Evaluation of an Enrichment-Plating Procedure for the Recovery of Campylobacter jejuni from Turkey Eggs and Meat

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ABSTRACT

A selective enrichment-plating procedure was tested for the recovery and enumeration of Campylobacter jejuni from turkey eggs and meat. Enrichment was in brucella broth with ferrous sulfate, sodium metabisulfite, sodium pyruvate and five antimicrobial agents. Plating was on brucella agar supplemented with equine blood and antimicrobial agents. Incubation of tubes and plates was at 42°C in an atmosphere of 5% O₂:10% CO₂:85% N₂. C. jejuni could be recovered from the enrichment broth when calculated initial cell numbers per ml of broth were as low as 0.3 to 3.3.

In recent years, water, raw milk and undercooked poultry contaminated with C. jejuni have been incriminated as potential sources of human gastroenteritis (3, 16, 18, 23, 25). Several reports indicate the presence of C. jejuni in fecal material, carcasses and giblets of poultry (10, 13, 18, 19). Until recently, isolation procedures for C. jejuni focused primarily on the recovery of this organism from fecal specimens. In most of these procedures direct plating of the specimen was carried out because high numbers of the organism were expected in the clinical specimen. These plating media as reported by Dekeyser et al. (5), Blaser et al. (2), Butzler et al. (4), Skirrow (20), Lauwers et al. (12) and Patton et al. (15) consist primarily of brucella agar or thioglycollate agar base, blood and a number of antimicrobial agents such as vancomycin, polymyxin, trimethoprim and cephalothin. Doyle and Roman (7) recovered small numbers of Campylobacter from raw milk, hamburger and chicken skin by selective enrichment in brucella broth supplemented with horse blood, sodium succinate, cysteine hydrochloride, vancomycin, trimethoprim, polymyxin B and cycloheximide.

The inoculated enrichment medium was plated on Campy-BAP agar. The present paper reports on an enrichment-plating procedure for the recovery and enumeration of C. jejuni from turkey eggs and turkey meat.

MATERIALS AND METHODS

Cultures and media

The following strains of C. jejuni were used: 6 (C. E. Park, Microbiology Research Division, Health and Welfare Canada, Ottawa), VDB 101 (V. D. Bokkenheuser, Department of Microbiology, St. Luke’s Hospital, New York, NY) and 29428 (American Type Culture Collection, Rockville, MD). Test cultures were maintained at 5°C in culture maintenance broth (CMB) consisting of brucella broth with 0.15% agar and 0.05% filter-sterilized sodium pyruvate. Cultures were transferred weekly. Micrococcus luteus, Alteromonas putrefaciens and a Lactobacillus sp. were from the departmental culture collection. These cultures were maintained on tryptic soy agar (Difco) slants. Inocula of M. luteus and A. putrefaciens consisted of 18-24 h-old cultures in TS broth, those of the Lactobacillus sp. were 24-h cultures in lactobacilli MRS broth (Difco). Cultures were incubated at 25°C under stationary conditions. Inocula consisted of cells in the late log phase. Composition and method of preparation of brucella-FBP-AM broth (brucella broth supplemented with ferrous sulfate, sodium metabisulfite, sodium pyruvate and five antimicrobial agents), brucella-campylobacter agar and brucella broth diluent are presented in a previous paper (8).

Enrichment-plating procedure

Known numbers of C. jejuni 6, VDB 101 and 29428 were inoculated separately into sterile 600-ml glass jars containing 1:10 dilutions of either turkey eggs or turkey meat in brucella-FBP-AM broth. The meat used consisted of interior portions of the pectoralis major muscle aseptically removed from freshly eviscerated turkey carcasses. Turkey eggs were obtained from large commercial hatcheries in Texas. Inocula for the turkey eggs or turkey muscle-brucella-FBP-AM broth mixtures consisted of cultures grown in CMB for 48 h at 42°C in 5% O₂:10% CO₂:85% N₂ in an Anaerobic Environment Enclosure (ANEE, National Appliance Co., Portland, Oregon). Initial cell numbers were established by direct plating on brucella-campylobacter agar plates. The highest levels of C. jejuni ranged from 1 to 3.3 x 10⁶ cells per ml (in 6 of 7 trials) and descended through

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Table 1. Recovery of C. jejuni by an enrichment-plating (MPN) procedure from mixtures of turkey eggs or turkey muscle and brucella-FBP-AM broth inoculated with different levels (A-I) of this organism.

