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Selective Enrichment Broth Medium for Isolation of *Campylobacter jejuni*

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A new selective *Campylobacter* enrichment broth for the isolation of *Campylobacter jejuni* has been tested with fecal specimens of human, poultry, and bovine origin. We compared the isolation rate with that from the simultaneous direct plating of a duplicate specimen. Of the 380 duplicate specimens examined comparatively, we obtained a 46.3% increase in isolation of *C. jejuni* by using the new *Campylobacter* enrichment broth medium.

Campylobacter jejuni has been previously reported to be an important etiological agent of human diarrheal illness (3, 8, 13). The selective plating media for the isolation of *C. jejuni*, especially Campy-BAP (1) and the medium of Butzler (4), are highly selective and have good sensitivity. Selective broths such as alkaline peptone (15), Campy-thio (1), and others (6, 9, 10) have also been used to improve the isolation of *C. jejuni* from fecal specimens. In this paper we describe a new selective *Campylobacter* enrichment broth (CEB) and compare its effectiveness with that of direct plating for the isolation of *C. jejuni* from bovine, human, and poultry feces.

MATERIALS AND METHODS

The specimens cultured in these studies were 193 bovine fecal specimens from animals with no apparent disease, 50 fecal specimens from broiler chickens with no apparent disease, and 137 human fecal specimens from hospitalized patients with diarrhea.

CEB was composed of brucella broth (Albimi) medium (GIBCO Diagnostics) with the following antimicrobial agents added individually: 5-fluorouracil (Roche Diagnostics, Div. Hoffman-La Roche, Inc.), 333 μg/ml; cefaperazone (Pfizer Inc.), 32 μg/ml; and trimethoprim (Burroughs Wellcome Co.), 32 μg/ml. The 5-fluorouracil was a commercially prepared sterile solution. The cefaperazone and trimethoprim solutions were filter sterilized. The CEB was dispensed in 4.5-ml aliquots into screw-capped tubes (16 by 125 mm).

Campy-BAP, modified Butzler medium containing 40 μg of colistin per ml (BU40) (11), and BU40 containing 15 g of agar per liter (BU40/15) were used as plating media. The use of plating media varied as follows: (i) all human specimens were inoculated to Campy-BAP only, whether plated directly to agar medium or subcultured from broth; (ii) all poultry specimens and 100 bovine specimens were inoculated to Campy-BAP and BU40/15 from both direct culture and enrichment broth; and (iii) the 93 remaining bovine specimens were inoculated to Campy-BAP and BU40 for the direct culture and to BU40/15 only for the enriched specimens.

We used cotton-tipped swabs with plastic sticks to process all specimens, as wooden sticks were found to be inhibitory to low numbers of *C. jejuni* in broth culture. With wooden sticks a 1- to 2-log-larger inoculum was required to initiate growth. Duplicate swab specimens were collected from bovine and poultry feces. One swab was placed in a tube of CEB and transported to the laboratory at ambient temperature, whereas the other swab was placed in a tube of Cary-Blair medium (5) and transported chilled with ice packs. Specimens arrived in the laboratory on the day of collection and were inoculated to media at the time of arrival. The CEBs were incubated with the swabs in the tubes. The swabs in Cary-Blair medium were inoculated directly to plates.

We prepared duplicate swabs from human fecal specimens that had been refrigerated for about 3 weeks in Cary-Blair transport medium, inoculated one swab from each specimen to CEB, and directly plated the other swab to Campy-BAP.

Plates from specimens inoculated directly and cultures in tubes of CEB with screw caps loosened were incubated at 42 to 43°C in a microaerophilic atmosphere of approximately 5% O_2, 7.5% CO_2, and 87.5% N_2. Tubes of CEB were incubated in a stationary position, except for 93 bovine specimens which were rotated in a nearly horizontal position during incubation. Roller incubation was done on a tube roller installed in a custom-built anaerobic incubator that allowed the control of atmosphere and temperature. The microaerophilic atmosphere was the same as described above. Tubes were rotated at 10 rpm. After 36 to 48 h of incubation, the CEB was subcultured to plating medium which was then incubated as described above for 48 h. Specimens plated directly were examined for suspect colonies after 24, 48, and 72 h of incubation. *Campylobacter*-like colonies that developed on plating medium were examined by dark-field microscopy and if compatible with *C. jejuni* were

subcultured and identified by standard procedures (14).

