Isolation, Characterization, and Serotyping of Campylobacter jejuni and Campylobacter coli from Slaughter Cattle

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A total of 525 specimens from 100 slaughter beef cattle were examined for the presence of Campylobacter jejuni and Campylobacter coli by direct plating and enrichment techniques. Isolates were identified by cultural, biochemical, antibiotic sensitivity, and immunofluorescence tests and further characterized with the aid of recently developed biotyping and serotyping methods. Fifty animals were positive for C. jejuni; only one was positive for C. coli. The distribution pattern of C. jejuni-positive animals, in decreasing order, was steers (55%), bulls (40%), heifers (40%), and cows (22%). Significantly higher isolation rates were obtained from the gall bladders (33%), large intestines (35%), and small intestines (31%) than from the livers (12%) or the lymph nodes (1.4%). C. jejuni isolation by the enrichment technique was 40.2% more frequent than by direct plating; 24-h enrichment resulted in 24% more isolations than 48-h enrichment. Eighty-four of 105 C. jejuni cultures were typable serologically and represented 13 serogroups. Biotype I accounted for 71% of biotyped cultures. Serogroup 7 biotype I was the most commonly encountered (24%) isolate. About one in three positive animals had C. jejuni strains representing more than one serogroup. C. jejuni serogroups encountered in slaughter cattle were similar to those commonly isolated from human sources.

In recent years, reports from around the world have demonstrated beyond doubt the importance of Campylobacter jejuni and Campylobacter coli in human enteritis. There has also been an increasing concern over the role of food animals as reservoirs of these organisms with the implication that Campylobacter diarrhea is a zoonotic infection. Indeed, a number of studies have shown that C. jejuni and C. coli are commonly found in healthy as well as diarrheic animals (1, 18, 21, 27) and that the organisms can be easily isolated from gall bladders and intestinal contents of pigs, sheep, and cattle (3, 23). The objective of this study was to examine, by direct plating and enrichment techniques, the prevalence and distribution of C. jejuni and C. coli among various sites of the digestive tract of slaughter cattle. Recently developed biochemical and serological tests were also used to compare these isolates with those reported in humans.

MATERIALS AND METHODS

Animals. Specimens were obtained from 100 apparently healthy cattle systematically selected from those slaughtered in a meat packing plant in Ottawa, Ontario. On the same day each week for a period of 20 weeks, samples were collected immediately after slaughter from five cattle, which constituted approximately 5% of the daily kill. Animals coming to slaughter on this day were considered by the meat inspectors to be representative of the slaughter population in the plant. The majority of these animals had been transported directly to the packing plant from stockyards 350 to 500 km distant.

Specimens. Laboratory processing of specimens was done on the same day they were collected. The specimens obtained from each animal included: (i) the intact gall bladder, (ii) a section of the large intestine, (iii) a section of the small intestine, (iv) a swab from a fresh cut of the liver, (v) a section of the spleen, and (vi) lymph nodes. Except for the liver swab, all specimens were processed in the laboratory before inoculation of appropriate media. To preserve the commercial value of the whole liver, the swab had to be taken at the plant. The liver was removed from the carcass and placed on a steel pan, and a site remote from the gall bladder was swabbed with 80% alcohol. A clean, deep incision was made with a sterile scalpel. A sterile swab was then rubbed along the incision and placed into a tube containing 5 ml of enrichment medium.

Media. For direct plating and transferring inocula from enrichment medium, we used Mueller-Hinton agar containing 10% sheep blood and the following supplements: trimethoprim lactate (5 mg/liter), vancomycin (10 mg/liter), polymyxin B (2,500 IU/liter), and 0.05 sodium pyruvate. For transfers of pure cultures, Mueller-Hinton blood agar without supplements was used. For initial enrichment, the enrichment broth described by Rosef (23) was used. One liter of this medium consisted of the following: peptone, 10 g; Lab-Lemco powder (Oxoid), 8 g; yeast extract, 1 g; NaCl, 5 g; 0.025% resazurin, 16 ml; distilled water, ca. 1 liter; plus the same supplements as in Mueller-Hinton blood agar.

