A Survey of *Campylobacter jejuni* in Broilers from Assignment to Slaughter Using DNA-DNA Hybridization

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**ABSTRACT.** A survey of *Campylobacter jejuni* in the cecal contents of broilers raised on a farm was carried out by the DNA-DNA hybridization method from the day of assignment to slaughter at about 1-week intervals. *C. jejuni* was detected in chickens as early as 1 week of age, and was widely detected at each week of age throughout the growing period. In addition, of 20 chickens tested just after assignment, 7 (35%) were *C. jejuni* positive. It is suggested that newly introduced chickens may have already been contaminated with *C. jejuni*. The hybridization method was able to detect *C. jejuni* in the chickens from the day of assignment to 3 weeks of age, but *C. jejuni* was never detected in the same chickens by the enriched culture method. Therefore, it is suggested that the hybridization method is more sensitive than the enriched culture method.—**KEY WORDS:** broiler, *Campylobacter jejuni*, DNA-DNA hybridization.


In recent years, *Campylobacter jejuni* has been implicated as an important cause of human gastroenteritis [3, 21]. Poultry meats have been reported to be a major source of *Campylobacter* infection in man [1, 11, 12, 22, 28]. *C. jejuni* contamination of poultry meat during processing has been well documented [2, 14, 18, 19, 29]. Some investigators [10, 15, 20, 27] found that *C. jejuni* had already spread at the farm level. *C. jejuni* infection has been reported in broilers about as young as 3 to 5 weeks of age, and the probability of infection increased with age. However, the sources of *C. jejuni* infection at the broiler farm level still remain unclear. To know the sources of *C. jejuni* contamination in these young broilers, it is necessary to survey in detail. Therefore, the need for improvements in isolation techniques for detecting low levels of *C. jejuni* are thought to be essential to clarify the primary sources of *C. jejuni* contamination [9, 10].

In a previous study, we established a sensitive method for the detection of small numbers of *C. jejuni* and *C. coli* in chicken fecal samples by DNA-DNA hybridization [4].

In the present study, a survey of *C. jejuni* in cecal contents of chickens that were raised in a broiler farm was carried out at about 1-week intervals by the DNA-DNA hybridization method, which is more sensitive than the routine culture method.

**MATERIALS AND METHODS**

**Sample collection:** A typical grow-out farm for broilers in Kagoshima prefecture consisting of 6 growing houses was investigated. Newly hatched chicks were introduced to the houses generally at 1 day of age and were grown in the same house for about 55 days until processing. The flock sizes in the 3 houses monitored in this study were 15,000 (house A), 10,000 (house B), and 15,000 (house C) birds. Each house had an automatic water supply system, an automatic feed system, atomizers and fan blowers. Newly hatched chicks were supplied from hatcheries to growing house A and also supplied to growing houses B and C at about 1-week intervals. All the houses used an all-in-all-out system. Twenty-five percent of the 40-day-old chickens were shipped to a poultry processing plant, and the rest were reared until 60 days old. Before assignment of newly hatched chickens, each house was washed with water and disinfected with o-Dichlorobenzene and cresol.

At about 1-week intervals, chickens were collected from each house and transported to the laboratory for the examination. Newly introduced chickens were also obtained within 1 hr after assignment. They were given no food or water until the examination. The cecal contents were collected from the chickens, following ether anesthesia and exsanguination.

**Isolation of *C. jejuni***: The cecal contents (0.1 g) were inoculated in Preston's medium consisting of nutrient broth, 5% sheep defibrinated blood, and Campylobacter-selective supplement (SR117E: OXoid). After incubation under microaerophilic conditions at 42°C for 24 hr, three loopfuls from each enrichment were streaked onto Bultzer's agar plates and cultured as described above for 2 days. Typical colonies of *Campylobacter* were streaked on to 5% sheep defibrinated blood agar plates and the isolates from the plates were identified by the following tests: Gram staining, cell morphology, catalase and oxidase reactions, motility, H₂S production, sensitivity to 30 μg of nalidixic acid, failure to grow under aerobic conditions and hippurate hydrolysis.

