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Recovery of *Campylobacter jejuni* and *Campylobacter coli* from Inoculated Foods by Selective Enrichment

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A direct enrichment procedure was developed to selectively recover small numbers of *Campylobacter jejuni, C. coli*, and nalidixic acid-resistant thermophilic *Campylobacter* from foods. The procedure includes an enrichment medium composed of brucella broth, 7% lysed horse blood, 0.3% sodium succinate, 0.01% cysteine hydrochloride, vancomycin (15 μg/ml), trimethoprim (5 μg/ml), polymyxin B (20 IU/ml), and cycloheximide (50 μg/ml) that is inoculated with 10 or 25 g of food and incubated with agitation under microaerophilic conditions at 42°C for 16 to 18 h. After incubation, the medium is plated directly onto Campy-BAP agar plates (M. J. Blaser et al., Ann. Intern. Med. **91**:179–185, 1979), and resulting colonies that resemble *Campylobacter* are identified by conventional tests. The foods evaluated included raw milk, hamburger, and chicken skin which had aerobic plate counts of 10^5 to 10^9 becteria/g. The procedure was effective in recovering as few as 0.1 cell of *Campylobacter* per g of food. Of the 50 isolates of *Campylobacter* evaluated, all were recovered from raw milk and hamburger at a level of 1 to 4 cells/g, and 41 and 40 isolates were recovered from the hamburger and milk, respectively, at 0.1 to 0.4 cell/g. The enrichment was least effective for recovering campylobacters from chicken skin, as 7 and 26 of 50 isolates were not recovered at 1 to 4 and 0.1 to 0.4 cell/g, respectively. This new procedure is more rapid, direct, and effective than other enrichment or direct plating procedures for recovering small numbers of campylobacters from foods.

Campylobacter jejuni is now recognized as a prominent cause of acute bacterial gastroenteritis in humans (5, 7). It is commonly associated with normal, apparently healthy animals (7, 10, 19, 38) and is often present in raw foods of animal origin (4, 11, 16, 18, 21, 28, 34, 36, 37). Several types of foods, including pork (22), poultry (3, 12, 27, 32), and, most commonly, raw milk (2, 23–26, 40–42) have been implicated as vehicles of outbreaks of *Campylobacter* enteritis. In Great Britain alone, at least 14 milk-related outbreaks involving more than 4,000 cases have been investigated (24). A number of milk-related outbreaks have also been reported in the United States (2, 40–42).

Robinson (24) has recently shown the human infective dose of *C. jejuni* to be very low. He was able to infect himself and develop symptoms of *Campylobacter* enteritis after ingesting 180 ml of milk containing 500 cells of a strain of *C. jejuni* that was originally isolated from a milk-borne outbreak. This study indicates that as few as 2 to 3 cells per ml of milk can infect an individual who consumes an 8-oz (ca. 240-ml) serving of contaminated milk.

Many of the media and procedures currently used for isolating *C. jejuni* from foods were originally developed to isolate campylobacters from stool specimens. These methods generally involve passing the supernatant fluid of a centrifuged extract of stool specimen through a series of filters and plating the filtrate onto blood or chocolate agar plates (6, 7, 11) or directly plating a few drops (~0.1 ml) of a stool suspension onto plates of blood agar containing antibiotics (1, 7, 29). Grant et al. (11) reported that approximately 99% of *C. jejuni* organisms are retained by the 0.65-μm filter used for the filtration technique. Direct plating of specimens onto antibiotic-containing blood agar plates is limited by the fact that only a small amount of sample can be surface plated, and solid specimens must be suspended in liquid and homogenized, thereby diluting the campylobacters which may be present. Hence, neither method is conducive to recovering small numbers of campylobacters. In addition, several types of enrichments have been developed for recovering campylobacters from foods and feces; however, those that have been proposed have limitations in that they involve an excessive amount of time and manipulation or are not effective in recovering small numbers of campylobacters (1, 9, 14, 21, 39).

Since relatively low numbers of *C. jejuni* can produce enteritis in humans and because meth-

ods currently available for isolating campylobacters from environments containing large numbers of other microorganisms are not direct or are not conducive to recovering small numbers of organisms, a need exists for a direct enrichment procedure that can detect small numbers of *C. jejuni* in foods. The objective of this study was to develop and verify the efficacy of such an enrichment procedure.

MATERIALS AND METHODS

Bacterial strains. Human isolates, FRI-CF 1, 2, 3, 5, 6, 7, 8, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, and 30, were obtained from A. Helstad (Wisconsin State Laboratory of Hygiene, Madison, Wis.). Isolates FRI-CF 31P and 33P were obtained from cecal contents of freshly slaughtered pigs. Strains FRI-CF 142B, 143B, 144B, 145B, 146B, and 147B were obtained from anal swabs of milk cows. FRI-CF 97D, 98D, and 99D were isolated from fecal specimens of dogs. FRI-CF 74C, 75C, 108C, 115C, 119C, 122C, 128C, 148C, 149C, 150C, and 151C were isolated from fecal specimens of laying hens. All animals were apparently healthy and did not appear diseased at the time of sampling. Strains NCTC 11168, isolated from the feces of an infected human; NCTC 11353, isolated from the placenta of a pig; and NCTC 11352, isolated from a seagull, were obtained from the National Collection of Type Cultures, London, England.

Strains NCTC 11352 and FRI-CF 31P were nalidixic acid-resistant thermophilic *Campylobacter* (NARTC) as defined by Skirrow and Benjamin (30). Strains NCTC 11353 and FRI-CF 21 were *C. coli* (30, 31). All other strains were *C. jejuni* (30, 31). After receipt or isolation, cultures were subcultured once, then frozen in skim milk, and held at −70°C until required.

Preparation of inoculum. A vial of the required bacterial strain was thawed at room temperature, and 1 ml was inoculated into 2 ml of brucella broth (GIBCO Diagnostics, Madison, Wis.) containing 0.1% agar (semisolid brucella broth). The culture was incubated at 42°C for 16 to 18 h and then streaked onto blood agar plates composed of 5% defibrinated sheep blood and brucella agar (BBA). BBA plates were incubated microaerophilically (5% O_2–10% CO_2–85% N_2) at 42°C for 48 h. After visually determining that the culture was pure, an isolated colony was transferred from BBA into semisolid brucella broth and incubated at 42°C for 24 h. This culture was subsequently inoculated into a 250-ml side-arm Erlenmeyer filter flask containing 90 ml of brucella broth supplemented with 0.3% sodium succinate (Mallinckrodt, Paris, N.Y.) and 0.01% cysteine hydrochloride (Eastman Organic Chemicals, Rochester, N.Y.). The atmosphere in the flask was replaced with 5% O_2–10% CO_2–85% N_2, and then the flask was placed in a gyratory water bath (model G76; New Brunswick Scientific Co., New Brunswick, N.J.) and shaken at 100 gyrations/min for 16 to 18 h at 42°C.

