Chicken-to-Human Infection with Campylobacter jejuni and Campylobacter coli: Biotype and Serotype Correlation

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ABSTRACT

Isolates of Campylobacter jejuni and Campylobacter coli isolated from two flocks of parent hens and their progeny which were followed from hatch to slaughter in 10 different farms within a 6-month period in the area of Ljubljana, Yugoslavia were bio- and serotyped. They were compared to those isolated from diarrheic patients within the same period of time. C. jejuni biotype I of Lior's biotyping scheme was found most predominant. Using 25 unabsorbed antisera raised against live biotype I of Lior's biotyping scheme was found most predominant. Using 25 unabsorbed antisera raised against live C. jejuni cultures, 62.2% and 44.8% of the isolates from patients and chickens, respectively, could be serotyped. Penner serogroups (PG) 1, 2, 5, 7, 9, and 22 were found common to both patients and chickens. PG 2 was the most common isolate. PG 8, which was the second most frequently isolated serogroup from patients was not isolated from chickens. No Campylobacters were isolated from the second most frequently isolated serogroup from patients within a 6-month period in the area of Ljubljana, Yugoslavia from 71 farm family members.

C. jejuni and C. coli have become established as common etiological agents in human diarrhea (2,16). Epidemiological evidence has implicated contaminated chicken meat as a potential source for human infection (6,14). The aim of this study was to use bio- and serotyping to find the correlation between human and chicken isolates of C. jejuni and C. coli. Campylobacter isolates obtained from 2 flocks of parent hens and their progeny reared on 10 different farms were compared to those obtained from the feces of diarrheic patients within the same period of time. This study was conducted in the area of Ljubljana, Yugoslavia.

MATERIALS AND METHODS

Samples

Fecal samples of diarrheic patients were collected during the initial stage of the disease, before any treatment, especially with antibiotics. Fetal samples of farm family members were collected 10 to 30 d after their farms received 1-d-old chicks from the hatchery. Fetal samples of broilers were removed from their cloaca by means of sterilized cotton swabs. All samples were cultured within 2 h of their collection.

Culturing

All fecal samples were surface-plated directly on modified Blaser-Wang medium: 20 g of peptone (DIFCO, Detroit, MI, USA), 1 g of dextrose (DIFCO, Detroit, MI, USA), 2 g of yeast extract (Tolrak, Belgrade, Yugoslavia), 5 g of NaCl (Kemika, Zagreb, Yugoslavia), 15 g of agar (Bio-Merieux, Charbonnieres des Bains, France), 1 L of distilled water, 10% lysed, defibrinated bovine blood and 5% ascites fluid from patients not treated with antibiotics. The following antimicrobials were added: 2 mg of amphotericin (Sigma, St. Louis, MO, USA), 15 mg of cephalothin (Lilly & Co., Indianapolis, IN, USA), 2,500 IU of trimethoprim (Pfizer, Karlsruhe, FRG) and 10 mg of vancomycin (Lilly & Co., Indianapolis, IN, USA).

Plated samples were incubated at 42°C for 2 d under microaerobic conditions made up of 5% O2, 10% CO2, 18% N2, and 67% H2.

Identification and biotyping

C. jejuni and C. coli were identified and biotyped on the basis of their colonial and microscopic morphology, catalase and oxidase activity, hippurate hydrolysis, production of H2S in FBP medium, DNase production and sensitivity to nalidixic acid and cephalothin. The isolates were placed in the 6- biotype scheme for C. jejuni and C. coli proposed by Lior (9).

Serotyping

Isolates were serotyped by slide agglutination using 25 unabsorbed antisera raised against whole cells of Penner's reference strains of C. jejuni and C. coli. The reference strains were selected because they were most commonly encountered (13). The antisera had been prepared by 3-d interval successive intravenous injections of cell suspensions (reference strains were kindly supplied by Mr. E. Falsen, Culture Collection EF, Goteborg, Sweden) into rabbits over a period of 14 d. The cultures, harvested from blood agar plates, had been suspended in physiological saline solution to give approximately 1010 bacterial cells per ml. The rabbits were bled 10 d after the last injection, the blood was collected and the antiserum extracted. Slide agglutination was done according to Lior's method (10). Where isolates agglutinated strongly in one antiserum but weakly in another or others, the serogroup of the isolate was determined by tube titration using double dilutions of the antiserum, starting from 1:10 in saline solution to the titer of each antiserum used. An isolate was considered untypable if it still agglutinated in more than one antiserum using the tube titration method. Isolates that agglutinated in 2% NaCl solution and in all antisera were classified as rough strains.
TABLE 1. Incidence of C. jejuni/coli in broilers and materials relevant to Campylobacter epidemiology.

