe from the potential hazards of this prevalent pathogen.

**MATERIALS AND METHODS**

Organisms and cultural conditions

All cultures of *C. fetus* subsp. *jejuni* used for this study were isolated from human stool specimens and were obtained from A. Helstad (Wisconsin State Laboratory of Hygiene, Madison, WI). The cultures were identified as FRI-CF3, FRI-CF6, FRI-CF8, FRI-CF12 and FRI-CF16.

In preparation for growth and survival studies, organisms were first cultured on blood agar plates containing Brucella agar (Difco) and 5% defibrinated sheep blood (Gibco). The plates were incubated for 3 days at 42 C in an atmosphere of 5% O₂:10% CO₂:85% N₂. Loopfuls of these cultures were transferred to 2 ml of Brucella broth (Gibco) containing 0.1% agar. Cultures were inoculated into the upper 1 cm of the semisolid agar and were incubated at 42 C for 24 h. These cultures were subsequently transferred into 500-ml filter flasks, each containing 100 ml of Brucella broth supplemented with 0.3% sodium succinate (Mallinckrodt, Paris, KY) and 0.01% cysteine hydrochloride (Eastman, Rochester, NY). The atmosphere in the flasks was replaced with a gas mixture of 5% O₂:10% CO₂:85% N₂, then flasks were placed in a gyratory water bath (New Brunswick Scientific, New Brunswick, NJ, Model G76) and shaken at 100 gyrations/min for 10 to 18 h at 42 C. Cells that were in the late logarithmic phase of growth were used as inocula for growth, survival and thermal inactivation studies.

**Growth and survival studies**

Studies were done in a series of screw-capped test tubes (16 mm × 125
mm) containing 1.9 ml of Brucella broth supplemented with 0.1% agar. The pH of this medium was adjusted to 3.0, 3.5, 4.0, 4.5, 4.7, 4.9, 5.0, 5.3, 5.5, 6.0, 6.5, 6.9, 7.5, 8.0, 8.5, 9.0, or 9.5 with 1 N HCl or 1 N NaOH. After the media were autoclaved, pH values were within 0.05 pH unit of those desired.

Cells of _C. fetus_ subsp. _jejuni_ were diluted in 0.1% peptone-water and 0.1 ml of the appropriate dilution was added to each tube of semisolid agar. The tubes were incubated at 4, 25, 27, 30, 32, 35, 37, 42, 45, or 47 C in an atmosphere of 5% O_2 : 10% CO_2 : 85% N_2. At selected times, the contents of a tube were serially diluted in 0.1% peptone-water and surface-plated onto blood agar plates for enumeration of cells. Media adjusted to pH 4.5 or below were neutralized to pH 6.0 with 0.1 N NaOH at the time of sampling. Blood agar plates were incubated at 42 C for 2 to 3 days in the presence of the previously described mixture of gases. Duplicate determinations were done for each treatment studied.

**Thermal inactivation studies**

Cells of _C. fetus_ subsp. _jejuni_ were heated at 48, 50, 53 and 55 C. Heating menstrua each consisted of 9.9 ml of sterile skim milk (pH 6.8) contained in a 50-ml Erlenmeyer flask. The milk was heated to the appropriate temperature while under constant agitation (150 gyrations/min) in a New Brunswick Scientific Model G76 shaking water bath. An inoculum of 0.1 ml of a suspension containing approximately 10^8 cells/ml was introduced into each heating menstruum, giving a final cell concentration of approximately 10^9/ml. A duplicate series of at least six flasks was used for each strain and temperature studied. At selected times throughout the heat treatment, duplicate flasks were removed from the water bath and immersed in ice water. Upon cooling, the milk was serially diluted in 0.1% peptone-water and cells were enumerated on blood agar plates, using conditions previously described. Counts of survivors reported were derived from averages of duplicate samples. The line of best fit for data in the survivor curves was determined using linear regression analysis (19). Decimal reduction times (D-values) were determined from these data, using the methods described by Stumbo (20).

### RESULTS

**Effect of temperature on growth**

To determine the minimum, maximum and optimum temperatures for growth of _C. fetus_ subsp. _jejuni_, three strains were incubated in semisolid Brucella agar, pH 6.9, at temperatures ranging from 25 to 47 C. It has been reported that _C. fetus_ subsp. _jejuni_ can grow at 37 and 42 C but not at 25 C (6) and that growth is better at 42 or 43 C than at 37 C (8); however, nothing more specific in terms of kinetics of growth has been documented.