<table>
<thead>
<tr>
<th>Initial level of cells and MPN/ml</th>
<th>Sample&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C. jejuni strains 6, VDB 101 and 29428 from inoculated or muscle-broth mixtures</th>
<th>Initial number of cells (CFU/ml) in and recovery (MPN/ml) of</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial level&lt;sup&gt;b&lt;/sup&gt;-6</td>
<td>Egg</td>
<td></td>
<td></td>
<td>10,000</td>
<td>5,000</td>
<td>1,000</td>
<td>500</td>
<td>100</td>
<td>50</td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>MPN/ml</td>
<td></td>
<td></td>
<td></td>
<td>4,600</td>
<td>4,600</td>
<td>1,100</td>
<td>430</td>
<td>110</td>
<td>7.5</td>
<td>2.3</td>
<td>0.4</td>
<td></td>
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<tr>
<td>Initial level-VDB 101</td>
<td>Egg</td>
<td></td>
<td></td>
<td>32,000</td>
<td>16,000</td>
<td>3,200</td>
<td>1,600</td>
<td>320</td>
<td>160</td>
<td>32</td>
<td>16</td>
<td>3.2</td>
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<tr>
<td>MPN/ml</td>
<td>≥24,000</td>
<td></td>
<td></td>
<td>11,000</td>
<td>460</td>
<td>1,100</td>
<td>75</td>
<td>110</td>
<td>15</td>
<td>15</td>
<td>1.5</td>
<td></td>
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<tr>
<td>Initial level-29428</td>
<td>Egg</td>
<td></td>
<td></td>
<td>54</td>
<td>27</td>
<td>6</td>
<td>3</td>
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<td>0.3</td>
<td>0.06</td>
<td>0.03</td>
<td>0.006</td>
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<tr>
<td>MPN/ml</td>
<td>15</td>
<td>43</td>
<td>9.3</td>
<td>4.3</td>
<td>0.4</td>
<td>1.5</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>&lt;0.3&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td></td>
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<tr>
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<td></td>
<td>16,000</td>
<td>7,800</td>
<td>1,600</td>
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<td>160</td>
<td>78</td>
<td>16</td>
<td>7.8</td>
<td>1.6</td>
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<td>1,100</td>
<td>460</td>
<td>43</td>
<td>24</td>
<td>110</td>
<td>24</td>
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<td></td>
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<td>140</td>
<td>70</td>
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<td>7</td>
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</tr>
<tr>
<td>MPN/ml</td>
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<td>2,400</td>
<td>1,100</td>
<td>460</td>
<td>240</td>
<td>110</td>
<td>9.3</td>
<td>7.5</td>
<td>0.7</td>
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<tr>
<td>Initial level-29428&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Muscle</td>
<td></td>
<td></td>
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<td>8,100</td>
<td>1,600</td>
<td>810</td>
<td>160</td>
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<td>16</td>
<td>8.1</td>
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<td>MPN/ml</td>
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<td>1,500</td>
<td>460</td>
<td>460</td>
<td>46</td>
<td>46</td>
<td>9.3</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
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</tbody>
</table>

<sup>a</sup>50 g of eggs or 40 g of muscle were mixed with brucella-FBP-AM broth to give a 1:10 dilution.

<sup>b</sup>Counts were determined on brucella-campylobacter agar.

<sup>c</sup>After incubation of sample-broth mixtures (A-I) for 48 h at 42°C, a loopful was tested for C. jejuni on brucella-campylobacter agar.

Of 63 mixtures tested, only one did not yield (-) C. jejuni.