Preparation of foods. Raw milk was obtained from the University of Wisconsin dairy herd. Hamburger and fresh fryer chickens were purchased from a local grocery store. Skin was removed from all areas, except the wings, of each chicken and ground through a template of 5-mm holes attached to a model FG food grinder (Kitchen Aid, Troy, Ohio; model K5-A). Milk, hamburger, and ground skin were placed in Whirlpak bags and held at 4°C until the bacterial population of each developed an aerobic plate count (APC) of approximately 10^6 to 10^7 per ml or g. Samples of hamburger and chicken skin that were not used within 2 days after the microbial population reached the desired level were held at −20°C and used within 3 weeks. Immediately before being used for enrichment studies, a representative sample consisting of 10 ml of milk or 10 g of chicken skin or hamburger was homogenized, serially (10-fold) diluted in 0.1% peptone, and enumerated on plate count agar (Difco Laboratories, Detroit, Mich.) to obtain an APC. Plates inoculated with milk were incubated at 32°C (17), and those inoculated with hamburger or chicken skin were incubated at 35°C for 48 h (35).

Enrichment procedure. Enrichment broth (EB) comprised brucella broth (GIBCO) plus 7% lysed horse blood, 0.3% sodium succinate, 0.01% cysteine hydrochloride, vancomycin (15 μg/ml), trimethoprim (5 μg/ml), polymyxin B (20 IU/ml), and cycloheximide (50 μg/ml). Initial studies used 10 ml of milk or 10 g of hamburger or chicken skin in 90 ml of EB. To evaluate the effect of increasing sample size, a later study used 25 ml of milk or 25 g of hamburger or chicken skin in 100 ml of EB. All samples were stomached (Lab-Blender 400; Seward Laboratory, London, England) in the presence of EB for approximately 15 s to suspend meat particles and homogeneously distribute the food. These homogenates of EB and food were transferred to 250-ml side-arm Erlenmeyer filter flasks and inoculated with 1.0 ml of inoculum containing approximately 1 to 4 or 10 to 40 campylobacters/ml (for initial studies) and 8 to 28 campylobacters/ml (for the later study). Initial studies used both levels of inoculum of each strain of *Campylobacter* for each type of food tested. The actual number of campylobacters inoculated into the EB-food homogenate was determined by serially diluting cultures in 0.1% peptone and plating onto BBA. Plates were counted after 48 h at 42°C under microaerophilic conditions (5% O_2–10% CO_2–85% N_2). Uninoculated controls composed of EB and 10 or 25 g of food were included to confirm that none of the foods contained *C. jejuni*, *C. coli*, or NARTC as part of their flora.

After inoculation, flasks were evacuated three times to 20 inches of Hg and replaced with an atmosphere of 5% O_2–10% CO_2–85% N_2. Flasks were placed in a New Brunswick model G76 gyratory water bath and shaken at 100 gyrations/min for 16 to 18 h at 42°C. After incubation, enrichments were serially diluted (1:10) in 0.1% peptone and plated onto Campy-BAP agar, the selective medium of Blaser et al. (1). Plates were incubated in a microaerophilic atmosphere at 42°C for 48 h and counted. Characteristic colonies, i.e., those which were gray (some tan or slightly pink), small (1 to 1.5 mm), nonhemolytic, and/or mucoid, were counted, and at least one representative colony from each plate was observed under wet mount preparations with a phase-contrast microscope. Cells having vibrioid morphology and corkscrew, darting movements were presumptively confirmed as being campylobacters. All cultures were identified according to biochemical and growth characteristics, using procedures of Smibert (33), Holdeman et al. (13), and Skirrow and Benjamin (30, 31).

RESULTS

Initial studies were done by using two levels of *Campylobacter* as inocula, i.e., approximately 0.1 to 0.4 and 1 to 4 cells/g or ml of food, and an enrichment medium composed of 90 ml of broth and inoculated with 10 g or ml of food. A total of 25 human, 11 avian, 6 bovine, 1 porcine, and 3 canine isolates of *C. jejuni*; 1 human and 1 porcine isolate of *C. coli*; and 1 porcine and 1 seagull isolate of NARTC were evaluated in three different types of food. The foods evaluated, i.e., raw milk, hamburger, and chicken, represent types of food likely to be contaminated with *C. jejuni* and involved in food-borne disease outbreaks.

Table 1 shows results for the recovery of human strains of *C. jejuni* from the different foods. The enrichment procedure was most effective in recovering *C. jejuni* from hamburger. All 25 strains were recovered at the higher (1 to 4 cells/g) level of inoculum, and 20 of 25 were recovered from foods receiving a lower inoculum (0.1 to 0.4 cell/g). Similarly, all strains were recovered from raw milk containing the higher level of inoculum; however, 6 strains were not recovered with an inoculum of 0.1 to 0.4 cell/ml. Small numbers of *C. jejuni* were most difficult to recover from chicken skin, as 11 and 4 of the 25 strains were not recoverable with inocula of 0.1 to 0.4 and 1 to 4 cells/g, respectively.

Similar results were obtained for recovering avian (Table 2), bovine, canine, and porcine (Table 3) isolates of *C. jejuni*. All of the avian isolates were recovered from all three types of foods when present at 1 to 4 cells/g; however, less, i.e., 10, 9, and 6 of 11 isolates, were recovered from hamburger, raw milk, and chicken skin, respectively, when 0.1 to 0.4 cell/g was present (Table 2). When present at a level of 1 to 4 cells/g, all of the bovine, canine, and porcine isolates were recovered from hamburger and raw milk; however, the porcine and two of six bovine isolates were not recovered from the chicken skin (Table 3). At 0.1 to 0.4 cell/g, all except one and two bovine isolates were recovered from milk and hamburger, respectively. However, at this level of inoculum, recovery of *C. jejuni* from chicken skin was less effective, with four of six bovine, two of three canine, and the single porcine isolate not being recovered.