The specimens for direct culture were processed by personnel in the Enteric Laboratory, and the CEB was developed and tested in the Bacterial Zoonoses Laboratory. Personnel in both laboratories routinely worked with campylobacters. The results were not compared until the work was completed.

RESULTS AND DISCUSSION

A total of 380 specimens were examined for *C. jejuni*, and 149 were positive. The results of the comparative yield by direct plating and by plating after enrichment are shown in Table 1. A 46.3% increase in the isolation rate by the use of an enrichment broth was statistically significant ($P < 0.0001$ by the McNemar paired specimen test).

The following differences were observed, depending on the source of specimens. For poultry fecal specimens, the enrichment broth showed little advantage over direct plating for the isolation of *C. jejuni*; there were 48 and 47 positive specimens for enrichment and direct plating, respectively. Apparently, the numbers of *Campylobacter* organisms in poultry feces were large enough to allow recovery by direct plating from 47 specimens. Mean counts of 4.4×10^6 *C. jejuni* organisms per g of feces were previously reported for healthy broilers (7); therefore, it is not surprising that the two procedures were equally effective for the recovery of *C. jejuni* from poultry specimens in this study.

A total of 69% (13 versus 22) more *Campylobacter*-positive specimens were detected from human specimens by the CEB method than by direct plating. However, these specimens had been held in Cary-Blair medium at 4°C for 3 to 4 weeks, and many specimens were overgrown by *Pseudomonas* sp. when plated directly. When these samples were first examined by direct plating after only 7 to 10 days in Cary-Blair medium, the *Pseudomonas* sp. was not a problem. At that time 19 specimens instead of 13 were found to be positive. Both results were inferior to those with the 22 isolates made through CEB. The long-term holding of these specimens in transport medium is much longer than usual. It is not expected that the difference in yield between direct plating and the enrichment broth procedure would have been as great with fresh specimens. The effectiveness of the CEB was greatest with bovine specimens. Low numbers of *C. jejuni* ($\leq 10^3$) have previously been reported in feces from healthy bovine species (6a).

Patton et al. have previously reported BU40 medium to be superior to Butzler medium and Skirrow medium for the isolation of *C. jejuni* from dogs and cats (11). In these studies, they included 3% agar in both the Butzler and the BU40 media. Since the introduction of his medium, Butzler has reduced the agar content to 1.5% (2). The concentration of agar in BU40 for this study was also reduced to 1.5% (BU40/15) and compared with Campy-BAP for the isolation of *C. jejuni* from bovine and poultry specimens after CEB enrichment and direct plating. A similar comparison of media had already been made with human specimens (16). In our study we found no difference between isolation rates with BU40/15 and Campy-BAP after enrichment in CEB. There were 80 positive specimens with BU40/15 versus 78 positive specimens with Campy-BAP, of a total of 81 positive specimens. However, more isolates were obtained on Campy-BAP than on BU40/15 when specimens were directly plated, 66 versus 56 of 69 positive specimens ($P = 0.01$). If direct plating alone is the method chosen for isolation from poultry and bovine specimens and only one medium can be used, these results indicate that Campy-BAP would be better than BU40/15.

