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Evaluation of Transport and Storage Techniques for Isolation of *Campylobacter fetus* subsp. *jejuni* from Turkey Cecal Specimens

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Immediate culturing of fecal specimens is not always possible, and appropriate methods for transport and storage of *Campylobacter fetus* subsp. *jejuni* specimens have not been fully evaluated. Using nine techniques, we studied the survival of *C. fetus* subsp. *jejuni* in cecal specimens from infected turkeys. The organisms survived in specimens held without transport medium for 3 to 15 days (median, 9 days) at 4°C, and 2 to 9 days (median, 4 days) at 25°C. Only 20% of specimens frozen for 24 h at either −20 or −70°C yielded *C. fetus* subsp. *jejuni*. Specimens dried on filter paper strips were negative for *C. fetus* subsp. *jejuni* within 1.5 h. Cary-Blair medium with decreased agar was the best of the six transport media tested, it enabled recovery of the organism from 100% (3 days) and 71% (7 days) of cecal samples held at 4°C and 94% (3 days) and 85% (7 days) of cecal specimens held at 25°C. In contrast, more than half of all cecal specimens held at 4 or 25°C in Culturettes or buffered glycerol saline were negative by 3 days, and all were negative at 7 days. Results with the other three media studied (Campy-thio, thioglycolate medium, and alkaline peptone water) were intermediate. Overnight incubation of specimens in alkaline peptone water at 37 or 42°C did not enhance recovery of *C. fetus* subsp. *jejuni*. Therefore, refrigeration without a transport medium is satisfactory for up to 3 days for recovery of *C. fetus* subsp. *jejuni* from specimens; however, we recommend the use of Cary-Blair medium with decreased agar for specimens that must be transported or stored for longer than 3 days and for rectal swabs, to prevent drying.

Campylobacter fetus subsp. *jejuni* is now recognized as a common cause of diarrheal illness in humans and has been isolated from 3 to 14% of unselected patients with diarrhea in Europe, North America, and Australia (2, 4, 5, 25). Selective culture techniques, including use of antibiotic-containing media, an atmosphere of 5% oxygen, and an incubation temperature of 42°C, are recommended for the primary isolation of this organism from clinical specimens. Even optimum culture techniques, however, will fail to grow *C. fetus* subsp. *jejuni* if methods of transport and storage of fecal specimens allow death of the organisms.

We evaluated six possible transport media, including buffered glycerol saline (10), Cary-Blair medium with decreased agar (6), alkaline peptone water (APW) (17), thioglycolate medium (10), Campy-thio (2), and Culturettes (Marion Scientific Corp., Rockford, Ill.) with modified Stuart medium (7) for preservation of *C. fetus* subsp. *jejuni* in turkey cecal specimens. We also tested the survival of the organism in cecal specimens held without transport media at −70, −20, 4, and 25°C. Finally, we tested its survival in cecal samples that had been dried on filter paper strips, a method which reportedly works well for transporting stool specimens with other enteric pathogens such as *Salmonella*, *Shigella*, and *Vibrio cholerae* (1, 9).

MATERIALS AND METHODS

Source of specimens. All samples tested in this study were obtained from the ceca of 18- to 24-week-old turkeys shortly after slaughter at a turkey processing plant. Turkeys were chosen as a specimen source because they were readily available to us and because all cecal samples were initially positive for *C. fetus* subsp. *jejuni*, as confirmed by identification methods described previously (2). Cecal specimens were collected by cutting with sterile scissors one cecum from the intestines of each bird; cecal material was then expressed from the ceca into sterile test

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tubes and transported within 1 h to the laboratory; all transport media were inoculated and quantitative cultures were plated within 4 h after collection of the specimens.

Media. Cary-Blair medium (10) was modified by decreasing the agar from 5.0 to 1.6 g/liter.

Campy-thio (Pasco Laboratories, Inc., Wheatridge, Colo.) consisted of thioglycolate broth without indicator, 0.1% dextrose, 0.16% agar, and the following antimicrobial agents per liter: vancomycin, 10.0 mg; polymyxin B, 2,500 U; trimethoprim, 5.0 mg; amphotericin B, 2.0 mg; and cephalothin, 15.0 mg (2).

The plating medium used in all instances was Campy-BAP (Pasco), prepared from tryptose agar base for Brucella (Difco Laboratories, Detroit, Mich.), 5% sheep blood, and the same concentrations of antimicrobial agents as found in Campy-thio (2).

