Research Note

Intestinal Carriage and Excretion of Campylobacter jejuni in Chickens Exposed at Different Ages

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ABSTRACT

Campylobacter jejuni is usually recovered from chickens in commercial broiler farms after 2 to 3 weeks of age. This study was conducted to clarify whether fecal excretion is associated with the age of exposure to this bacterium. Day-of-hatch broiler chickens were separated from a flock in a local commercial farm, kept in isolation rooms, and esophageally inoculated with C. jejuni (5.5 × 10⁵ to 5.4 × 10⁸ CFU) at 0, 7, 14, 21, 28, and 35 days of age. The remaining chicks were placed on the farm. Fecal samples obtained from the birds with the experimental infection and those reared on the farm were monitored for C. jejuni. Cecal contents obtained on necropsy were also cultured. In chickens inoculated with C. jejuni at 0 to 14 days of age, fecal excretion of C. jejuni was not observed until 42 days of age, although the organism was recovered from the cecal contents of these birds. When chickens were inoculated at 21 to 35 days of age, C. jejuni was isolated from fecal samples 2 or 3 days after inoculation, and the birds continually shed the organism until they reached 49 days of age, with the maximal numbers of the organism ranging from 1.7 × 10⁸ to 1.0 × 10¹⁰ CFU/g. In the commercial broiler farm, C. jejuni was first isolated from fecal samples obtained from two of five chickens at 28 days of age, and the organism was isolated from all five birds tested at 43 days of age. Restriction fragment length polymorphism analysis of the fla gene of C. jejuni isolates revealed that birds on the farm were colonized with C. jejuni after placement of the chickens on the farm. These observations indicate that chickens younger than 2 to 3 weeks old may carry C. jejuni in the ceca if they were exposed to this organism. Our results also suggest that fecal excretion of C. jejuni in commercial broiler chickens older than 3 to 4 weeks of age may be mainly caused by exposure of chickens at this age to this organism.

Campylobacter jejuni is a major cause of enteric disease in humans. Broiler meat may play an important role in the transmission of this bacterium to humans because commercial broiler chickens frequently carry C. jejuni in their gastrointestinal tracts (6). In infected broiler chickens, C. jejuni organisms are primarily found in the ceca, where their numbers generally range from 10⁵ to 10⁸ CFU/g (1, 9).

Usually, C. jejuni is first isolated from fecal samples of commercial broiler chickens after 2 to 3 weeks of age (11, 13), although the time of chicken exposure to this organism is unclear. That is, it is possible that the organism colonized chickens before they reached 2 weeks of age. Ringoir et al. (10) have previously revealed that various C. jejuni strains were able to colonize the chicken gastrointestinal tract in 2- and 14-day-old chicken colonization models. However, why colonization of broiler chickens in commercial farms is only detectable at more than 2 weeks of age remains unclear. To determine whether fecal excretion is associated with the age of exposure to C. jejuni, we experimentally inoculated chickens at different ages with C. jejuni and monitored fecal samples for the organism. Chickens originating from the same flock were placed at a commercial broiler farm, which was naturally contaminated with C. jejuni, and the fecal samples were also monitored.

MATERIALS AND METHODS

Chickens. Day-of-hatch broiler chickens used in the present study were acquired from a commercial poultry hatchery in August 2009. The chicks were divided into seven groups of four birds each and kept in isolation rooms until required. The birds were given a commercial chick starter diet containing 10 g/ton (grams per 10³ kg) colistin sulfate and 5 g/ton (grams per 10³ kg) nosithecide until 20 days of age. For the next 20 days, the birds received a grower diet containing 5 g/ton nosithecide, and after that, a nonmedicated finishing diet was given. Feed and water were available ad libitum. The chicks were confirmed to be uninfected with Campylobacter spp. through bacterial examination of feces collected on the day of hatching.

