Experimental Hepatitis Induced by Campylobacter jejuni Infection in Japanese Quail (Coturnix coturnix japonica)

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ABSTRACT. To establish an experimental model for vibrionic hepatitis caused by Campylobacter jejuni, Japanese quails (Coturnix coturnix japonica) were inoculated with C. jejuni strains isolated from chicken hepatitis (BL107) and human diarrhea (HP5113). Necrotic liver lesions were formed by intra-pancreatic duodenal vein injection by which the bacteria reached the liver directly via the portal vein, but not by intra-gastric infection. These liver lesions were observed from day 1 to 7 after the infection. The pathological changes were weak and no clinical signs were observed throughout the experimental period. By immunohistochemistry, the bacterial antigens were detected in the hepatocytes, and intercellular spaces between the hepatocytes, and in the macrophages during the early stage of the infection. When focal hepatocyte necrosis was formed, the antigen was detected more frequently in the intact hepatocytes at the periphery of the lesions than within necrotic foci. The bacteria were not detected from the liver, spleen or blood according to raising the serum agglutination titer. In contrast, the bacteria immediately invaded the bile in 5 min post-infection and were retained in the gallbladder for long periods. The present study showed that necrotizing hepatitis was formed by intra-pancreatic duodenal vein infection of the quail with C. jejuni. — KEY WORDS: Campylobacter jejuni, experimental infection, hepatitis, Japanese quail, translocation.

Campylobacter jejuni is recognized as the most common human enteropathogen throughout the world [27, 33]. Intestinal contents of chickens and contaminated chicken meat products are considered epidemiologically as one of the most important sources of the infection in humans [8–10]. C. jejuni is widely colonized in the intestinal tract of chickens, however, the birds usually exhibits no clinical signs. Although chickens have been used as an experimental animal for the analysis of certain virulence factors of Campylobacter strains [14, 21, 24, 30], only the chick embryo has been reported as a useful model for invasion or lethality [6, 7, 18]. On the other hand, Peckham [22] reported that C. jejuni was a causative agent of so-called vibrionic hepatitis in chickens. Several studies on chicken hepatitis were reported also in U.S.A., Canada, Europe, and Japan from 1950’s to 1960’s [2, 11, 20, 29, 32]. However, the participation of C. jejuni in the formation of hepatitis in chickens is not well understood since there are few suitable experimental models [5, 20, 22, 25].

Japanese quail is easy to maintain and handle in laboratories and the biological characteristics are similar to that of the chicken. Therefore, we used Japanese quails to establish an experimental infection model for studies of vibrionic hepatitis caused by C. jejuni. In this study, we investigated the effective infection route on the formation of vibrionic hepatitis and the translocation of the inoculated organisms.

MATERIALS AND METHODS

Bacterial strains: Two strains of C. jejuni (BL107 and HP5113) were used in this study. Strains BL107 and HP5113 were isolated from the liver of a broiler chicken with necrotizing hepatitis, and human campylobacteriosis (kindly supplied by Dr. T. Itoh, Tokyo Metropolitan Research Laboratory of Public Health, Tokyo), respectively. The strains were suspended in Nutrient Broth No. 2 (Oxoid Ltd., Basingstoke, England) containing 10% dimethylsulfoxide (Sigma, Chemical Co., St. Louis, Mo, U.S.A.) and stored at −80°C until experiment.