Figure 1 illustrates the response of strain FRI-CF8 to different temperatures of incubation. It is evident that at temperatures of 30 C and below no growth occurred. Furthermore, when the temperature of incubation was decreased from 30 to 25 C, there was an increase in the rate of cell death. For example, when the culture was incubated at 30 C, a 0.5-log decrease in number of surviving cells was observed after 72 h. When the same culture was incubated at 25 or 27 C, a decrease of ~1.5 log of cells occurred after 72 h. Growth was observed at 32 C; however, in comparison to higher temperatures of incubation, the rate of cell replication was minimal. After 72 h at 32 C there was only a 1.5-log increase in number of cells.

Rates of growth at 35 and 37 C were similar; however, growth at 37 C was slightly better. Cells grew best at 42 and 45 C. In contrast to 48 h that were required to obtain a 6-log increase in cells grown at 35 or 37 C, only 24 h of incubation were needed to obtain a similar increase when cells were grown at 42 or 45 C. None of 6 x 10^9/ml of cells was recovered after 12 h at 47 C.

A comparison of rates of growth for _C. fetus_ subsp. _jejuni_ strains FRI-CF3, FRI-CF6, and FRI-CF8 is presented in Table 1. All three strains grew at comparable rates at 35 to 45 C. At 32 C, FRI-CF8 grew at approximately one-half the rate of the other two strains. At the temperatures used, all three strains had the same minimum, maximum and optimum temperatures for growth, i.e., 32, 45, and 42 to 45 C, respectively.

**Effect of pH on growth**

The same three strains of _C. fetus_ subsp. _jejuni_ were studied to determine what effect pH of the growth
medium had on their ability to proliferate. Each strain was incubated at 42°C in semisolid Brucella agar that had been adjusted to the desired pH with HCl or NaOH. Results from these experiments are summarized in Table 2. The doubling time for each strain was calculated from viable cell counts obtained during the first 12 h of incubation. Although the initial pH of the media was carefully adjusted and checked at the beginning of each experiment, the pH of media adjusted to pH 7.5 and above decreased during incubation. Hence the pH of each medium after 12 h of incubation is also included in Table 2.

**TABLE 2. Rate of growth at 42°C of selected strains of C. fetus subsp. jejuni in response to pH of the growth medium. The medium for growth was Brucella broth + 0.1% agar.**

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>pH after 12 h incubation</th>
<th>Doubling time (h) for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRI-CF3</td>
<td>FRI-CF6</td>
</tr>
<tr>
<td>4.7</td>
<td>4.7</td>
<td>NG¹</td>
</tr>
<tr>
<td>4.9</td>
<td>4.9</td>
<td>NG</td>
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<tr>
<td>5.3</td>
<td>5.3</td>
<td>1.71</td>
</tr>
<tr>
<td>5.5</td>
<td>5.5</td>
<td>1.45</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0</td>
<td>1.35</td>
</tr>
<tr>
<td>6.5</td>
<td>6.5</td>
<td>1.31</td>
</tr>
<tr>
<td>6.9</td>
<td>6.9</td>
<td>1.31</td>
</tr>
<tr>
<td>7.5</td>
<td>7.3</td>
<td>1.33</td>
</tr>
<tr>
<td>8.0</td>
<td>7.7</td>
<td>1.33</td>
</tr>
<tr>
<td>8.5</td>
<td>8.0</td>
<td>1.75</td>
</tr>
<tr>
<td>9.0</td>
<td>8.4</td>
<td>R</td>
</tr>
<tr>
<td>9.5</td>
<td>8.6</td>
<td>R</td>
</tr>
</tbody>
</table>

¹NG = no growth when incubated for up to 72 h.
²R = reduction in number of organisms during first 12 h of incubation; however, the number of organisms increased after 12 h of incubation.

The data indicate that the minimum and maximum pH at which *C. fetus* subsp. *jejuni* can grow is strain-dependent. Of the strains evaluated, FRI-CF8 was the most tolerant at both extremes. It was able to grow in a medium having an initial pH as low as 4.9 and as high as 9.5. Neither strain FRI-CF3 nor FRI-CF6 grew at pH 4.9. However, FRI-CF8 could not grow at pH 4.7, suggesting that pH 4.9 is close to the minimum pH at which exceptionally tolerant strains of *C. fetus* subsp. *jejuni* can grow.