The enrichment procedure was also effective in recovering *C. coli* and NARTC from foods. Two strains each of *C. coli* and NARTC were successfully recovered from both hamburger and raw milk when present at either 0.1 to 0.4 or 1 to 4 cells/g (Table 4). Similar results were obtained for recovering the NARTC strains from chicken skin; however, neither strain of *C. coli* was recovered from chicken skin when 0.1 to 0.4 cell/g was present, and only one of the two strains was recovered at 1 to 4 cells/g.

In an effort to determine if the size of the sample of food could be increased without substantially increasing the amount of EB and still effectively recover small numbers of campylobacters, a study was done in which the amount of food tested was increased from 10 to 25 g and EB was increased from 90 to 100 ml. Of the 11 strains of *Campylobacter* evaluated, as few as 0.3 to 1.0 *Campylobacter* per g of food was effectively recovered from 25 g of food and 100 ml of EB (Table 5). All strains were recovered from hamburger and raw milk, and only one strain, FRI-CF22, was not recovered from the chicken skin.

Of the three foods evaluated, it appeared that the indigenous flora of chicken skin provided the greatest competition for survival and growth of *C. jejuni, C. coli*, and NARTC in this enrichment medium and on selective plating media. Comparatively, considerably less bacteria developed as background colonies on plates of Campy-BAP that were plated with enrichment cultures of raw milk or hamburger than enrichment cultures of chicken skin. A total of 97% of the raw milk and 68% of the hamburger enrichments had $\leq$100 background colonies on Campy-BAP agar plates surface plated with 0.1 ml of a 1:10 dilution of enrichment medium (Table 6). This level of background growth had little effect on our ability to recovery campylobacters from such enrichments. In almost all instances, campylobacters were easily recovered from enrichments of milk or hamburger by surface plating 0.1 ml of enrichment medium directly onto Campy-BAP. However, because of the large number of background organisms that generally develop on Campy-BAP agar surface plated with 0.1 ml of undiluted enrichment chicken skin (Table 6), it was necessary to also plate 0.1 ml of a 1:10 and 1:100 dilution of enrichment medium. By diluting out the background flora, we were able to recover 24% more campylobacters that would not have otherwise been detected (Table 7).

Streaking a 3-mm loopful of enrichment of chicken skin directly onto Campy-BAP agar would be more rapid than surface plating 0.1-ml dilutions; however, streak plating was 24% less effective in recovering campylobacters from enrichments of chicken skin than was the other method (Table 8). Streak plating enrichments of hamburger or raw milk was as effective in recovering campylobacters as surface plating 0.1-ml portions of enrichments.

DISCUSSION

A number of procedures have been proposed to enrich for campylobacters; however, many

TABLE 1. Recovery of different human isolates of *C. jejuni* from inoculated foods by enrichment[a]

Strain	Milk			Hamburger			Chicken skin		
	APC (CFU/ml)	Inoculated (campy/ml)	Recovered (campy/ml)	APC (CFU/g)	Inoculated (campy/g)	Recovered (campy/ml)	APC (CFU/g)	Inoculated (campy/g)	Recovered (campy/ml)
FRI-CF 1	1.7×10^8	0.21	<10	9.2×10^6	0.21	5.0×10^5	6.2×10^5	0.21	<10
	1.7×10^8	2.1	1.7×10^2	9.2×10^6	2.1	8.7×10^4	6.2×10^5	2.1	4.1×10^4
FRI-CF 2	8.5×10^5	0.08	2.0×10^2	4.6×10^6	0.08	6.0×10^3	2.9×10^6	0.08	5.0×10^2
	8.5×10^5	0.80	1.2×10^3	4.6×10^6	0.80	6.0×10^4	2.9×10^6	0.80	3.3×10^3
FRI-CF 3	3.7×10^5	0.13	<10	4.6×10^6	0.13	4.0×10^4	1.7×10^6	0.22	2.1×10^3
	3.7×10^5	1.3	1.6×10^5	4.6×10^6	1.3	7.0×10^5	1.7×10^6	2.2	2.7×10^4
FRI-CF 5	9.9×10^5	0.19	1.0×10^3	8.3×10^6	0.19	1.0×10^4	1.2×10^7	0.19	3.5×10^3
	9.9×10^5	1.9	1.1×10^4	8.3×10^6	1.9	1.6×10^5	1.2×10^7	1.9	4.3×10^3
FRI-CF 6	2.4×10^6	0.36	<10	6.9×10^7	0.36	5.5×10^4	1.7×10^6	0.23	1.3×10^4
	2.4×10^6	3.6	3.8×10^5	6.9×10^7	3.6	4.2×10^5	1.7×10^6	2.3	2.3×10^4
FRI-CF 7	9.8×10^7	0.16	1.6×10^3	7.4×10^5	0.16	2.7×10^4	1.8×10^7	0.16	4.0×10^2
	9.8×10^7	1.6	1.6×10^4	7.4×10^5	1.6	6.4×10^4	1.8×10^7	1.6	2.2×10^3
FRI-CF 8	1.5×10^5	0.18	7.1×10^3	8.5×10^5	0.18	5.1×10^3	1.7×10^6	0.28	<10
	1.5×10^5	1.8	1.0×10^4	8.5×10^5	1.8	2.8×10^4	1.7×10^6	2.8	3.6×10^3
FRI-CF 11	5.8×10^6	0.18	1.3×10^3	8.9×10^6	0.18	4.5×10^4	3.1×10^7	0.18	<10
	5.8×10^6	1.8	4.4×10^3	8.9×10^6	1.8	7.7×10^4	3.1×10^7	1.8	2.6×10^2
FRI-CF 12	2.2×10^6	0.20	<10	4.7×10^7	0.20	4.4×10^3	1.7×10^6	0.20	1.3×10^2
	2.2×10^6	2.0	2.8×10^5	4.7×10^7	2.0	2.5×10^5	1.7×10^6	2.0	1.5×10^2
FRI-CF 13	1.3×10^6	0.18	2.5×10^4	2.6×10^7	0.18	4.0×10^3	1.9×10^7	0.18	<10
	1.3×10^6	1.8	1.0×10^2	2.6×10^7	1.8	8.2×10^4	1.9×10^7	1.8	1.5×10^3
FRI-CF 16	4.2×10^5	0.05	<10	4.9×10^6	0.05	1.4×10^3	1.2×10^6	0.07	<10
	4.2×10^5	0.52	1.3×10^3	4.9×10^6	0.52	9.6×10^3	1.2×10^6	0.74	<10
FRI-CF 17	5.2×10^6	0.24	2.0×10^3	1.6×10^7	0.24	<10	1.1×10^8	0.24	<10
	5.2×10^6	2.4	2.0×10^3	1.6×10^7	2.4	2.1×10^4	1.1×10^8	2.4	4.7×10^3