Experimental animals and inoculation: Japanese quails (Coturnix coturnix japonica) were bred in our laboratory, and 123 of 2 month-old birds were used for the experimental infection. The inocula were prepared as follows: strains were grown on horse blood agar (HBA) plates for 48 hr at 42°C under a gas mixture containing 5% O2, 5% H2, 10% CO2 and 80% N2. A loopful of bacteria was inoculated into 20 ml of Brucella broth (BBL Becton Dickinson Microbiology Systems, Cockeysville, Md, U.S.A.) in an Erlenmeyer flask, and incubated at 42°C for 2 days by stationary culture. To determine the effective route for experimental hepatitis, a volume of 0.2 ml of culture broth containing 5.0 × 107 to 1.0 × 109 colony forming units (cfu) of C. jejuni was inoculated into the basilic vein, pancreaticoduodenal (PD) vein or stomach. The injection into the PD vein was followed by Shinjo et al. [26]. The organisms inoculated by this route are considered to reach the liver directly via the portal vein. Control animals received the same volumes of Brucella broth. The livers, blood, bile and cecum contents of five quails randomly selected were cultured for C. jejuni, and the birds were confirmed to be free of the bacteria.
**Bacteriological examination:** The quails were autopsied at various intervals post-infection. The liver, spleen, blood and bile were examined for viable counts of the inoculated C. jejuni. One gram of liver was homogenized with 10 mM sterile phosphate-buffered saline (pH 7.2) and 10-fold serial dilutions were made. A volume of 0.05 ml of each dilution was plated onto HBA and cultured by the method stated above. The spleen, blood and bile were placed directly onto blood agar plates. The plates were incubated at 42°C under microaerobic conditions. C. jejuni was recovered from fecal samples on Skirrow’s selective medium plates [27] after enrichment culture with Preston medium [4]. The confirmation of the recovered organisms was followed by the slide-agglutination test of Lior et al. [17].

**Pathological examination:** The liver of the quail was fixed in 10% neutral buffered formalin or Bouin’s solution and embedded in paraffin. Then, 4-μm sections were stained with hematoxylin and eosin (HE). Immunostaining for the detection of C. jejuni in situ was performed by avidin-biotin peroxidase complex (ABC) methods with a Vectastain®, ABC Kit (Vector Labo., Burlingame, Ca, U.S.A.). As primary antibody, rabbit IgG fraction against heat-labile whole cells of C. jejuni strain BL107 or HP5113 was employed. Biotinylated goat serum against rabbit immunoglobulin (Dako Japan Co., Ltd., Kyoto) was used as the secondary antibody. The reaction products were visualized with 3,3′-diaminobenzidine (Sigma). As counterstain, Mayer’s hematoxylin was used.

For the transmission electron microscopy, tissue blocks of the liver were collected 1 hr after PD-infection and fixed in 2.5% glutaraldehyde in 100 mM cacodylate buffer (pH 7.4) for 2 hr. They were postfixed in 1% osmium tetroxide in 100 mM cacodylate buffer for 2 hr and dehydrated through a graded series of ethanol. Then, they were embedded in Quetol 812 (Nissin EM Co., Ltd., Tokyo). Thick sections for light microscopy were stained with toluidine blue. Ultrathin sections stained with uranyl acetate and lead citrate were observed under a Hitachi H-800 MU transmission electron microscope.

**Serological examination:** Before autopsy, the serum taken from the quails was examined for antibodies against the inoculated strain by the microplate agglutination test.

**Translocation of C. jejuni from the blood to bile:** Translocation of the inoculated bacteria from the blood stream into the bile was examined. A portion of bile was taken from the gallbladder with a 27-gauge needle at various intervals (5, 10, 20 and 30 min) after infection into the basilic or PD vein. Three quails were used at each interval. The bile was directly placed onto HBA plates and incubated at 42°C for 2 days under the microaerobic conditions.

**Statistical analysis:** The χ² test was used to assess significant differences in the frequency of occurrence of necrotic lesions among the routes of infection.

**RESULTS**

**Experimental infection:** The most effective route to cause vibrionic hepatitis was determined by comparing three different infection routes. One or 2 days after infection with C. jejuni strain BL107, small white spots about 0.5 mm in diameter were observed in most hepatic lobules by intravenous injection (Fig. 1A). Although the occurrence rate of necrotic lesions in the liver by PD vein injection was higher than that by basilic vein injection, there was no significant difference (P>0.05). The organism inoculated via the PD vein was more frequently recovered from the liver, spleen, bile, and blood than that via the basilic vein. In contrast, no obvious lesion was observed up to 14 days after intra-gastric infection and the inoculated bacteria were recovered only from the cecal contents (Table 1).