It is difficult to identify the maximum pH at which *C. fetus* subsp. *jejuni* can grow because of the decrease in pH that occurred as the cultures were incubated. However, there appeared to be more variation in the ability of different strains to grow at high pH than at low pH. For example, FRI-CF3 could not grow in media having an initial pH of ≥ 9.0; FRI-CF6 could not grow at an initial pH of ≥ 9.5; however, FRI-CF8 could grow in media having an initial pH of 9.5. The best growth for all three strains occurred in media adjusted from pH 6.0 to pH 8.0, with the optimum pH ranging from 6.5 to 7.5.

**Effect of temperature and pH on survival**

Experiments were done to determine what effect temperature had on the ability of *C. fetus* subsp. *jejuni* to survive in a growth-supporting medium that was adjusted to different pH values. Since strain FRI-CF8 was determined to be the most acid- and alkaline-tolerant isolate of the strains of *C. fetus* subsp. *jejuni* tested, it was selected for this study. Temperatures selected for evaluation included 4, 25 and 42°C.

The ability of FRI-CF8 to survive at 42°C in semisolid Brucella agar adjusted to pH values ranging from 3.0 to 5.0 is illustrated in Fig. 2. Cell death was observed at every pH except 5.0. During the first 4 h at pH 5.0 there...
was a slight decline in the number of viable cells; however, growth, albeit at a very slow rate, was observed thereafter. A 3-log$_{10}$ decrease was observed within 8 h for cells incubated at pH 4.5 while a 3.5-log$_{10}$ decrease occurred within 3 h for cells incubated at pH 4.0. The broken line in this and other figures indicates that < 10 cells/ml were recovered at the final sampling time. At pH 3.0 and 3.5, a 3.5-log$_{10}$ decrease occurred after approximately 15 and 30 min, respectively.

When FRI-CF8 was incubated in media that were adjusted to the same pH values but held at lower temperatures, there was a marked increase in survival. Figures 3 and 4 illustrate rates at which FRI-CF8 was inactivated in semisolid Brucella agar that was incubated at 25 and 4 C, respectively. At pH 3.0, a 3.5-log$_{10}$ decrease in viable cells occurred after 35 and 50 min at 25 and 4 C, respectively. In media adjusted to pH 3.5, the same 3.5-log$_{10}$ decrease took 3 h at 25 C and 5 h at 4 C. At pH 4.0, a 3.5-log$_{10}$ decrease occurred after 24 h for cells incubated at 4 C while more than 5 log$_{10}$ cells were inactivated during the same period at 25 C.

When the pH of the medium was > 4.5, the effect that lower temperatures of incubation had on survival of C. fetus subsp. jejuni was more pronounced. At pH 4.5, more than 5 log$_{10}$ cells were inactivated after 2 days at 25 C; however, after 4 days at 4 C a decrease of less than 3 log$_{10}$ cells was observed. Even more dramatic were the differences in cell survival when FRI-CF8 was incubated at 4 and 25 C in a medium adjusted to pH 5.0. In a medium at this pH, a 3-log$_{10}$ decrease of cells occurred after 2 days at 25 C; however, at 4 C 14 days were required to inactivate the same number of cells.

**Thermal inactivation in skim milk**

Since C. fetus subsp. jejuni may be present in milk (1, 7, 11, 12, 21), a study was done to determine what times and temperatures are required to inactivate the organism in milk. Five strains of C. fetus subsp. jejuni, FRI-CF3, FRI-CF6, FRI-CF8, FRI-CF12, and FRI-CF16, were selected for this study. Each was heated in skim milk at 48, 50, 53 and 55 C.

Figure 5 illustrates rates of inactivation at 48 C. The strain most resistant to this temperature, FRI-CF12, yielded a 4-log$_{10}$ decrease after 50 min while only 30 min were required for a comparable decrease in cells of the least resistant strain, FRI-CF6. Hence the resistance of different strains to heat is quite varied. This can be more fully appreciated when one compares the D-values, i.e., time required to inactivate 90% of the cells obtained for each strain. D-values calculated for each temperature are reported in Table 3. It is evident that at 48 C, a wide range exists among the strains in the time required to inactivate 1 log$_{10}$ of the population. For instance, the D-value for FRI-CF6 was 7.2 min while that for FRI-CF12 was 12.8 min.

As the temperature was increased, the range of rates of inactivation among the strains narrowed. D-values calculated for each strain clearly demonstrate this effect. At 50 C, the D-values ranged from 3.5 to 5.4 min while at 53 C, the range was narrowed to D-values of 1.56 to 1.95 min. At 55 C, the D-values for the five strains ranged from 0.74 to 1.00 min.