FRI-CF 18	2.7×10^7	0.08	4.7×10^3	1.5×10^7	0.08	4.0×10^1	4.7×10^7	0.08	1.3×10^2
	2.7×10^7	0.80	3.3×10^4	1.5×10^7	0.80	7.9×10^4	4.7×10^7	0.80	2.7×10^3
FRI-CF 19	8.3×10^7	0.10	8.6×10^3	1.3×10^7	0.10	2.0×10^1	1.7×10^7	0.10	2.1×10^3
	8.3×10^7	0.95	8.1×10^4	1.3×10^7	0.95	3.9×10^4	1.7×10^7	0.95	4.2×10^3
FRI-CF 20	8.3×10^7	0.12	<10	1.3×10^7	0.12	6.1×10^3	1.7×10^7	0.12	2.2×10^3
	8.3×10^7	1.2	1.0×10^4	1.3×10^7	1.2	3.9×10^4	1.7×10^7	1.2	2.1×10^3
FRI-CF 22	4.4×10^5	0.38	2.4×10^4	1.1×10^7	0.15	<10	2.2×10^5	0.15	<10
	4.4×10^5	3.8	2.8×10^5	1.1×10^7	1.5	1.5×10^4	2.2×10^5	1.5	1.6×10^3
FRI-CF 23	2.7×10^5	0.19	4.0×10^1	1.1×10^7	0.19	1.2×10^4	1.2×10^7	0.19	1.1×10^2
	2.7×10^5	1.9	1.4×10^3	1.1×10^7	1.9	1.2×10^5	1.2×10^7	1.9	1.4×10^3
FRI-CF 24	4.4×10^5	0.42	1.8×10^3	1.1×10^7	0.31	<10	1.2×10^6	0.17	<10
	4.4×10^5	4.2	6.2×10^4	1.1×10^7	3.1	6.9×10^4	1.2×10^6	1.7	<10
FRI-CF 25	2.1×10^5	0.09	1.5×10^3	5.4×10^6	0.09	5.0×10^1	5.0×10^6	0.09	<10
	2.1×10^5	0.93	4.8×10^4	5.4×10^6	0.93	3.2×10^4	5.0×10^6	0.93	4.0×10^3
FRI-CF 26	1.1×10^5	0.09	<10	1.2×10^7	0.32	<10	3.0×10^6	0.09	<10
	1.1×10^5	0.92	6.4×10^3	1.2×10^7	3.2	3.3×10^4	3.0×10^6	0.92	<10
FRI-CF 27	2.1×10^5	0.08	2.0×10^2	5.4×10^6	0.08	2.0×10^3	5.0×10^6	0.08	7.0×10^2
	2.1×10^5	0.82	3.1×10^3	5.4×10^6	0.82	1.1×10^5	5.0×10^6	0.82	4.0×10^3
FRI-CF 28	1.1×10^5	0.08	1.8×10^3	1.5×10^7	0.13	<10	3.0×10^6	0.08	<10
	1.1×10^5	0.82	2.2×10^5	1.5×10^7	1.3	1.9×10^4	3.0×10^6	0.82	<10
FRI-CF 29	5.5×10^5	0.22	6.8×10^3	4.4×10^6	0.22	2.0×10^4	1.0×10^9	0.22	<10
	5.5×10^5	2.2	2.6×10^5	4.4×10^6	2.2	1.1×10^5	1.0×10^9	2.2	2.0×10^2
FRI-CF 30	5.5×10^5	0.19	9.0×10^3	4.4×10^6	0.19	1.7×10^3	1.0×10^9	0.19	2.0×10^2
	5.5×10^5	1.9	9.3×10^4	4.4×10^6	1.9	2.4×10^5	1.0×10^9	1.9	2.4×10^4
NCTC 11168	2.1×10^5	0.24	2.0×10^1	2.9×10^6	0.24	8.3×10^3	2.3×10^7	0.24	4.2×10^3
	2.1×10^5	2.4	1.5×10^4	2.9×10^6	2.4	1.3×10^5	2.3×10^7	2.4	3.5×10^4

[a] Enrichment consisted of 10 g or ml of food in 90 ml of EB. CFU, Colony-forming units. "Inoculated" values are number of bacteria per milliliter or gram of food tested. "Recovered" values are number of cells per milliliter of EB.

TABLE 2. Recovery of different avian isolates of *C. jejuni* from inoculated foods by enrichment[a]