The pathogenicities of the two isolates from chicken hepatitis and human diarrhea were compared by inoculating via the PD vein. There were no significant differences (P>0.05) in the frequency of liver lesions between the two strains. The liver lesions were observed at high frequency up to 5 or 7 days after infection of either strain. The necrotic lesions of the liver were hardly observed 10 days after infection. There were no obvious lesion in other organs except for slight swelling of the spleen in an early stage after infection (Table 2). C. jejuni was recovered from the liver at a level from 10⁴ to 10⁵ cfu/g during 3 days after infection. The bacteria recovered from the liver decreased in number as the agglutination antibody titer increased from 5 days after infection (Fig. 2). Similarly, the detection of bacteremia was limited to the earliest stage of the infection and the inoculated bacteria were not isolated from the spleen or blood 5 days after infection. In contrast, C. jejuni was detectable for up to 14 days from bile and feces (Table 2). However, no clinical signs were observed in the infected quails throughout the 2 weeks after infection. C. jejuni was not isolated from any control bird receiving Brucella broth.

Histopathologically, the lesions in the liver consisted of focal hepatocyte necrosis associated with marked accumulation of heterophils and macrophages (Fig. 1B). The inoculated bacteria were detected in the hepatocytes and the intercellular spaces between hepatocytes (Fig. 1C) and in Kupffer’s cells by the immunohistochemical technique 1 hr after infection. The inoculated bacteria were found in the vacuoles of hepatocytes, intercellular spaces between the hepatocytes and Kupffer’s cells 1 hr after the PD vein infection by transmission electron microscopy (Fig. 3). When the focal necrosis of hepatocyte was formed, the antigen was detected more frequently in intact hepatocytes at the periphery of the lesions than within necrotic foci.

**Detection of C. jejuni from the bile:** As shown in Table 3, both human and animal strains were detected from bile in the gallbladder 5 min after intra-PD and basilic vein injection.

**DISCUSSION**

In the present study, avian vibrionic hepatitis was induced in Japanese quails by the experimental infection with C. jejuni. Intra-PD vein injection was the most effective route to form necrotic lesions in the liver, however, the quails did not show any clinical sign throughout the experiment.
Histopathological findings of the hepatitis in quails resembled those in the chickens described in other reports [5, 11, 25]. These aspects of infection with C. jejuni were distinct from those with Pasteurella multosida in which the animals revealed acute sepsis even though focal necrosis was observed [23].

The organisms injected by the intravenous route were immediately excluded from the bloodstream. Other reports also demonstrated that clearance of C. jejuni occurred immediately after intravenous infection of mice [1, 3] and the organism was engulfed by granulocytes and eliminated from the liver [1]. In the present study, it was shown that the clearance of the bacteria from the liver, spleen and blood occurred immediately as the agglutination titer increased. In contrast, it appeared that C. jejuni was transferred to the gallbladder immediately after PD vein injection and persisted in bile for a long time. Although we could not demonstrate the actual infection route from bloodstream into the bile, it seemed that direct transfer of the organisms from the bloodstream to bile was impossible on account of the
anatomical structure of the liver and gallbladder. *C. jejuni* detected in quail bile may have passed through the hepatocytes from the bloodstream and not via the cystic duct because the organism given by the oral route was not isolated from bile. From these findings, it was considered that the focal necrosis may have been formed by the bacterial passage through the hepatocytes because the bacterial antigen was detected more frequently in intact hepatocytes at the periphery of the lesions than within necrotic foci. In addition to the physical disruption of the liver tissue by bacterial invasion, some toxic factors such as a cytotoxin [19], hepatotoxin [16], endotoxin [31], or chemotactic behavior with respect to bile and mucin [12] may also be implicated in inducing the hepatic lesions.

