Invasion and Viability of Campylobacter jejuni in Experimentally Contaminated Japanese Quails’ Eggs
Soichi MARUYAMA, Yukio MORITA, and Yasushi KATSUBE
Laboratory of Veterinary Public Health, College of Agriculture and Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252, Japan
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ABSTRACT. In Japanese quail eggs experimentally immersed in a suspension of Campylobacter jejuni from human patients (Y6817, Y6878), strain Y6817 was recovered from 15 (18.8%) eggs among 80 between 0.5 hr and 72 hr after immersion. Invasion of the eggs by strain Y6878 was also seen in 10 (12.5%) of 80 eggs between 0.5 hr and 24 hr after immersion. When egg yolks was inoculated with both strains and held at 4°C, the organisms were detectable for a longer time than the ones kept at 20°C. On the other hand, when albumen was inoculated with both strains, the organisms died within 96 hr at 4°C and within 24 hr at 20°C.—Key words: Campylobacter jejuni, Japanese quail egg, viability.

Campylobacter jejuni is now recognized as the major causative organism in food poisoning as well as sporadic diarrhea in man. It is reported that the organism distributed widely in raw meat [4], raw milk [2, 4], water [3], wild and domestic animals and birds [17, 20, 22]. Chickens and meat are suspected of being the most potentially infectious sources of the organism for humans [14, 20].

The authors reported previously that C. jejuni colonized easily in the intestines of Japanese quails inoculated orally and the organism was recovered from the egg shell surface and contents of eggs laid by the quails, though the frequency was rather low [15, 16], but, it was not clear whether the contamination of the egg contents was caused by on- or in-egg infection.

In this study, the authors studied whether C. jejuni on-egg infection occurred in quail eggs or not, when the egg surface was experimentally contaminated with the organism. Furthermore, when the egg contents were contaminated with the organism, the viability of the organism in the quail egg yolk and albumen was also investigated.

Modified Preston’s Broth (MPB) and Butzler’s agar plates (BAP) were prepared by the manner described previously [17]. Two strains (Y6817 and Y6878) of Campylobacter jejuni derived from infant diarrheal cases were used for this experiment. Strains Y6817 and Y6878 were identified as Penner’s serogroups 2 and 8 by passive hemagglutination, respectively [17]. The organisms were cultured on BAP at 42°C for 48 hr under microaerophilic conditions (BBL CampyPak System). The suspension were then harvested from the agar plates and suspended in sterile phosphate buffered saline (PBS). The suspension was washed three times with PBS by centrifugation at 3,000 rpm for 20 min and resuspended in PBS.

For each strain of C. jejuni, 13 cases of Japanese quails eggs packed in a plastic case (10 eggs/case) were purchased from retail food stores or supermarkets. One egg was selected at random from each case, and the shell surface and contents of the eggs were examined for the contamination by thermophilic campylobacters by the method reported previously [16]. And a total of 15 eggs selected randomly from three cases were investigated for the presence of any antibiotics by high performance liquid chromatography [13, 19]. The remaining eggs were used for the experiment mentioned below.

The bacterial suspensions of strain Y6817 containing 8.0 \( \log_{10} \) colony forming units (CFU)/ml and Y6878 containing 8.2 \( \log_{10} \) CFU/ml were used for egg immersion. Eighty eggs were immersed in each bacterial suspension for 30 sec. The eggs were removed and placed under aerobic condition for 24 hr at room temperature, and then the eggs were kept at 4°C. The isolation of C. jejuni from the egg surface, egg yolk and albumen were carried out at 0.5, 1, 3, 6, 12, 24, 48 and 72 hr after the immersion. At each time of examination, the shell surface of 10 eggs was swabbed with a sterile cotton swab immersed in MPB at each time examined. After the swabbing, the eggs were disinfected by immersing in 50% (v/v) isopropanol for 1 min and swabbing thoroughly with a cotton swab moistened with 50% isopropanol. Furthermore, the eggs were dried and placed under a UV germicidal lamp for 30 min while turning them around several times. The eggs were opened ascetically with forceps, and the albumen and egg yolk were obtained separately. The isolation of the organism from the swab specimen and egg contents was followed by the method reported previously [16].