The rest of the chicks, which were not used in the experimental infection, were placed in a chicken house on a commercial broiler farm in August 2009. Birds on this farm were naturally contaminated with C. jejuni.
EXCRETION OF *C. JEJUNI* IN CHICKENS

TABLE 1. Isolation of *C. jejuni* from fecal samples and cecal contents of experimentally inoculated chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (day) of inoculation</th>
<th>Dose</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>6.3 × 10^7</td>
<td>2/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>4.0 × 10^8</td>
<td>0/4</td>
<td>ND</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>NA</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>5.5 × 10^7</td>
<td>0/4</td>
<td>ND</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>D</td>
<td>21</td>
<td>2.9 × 10^8</td>
<td>0/4</td>
<td>ND</td>
<td>4/4</td>
<td>1/4</td>
<td>0/4</td>
<td>0/4</td>
<td>4/4</td>
<td>4/4</td>
<td>NA</td>
</tr>
</tbody>
</table>

a Number of birds positive for *C. jejuni*/number of birds inoculated. ND, not done; NA, not applicable. Numbers in italics represent isolation results from cecal contents.

b Values in columns that share no common letters differ significantly (*P* < 0.05).

Inoculum. The *C. jejuni* strain Cj17-53 isolated from a fecal sample collected in a commercial egg-producing farm in 2005 (16) was used. Stock cultures of the isolate were grown on Mueller-Hinton agar (BD, Sparks, MD) plates supplemented with 5% defibrinated horse blood for 48 h at 42°C under microaerobic conditions (85% N₂, 10% CO₂, 5% O₂). Bacteria were harvested in saline to make suspensions of 5.5 × 10⁷ to 5.4 × 10⁸ CFU/ml, with 0.25-ml amounts used as inocula. Each bird was inoculated by oral gavage with the bacterial suspension. Viable cell counts were determined immediately after inoculation by spreading the suspensions on the culture plates. The actual dose per bird is given in Table 1.

Experimental procedure. Birds in groups A through F were inoculated with live bacterial cells at 0, 7, 14, 21, 28, and 35 days old, respectively. A control group of chicks received saline at the age of day 0. After treatments, the birds in each of the groups were returned to the previous rooms. Fresh fecal samples were tested for the presence of *Campylobacter* on days 1, 2, 3, 5, and 7 post-inoculation, and every 7 days thereafter up to 35 days old in groups A, B, and C, and up to 49 days old in groups D, E, and F and the control group.

A portion of the fecal samples was streaked directly onto modified charcoal cefoperazone deoxycholate agar plates, consisting of *Campylobacter* blood-free selective agar base CM0739 (Oxoid Ltd., Cambridge, UK) and charcoal cefoperazone deoxycholate agar selective supplement SR0155 (Oxoid), and incubated for 48 h at 42°C under microaerobic conditions. Two suspect colonies from one fecal sample were picked and screened for *Campylobacter* species by negative Gram staining and the absence of growth in the aerobic condition. Identification of *C. jejuni* was performed by the hippuricase gene–based PCR assay (8). The remaining feces were weighed, 10-fold dilutions were prepared in sterile saline, and then viable counts were determined. The birds were euthanized when 35 or 49 days old with an excess dose of anesthetic ether, and cecal contents were examined for the presence of viable *Campylobacter* cells.

To test the reproducibility of the results obtained from group C with birds infected at 7 days old, eight additional chicks were divided into two groups of four each and kept as described above. Each bird in one group was inoculated with 6.3 × 10⁷ CFU of *C. jejuni* via the esophagus at 7 days old. The other group of birds received saline. Fecal samples were monitored for *C. jejuni* as described above. Two of the birds were kept for 63 days, and the remaining two birds were kept for 84 days to determine possible shedding of *C. jejuni* from aged birds. After euthanization, the liver, bile, and contents of the jejunum, ceca, and colon were subjected to bacterial examination.