Kita *et al.* [15] observed that hepatitis occurred in mice after oral infection of *C. jejuni*. Humphrey [13] also reported that a man with *C. jejuni* diarrhea showed swelling and dysfunction of the liver. These findings suggest that *C. jejuni* translocates from the intestinal tract to the liver via the portal vein or lymphatic circulation and is transferred to bile after passing through the hepatocytes. In this study, we failed to reproduce hepatitis by intragastric injection. Soerjadi *et al.* [28] reported that only chickens pretreated with cyclophosphamide, an immunosuppressive agent, showed gross lesions in the liver after intragastric infection. These findings suggest that the reduced host defense status may promote *C. jejuni* translocation from the intestine to the liver and induce hepatitis. In the experimental hepatitis in normal quails, intra-PD vein injection might be an effective infection route for the induction of hepatitis.

**Table 1.** Comparison of three different infectious routes in the formation of necrotic lesions in the liver and recovery of *C. jejuni* strain BL107

<table>
<thead>
<tr>
<th>Infectious route</th>
<th>Rate of necrosis in the liver (%)</th>
<th>Bacterial recovery (%) from Liver</th>
<th>Bile</th>
<th>Spleen</th>
<th>Blood</th>
<th>Cecum</th>
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<tr>
<td>PD vein&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10/16&lt;sup&gt;1&lt;/sup&gt; (71.4)</td>
<td>100</td>
<td>100</td>
<td>64.3</td>
<td>50.0</td>
<td>100</td>
</tr>
<tr>
<td>Basilic vein</td>
<td>7/15 (46.7)</td>
<td>86.7</td>
<td>93.3</td>
<td>40.0</td>
<td>40.0</td>
<td>100</td>
</tr>
<tr>
<td>Oral</td>
<td>0/9 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>88.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> A volume of 0.2 ml containing 1.0 × 10<sup>8</sup> cfu/ml of BL107 strain was inoculated via three different routes.

<sup>b</sup> PD vein: pancreaticoduodenal vein.

c) Number of quails showing necrotic lesions in the liver/Number of quails examined. One to 4 quails were sacrificed on days 1, 2, 3 and 5. *C. jejuni* was not isolated from any control animal inoculated with Brucella broth.

**Fig. 2.** Number of *C. jejuni* (― ●― HP5113, — ○— BL107) recovered from the liver, and the serum agglutination titer (― △— HP5113, — △— BL107) after experimental infection via the PD vein of quails. Each point indicates the mean of three experiments.

**Table 2.** Recovery of *C. jejuni* from infected quails and formation of necrotic lesions in the liver after the PD vein inoculation

<table>
<thead>
<tr>
<th><em>C. jejuni</em> strain (source)</th>
<th>Days after infection</th>
<th>Recovery of <em>C. jejuni</em> from:</th>
<th>Necrotic lesion</th>
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<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Bile</td>
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<tr>
<td>HP5113 (human)</td>
<td>1</td>
<td>4/4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3/3</td>
<td>3/5</td>
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<td>3</td>
<td>3/3</td>
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<td>7</td>
<td>1/3</td>
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<td>10</td>
<td>0/2</td>
<td>1/3</td>
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<tr>
<td></td>
<td>14</td>
<td>0/3</td>
<td>1/3</td>
</tr>
<tr>
<td>BL107 (chicken)</td>
<td>1</td>
<td>4/4</td>
<td>4/4</td>
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<tr>
<td></td>
<td>2</td>
<td>3/3</td>
<td>3/5</td>
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<tr>
<td></td>
<td>14</td>
<td>1/3</td>
<td>2/3</td>
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</table>

<sup>a</sup> Number of quails showing necrotic lesions in the liver/Number of quails examined.

<sup>b</sup> Number of *C. jejuni*-positive quails/Number of quails examined.

<sup>c</sup> Not done.
Our data strongly suggest that Japanese quails served as one of useful experimental models for vibrionic hepatitis in the chicken, but further studies are needed to clarify the mechanism of formation of necrotic lesions in the liver.

REFERENCES