Isolation. (i) Gall bladder. A sterile swab was inserted aseptically through an incision into the gall bladder and rubbed against the mucosal wall. The swab was used for direct plating. The bile was emptied, and two or three strips (1 by 4 cm) of the gall bladder lining were put into a flask containing 100 ml of enrichment medium.

(ii) Large intestine. A swab was inserted into both openings of a section of large intestine and rubbed vigorously against the interior surfaces.

(iii) Small intestine. The same procedure was used as was described for the large intestine.

(iv) Liver. Swabs were obtained as described above.

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(v) Spleen. The surface of the spleen was seared, and an incision was made in the seared area. A swab was rubbed against the cut area.

(vi) Lymph nodes. Defatted mesenteric lymph nodes were stomached in ca. 30 ml of sterile saline for about 30 s with a Colworth Stomacher 400. A swab was inserted into the stomacher bag containing the tissue suspension.

For direct plating, the swabs from the gall bladder, intestines, livers, spleens, and lymph nodes were streaked directly onto duplicate plates of Mueller-Hinton agar with supplements. Inoculated plates were incubated at 43°C in a microaerobic gas mixture of 10% CO2, 4% O2, and 86% N2 and examined after 48 h. For the enrichment technique, the organ or swab samples were inoculated into tubes and bottles of enrichment broth and incubated at 43°C in the microaerobic gas mixture. After 24 and 48 h, each inoculated enrichment broth was streaked onto duplicate plates of Mueller-Hinton blood agar with supplements. Plates were incubated at 43°C in the microaerobic gas mixture and examined after 48 h. Initial plates containing nonswarming suspect colonies were subcultured to Mueller-Hinton blood agar plates. For subculturing swarming-type colonies, the agar content of Mueller-Hinton blood agar was increased from 1.5 to 2.0%.

Identification, characterization, and biotyping. Unless otherwise specified, cultures used in the various tests were grown in Mueller-Hinton blood agar at 43°C for 24 h in the microaerobic gas mixture. The following tests were employed: Gram stain, motility, incubation at 25°C, catalase, oxidase, and sensitivity to nalidixic acid and cephalothin (12). Triple sugar iron agar, a relatively insensitive medium for hydrogen sulfide production, was used to detect strong H2S-producing thermophilic campylobacters such as C. f. cals (6).

Biotyping was performed on the basis of three metabolic tests: sodium hippurate hydrolysis, H2S production in the modifed FBP agar (described in reference 8 as modified in reference 15), and DNA hydrolysis (15). The sodium hippurate hydrolysis test was based on the rapid test of Hwang and Ederer (11). The cell suspension used in this test was an overnight blood agar growth at 37°C suspended in sterile 0.01 M phosphate buffer (pH 7.0) and adjusted to an optical density equivalent to McFarland tube no. 4. The rapid H2S test was modified from that described by Skirrow and Benjamin (26). With the use of a 5-mm wire loop, a large inoculum was deposited as a ball into the upper third of FBP medium (8) buffered at pH 7.4. The tube was incubated in a 37°C water bath for 2 h. A blackening around the bacterial mass represented a positive reaction. For the DNA hydrolysis test, a heavy inoculum was applied to a circular area (diameter, ca. 1 cm) of a well-dried DNA test plate. A maximum of five test cultures plus one positive control culture could be inoculated onto the one plate (one segment per culture). The plates were incubated at 37°C for 3 to 5 days under microaerobic conditions. A clear, colorless zone around the growth was considered a positive reaction.

Immunofluorescence. Common and uncommon serogroups based on the serotyping of heat-labile antigens (16) were grown on Mueller-Hinton blood agar for 48 h at 37°C in the microaerobic gas mixture. The common serogroups included 1, 2, 4, 7, 8, 9, 11, and 17, and the uncommon serogroups included 5, 6, 10, 12, 13, 14, 15, 16, 38, 19, 20, and 22. Growth was harvested, washed twice, and suspended in saline. An antigen pool composed of common serogroups and another pool of uncommon serogroups were each adjusted to a turbidity equivalent to McFarland tube no. 5 and injected into at least two young adult New Zealand rabbits following the immunization schedule described by Ruckebauer and co-workers (24).