Typing of *C. jejuni* isolates. Restriction fragment length polymorphism (RFLP) analysis of the flaA gene was undertaken by digestion of the PCR product with *DdeI* (TaKaRa Bio Inc., Otsu, Shiga, Japan) based on the method described previously (3).

Monitoring a commercial broiler farm for *C. jejuni*. After the chicks were placed in a chicken house on a commercial broiler farm, the flock in this house was monitored for *C. jejuni* during August to October 2009. Freshly dropped feces from five birds in a house were collected and kept in Cary-Blair medium (Nissui Pharmaceutical Co., Tokyo, Japan) at 4°C during transportation. Isolation and typing of *C. jejuni* were carried out as described above. Monitoring of the previous flock immediately before the present flock was conducted during June to August 2009.

Statistical analysis. The proportion of birds, from which *C. jejuni* was isolated, were compared among groups A to F at 7 and 14 days postinoculation, using Fisher’s exact test with *P* < 0.05 as significant. Relation between the number of days after inoculation and the number of isolated bacteria were analyzed among groups D to F, because the numbers of the bacteria were counted in these groups only. For visual evaluation, the linear regression line between logarithmic values of bacterial numbers (CFU per gram) and the number of days postinoculation was drawn for each group.

RESULTS

Isolation of *C. jejuni* from chickens with experimental infection. *C. jejuni* was isolated on the day after the inoculation from two of the four birds inoculated at 0 day of age (group A) (Table 1). After that, the organism was not isolated until 28 days of age. At 35 days postinoculation, *C. jejuni* was isolated from the fecal contents of a bird at necropsy but was not isolated from fecal samples. In groups B and C of birds inoculated at 7 and 14 days of age, respectively, all fecal samples were negative for *C. jejuni*, although the organism was isolated from the cecal contents.
of two birds at necropsy in each group. In contrast, all chickens in groups D, E, and F inoculated at 21, 28, and 35 days of age, respectively, continually shed \textit{C. jejuni} until they reached 49 days of age. Additionally, the organisms were isolated from all cecal contents obtained at 49 days of age. RFLP patterns of all isolates recovered from the chickens experimentally infected with \textit{C. jejuni} were identical to that of the inoculum (Fig. 1). \textit{C. jejuni} was not isolated from any chickens administered saline.

The proportion of birds, from which \textit{C. jejuni} was isolated, in group F was significantly higher than the proportions in groups A to D at 7 days postinoculation \((P < 0.05)\), while the differences between group E and groups A to D were not significant \((P \approx 0.14; \text{Table 1})\). At 14 days postinoculation, the proportions of birds with \textit{C. jejuni} in groups D and E were significantly higher than those in groups A to C.

The numbers of \textit{C. jejuni} in fecal samples obtained 2 to 3 days postinoculation reached \(4.0 \times 10^8\) to \(1.7 \times 10^9\) CFU/g in the birds in groups D, E, and F, and the maximal numbers of the organism in each of the groups ranged from \(1.7 \times 10^8\) to \(1.0 \times 10^{10}\) CFU/g at 3 to 21 days after inoculation (Fig. 2). The numbers of \textit{C. jejuni} in the cecal contents were \(8.4 \times 10^8\) to \(1.9 \times 10^9\) in group D, \(2.0 \times 10^8\) to \(1.0 \times 10^{10}\) in group E, and \(1.3 \times 10^7\) to \(2.0 \times 10^9\) in group F.

Regression lines showing associations between the log CFU per gram of isolated \textit{C. jejuni} and the postinoculation days in groups D to F are shown in Figure 2. These lines suggest that the numbers of \textit{C. jejuni} obtained from the cecal contents at necropsy were high in comparison to those within 7 days postinoculation.

In four chickens inoculated at 7 days of age in the additional experiment, \textit{C. jejuni} was isolated from a fecal sample obtained from a bird at 77 days of age, proving that the bird was carrying \textit{C. jejuni} in its intestine. The organism was also recovered from a bile sample from one of the birds and from the cecal and colon contents of another bird at 63 days of age. In the remaining two birds euthanized at 84 days of age, \textit{C. jejuni} was not recovered from the organs.