<sup>d</sup>Micrococcus luteus (2.2 × 10<sup>5</sup> per ml), Alteromonas putrefaciens (1.6 × 10<sup>6</sup> per ml) and a Lactobacillus sp. (3.3 × 10<sup>5</sup> per ml) were added to the muscle-broth C. jejuni mixture.
RESULTS AND DISCUSSION

The present method for the recovery and enumeration of C. jejuni consists of a combination of an enrichment and plating procedure. The plating protocol is essentially the same as that described by Blaser et al. (2) for the recovery of this organism from fecal specimens. A selective enrichment phase in brucella-FBP-AM broth was included because C. jejuni may be present in some foods in low numbers (6), and often in the presence of a large variety of other microorganisms.

Data from the present study (Table 1) indicate that the MPN enrichment-plating procedure could detect C. jejuni when the number of viable cells in the egg/muscle-brucella-FBP-AM broth mixture was as low as 0.3 to 3.3 cells per ml. This range represents a contribution of 3 to 33 cells per g of egg or muscle. When C. jejuni was isolated from a turkey muscle-brucella-FBP-AM broth mixture including a mixed microbial population, the organism was recovered by the MPN procedure from mixtures containing as few as 1.6 viable cells per ml.

A second approach for the recovery of C. jejuni consisted of streaking a loopful of the sample-brucella-FBP-AM broth mixture, which had been incubated for 48 h at 42°C, on brucella-campylobacter agar. Whenever the presence of C. jejuni was evident with the MPN procedure, the organism was also isolated from the incubated sample-brucella-FBP-AM broth mixtures. In addition, in one experiment (strain 29428 in eggs), C. jejuni was recovered from two samples (G, H) by the direct streaking of a loopful of incubated egg-brucella-FBP-AM broth mixtures on brucella-campylobacter agar and not by the MPN (enrichment-plating) procedure. In these cases the initial numbers of C. jejuni in the egg-brucella-FBP-AM broth mixtures were 0.06 and 0.03 cells per ml, representing a total C. jejuni population of 30 and 15 cells (per 500 ml of broth), respectively. In terms of quantity of egg, these populations represent 0.6 and 0.3 cells of C. jejuni per g of egg.

In 45 of 63 samples, the initial number of C. jejuni per ml of sample-broth mixture fell between the upper and lower limit (95% confidence limit) of the MPN calculated from the results of the 3-tube MPN technique. In an additional nine samples, the initial number of C. jejuni was slightly higher than the upper limit of the MPN value. Stern (22) reported that a swabbing procedure could detect as few as 0.3 to 3.2 cells of C. jejuni per cm² on the surface of lamb carcasses in two of five trials. He reported consistent recovery when the organism was present at a concentration of 32 cells per cm² of lamb surface (22). The selective enrichment-plating procedure of Doyle and Roman (7) was effective in recovering as few as 0.1 cell of Campylobacter per g of food.

The present enrichment-plating procedure is applied in a companion paper (7) for the recovery of C. jejuni from fertile turkey eggs, newly-hatched poults and brooder house facilities.

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REFERENCES


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tained in a *Campylobacter*-free environment indicate that newly-hatched turkey poults can be maintained for 19 to 21 days without the presence of *C. jejuni* in fecal swabs. Limited data obtained from a brooder house that had been cleaned extensively indicate that some of the sources of contamination with *C. jejuni* had been removed, most likely those pertaining to the drinking water and litter.

Drinking water troughs were often contaminated with litter and manure. There is some evidence (12,16) that small animals, such as rodents, cats, dogs and birds, can harbor *Campylobacter* species. It is conceivable that these animals bring these organisms into the brooder house. Sparrows were seen in the brooder houses eating feed and drinking at the waterers. Fecal swabs from two of three sparrows caught in brooder house No. 2 were positive for *C. jejuni*. Cats were kept on the premises as a rodent control. In addition, man, through contaminated footwear, may transfer organisms from one area to another. Additional experiments on the influence of small animal and human involvement in the dissemination of *C. jejuni* in brooder house facilities may contribute additional information on the exact source of *C. jejuni* infection in the turkey.

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**REFERENCES**


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