Fluorescent-labeled antibody conjugates were prepared from the preinoculation serum and the antisera to the antigen pool of common or uncommon serogroups. For general-purpose examination, a conjugate made from the combined antisera to the common or uncommon serogroups was titrated before use against each serogroup. The preparation and assessment of the conjugates as well as the staining procedure are described in a previous report (25).

Serotyping. Frozen cultures of C. jejuni were revived on Mueller-Hinton blood agar plates. About five to seven colonies from each plate were randomly selected, and each colony was streaked onto a plate of the same medium. These plates were incubated at 43°C for 48 h under microaerobic conditions. Growth from five plates per culture was used for serotyping. Slide agglutinations with both crude and absorbed sera were performed on glass slides (16). A loopful of DNase-treated bacteria was mixed with 1 drop (50 μl) of antiserum. The mixture was allowed to react for 30 to 45 s, after which agglutination was read.

Statistical analysis. Tests on binomial proportions were done at a 5% level of significance or, equivalently, at the 95% level of confidence. These included testing for a specific value (P0) of a binomial proportion P in which the established null hypothesis (H) was P = P0 and the alternative hypothesis (A) was P ≠ P0. In testing for the difference between two binomial proportions, P1 − P2, the established null and alternative hypotheses were P1 = P2 and P1 ≠ P2 (or P1 > P2 where appropriate), respectively.

RESULTS

Identification and immunofluorescence. All cultures identified as C. jejuni were gram negative, predominantly curved or spiral with occasional coccoid forms, motile, catalase and oxidase positive, H2S negative in triple sugar iron agar, sensitive to nalidixic acid, and resistant to cephalothin.

None grew microaerobically at 25°C or in air at 37°C. Complete results of biotyping are given elsewhere in this paper. In specimens from one C. jejuni-negative animal, an isolate from the gall bladder corresponded to characteristics of C. coli biotype 1. None of the nalidixic acid-resistant strains (Campylobacter laridis) was encountered. Consequently, most of the results presented and discussions will deal mainly with C. jejuni strains unless otherwise indicated.

Immunofluorescence results with the cattle isolates demonstrated the specificity of the conjugates to C. jejuni cultures. None of the conjugates reacted strongly with C. fetus subsp. fetus, C. fetus subsp. venerealis, or C. sporum subsp. babulcus cultures. Most of the C. jejuni isolates showed 3+ to 4+ reactions (on a 1+ to 4+ scale), giving intense fluorescence particularly along the periphery of the cells. Tissues deposited in the field were tested at 4°C as early as possible.

Frequency of occurrence and distribution of C. jejuni. Isolation rates of C. jejuni from the four categories of cattle and from the different specimens are presented in Table 1. The figures represent the combined isolations from the direct plate and enrichment methods. Of the total of 525 specimens processed, 112 (21%) were found to contain C. jejuni. Based on the possibility that lymph nodes might harbor C. jejuni, these tissues were included after the first 30 animals were tested; only one sample was positive for C. jejuni.

Animals with only one specimen positive (36% of positive animals) were significantly more prevalent than those with four positive specimens but only slightly more so than those...
with two or three (Table 2). The gall bladder, large intestine, and small intestine were equally good as a source for *C. jejuni* isolation. In the case of combinations of two positive specimens, the frequency of isolation from the combination of gall bladders and large intestines or large and small intestines was higher than that from gall bladders and small intestines.

**Comparison of direct plating and enrichment techniques.** The use of enrichment medium improved the recovery of *C. jejuni*. Isolation rates from gall bladders, large and small intestines, and liver swabs were significantly higher with the enrichment technique as compared with direct plating (Table 3). Differences in isolation rates between the two methods were greater for the large and small intestines and the liver than for the gall bladder. Except for the single isolation from the lymph node, the gall bladder was the only site where direct plating showed a high recovery rate; isolation rates from the other specimens were less than 50%. Only 3.6% of the isolations were made on the direct plate alone compared with 43.8% obtained with the enrichment method alone (Table 3). Over half of the positive *C. jejuni* isolations from the large and small intestines were due to enrichment alone. When the latter method was compared with the two methods together, only marginal differences were observed with either the large or the small intestines. Conversely, more livers were found to be positive for *C. jejuni* by the enrichment method alone than were commonly recovered in both techniques (75.0 versus 16.7%).