Strain	Milk			Hamburger			Chicken skin		
	APC (CFU/ml)	Inoculated (Campy/ml)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)
FRI-CF 74C	2.4×10^6	0.12	2.0×10^4	6.9×10^7	0.12	<10	8.2×10^7	0.18	4.0×10^3
	2.4×10^6	1.2	3.7×10^4	6.9×10^7	1.2	3.3×10^5	8.2×10^7	1.8	2.8×10^3
FRI-CF 75C	2.4×10^6	0.28	4.4×10^4	6.9×10^7	0.28	3.4×10^4	1.7×10^6	0.35	1.6×10^4
	2.4×10^6	2.8	3.0×10^4	6.9×10^7	2.8	1.3×10^5	1.7×10^6	3.5	3.6×10^4
FRI-CF 108C	4.1×10^7	0.13	4.6×10^3	7.2×10^5	0.13	5.4×10^4	1.4×10^6	0.13	<10
	4.1×10^7	1.3	8.7×10^4	7.2×10^5	1.3	6.0×10^4	1.4×10^6	1.3	2.5×10^3
FRI-CF 115C	3.0×10^6	0.25	3.8×10^3	6.5×10^5	0.25	1.7×10^5	6.2×10^5	0.22	3.0×10^2
	3.0×10^6	2.5	1.2×10^4	6.5×10^5	2.5	3.8×10^5	6.2×10^5	2.2	1.6×10^4
FRI-CF 119C	4.1×10^7	0.10	7.9×10^3	7.2×10^5	0.10	4.7×10^3	1.4×10^6	0.10	<10
	4.1×10^7	1.0	8.8×10^3	7.2×10^5	1.0	5.3×10^3	1.4×10^6	1.0	2.7×10^3
FRI-CF 122C	5.8×10^7	0.16	1.6×10^3	4.7×10^6	0.16	4.8×10^2	2.6×10^7	0.16	1.0×10^1
	5.8×10^7	1.6	2.7×10^4	4.7×10^6	1.6	5.6×10^4	2.6×10^7	1.6	7.2×10^2
FRI-CF 128C	5.8×10^7	0.14	1.2×10^4	4.7×10^6	0.14	4.9×10^3	2.6×10^7	0.14	4.0×10^1
	5.8×10^7	1.4	3.0×10^4	4.7×10^6	1.4	3.3×10^4	2.6×10^7	1.4	2.9×10^3
FRI-CF 148C	4.3×10^7	0.11	1.5×10^3	1.1×10^8	0.10	6.9×10^2	2.1×10^6	0.10	<10
	4.3×10^7	1.1	2.3×10^3	1.1×10^8	1.0	1.3×10^2	2.1×10^6	1.0	7.2×10^2
FRI-CF 149C	4.3×10^7	0.13	1.7×10^3	1.1×10^8	0.14	2.4×10^4	2.1×10^6	0.14	2.6×10^3
	4.3×10^7	1.3	2.6×10^3	1.1×10^8	1.4	1.7×10^4	2.1×10^6	1.4	1.8×10^3
FRI-CF 150C	4.3×10^7	0.10	<10	1.1×10^8	0.15	3.1×10^3	3.6×10^8	0.10	<10
	4.3×10^7	1.0	3.5×10^3	1.1×10^8	1.5	1.2×10^5	3.6×10^8	1.0	3.0×10^2
FRI-CF 151C	2.3×10^6	0.06	<10	6.2×10^6	0.06	7.2×10^2	8.2×10^7	0.12	<10
	2.3×10^6	0.64	8.5×10^3	6.2×10^6	0.64	7.8×10^4	8.2×10^7	1.2	1.2×10^1

[a] See Table 1.

TABLE 3. Recovery of different bovine, canine, and porcine isolates of *C. jejuni* from inoculated foods by enrichment[a]

Strain	Origin	Milk			Hamburger			Chicken skin		
		APC (CFU/ml)	Inoculated (Campy/ml)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)
FRI-CF 142B	Cow	4.8×10^5	0.07	<10	3.6×10^6	0.07	<10	2.7×10^6	0.07	<10
		4.8×10^5	0.65	3.0×10^3	3.6×10^6	0.65	3.0×10^4	2.7×10^6	0.65	1.0×10^2
FRI-CF 143B	Cow	4.8×10^5	0.14	1.3×10^4	3.6×10^6	0.14	2.7×10^4	2.7×10^6	0.14	3.1×10^2
		4.8×10^5	1.4	8.5×10^4	3.6×10^6	1.4	3.8×10^5	2.7×10^6	1.4	1.4×10^3
FRI-CF 144B	Cow	9.1×10^6	0.17	4.2×10^3	9.3×10^6	0.17	2.9×10^3	3.0×10^6	0.11	<10
		9.1×10^6	1.7	1.2×10^3	9.3×10^6	1.7	6.3×10^3	3.0×10^6	1.1	<10
FRI-CF 145B	Cow	2.3×10^6	0.11	9.0×10^2	6.2×10^6	0.11	<10	8.2×10^7	0.24	<10
		2.3×10^6	1.1	1.6×10^2	6.2×10^6	1.1	1.9×10^3	8.2×10^7	2.4	1.5×10^4
FRI-CF 146B	Cow	9.1×10^6	0.21	2.7×10^3	9.3×10^6	0.21	3.5×10^4	3.6×10^8	0.13	1.0×10^2
		9.1×10^6	2.1	4.0×10^3	9.3×10^6	2.1	5.0×10^5	3.6×10^8	1.3	1.2×10^2
FRI-CF 147B	Cow	9.1×10^6	0.08	1.1×10^2	9.3×10^6	0.08	5.9×10^3	4.2×10^7	0.09	<10
		9.1×10^6	0.07	7.1×10^3	9.3×10^6	0.76	7.2×10^3	4.2×10^7	0.92	<10
FRI-CF 97D	Dog	9.8×10^7	0.10	7.2×10^2	7.4×10^5	0.10	1.1×10^3	1.8×10^7	0.10	<10
		9.8×10^7	1.0	7.9×10^3	7.4×10^5	1.0	1.2×10^4	1.8×10^7	1.0	8.0×10^1
FRI-CF 98D	Dog	3.0×10^6	0.27	3.7×10^2	6.5×10^5	0.27	7.5×10^4	1.7×10^6	0.18	<10
		3.0×10^6	2.7	5.7×10^4	6.5×10^5	2.7	$>1.0 \times 10^6$	1.7×10^6	1.8	1.0×10^2
FRI-CF 99D	Dog	3.0×10^6	0.21	3.1×10^4	6.5×10^5	0.21	9.3×10^4	8.2×10^7	0.17	3.0×10^2
		3.0×10^6	2.1	8.7×10^4	6.5×10^5	2.1	4.2×10^5	8.2×10^7	1.7	3.2×10^4
FRI-CF 33P	Pig	3.0×10^6	0.21	2.2×10^4	6.5×10^5	0.21	<10	3.0×10^6	0.09	<10
		3.0×10^6	2.1	5.2×10^4	6.5×10^5	2.1	$<1.0 \times 10^6$	3.0×10^6	0.85	<10

[a] See Table 1.