The viability of C. jejuni inoculated into egg yolk and albumen was examined as follows. The surface of the quail eggs was disinfected in the manner stated above. The eggs were opened ascetically with forceps and the egg yolk and albumen were obtained separately. Egg yolk and albumen were pooled separately. Four ml of each bacterial suspension measuring 2.4 \( \log_{10} \) to 6.0 \( \log_{10} \) CFU/ml was inoculated into 36 ml of egg yolk and 36 ml of albumen, and then mixed well. The mixtures were kept at 4°C or 20°C under aerobic conditions. The viability of the organisms in the egg yolk and albumen was examined from 1.5 hr to 86 days post inoculation. A volume of 0.5 ml of each specimen was inoculated into 4.5 ml of MPB and mixed well. Ten fold serial dilution from 10 to 10^3 was made in MPB. A set of 3 test tubes each containing 4.5 ml of MPB was prepared for each dilution. Five tenth ml of each dilution was inoculated into a tube and incubated at 42°C for 24 hr under aerobic conditions. After the incubation, a loopful to MPB from each tube was streaked onto BAP and incubated at 42°C for 48 hr under microaerophilic conditions. The plates growing colonies of C. jejuni with the typical cell shape, motility...
Table 1. Recovery of *C. jejuni* strain Y6817 and Y6878 from Japanese quail eggs immersed with the bacterial suspension

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Time (hr) after immersion</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Y6817</td>
<td>Shell</td>
<td>9/10/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Albumen</td>
<td>1/10</td>
<td>2/10</td>
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<tr>
<td></td>
<td>Yolk</td>
<td>2/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Y6878</td>
<td>Shell</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Albumen</td>
<td>1/10</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Yolk</td>
<td>0</td>
<td>2/10</td>
</tr>
</tbody>
</table>

(1) The number of *C. jejuni* positive eggs/ The number of eggs examined.

and biochemical characteristics were regarded as positive. The number of *C. jejuni* per ml in each specimen was expressed as the most probable number (MPN). On each day of examination, the pH values for the egg yolk and albumen inoculated with the organism were also measured.

In the experimental immersion of quail eggs, the recovery of the organisms from each egg specimen is given in Table 1. Strain Y6817 was recovered from 8 lots of albumen (10%) and 12 egg yolks (15.0%) from 80 eggs between 0.5 hr and 72 hr after immersion. Invasion of the organisms from the egg shell into the egg contents was observed in 15 (18.8%) of the 80 eggs. Strain Y6878 was recovered from 9 lots of albumen (11.3%) and 7 egg yolks (8.8%) from 80 eggs between 0.5 hr and 24 hr after immersion. Invasion by the organisms was seen in 10 (12.5%) of 80 eggs. Most cases of invasion of the egg contents were observed during 24 hr post immersion.

Figure 1 illustrates the viability of strains Y6817 and Y6878 inoculated into egg yolk at 4°C or 20°C. When 3.5 log<sub>10</sub> CFU/ml of strain Y6817 was inoculated into egg yolk and kept at 4°C, the organisms were detected until 22 days after inoculation. When 6.0 log<sub>10</sub> CFU/ml of strain Y6817 was inoculated into egg yolk and kept at 4°C, the organisms could survive for up to 86 days.

When 3.0 log<sub>10</sub> CFU/ml of strain Y6878 was inoculated into egg yolk and kept at 4°C, the organisms were detectable until 13 days after the inoculation. When 5.4 log<sub>10</sub> CFU/ml of strain Y6878 was inoculated into egg yolk and kept at 4°C, the organisms were recovered for up to 19 days.

When 3.0 log<sub>10</sub> or 5.1 log<sub>10</sub> CFU/ml of strain Y6817 was inoculated into egg yolk at 20°C, the organism was detectable until 14 or 20 days after inoculation, respectively. When 3.2 log<sub>10</sub> or 5.1 log<sub>10</sub> CFU/ml of strain Y6878 was inoculated into egg yolk, the organisms were recovered for up to 9 or 11 days, respectively. The viability of strains Y6817 and Y6878 at 20°C was lower than that at 4°C. The pH value in egg yolk at 4°C or 20°C varied from 6.2 to 6.3.

Figure 2 shows the viability of the organisms inoculated into the albumen at 4°C or 20°C. When 3.5 log<sub>10</sub> or 6.0 log<sub>10</sub> CFU/ml of strain Y6817 and 3.0 log<sub>10</sub> or 4.2 log<sub>10</sub> CFU/ml of strain Y6878 were inoculated and the albumen was kept at 4°C, both of the strains were recovered within 48 hr.