Methods of quantitation. The number of colony-forming units per gram of feces was determined by diluting 0.1 g of stool with 0.9 ml of sterile saline. Serial 10-fold dilutions were then made to obtain an optimal range for counting colonies, and dilutions were inoculated onto Campy-BAP with a 0.05-ml pipette as described by Miles and Misra (16).

Culture methods for temperature studies. Approximately 20 g of cecal material was obtained from each of 12 turkeys; each 20-g sample was then divided equally between two sterile tubes. One tube from each of the 12 turkeys was held at room temperature, and the other tube was held at 4°C. Tubes were subcultured by dipping a sterile swab into the cecal material, emulsifying the material in 0.5 ml of Campy-thio, inoculating one-fourth of a plate of Campy-BAP, and streaking for isolation. These tubes were subcultured daily until *C. fetus* subsp. *jejuni* was no longer recovered. At the same time, 12 tubes of Cary-Blair medium with decreased agar were inoculated heavily with the same 12 cecal samples, placed at 4°C, and subcultured daily as above.

The colony-forming units of *C. fetus* subsp. *jejuni* in cecal samples from each of 20 turkeys were counted within 4 h of collection as a base line before freezing. Seven replicate samples from each of the 20 turkeys were then placed into plastic tubes with air-tight caps at −20°C , and another set of seven tubes from each of 10 of these turkeys was placed at −70°C. For each turkey studied, one tube from each temperature was thawed out and counted every other day for the first 8 days and then twice a week thereafter until two consecutive negatives for *C. fetus* subsp. *jejuni* were obtained from a given set of tubes.

Culture methods for transport media. Cecal contents from 30 turkeys were made individually into heavy suspensions in sterile saline (approximately 1 part of sample to 3 parts of saline). Three drops of each suspension was added to sterile screw-cap glass tubes containing 1.5 ml of one of the following media, then mixed thoroughly: buffered glycerol saline, Campy-thio, APW, thioglycolate medium, and Cary-Blair medium with decreased agar. In addition, 2 drops of each suspension were placed on swabs from the Culturette tubes. The swabs then were replaced in their plastic containers, the vials containing 0.5 ml of the carrier medium were crushed to immerse the swabs, and the Culturette tubes were resealed with their respective caps. Duplicate tubes of all media were placed at 4 and 25°C. All tubes were subcultured daily by dipping a sterile swab into a tube and inoculating a plate of Campy-BAP. Five Culturette tubes were inoculated originally for each condition so that no tube was subcultured more than twice. All inoculated plates were then streaked for isolation with a bacteriological loop and incubated for 48 h at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. Each tube was subcultured daily. The endpoint of survival was the first day on which the

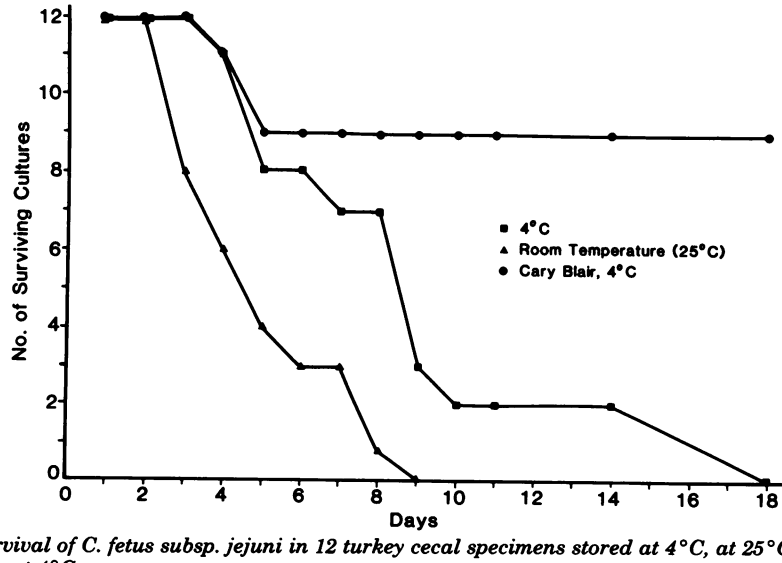

FIG. 1. *Survival of C. fetus subsp. jejuni in 12 turkey cecal specimens stored at 4°C, at 25°C, and in Cary-Blair medium at 4°C.*

subculture from a given tube or set of Culturettes was negative for *C. fetus* subsp. *jejuni*.