TABLE 4. Recovery of *C. coli* and NARTC from inoculated foods by enrichment[a]

Strain	Origin	Milk			Hamburger			Chicken skin		
		APC (CFU/ml)	Inoculated (Campy/ml)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)
C. coli										
FRI-CF 21	Human	2.8×10^5	0.11	1.6×10^3	1.4×10^6	0.11	1.0×10^1	1.2×10^6	0.11	<10
		2.8×10^5	1.1	1.1×10^4	1.4×10^6	1.1	6.6×10^2	1.2×10^6	1.1	<10
NCTC 11353	Pig	2.1×10^5	0.15	6.0×10^1	2.9×10^6	0.15	1.2×10^2	2.3×10^7	0.15	<10
		2.1×10^5	1.5	8.0×10^2	2.9×10^6	1.5	1.3×10^4	2.3×10^7	1.5	1.5×10^4
NARTC										
FRI-CF 31P	Pig	4.1×10^7	0.18	5.6×10^3	7.2×10^5	0.18	1.0×10^1	1.4×10^6	0.18	2.1×10^3
		4.1×10^7	1.8	1.4×10^3	7.2×10^5	1.8	2.4×10^5	1.4×10^6	1.8	9.8×10^4
								1.7×10^6	0.22	1.9×10^4
								1.7×10^6	2.2	2.1×10^5
NCTC 11352	Seagull	2.1×10^5	0.37	1.8×10^5	2.9×10^6	0.37	2.9×10^2	2.3×10^7	0.37	3.7×10^3
		2.1×10^5	3.7	2.1×10^5	2.9×10^6	3.7	8.9×10^3	2.3×10^7	3.7	3.0×10^5

[a] See Table 1.

were developed to recover the organism from specimens, such as feces, which contain large numbers of campylobacters. Blaser et al. (1) developed a liquid medium (Campy-thio), composed of thioglycolate broth plus 0.16% agar and antibiotics, i.e., vancomycin (10 μg/ml), trimethoprim (5 μg/ml), polymyxin B (2.5 IU/ml), and amphotericin B (2 μg/ml), which provided greater rates of recovery of campylobacters when *Campylobacter*-positive stool specimens were held in this medium for 8 h at refrigeration temperature before plating onto selective agar (Campy-BAP) than resulted when stools were plated directly onto Campy-BAP. However, this procedure would only be useful for recovering campylobacters from specimens containing relatively large numbers (several hundred per milliliter) of *Campylobacter* (1) because such organisms will not grow but rather progressively die, albeit slowly, at refrigeration temperature (8).

Tanner and Bullin (39) reported alkaline peptone water (pH 8.4) incubated at 43°C in an atmosphere of 5% O_2–10% CO_2–85% H_2 to be a satisfactory method for enrichment of campylobacters. Preliminary studies indicated that this medium could support the growth of as few as 1 to 10 campylobacters and that 2 samples of feces which were negative by direct plating yielded campylobacters after enrichment in alkaline peptone water and subsequent plating on selective agar. However, others (43) have reported that enrichment in alkaline peptone water is less effective in isolating *C. jejuni* from patients' stool specimens than is direct plating on selective medium. The effectiveness of enrichment of *Campylobacter* in alkaline peptone water may be due to this organism's relatively slow rate of growth, even under apparently optimal conditions (1), which allows other fecal microorganisms to outgrow campylobacters in nutrient-rich conditions.

A selective enrichment medium composed of veal infusion broth plus 7% lysed horse blood, 1% bacterial charcoal, vancomycin (40 μg/ml), trimethoprim (20 μg/ml), polymyxin B (10 IU/ml), cycloheximide (100 μg/ml), and 5-fluorouracil (500 μg/ml) was described by Lander and Gill (14) and Fitzgeorge et al. (9) for isolating *C. jejuni* from feces of cows infected with *Campylobacter* by intramammary inoculation and from tissues and feces of monkeys experimentally infected with *C. jejuni*. In one instance the medium was incubated in air at 37°C for 2 days (14), and in the other it was incubated anaerobically at 42°C for 18 to 24 h (9). In our hands this medium, when incubated under microaerophilic conditions at either temperature, proved inhibitory to some of our strains of *C. jejuni* (data not shown), possibly due to the large concentrations of antibiotics present.

Oosterom et al. (20) were able to recover 100 to 1,000 cells of *C. jejuni* per g of artificially contaminated minced meat by using an enrichment medium composed of thioglycolate broth, 7% lysed horse blood, vancomycin (40 μg/ml), trimethoprim (30 μg/ml), polymyxin B (10 IU/ml), and cycloheximide (100 μg/ml) and incubated at 37°C for 24 h in a microaerophilic atmosphere. Adding 1.5% ox bile to the enrichment broth improved the sensitivity of the medium, allowing recovery of 3 to 10 cells of *C. jejuni* per g of meat. The authors did not report how many strains of *C. jejuni* were evaluated; however, to fully assess the efficacy of this medium, it is important to test several strains as the concentrations of antibiotics used in their broth were similar to those used in the medium described by Lander and Gill (14) and Fitzgeorge et al. (9), which was found to be inhibitory to some of our strains of *C. jejuni*.

In studying the incidence of *C. jejuni* in fresh, eviscerated chickens, Park et al. (21) used an enrichment procedure that proved to be approximately twice as effective in recovering *Campylobacter* from poultry carcasses as direct plating onto selective agar. It was reported that this enrichment procedure is effective in recovering up to 0.2 cell of *Campylobacter* per g of food in the presence of 10^4 to 10^6 of contaminants per g. However, one limitation is that the procedure is rather involved. It requires that each chicken be washed in nutrient broth; that the nutrient broth be filtered through cheesecloth and centrifuged; and that the sediment be suspended in brucella broth which is transferred to EB composed of 100 ml of brucella broth, vancomycin (8 μg/ml), trimethoprim (4 μg/ml), and polymyxin B (8 U/ml). EB must then be incubated for 3 days at 37°C under a constant flow of 5% O_2–10% CO_2–85% N_2 (5 to 7 ml/min). After incubation, approximately 5 ml of enrichment culture is filtered through a 0.65-μm membrane filter, and several dilutions of filtrate are plated on two selective agars.

Although this procedure is purported to be effective in recovering small numbers of *C. jejuni*, it requires a large amount of time, labor, and equipment. Our procedure is at least as effective as the method of Park et al. (21), as it can recover as few as 0.1 cell per g of food containing as many as 10^6 to 10^9 indigenous bacteria per g; is more direct in that the enrichment medium is simply inoculated with food and plated directly onto selective agar after incubation; and requires less time and gas mixture, i.e., 18 h versus 3 days and a simple one-time exchange of the atmosphere versus a continual flow of gas through the enrichment culture.