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>A/B</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diarrheic farmers (feces)</td>
<td>0/71</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrheic patients</td>
<td>64/**</td>
<td>-</td>
<td>59</td>
<td>92.2</td>
<td>5</td>
<td>7.8</td>
</tr>
<tr>
<td>Chickens</td>
<td>125/1238</td>
<td>10</td>
<td>120</td>
<td>96.0</td>
<td>5</td>
<td>4.0</td>
</tr>
<tr>
<td>Totals</td>
<td>189</td>
<td>179</td>
<td>94.7</td>
<td>10</td>
<td>5.3</td>
<td></td>
</tr>
</tbody>
</table>

`*A/B* - Number of isolates/number of samples. % - percentage of isolates. No. - Number of isolates which are C. jejuni or C. coli. % - percentage of isolates which are C. jejuni or C. coli. ** - The 64 isolates were picked at random from 168 isolates obtained from patients within the same period of time.

TABLE 2. Distribution of biotypes.

<table>
<thead>
<tr>
<th>Source of isolate</th>
<th>Campylobacter jejuni</th>
<th>Campylobacter coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Patients</td>
<td>55</td>
<td>2</td>
</tr>
<tr>
<td>Chicken</td>
<td>91</td>
<td>13</td>
</tr>
<tr>
<td>Totals</td>
<td>146</td>
<td>15</td>
</tr>
</tbody>
</table>

Biotype as % of genus: 77.2 | 7.9 | 7.4 | 2.1 | 5.2 | 0  | 100  |

Biotype as % of species: 93.2 | 3.4 | 3.4 | 0  | 100 | 0  |

TABLE 3. Distribution of serotypable isolates of C. jejuni/coli.

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Penner serogroups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Patients</td>
<td>2</td>
</tr>
<tr>
<td>Chicken</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>8</td>
</tr>
</tbody>
</table>

c - C. coli  R - rough, U - untypable with antisera used, L - lost after biotyping.

RESULTS

Table 1 shows that isolation of C. jejuni is far more frequent than isolation of C. coli from both human and chicken sources. No campylobacters were isolated from the feces of healthy members of poultry farm families, though they were in contact with infected chickens.

Table 2 shows that at the biotype level, C. jejuni biotype I was isolated most frequently from both human and poultry sources at both genus and species levels. C. coli biotype II was not isolated during this study.

Table 3 shows that serotypes 1, 2, 5, 7, 9, and 22 were common to both patients and infected chickens. Serogroup 8, the 2nd most common isolate from patients, was not isolated from chickens. The situation is reversed for serogroup 13. Rough strains accounted for 9.4% (6/64) and untypable strains accounted for 29.7% (19/64) of isolates from patients. For chickens, rough strains accounted for 1.6% (2/125) and untypable strains 32% (40/125) of the isolates.

DISCUSSION

The results agree with the general finding that the overall incidence of C. jejuni exceeds by far that of C. coli. Most of the isolates fell into C. jejuni biotype I of the 6 biotype scheme proposed by Lior (9). The order of frequency of biotypes agrees with his findings.

A comparison of biotypes of isolates from patients and chickens suggest epidemiological links. Shanker (17) also suggested an epidemiological link between human and chicken isolates on the basis of biotyping only. However, such comparison is not definitive because isolates of the same biotype may not be necessarily of the same serotype. Bio-serotyping has a greater value as an epidemiological tool. The finding that serogroups 1, 2, 5, 7, 9, and 22 were common to both patients and chickens suggests that infected chicken meat is a source from which humans can be infected. Using Pearson’s correlation coefficient method and testing by Student’s t-distribution test, the correlation coefficient of frequency distribution of serogroups in patients and chicken was not found significant. This may be due to the small number of isolates obtained during this study and also because some serogroups were isolated from patients but not from chickens and vice-versa.

Although serogroups 3, 8, 15, 16, 23, and 39 were isolated from diarrheic patients, they were not isolated from chickens. This study covered only 14 flocks with 186,652 chickens. This represents only a small proportion (approx. 0.4%) of the actual production of broilers in the area where the study was carried out. Thus, one can speculate that sources of human infection with serogroups not found in broilers in this study are either from chickens from other places or from other sources such as pork, pets, surface water, etc. Milk is not a likely source of campylobacters in this part of the country, since pasteurization of milk for sale is compulsory and is traditionally cooked in farm homes. The same is true for
fermented milk products, like yogurt, which contain bactericidal amounts of weak organic acids (4).

The evidence (3,6,7,12) that vertical transmission of campylobacters is rare suggests that chickens could be raised to slaughter campylobacter-free by strictly adhering to sanitary and health-care principles. Since industrial slaughter (15,17,18) and freezing (11) do not eliminate Campylobacters from chicken, undercooked chicken (1) can contain Campylobacters. Poultry meat producers must be encouraged to educate consumers to cook chickens properly before consumption.

The fact that fecal samples of 71 healthy members of farm families were negative for C. jejuni and C. coli indicates that they are not necessarily carriers, though they had worked with infected flocks.

Although 25 selected antisera were used, 40% of chicken and 29.7% of patient isolates could not be serotyped. This suggests that in a geographical region where the frequency of serotypes has not been fully studied, the use of a selected number of antisera selected according to the distribution of serogroups in another part of the world may give a blurred serogroup frequency picture. The work of Lastovica et al. (8) shows that if more antisera were used the percentage of untypable strains would be lower.

REFERENCES