To test whether overnight incubation in APW or Campy-thio would increase the isolation rate of *C. fetus* subsp. *jejuni* from cecal samples, suspensions of 30 samples were made in saline, and 5 drops was added to duplicate tubes containing 3 ml of either APW or Campy-thio. Samples were emulsified and then incubated at 42 or 37°C in an atmosphere of 5% oxygen and 10% carbon dioxide. In addition, a full set of inoculated Campy-thio tubes was also placed at 4°C. All tubes were then subcultured as described above after overnight incubation or refrigeration.

Drying on filter paper. Ten cecal specimens were smeared onto multiple strips of sterile filter paper and dried at room temperature as described by Bailey and Bynoe (1). Timing of the experiment began as soon as the specimens were placed on the filter paper strips. At 0, 0.5, 1, 1.5, and 2 h thereafter, the strips were placed into vials or wells containing 0.5 ml of Campy-thio medium, then stirred and mixed with a sterile swab; the resulting suspension was subcultured onto Campy-BAP.

RESULTS

Quantitation of 36 turkey cecal specimens showed 1.2×10^4 to 1.5×10^7 (median, 2.7×10^6) colony-forming units of *C. fetus* subsp. *jejuni* per g of cecal material.

Figure 1 shows that *C. fetus* subsp. *jejuni* survived in cecal specimens for a median of 9 days at 4°C and 4 days at 25°C (without transport media); in duplicate samples the organism survived in Cary-Blair medium with decreased agar at 4°C for over 24 days (median).

After only 24 h of freezing at −20°C, 16 of 20 cecal samples (80%) were negative for *C. fetus* subsp. *jejuni*. Similarly, 8 of 10 specimens frozen for 24 h at −70°C were negative. The six specimens that were still positive after 24 h of freezing dropped 3 to 4 logs of colony-forming units per g of specimen. On subsequent thawing of duplicates of these six specimens, all were negative by 17 days.

Figures 2 and 3 show the recovery of *C. fetus* subsp. *jejuni* from specimens held in six transport media at 25 and 4°C. Of the media we tested, Cary-Blair medium with decreased agar was the best for recovery of the organism from cecal specimens held at both temperatures.

Table 1 shows that overnight incubation of samples in APW or Campy-thio decreased the number of positives compared with direct plating or overnight refrigeration in Campy-thio; also, plates streaked from incubated APW showed significantly fewer *Campylobacter* colonies per plate.

Ten specimens dried on filter paper strips were positive for *C. fetus* subsp. *jejuni* at 0 and 0.5 h; all specimens were negative by 2 h.

DISCUSSION

Little work has been published evaluating common transport and storage techniques for use with *Campylobacter* specimens. Blaser et al. (3) reported that *C. fetus* subsp. *jejuni* was recoverable from five positive human stools for 9 to 22 days when refrigerated at 4°C, but for only 2 to 8 days when left at room temperature. Tanner and Bullin (26) kept aliquots of seven positive fecal specimens at room temperature and at 4°C; after 48 h, six of the seven samples stored at room temperature were negative, whereas all seven samples stored at 4°C were still positive. Our data with 12 turkey cecal specimens also show that *C. fetus* subsp. *jejuni* survives longer at 4°C than at room temperature.

Pure cultures of *Campylobacter* frozen at −70°C in glycerol-Brucella medium have remained viable for several years (W.-L. L. Wang, unpublished data). Certain foods such as meat, fish, and eggs are known to protect bacteria against injury at low temperature (12), and *Campylobacter* has been isolated from poultry which had been frozen at −20°C for 3 weeks (24). However, in the current study we found that the recovery of *C. fetus* subsp. *jejuni* from turkey cecal samples held at −20 or −70°C was generally quite poor after only 24 h of freezing.