The formulation of our enrichment medium was contrived to include the appropriate combi-

TABLE 5. Recovery of different isolates of *C. jejuni*, *C. coli*, and NARTC from inoculated foods by enrichment[a]

Strain	Milk			Hamburger			Chicken skin		
	APC (CFU/ml)	Inoculated (Campy/ml)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)	APC (CFU/g)	Inoculated (Campy/g)	Recovered (Campy/ml)
C. jejuni									
FRI-CF 1	8.0×10^5	0.84	7.2×10^4	4.2×10^7	0.84	3.1×10^4	5.5×10^6	0.84	3.0×10^2
FRI-CF 17	1.1×10^6	0.76	5.1×10^4	3.1×10^7	0.76	6.6×10^4	1.2×10^6	0.76	1.2×10^3
FRI-CF 22	8.0×10^5	0.80	1.5×10^5	4.2×10^7	0.80	8.8×10^4	5.5×10^6	0.80	<10
FRI-CF 24	2.7×10^5	0.80	3.2×10^4	1.8×10^8	0.80	1.9×10^3	4.8×10^5	0.80	1.0×10^2
FRI-CF 33P	8.0×10^5	0.96	4.3×10^4	4.2×10^7	0.96	2.7×10^5	5.5×10^6	0.96	1.8×10^4
FRI-CF 142B	2.7×10^5	0.48	7.2×10^3	1.8×10^8	0.48	3.0×10^2	4.8×10^5	0.48	2.0×10^2
FRI-CF 150C	1.1×10^6	0.32	1.7×10^3	3.1×10^7	0.32	8.9×10^2	1.2×10^6	0.32	5.5×10^3
NCTC 11168	2.7×10^5	0.33	3.0×10^3	1.8×10^8	0.33	2.0×10^2	4.8×10^5	0.33	2.5×10^3
C. coli									
NCTC 11353	1.1×10^6	0.80	8.8×10^2	3.1×10^7	0.80	1.2×10^5	1.2×10^6	0.80	2.1×10^2
NARTC									
FRI-CF 31P	2.7×10^5	1.1	2.3×10^5	1.8×10^8	1.1	1.5×10^4	4.8×10^5	1.1	1.0×10^6
NCTC 11352	1.1×10^6	0.96	$<1.0 \times 10^6$	3.1×10^7	0.96	5.0×10^5	1.2×10^6	0.96	8.0×10^2

[a] Enrichment consisted of 25 g of ml of food and 100 ml of EB. Other details as in Table 1.

TABLE 6. Number of background colonies present on Campy-BAP agar plated with 0.1 ml of different dilutions of enrichment medium

Inoculum	No. of background colonies	Dilution	No. of enrichment cultures	% of enrichment cultures
Raw milk	0	Undiluted	42	45
	1–100	Undiluted	31	34
	1–100	10^1	17	18
	1–50	10^2	3	3
Hamburger	0	Undiluted	15	17
	1–100	Undiluted	15	17
	1–100	10^1	30	34
	1–50	10^2	21	24
	>50	10^2	7	8
Chicken skin	>100	10^2	88	100

nation of antibiotics necessary to repress the growth of organisms other than *C. jejuni* and *C. coli* and include growth factors that would promote the proliferation of campylobacters. Brucella broth and blood are both well-established growth media for campylobacters. Although not commonly used, both cysteine and succinate are also known growth substrates for campylobacters. Zemjanis and Hoyt (44) have shown that adding 0.002 to 0.03% cysteine to brucella broth markedly stimulates the growth of *Vibrio (Campylobacter) fetus*. Lecce (15) has shown that, of 30 substrates tested, succinate was 1 of only 5 which could be used as an electron donor by *V. fetus* or other related vibrios.

Epidemiological data have implicated raw milk as the vehicle of several outbreaks of *Campylobacter* enteritis, yet *C. jejuni* or *C. coli* has seldom been recovered from suspect samples of milk. One possible explanation may be that small numbers of *Campylobacter* were present in the milk, and the methods used for their recovery were not sensitive enough to detect these few cells. The study of Robinson (24) demonstrates that very few *C. jejuni* in milk (2 to 3 cells/ml) can infect an adult human and produce symptoms of gastroenteritis, thus illustrating the need for a procedure that can recover small numbers of *Campylobacter* from food. The enrichment procedure we have developed should fulfill this need by providing a direct, effective, and relatively rapid method for recovering small numbers of *C. jejuni* from food.

TABLE 7. Dilutions of enrichment medium from which *C. jejuni* and *C. coli* were recoverable from inoculated chicken skin when plated (0.1 ml) on Campy-BAP agar

Dilution	No. of enrichment cultures[a]	% Recovery
Undiluted (and often 10^1, 10^2)[b]	67	76
10^1 (and 10^2); not undiluted	13	15
10^2 only	8	9

[a] Number of campylobacter-positive enrichment cultures from which *Campylobacter* was recovered. A total of 88 campylobacter-positive enrichments are compared.

[b] *C. jejuni* and *C. coli* were not always recovered at the 10^1 or 10^2 dilution.

TABLE 8. Comparison of streak plating versus surface plating 0.1-ml serial dilutions (1:10) of enrichment medium for recovering *C. jejuni* and *C. coli* from inoculated chicken skin[a]

C. jejuni and *C. coli* recovered by:		No. of enrichment cultures	% Recovery
Streak plating	Plating dilutions		
+	+	17	68
−	+	7	28
+	−	1	4

[a] Enrichment medium was plated on Campy-BAP agar.

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LITERATURE CITED

1. **Blaser, M. J., I. D. Berkowitz, F. M. LaForce, J. Cravens, L. B. Reller, and W.-L. L. Wang.** 1979. *Campylobacter* enteritis: clinical and epidemiological features. Ann. Intern. Med. **91**:179–185.
2. **Blaser, M. J., J. Cravens, B. W. Powers, F. M. LaForce, and W.-L. L. Wang.** 1979. *Campylobacter* enteritis associated with unpasteurized milk. Am. J. Med. **67**:715–718.
3. **Brouwer, R., M. J. A. Mertens, T. H. Siem, and J. Katchaki.** 1979. An explosive outbreak of *Campylobacter* enteritis in soldiers. Antonie van Leeuwenhoek. J. Microbiol. Serol. **45**:517–519.
4. **Bruce, D., W. Zochowski, and I. R. Ferguson.** 1977. *Campylobacter* enteritis. Br. Med. J. **2**:1219.
5. **Butzler, J. P., and M. B. Skirrow.** 1979. *Campylobacter* enteritis. Clin. Gastroenterol. **8**:737–765.
6. **Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon.** 1972. Acute enteritis due to related vibrio: first positive stool cultures. J. Infect. Dis. **125**:390–392.
7. **Doyle, M. P.** 1981. *Campylobacter fetus* subsp. *jejuni*: an old pathogen of new concern. J. Food Prot. **44**:480–488.
8. **Doyle, M. P., and D. J. Roman.** 1981. Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. J. Food Prot. **44**:596–601.
9. **Fitzgeorge, R. B., A. Baskerville, and K. P. Lander.** 1981. Experimental infection of Rhesus monkeys with a human strain of *Campylobacter jejuni*. J. Hyg. **86**:343–351.
10. **Goren, E., and W. A. deJong.** 1980. *Campylobacter fetus* subsp. *jejuni* by pluimvee. Tijdschr. Diergeneesk. **105**:724–726.
11. **Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser.** 1980. Broiler chickens as potential source of *Campylo-*