We developed an enrichment medium that improved the recovery of *C. jejuni* from fecal specimens in the presence of normal fecal flora and from those specimens that were overgrown by contaminants after being held in transport medium for 3 to 4 weeks. It is possible that this medium may offer little advantage for fecal specimens with high numbers of *Campylobacter* organisms, such as might be seen with freshly collected clinical specimens (12) and possibly some poultry specimens. It is more likely to be advantageous when the number of *Campylobacter* organisms in the specimen is low and the

TABLE 1. *C. jejuni* isolations from duplicate fecal specimens by enrichment and direct isolation procedures

Source	No. tested	No. positive	No. positive by the following method:				
			Direct only[a]	Enrichment only[b]	Direct and enrichment	Total direct	Total enrichment
Bovine	193	77	8	42	27	35	69
Human	137	22	0	9	13	13	22
Poultry	50	50	2	3	47	47	48

[a] Specimens positive when plated directly but negative when cultured by enrichment broth.
[b] Specimens positive when cultured by enrichment broth but negative by direct plating.

number of competing microflora is high. CEB is easy to prepare and does not require special equipment for incubation.

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LITERATURE CITED

1. **Blaser, M. J., D. Ivor, M. B. 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. **Butzler, J. P.** 1981. General discussion on culture media, p. 83–84. *In* D. G. Newell (ed.), *Campylobacter*, epidemiology, pathogenesis and biochemistry. Proceedings of an International Workshop on *Campylobacter* Infections, March 24–26, 1981. MTP Press Ltd., Lancaster, England.
3. **Butzler, J. P., P. Dekeyser, M. Detrain, and F. Dehaen.** 1973. Related vibrio in stools. J. Pediatr. **82**:493–495.
4. **Butzler, J. P., and M. B. Skirrow.** 1979. Campylobacter enteritis. Clin. Gastroenterol. **8**:737–765.
5. **Cary, S. B., and E. L. Blair.** 1964. A new transport medium for shipment of clinical specimens. J. Bacteriol. **88**:96–98.
6. **Chan, F. T. H., and A. M. R. MacKenzie.** 1982. Enrichment medium and control system for isolation of *Campylobacter fetus* subsp. *jejuni* from stools. J. Clin. Microbiol. **15**:12–15.

6a. **Gill, C. O., and L. M. Harris.** 1982. Contamination of red meat carcasses by *Campylobacter fetus* subsp. *jejuni*. Appl. Environ. Microbiol. **43**:977–980.

7. **Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser.** 1980. Broiler chickens as potential source of *Campylobacter* infections in humans. J. Clin. Microbiol. **11**:508–510.
8. **King, E. O.** 1975. Human infections with *Vibrio fetus* and a closely related vibrio. J. Infect. Dis. **101**:119–128.
9. **Lander, K. P.** 1981. A selective, enrichment and transport medium for campylobacters, p. 77–78. *In* D. G. Newell (ed.), *Campylobacter*, epidemiology, pathogenesis and biochemistry. Proceedings of an International Workshop on *Campylobacter* Infections, March 24–26, 1981. MTP Press Ltd., Lancaster, England.
10. **Oosterom, J., M. J. G. M. Vereijken, and G. B. Engels.** 1981. *Campylobacter* isolation. Vet. Q. **3**:104.
11. **Patton, C. M., S. W. Mitchell, M. E. Potter, and A. F. Kaufmann.** 1981. Comparison of selective media for primary isolation of *Campylobacter fetus* subsp. *jejuni*. J. Clin. Microbiol. **13**:326–330.
12. **Robinson, D. A.** 1981. Infective dose of *Campylobacter jejuni* in milk. Br. Med. J. **282**:1584.
13. **Skirrow, M. B.** 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. **2**:9–11.
14. **Smibert, R. M.** 1978. The genus *Campylobacter*. Annu. Rev. Microbiol. **32**:673–709.
15. **Tanner, E. I., and C. H. Bullin.** 1977. *Campylobacter* enteritis. Br. Med. J. **2**:579.
16. **Wells, J. G., C. A. Bopp, and M. J. Blaser.** 1981. Evaluation of selective media for isolation of *Campylobacter jejuni*, p. 80–82. *In* D. G. Newell (ed.), *Campylobacter*, epidemiology, pathogenesis and biochemistry. Proceedings of an International Workshop on *Campylobacter* Infections, March 24–26, 1981. MTP Press Ltd., Lancaster, England.