**DNA-DNA hybridization method:** The culture medium for *Campylobacter* species was modified Skirrow agar consisting of Blood Agar Base No. 2 (Oxoid) with 5% sheep defibrinated blood and Campylobacter-selective supplement (SR069E: Oxoid). Each sample of cecal contents was diluted 10-fold and 0.1 ml of the solution was cultured on the medium at 42°C for 48 hr under microaerophilic conditions.

Colonies were transferred onto nylon membranes (Oncor). Each membrane was soaked in alkaline solution
(0.5 M NaOH and 1.5 M NaCl) for 15 min, twice in neutralizing solution (0.5 M Tris-HCl and 1.5 M NaCl, pH 7.4) for 5 min, and then washed with 2 × SSC (0.15 M NaCl and 15 mM sodium citrate, pH 8.0). The membrane was then air-dried, baked for 30 min at 80°C, and treated with pronase solution (1 mg/ml pronase, 15 mM sodium citrate, and 0.15 M NaCl, pH 7.0) at 37°C for 30 min. The membrane was prehybridized in Membrane Blocking Solution (Oncor) at 42°C for 30 min and hybridized at 42°C for 16 hr in Hybrisol III (Oncor) containing the probe. The preparation of the probe has been described previously [4]. In brief, the probe was biotinylated by nick-translation from DNA of C. jejuni ATCC 33560. The hybridized membrane was washed with 0.16 × SSC containing 0.1% SDS and 0.08% Washing Enhancer (Oncor) at 50°C for 30 min and then twice with 1 × SSC for 3 min at room temperature. The membrane was then transferred to 1:1000-diluted Streptavidine Solution (Oncor) and kept for 10 min at room temperature, followed by washing 3 times with 1 × SSC for 5 min. The membrane was then soaked in 1:1000-diluted Biotin-labeled Alkaline Phosphatase Solution (Oncor) for 10 min and washed 3 times with 1 × SSC for 5 min. Finally it was immersed in staining buffer (Oncor) with nitro-blue-tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate at 37°C for 2 hr and the staining was stopped by washing with 75% ethanol. The positive samples showed blue spots on the membrane.

**RESULTS**

**Prevalence of C. jejuni in broilers during growth examined by DNA-DNA hybridization method:** Two consecutive rearing cycles from assignment to forwarding were monitored for the presence of C. jejuni in the cecal contents. Three chickens were obtained from each of the growing houses, A, B, and C, at each age in weeks. The results of the survey of broilers introduced in April and July are shown in Table 1.

In the first cycle, chickens were surveyed from 1 week old to 8 weeks old. The proportion of chickens carrying C. jejuni was 33% to 67% at 1 to 5 weeks of age, and 83% to 100% at 6 to 8 weeks of age.

In the second cycle, the cecal contents of 20 chickens within 1 hr of assignment were also examined. Thirty-five percent of newly introduced chickens were positive for C. jejuni. The transition pattern for C. jejuni was similar to that for the first cycle.

*Comparison of the results of DNA-DNA hybridization with those of the enriched culture method:* Twenty chickens were obtained from growing houses A and B from the day of assignment to 3 weeks of age at 1-week intervals. The enrichment culture was performed simultaneously with the DNA-DNA hybridization method. As shown in Table 2, 20% to 50% of chicks were positive by the DNA-DNA hybridization method, whereas no colonies of C. jejuni were detected on Butzler’s agar plates after enrichment with Preston’s medium.

### Table 1. Transition of C. jejuni carrier rate in chickens from the day of assignment to 8 weeks old

<table>
<thead>
<tr>
<th>Trial</th>
<th>House</th>
<th>Age in weeks</th>
<th>DA 10 &lt;sup&gt;a&lt;/sup&gt;</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>A</td>
<td>1/3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0/3</td>
<td>1/3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2/3</td>
<td>4/9</td>
<td>2/6</td>
<td>2/3</td>
<td>6/9</td>
<td>8/9</td>
<td>9/9</td>
<td>5/6</td>
<td>67</td>
<td>44</td>
</tr>
</tbody>
</table>
(%)    |       | 67  | 44  | 33  | 67 | 67 | 89 | 100 | 83 |    |    |
(%)    |       | 35  | 50  | 50  | 83  | 100 | 100 | 100 | 100 | 100 |

<sup>a</sup> Day of assignment.