Cary-Blair medium has performed well in field trials for transport of fecal specimens positive for *Salmonella*, *Shigella*, *V. cholerae*, and *Vibrio parahaemolyticus* (8, 11, 18). H. Lior and P. Krol (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C174, p. 339) used fresh human stools inoculated with *C. fetus* subsp. *jejuni* to compare buffered glycerol saline, "fluid" Cary-Blair, Cary-Blair with agar, Stuart with charcoal, and Amies with charcoal media. They found that fluid Cary-Blair medium was best; it gave recovery of seven strains of *C. fetus* subsp. *jejuni* for over 14 days in the inoculated stools. In a previous study, we reported that Cary-Blair medium as it was originally formulated (0.5% agar) is inferior to APW and Campy-thio in preservation of *C. fetus* subsp. *jejuni* from turkey cecal specimens held in these media overnight at 4°C (27). The modified Cary-Blair medium (0.16% agar) used in these current experiments gave significantly improved results and was the best of six transport media in preserving the organisms in turkey cecal samples at either 4 or 25°C. Wells et al. (J. G. Wells, T. J. Barrett, and H. E. Sours, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C175, p. 339), using specimens from infected humans obtained after a common-source outbreak, compared direct inoculation

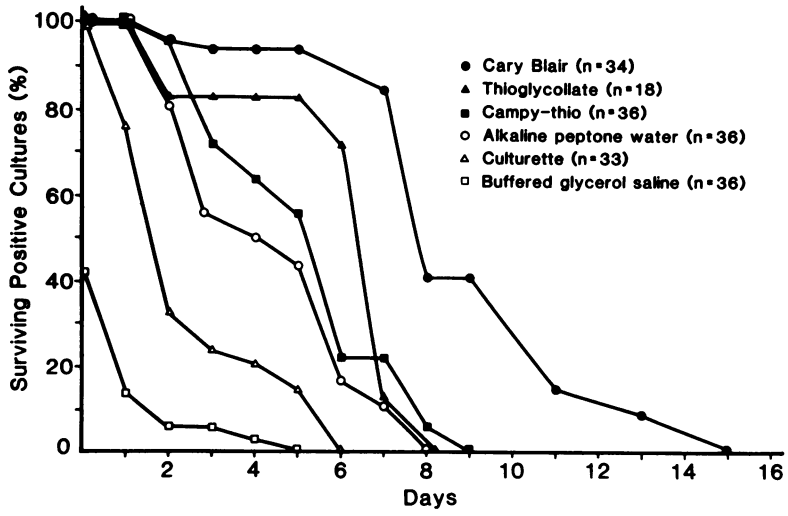

FIG. 2. *Survival of C. fetus subsp. jejuni in turkey cecal specimens stored in six transport media at room temperature (25°C) after initial subculture at 4 to 6 h.*

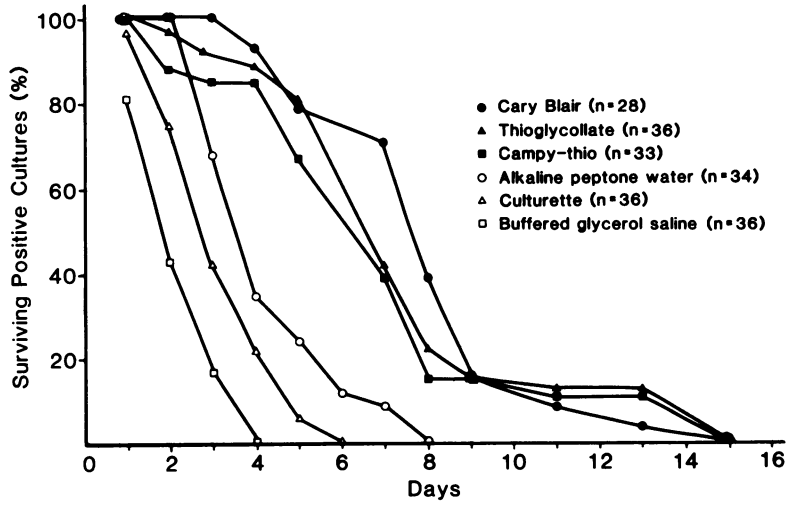

FIG. 3. *Survival of C. fetus subsp. jejuni in turkey cecal specimens stored in six transport media at 4°C after initial subculture at 24 h.*

onto plating media with transport in Cary-Blair medium at 4°C for 3 to 10 days, and found about equal recovery of *C. fetus* subsp. *jejuni* from the two methods. Since this was a common-source outbreak, probably only one strain of the organism was being tested. Our own experience with isolation of *C. fetus* subsp. *jejuni* from two patients whose fecal swabs were mailed in Cary-Blair medium from New Hampshire to Colorado (3 days transit time, one in winter and one in summer) supports the use of Cary-Blair for transport of fecal specimens (L. B. Reller, unpublished data). Modified Cary-Blair is a desirable transport medium in that storage and transport may be at room temperature, and other enteric pathogens besides *C. fetus* subsp. *jejuni* may be recovered as well.