When 3.0 log<sub>10</sub> CFU/ml or 5.1 log<sub>10</sub> CFU/ml of strain Y6817 was inoculated and the albumen was kept at 20°C, the organisms were recovered within 12 hr. When 2.4 log<sub>10</sub>
or 4.0 log_{10} CFU/ml of strain Y6878 was used, the organisms were recovered within 3 to 4.5 hr. The pH of the albumen at 4°C and 20°C varied from 8.6 to 9.2.

The cuticle covering the eggshell has the function of preventing the invasion of the egg contents from the eggshell by the bacteria. However, the cuticle is easily removed by scratching and brushing, and micro organisms are allowed to invade the inside of the egg. The diameter of the shell pore is 6–23 μm on the inside and 15–65 μm on the outside. On the other hand, the size of _C. jejuni_ is 0.2–0.8 μm in diameter and 1.5–5 μm long. It is therefore thought that the organism on the egg shell surface can easily invade the inside of the egg through the pore, if the cuticle is removed. In fact, both strains examined were recovered from 17 albumen and 19 egg yolk 0.5 hr and 72 hr respectively, after immersion of the bacterial suspension. These results showed that the organism on the quail eggs was able to invade the egg contents immediately through the pores.

It was reported that _C. jejuni_ was quite sensitive to drying and storage at room temperature [9, 12]. In this study, strains Y6817 and Y6878 on the egg shell surface were detected 72 hr and 48 hr after immersion, respectively. Since the shell surface isolation rate was rather low in the later stage compared with the early stage when stored, it seems that the organisms cannot survive for a long time on the shell surface of the quail eggs under drying and aerobic conditions.

The isolation status of the organisms from the albumen and yolk in immersed eggs was different from that of albumen and yolk inoculated with the organisms. In the present study, the eggs immersed in the bacterial suspension were placed at room temperature for 24 hr, and then, they were kept at 4°C. On the other hand, egg yolk and albumen inoculated with the organisms were immediately kept at 4°C. It is not yet clear how the viability of the organisms in immersed eggs is influenced by the storage conditions.

Clark and Bueschkens [5, 6] showed the possibility that _C. jejuni_ was able to infect fertile chicken eggs and in some cases, the organism was capable of surviving until the chicken hatched. Shanker _et al._ [21] also reported that 2 of 12 hatched chicks were colonized with _C. jejuni_ and the organism was isolated from the contents of all inoculated unhatched eggs, when the fertile chicken eggs were infected with various numbers of _C. jejuni_. These results suggest that the possibility of on-egg infection with _C. jejuni_, and the organism is carried for a long time inside the eggs.

When egg yolk was inoculated with _C. jejuni_ and kept at 4°C, the organism survived for a long time compared with storage at 20°C. Blaser _et al._[1] reported that _C. jejuni_ in feces, milk and water survived longer at 4°C than at 25°C. Furthermore, it was reported that the organisms survived longer at 4°C than at 25°C under dry conditions in the presence of skim milk or brucella broth [9]. It was shown that _C. jejuni_ could multiply at 42°C in the egg yolk of chicken [7], and egg yolk can be used as a maintenance

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Fig. 2. The viability of _C. jejuni_ strains Y6817 (●) and Y6878 (○) in quail albumen kept at 4°C or 20°C. +: _C. jejuni_ positive, -: _C. jejuni_ negative.
medium of the organism if the inoculum is tightly closed with rubber stoppers and stored at 4°C [18]. These data suggest that temperature was a major factor affecting the viability of the organism in egg yolk.

In this study, C. jejuni showed quite high sensitivity to the albumen of quail egg. It was reported that the organism was affected by a pH value as high as 9.0 [8, 11, 12] and that C. jejuni inoculated into albumen died in a short time and conalbumin in albumen was the major factor affecting the sensitivity of the organism [7]. The pH value of the quail albumen was about 9.0 during the period of experiment. The viability of the organism may be influenced by the high pH value of the albumen and some factors such as the conalbumin or lysozyme contained in the albumen.

This study suggested that C. jejuni could invade egg contents and survive for 2 to 3 days there, when the egg surface was contaminated with the organisms. Since Japanese people are in the habit of eating raw chicken and quail eggs, it is necessary to pay attention to contamination of poultry eggs with C. jejuni.

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REFERENCES