Subcultures from the 24-h enrichment of gall bladders and large and small intestines gave significantly higher recovery rates than did subcultures from the 48-h enrichment (Table 4). No difference was observed between the two enrichment periods with the liver swabs. Among the specimens tested, the presence of contaminants in the enrichment cultures was the least for liver swabs; no contaminants appeared on the direct plates for liver swabs. The fungus *Aspergillus cyclosporus* was occasionally encountered on antibiotic-containing plates inoculated with the enrichment culture, particularly from the large and small intestines. Colonies of *Bacillus* spp. were not uncommon in primary isolation plates of both the direct and enrichment methods.

**Number and distribution of serogroups and biotypes.** Of the 105 cultures serotyped, a total of 13 serogroups were identified.
TABLE 4. Comparison of 24- and 48-h enrichment culture incubation for the isolation of C. jejuni

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of positive isolations (%) in*:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h only</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>9 (28.1)</td>
</tr>
<tr>
<td>Large intestine</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>9 (29.0)</td>
</tr>
<tr>
<td>Liver</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>36 (33.3)</td>
</tr>
</tbody>
</table>

* Since the observations are paired for each specimen, the test procedure involves testing the null hypothesis H (P = 0) against the alternative hypothesis A (P > 0), P being the positive difference proportion. At the 5% level of significance, the 24-h period is significantly superior to the 48-h period in recovering C. jejuni for individual specimens of gall bladder, large intestine, and small intestine as well as for all of them collectively.

TABLE 5. Distribution of C. jejuni serogroups and biotypes among positive specimens

<table>
<thead>
<tr>
<th>Serogroup*</th>
<th>Bio-</th>
<th>Gall bladder</th>
<th>Large intestine</th>
<th>Small intestine</th>
<th>Liver</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (9)</td>
<td>I</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2 (5)</td>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (8)</td>
<td>I</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (12)</td>
<td>I</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7 (35)</td>
<td>I</td>
<td>10</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8 (3)</td>
<td>I</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9 (3)</td>
<td>II</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 (3)</td>
<td>I</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 (2)</td>
<td>I</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 (1)</td>
<td>III</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4, 17 (1)</td>
<td>I</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8, 29 (1)</td>
<td>I</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rough</td>
<td>I</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>U*</td>
<td>I</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses represent the total number of isolates of the serogroup.

* Number of serogroups represented: gall bladder, 7; large intestine, 10; small intestine, 10; liver, 2; lymph node, 1. The difference between the number of serogroups represented among specimens of gall bladder, large intestine, and small intestine and those of the liver and lymph node is significant at the 5% significance level.

U* Under investigation. serogroup not determined.
geography can account for significant differences in isolation rates. It is noteworthy that the steers brought to slaughter were feedlot finished, suggesting that the management and feeding practices to which they were subjected may have favored the development of *C. jejuni* in vivo. Moreover, the long distance involved in transporting these animals could have induced stress with overcrowding and might well have favored *C. jejuni* transmission, multiplication, or both. The enrichment technique has undoubtedly increased the efficiency of isolation from all tissues. With pigs, Rosef (23) has shown that 29 of 50 (58%) gall bladders together with the associated bile ducts were positive for *C. jejuni* with the use of a 48-h enrichment technique; no isolate was obtained from direct seeding on Skirrow selective agar. Our data corroborate the superiority of the enrichment technique over direct plating. But, contrary to the findings of Rosef with pig gall bladders, we observed that the 24-h enrichment favored significantly the recovery of *C. jejuni* from the gall bladder and the large and small intestines of cattle. This is probably due to differences in the initial flora of gall bladders between the two animal species. Also, the decreased recovery from 48-h enrichment could be due to changes that occurred during extended incubation at 43°C. These changes include the appearance of antibiotic-resistant contaminants or products inhibitory to the growth of *C. jejuni*. For instance, Mehliman and Romero (17) have noted that the production of acid from carbohydrates or of amines from the decarboxylation of amino acids by other populations may inhibit the growth of campylobacters. Of interest in this study, albeit circumstantial, is the similarity in the isolation rates for the 24- and 48-h enrichment cultures of liver swabs. Coincidentally, these cultures had the lowest contamination rate. Recently, Bolton and Robertson (2) have reported the effectiveness of a 24-h enrichment period in recovering *C. jejuni* from 47 of 400 cattle rectal swabs, although 24- and 48-h enrichment periods were not compared.