**Isolation of \textit{C. jejuni} from commercial chicken fecal samples.** On the commercial broiler farm, \textit{C. jejuni} was first isolated from fecal samples obtained from two of five chickens at 28 days of age. In the next week, four of five birds were positive for \textit{C. jejuni}, and the organism was isolated from all five birds tested at 43 days old. \textit{C. jejuni} was isolated from four of five chickens at 47 days in the previous flock. RFLP patterns of the isolates obtained from commercial chickens were not identical to the pattern in birds that were experimentally infected (Fig. 1).

**DISCUSSION**

The experimental infection results in this study suggest that young chicks exposed to \textit{C. jejuni} carry this organism in...
their ceca until they reach slaughter age. In chickens esophagally inoculated with *C. jejuni* at 0 to 14 days of age, the organism was recovered from the cecal contents of these birds when they reached 42 days of age, although fecal excretion was only found at 1 day postinoculation in two birds inoculated at 0 day of age but not observed thereafter. It is possible that the number of the organism was very low. Similar results were obtained from four chickens inoculated at 7 days of age in the additional experiment. Moreover, the RFLP type of *C. jejuni* isolates obtained from the cecal contents of the chickens was identical to that of the tested strain. *C. jejuni* was previously shown to be present in the ceca, liver and lymphoid-like organs 1 h, 1 day, and 1 week after oral inoculations of day-old broiler chicks (4). In *C. jejuni*–infected chickens, *C. jejuni* was found in the ceca, although the cecal mucosa displayed no inflammation (14). An inefficient inflammatory response may fail to clear the bacterium from the gut (5). The negative results for *C. jejuni* in fecal samples from the birds inoculated at 0 to 14 days of age is likely due to the isolation procedure because the bacteria were not isolated using enriched media in this study, resulting in an underestimation of the presence of *C. jejuni* in fecal samples from these birds.

When chickens were inoculated at 21 to 35 days of age, *C. jejuni* may have proliferated in the intestinal tract. Fecal excretion of this organism was observed 1 to 3 days after inoculation, and the birds continually shed *C. jejuni* until they reached 49 days of age. The amount of *C. jejuni* in fecal samples from these birds gradually increased day by day.

Chickens used in the experimental infection and those placed in the commercial broiler farm were free by methods used to test *C. jejuni* on the day of hatching. In birds that were administered saline as a negative control in the experimental infection, *C. jejuni* was not isolated from fecal samples or the cecal contents until slaughter age. This indicates that chicks put on the commercial broiler farm were colonized with *C. jejuni* after their placement on the farm. To prevent colonization of chicks after placement on commercial farms, routine monitoring of the farms with this organism using environmental samples, including litters and disinfection practices, may be important. All isolates recovered from birds on the farm, including the previous flock immediately before the present flock, belonged to an identical RFLP type. Strains with an identical RFLP type during successive production cycles have been isolated in previously studies (7, 11). The environment can be a source of *C. jejuni*, colonizing housed broiler flocks (2, 15).

On the commercial broiler farm, *C. jejuni* was isolated from chickens when they reached 28 days of age. This appears to be consistent with the intensive excretion of *C. jejuni* by the birds inoculated at 21 to 35 days of age in this study. However, the failure to detect *C. jejuni* in fecal samples from chickens younger than 28 days of age on the farm does not necessarily exclude the possibility that these birds were colonized with *C. jejuni* in the ceca. In a previous study, bacterial examination of the cecal contents demonstrated transmission of *C. jejuni* to uncolonized chickens at 1 week of age after exposure to colonized seeder chickens (12). Thus, when uncolonized chicks were placed on farms and exposed to *C. jejuni*, they may carry *C. jejuni* in the ceca.

**REFERENCES**