<sup>b</sup> C. jejuni-positive chickens/samples.

### Table 2. Comparison of results of DNA-DNA hybridization and enriched culture method

<table>
<thead>
<tr>
<th>Method</th>
<th>Age in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hybridization</td>
<td>4/20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Enrichment</td>
<td>0/20</td>
</tr>
</tbody>
</table>

<sup>a</sup> Day of assignment.

<sup>b</sup> C. jejuni-positive chickens/samples.
DISCUSSION

In the survey, chickens were found to become positive at 1 to 3 weeks of age. This is much earlier than has been reported by some previous investigators [10, 27]. In the study by Shanker et al. [26], placement of C. jejuni-colonized seeders resulted in rapid transmission of C. jejuni to Campylobacter-free chickens, and the chickens inoculated orally with C. jejuni were shown to be cloaca-positive within 3 days. Montrose et al. [17] reported that placing SPF chicks on contaminated litter resulted in intestinal shedding of C. jejuni within 5 days. Also in the present survey, once some chickens had turned positive for the organism, the contamination spread easily through the flock, and the isolation rate eventually became 83–100%. Chickens are therefore thought to be easily contaminated with C. jejuni by horizontal transmission.

In the broiler farm examined, the litter used was removed, and the feeding and drinking devices were removed and cleaned. The houses, especially walls and ceiling, were disinfected and dried before the next introduction (the vacant period was about 14 days). Poor survivability of C. jejuni in a dry environment [7] and its high sensitivity to disinfectants have been reported previously. Therefore, the possibility that the C. jejuni contamination was carried over to the next flock through these environmental factors seems unlikely. Shane et al. [24] demonstrated that flies could transmit C. jejuni under controlled laboratory conditions, and suggested that these were the primary source of infection. The possibility of transmission by flies cannot be ruled out in our study because the windows of the houses were sometimes open.

The survey of C. jejuni in the cecal contents of newly introduced chickens revealed that the carrier rate of C. jejuni was as high as 35%, suggesting that there was C. jejuni infection in the chickens before introduction to the houses. It is suspected that chickens are infected at hatcheries or during transportation to the farm.

Egg transmission of C. jejuni as a possible source of contamination has been considered [5]. Certainly, in the case of isolation of C. jejuni in newly introduced chickens, egg contamination would be suspected as a possible source. Doyle [8] reported that 226 eggs from hens excreting C. jejuni were isolated from the shell surface of 2 eggs penetration studies have revealed that the organism will not penetrate into the contents of eggs, but can be isolated occasionally from the inner shell and membranes of refrigerated eggs [5]. Shanker et al. [25] suggested that vertical transmission in the natural state was not easily effected. In view of the reports described above, C. jejuni seems unlikely to be transmitted to eggs from hens vertically. However, these examinations were carried out by a standard culture method, and there is still the possibility that vertical transmission might be revealed by a more sensitive method. In fact, Maruyama and Katsube [16] suggested that eggs were contaminated with C. jejuni while passing through the oviduct, based on an experiment by Japanese quails (Coturnix coturnix japonica). We were unable to detect C. jejuni in the cecal contents of chicks from the day of assignment to 3 weeks of age by the enrichment culture method, but some chickens were positive for C. jejuni by the DNA-DNA hybridization method, as shown in Table 2. This suggests that the hybridization method is more sensitive than the enriched culture method. In a previous study [4], this method has been able to detect small numbers of C. jejuni and had a weak cross-reaction only with C. coli.

In the present study, when the cecal contents were cultured on the plates, some plates had no colonies and others had very small colonies or diffused ones. Because no C. jejuni was isolated from these plates, the bacteria may be damaged or injured in the chicken cecal contents. The existence of viable but unculturable C. jejuni was reported previously by Rollins and Colwell [23]. In the present study, bacteria at this stage might have been detected by the DNA-DNA hybridization method.

The present findings suggest that some newly introduced chickens may already be contaminated with C. jejuni.

REFERENCES