The presence of organisms with spreading type of growth such as *Bacillus* spp. and *Absidia corymbifera* in some of our primary isolation plates suggests the need for more effective antibiotics. Fungal contamination may be diminished by the addition of cycloheximide (actidione) at 100 μg/ml. This was employed by Bolton and Robertson (2) and is a basic ingredient in the transport enrichment medium for *C. fetus* (5).

The isolation of *C. jejuni* from 12% of the livers is significant, considering the increasing emphasis on beef liver consumption in human nutrition. Unlike those found by Rosef (23), the isolates in our study were not obtained from the liver surface and therefore do not represent surface spills from the gall bladder. Furthermore, strict precautionary measures in sampling techniques and laboratory procedures minimized the chance of cross-contaminations between specimens. It should be pointed out, however, that the surface area swabbed along the incision of the liver is proportionally small and would limit the number of *C. jejuni* isolated from this organ. It is likely that there was internal migration of *C. jejuni* between the gall bladder, the bile duct, and the liver either in vivo or soon after slaughter. The high rate of isolation in these organs may be partly attributed to the presence of bile, which improves the growth of *C. jejuni* (19). It is of interest to note that 11 of the 12 positive livers were from animals with positive gall bladders.

With the increasing need to understand the epidemiology of Campylobacter enteritis, a number of serotyping and biotyping schemes for *C. jejuni* has been developed (10, 13–14, 16, 19, 20). The application of serotyping by the slide agglutination technique based on heat-labile antigens (16) in this study demonstrates that strains frequently found in humans are also encountered in cattle. Of the nine most common serogroups reported in humans (16) only serogroups 11, 17, and 57 were not found in cattle. Serogroup 7 was the most frequently encountered strain in cattle, whereas serogroup 4 was most common in humans. Another noteworthy finding of this study is that 36% of the positive animals possessed more than one serogroup. There was no indication that specific serogroups had predilections for specific organs or body sites. Caution should therefore be made in interpreting the serogroups of a fecal isolate as representing the serogroup(s) from the whole animal. Considering that feces and contaminated or infected milk have been pinpointed as possible vehicles for *C. jejuni* transmission, it might be worth monitoring the serogroup(s) of isolates from feces and milk samples of consistent *C. jejuni* shedders at different times.

The present biotyping method was performed to provide further discrimination between *C. jejuni* serogroups. Although the biotyping scheme may be considered an extension of that proposed earlier by Skirrow and Benjamin (26), it is, in fact, a new one with Roman numeral designations and based on improved methodology. The original FBP medium for the rapid H₂S test (26) was modified by buffering the medium to ensure a more consistent result (15). Furthermore, because tests that require positive or negative growth responses were excluded (10), ambiguous test results were avoided.

**TABLE 6. Distribution of *C. jejuni* serogroups and biotypes**

<table>
<thead>
<tr>
<th>No. of positive specimens per animal</th>
<th>No. of serogroups per animal</th>
<th>No. of biotypes per animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1⁴</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

⁴ The statistical analysis was performed at the 5% level of significance. Serotyping and biotyping of cultures were completed for 42 of 50 positive animals.

³ The number of animals from which only one serogroup was isolated was significantly higher than that with two or three serogroups.

² The number of animals from which only one biotype was found was significantly higher than that with two biotypes.

* The animals with one serogroup, those with only one positive specimen were significantly greater in number than those with two or more positive specimens.

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**LITERATURE CITED**