It might be noted that there is a great difference in the survival curves of Cary-Blair medium at 4°C in Fig. 1 and 3. In Fig. 1, 9 of 12 samples survived for over 18 days, whereas in Fig. 3, none of the 28 samples survived past 15 days. This discrepancy may be explained by the differences in dilutions of cecal material used in the two experiments. In Fig. 1, a heavy suspension (approximately 1:4 dilution of cecal material in

TABLE 1. *Effect of incubation on recovery of C. fetus subsp. jejuni from 30 stool specimens in APW or Campy-thio*[a]

Medium	Overnight temp before plating (°C)	% Positive
APW	37	83
APW	42	53
Campy-thio	37	0
Campy-thio	42	3
Campy-thio	4	100
Direct plating		100

[a] Campy-thio = thioglycolate medium with antibiotics (2).

Cary-Blair medium) was used, whereas in Fig. 3, the final dilution of cecal material was approximately 1:60. Although a direct comparison cannot be made between the two experiments (cecal material came from different turkeys), the data suggest that a heavy suspension of specimen may give longer recovery of *C. fetus* subsp. *jejuni* than its more dilute counterpart.

Preliminary data from this laboratory initially suggested that Campy-thio held at 4°C and subsequently plated onto Campy-BAP would supplement direct plating of fecal specimens onto Campy-BAP (2), but further studies showed that the number of positives (both human and animal specimens) was not substantially increased by using Campy-thio (W.-L. L. Wang, N. W. Luechtefeld, and M. J. Blaser, unpublished data). In the current study, we found that thioglycolate medium is comparable to Campy-thio for isolation of *C. fetus* subsp. *jejuni* from turkey cecal specimens; however, both were slightly inferior to Cary-Blair medium with decreased agar. It is possible that the plate medium (Campy-BAP) is so effective in inhibiting normal enteric flora that antimicrobial agents are not necessary in the transport medium.

Lior and Kroll (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C174, p. 339) found buffered glycerol saline and modified Stuart transport medium with charcoal to be inferior to Cary-Blair medium for preservation of *C. fetus* subsp. *jejuni* in artificially inoculated stools. Our tests support these data in that buffered glycerol saline and Culturette tubes were inferior to the other four media tested for preservation of the organism in cecal samples.

Tanner and Bullin (26) reported that two samples of feces which were negative for *C. fetus* subsp. *jejuni* on direct plating became positive after enrichment with APW (overnight incubation at 43°C in 5% oxygen), and that an inoculum of 1 to 10 organisms into APW allowed *C. fetus* subsp. *jejuni* to multiply in the presence of *Escherichia coli* and *Streptococcus faecalis*. Simmons and Gibbs (22) reported recovery of *Campylobacter* from 6 to 14 chickens which were previously positive and then frozen for 3 weeks, and they state that enrichment in APW was required for recovery of the organisms from three of the six birds. However, we found that overnight incubation of turkey cecal specimens in APW at 42°C in 5% oxygen and 10% carbon dioxide gave only 53% of the positives that direct plating yielded, and with fewer organisms. Thus, APW did not act as an enrichment medium with specimens from turkey ceca. It is possible that the organism is so sensitive to drying that organisms on a swab, directly streaked onto a plate without intermediate liquid medium, might die due to drying before they have a chance to multiply. In this case, the APW might simply protect the organism from drying rather than act as enrichment.

The use of filter paper strips as a means of transporting fecal specimens containing either *Salmonella* or *Shigella* has been found to be quite satisfactory for either organism (1, 9). Our experiments with turkey cecal specimens show that *C. fetus* subsp. *jejuni* did not survive more than 2 h of drying on filter paper strips. This organism's apparent sensitivity to drying may be a factor in the epidemiology of *Campylobacter* enteritis.

Poultry have been implicated as a source of human *Campylobacter* infection (4, 14, 15, 19–21), and the organisms isolated from chickens and from turkeys used in these experiments are biochemically identical to human stool isolates of *C. fetus* subsp. *jejuni* (13, 22, 24). The normal flora of the turkey gut and the content of turkey feces are somewhat different from those of humans, and this must be taken into consideration when interpreting these data for use with human specimens. Data from naturally infected human stools are needed to corroborate these results.

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