bacter infections in humans. J. Clin. Microbiol. **11**:508–510.
12. **Hayek, L. J., and J. G. Cruickshank.** 1977. *Campylobacter* enteritis. Br. Med. J. **2**:1219.
13. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
14. **Lander, K. P., and K. P. W. Gill.** 1980. Experimental infection of the bovine udder with *Campylobacter coli/jejuni*. J. Hyg. **84**:421–428.
15. **Lecce, J. G.** 1958. Some biochemical characteristics of *Vibrio fetus* and other related vibrios isolated from animals. J. Bacteriol. **76**:312–316.
16. **Luechtefeld, N. W., and W.-L. L. Wang.** 1981. *Campylobacter fetus* subsp. *jejuni* in a turkey processing plant. J. Clin. Microbiol. **13**:266–268.
17. **Marth, E. H. (ed.).** 1978. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Inc., Washington, D.C.
18. **Norberg, P.** 1981. Enteropathogenic bacteria in frozen chicken. Appl. Environ. Microbiol. **42**:32–34.
19. **Oosterom, J.** 1980. Het voorkomen van *Campylobacter fetus* subspecies *jejuni* by normale slachtvarkens. Tijdschr. Diergeneesk. **105**:49–50.
20. **Oosterom, J., M. J. G. M. Vereijken, and G. B. Engels.** 1981. *Campylobacter* isolation. Vet. Qr. **3**:104.
21. **Park, C. E., Z. K. Stankiewicz, J. Lovett, and J. Hunt.** 1981. Incidence of *Campylobacter jejuni* in fresh eviscerated whole market chickens. Can. J. Microbiol. **27**:841–842.
22. **Peel, R. N., and A. W. McIntosh.** 1978. The dog it was that died. Lancet **ii**:1212.
23. **Porter, I. A., and T. M. S. Reid.** 1980. A milk-borne outbreak of *Campylobacter* infection. J. Hyg. **84**:415–419.
24. **Robinson, D. A.** 1981. Infective dose of *Campylobacter jejuni* in milk. Br. Med. J. **282**:1584.
25. **Robinson, D. A., W. J. Edgar, G. L. Gibson, A. A. Matchett, and L. Robertson.** 1979. *Campylobacter* enteritis associated with consumption of unpasteurized milk. Br. Med. J. **1**:1171–1173.
26. **Robinson, D. A., and D. M. Jones.** 1981. Milk-borne *Campylobacter* infection. Br. Med. J. **282**:1374–1376.
27. **Schaefer, J. R., E. V. Conklin, D. F. M. Bunce, R. D. Storck, F. K. Arnold, J. P. Viner, F. B. Merritt, D. Krish, A. J. Roth, R. W. Currier, L. A. Wintermeyer, and J. P. Davis.** 1979. *Campylobacter* enteritis-Iowa. Morbid. Mortal. Weekly Rep. **28**:565–566.
28. **Simmons, N. A., and F. J. Gibbs.** 1979. *Campylobacter* spp. in oven-ready poultry. J. Infect. **1**:159–162.
29. **Skirrow, M. B.** 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. **2**:9–11.
30. **Skirrow, M. B., and J. Benjamin.** 1980. "1001" campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J. Hyg. **85**:427–442.
31. **Skirrow, M. B., and J. Benjamin.** 1980. Differentiation of enteropathogenic campylobacter. J. Clin. Pathol. **33**:1122.
32. **Skirrow, M. B., R. G. Fidoe, and D. M. Jones.** 1981. An outbreak of presumptive food-borne *Campylobacter* enteritis. J. Infect. **3**:234–236.
33. **Smibert, R. M.** 1974. *Campylobacter*, p. 207–212. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore, Md.
34. **Smith, M. V., II, and P. J. Muldoon.** 1974. *Campylobacter fetus* subsp. *jejuni* (*Vibrio fetus*) from commercially processed poultry. Appl. Microbiol. **27**:995–996.
35. **Speck, M. L. (ed.).** 1976. Compendium of methods for the microbiological examination of foods. American Public Health Association, Inc., Washington, D.C.
36. **Stern, N. J.** 1981. *Campylobacter fetus* subsp. *jejuni*: recovery methodology and isolation from lamb carcasses. J. Food Sci. **46**:660–661–663.
37. **Stern, N. J.** 1981. Recovery rate of *Campylobacter fetus* ssp. *jejuni* on eviscerated pork, lamb and beef carcasses. J. Food Sci. **46**:1291,1293.
38. **Sticht-Groh, V.** 1981. Campylobacters in pig faeces. Vet. Rec. **108**:42.
39. **Tanner, E. I., and C. H. Bullin.** 1977. *Campylobacter* enteritis. Br. Med. J. **2**:579.
40. **Taylor, P. R., W. M. Weinstein, and J. H. Bryner.** 1979. *Campylobacter fetus* infection in human subjects: association with raw milk. Am. J. Med. **66**:779–783.
41. **Terhune, C., E. Sazi, N. Kalishman, J. Bobst, B. Bonnlander, J. A. Googins, and P. Williams.** 1981. Raw milk-associated campylobacteriosis—Oregon. Morbid. Mortal. Weekly Rep. **30**:90–92.
42. **Tosh, F. E., G. A. Mullen, and D. E. Wilcox.** 1981. Outbreak of *Campylobacter* enteritis associated with raw milk—Kansas. Morbid. Mortal. Weekly Rep. **30**:218–220.
43. **Wang, W. L., M. Blaser, and J. Cravens.** 1978. Isolation of *Campylobacter*. Br. Med. J. **2**:57.
44. **Zemjanis, R., and H. H. Hoyt.** 1960. The effect of growth factors on the growth of *Vibrio fetus*. Am. J. Vet. Res. **21**:1109–1113.