**TABLE 3. D-values for different strains of C. fetus subsp. jejuni as determined at different temperatures.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>48°C</th>
<th>50°C</th>
<th>53°C</th>
<th>55°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRI-CF3</td>
<td>12.3</td>
<td>5.4</td>
<td>1.57</td>
<td>0.74</td>
</tr>
<tr>
<td>FRI-CF6</td>
<td>7.2</td>
<td>4.7</td>
<td>1.83</td>
<td>1.00</td>
</tr>
<tr>
<td>FRI-CF8</td>
<td>7.7</td>
<td>3.5</td>
<td>1.85</td>
<td>0.93</td>
</tr>
<tr>
<td>FRI-CF12</td>
<td>12.8</td>
<td>4.4</td>
<td>1.56</td>
<td>1.00</td>
</tr>
<tr>
<td>FRI-CF16</td>
<td>11.7</td>
<td>5.1</td>
<td>1.95</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Figure 3. Inactivation at 25 C of C. fetus subsp. jejuni FRI-CF8 in Brucella broth + 0.1% agar. The semisolid agar was adjusted to different pH values ranging from 3.0 to 6.9.

Figure 4. Inactivation at 4 C of C. fetus subsp. jejuni FRI-CF8 in Brucella broth + 0.1% agar. The semisolid agar was adjusted to different pH values ranging from 3.0 to 5.0.
DISCUSSION

Although data obtained from growth and survival studies done in semisolid agar cannot directly be extrapolated to foods, such information is useful in defining how C. fetus subsp. jejuni will respond to different environments. It appears that C. fetus subsp. jejuni will not grow at temperatures of 30°C and below or at 47°C and above. This suggests that foods maintained at room temperature or below would not allow growth of C. fetus subsp. jejuni. None of the strains studied could grow at pH 4.7 and only one could grow at pH 4.9. This suggests that C. fetus subsp. jejuni cannot grow in high acid foods.

Most interesting is the ability of C. fetus subsp. jejuni to survive in response to temperature and pH. At 42°C, which is within the range of the optimum temperatures for growth, the organism was inactivated more rapidly in a medium adjusted to a pH that is inhibitory to growth than in a medium having a comparable pH but incubated at 25°C. Similarly, in a medium having the same pH, C. fetus subsp. jejuni was inactivated more rapidly at 25°C than at 4°C. At low pH values, such as 3.0 or 3.5, the organism was readily inactivated at all temperatures, including 4°C. For example, the time required for a 4-log10 decrease of cells incubated at 4°C in a medium adjusted to pH 3.0 was <2h and in a similar medium adjusted to pH 3.5 was <7h. However, at a higher pH, such as 5.0, cells that were incubated at 4°C were stable for quite some time.

These studies revealed that after 2 weeks at 4°C in a medium having a pH of 5.0 there was less than a 3-log10 decrease in cells of C. fetus subsp. jejuni. This would suggest that C. fetus subsp. jejuni many remain viable for weeks in low-acid foods that are refrigerated. This would be particularly relevant to poultry because chickens harbor C. fetus subsp. jejuni after processing (14, 18). However, other factors such as the indigenous microflora of a food and the composition of the atmosphere (7) may also influence the organism’s ability to survive. At a temperature that would represent room temperature, i.e., 25°C, C. fetus subsp. jejuni was less tolerant to low-acid conditions as a 3.5-log10 decrease in cells occurred after 2 days at pH 5.0.

Several outbreaks of campylobacteriosis have been attributed to consumption of unpasteurized milk (1, 11, 12, 21). Investigators speculate that the time and temperature used to pasteurize milk is sufficient to inactivate C. fetus subsp. jejuni; however, this has not been demonstrated in the laboratory. Hence, a study was done to determine the time-temperature relationship for thermal inactivation of C. fetus subsp. jejuni. For the five strains of C. fetus subsp. jejuni that were evaluated, all had D-values of 1.00 min or less when heated in skim milk at 55°C. Even under extreme circumstances one would not expect more than 1010 cells of C. fetus subsp. jejuni/ml to be present in milk. Hence, 10 min at 55°C should be sufficient to inactivate even unusually large numbers of C. fetus subsp. jejuni in skim milk. Since milk is normally pasteurized at 62.7°C for 30 min or 71.7°C for 15 sec, properly pasteurized milk should not contain viable cells of C. fetus subsp. jejuni.

ACKNOWLEDGMENTS

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ADDED IN PROOF

Recently, Skirrow and Benjamin (J. Clin. Pathol. 33:1122 (1980)] have described a biotyping scheme that is useful for separating strains of C. fetus subsp. jejuni into different groupings. All five isolates used for this study were characterized using this scheme and were determined to belong to C. jejuni biotype 1.

REFERENCES


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