Horizontal Spread of Human and Poultry-Derived Strains of \textit{Campylobacter jejuni} among Broiler Chicks Held in Incubators and Shipping Boxes

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\textbf{ABSTRACT}

The first chick to hatch and dry in each of a series of incubators was fed a suspension of \textit{Campylobacter jejuni} via a stomach tube and returned to the incubator. Subsequently, all hatched chicks were taken out of the incubators and housed in standard transport boxes for a further 24 h, after which they were killed by carbon dioxide inhalation. The intestinal tracts of all hatched birds were excised, enriched in liquid media and then plated on media selective for \textit{C. jejuni}. 

\textit{Campylobacters} were cultured from up to 70\% of the chicks but this percentage varied depending on the strain originally fed to the initial chick. The spread of poultry-derived strains was as extensive as that of some human-derived strains, while other human strains showed little tendency to spread amongst chicks. A significant number of hatched, healthy chicks had distended intestinal tracts and showed abnormal gross liver pathology. This symptom was typical of those strains of \textit{C. jejuni} known to be invasive or toxigenic. However, the gross pathology occurred more frequently than did the incidence of viable \textit{C. jejuni} in the intestine.

The spread of \textit{C. jejuni} contamination of the birds has not yet been shown to be a reliable indicator of past or potential infection. The possible sources of barn infection have not been established. Rosef et al. (19) have suggested that flies can be an important source because \textit{C. jejuni} has been shown to spread from gulls to pigs, and via flies, to hens. Some broiler houses have neither rodent nor wild bird control. There also exists the possibility of egg infection at the time of laying, although vertical transmission in the field has never been recorded. Shanker et al. (23) have argued against vertical transmission as a source of flock infection and found no vertical spread when they raised chicks from breeder flocks known to be \textit{C. jejuni} carriers. Contamination of the laid egg by feces occurs with other bacteria (24), but infection of the egg in the field has never been established for \textit{C. jejuni}. Although eggs can be infected in the laboratory with \textit{C. jejuni} via temperature or pressure differential methods of inoculation (2,3), Doyle (4) could not establish egg infection by applying to the egg surface \textit{C. jejuni} mixed into feces.

\textit{Campylobacter jejuni} causes human intestinal infections and is derived from a broad range of animal and avian hosts. The most commonly implicated agricultural sources are milk (1,18) and poultry, but, although both cows and poultry are known to carry \textit{C. jejuni} (7,10,22), a full epidemiology of these foodborne infections has proven difficult to achieve despite the existence of acknowledged serotyping (13,15) and biotyping (8) schemes. There are two reasons for this difficulty: \textit{C. jejuni} exists in low numbers in foods, so isolation must be achieved in the face of the normal resident flora, and second the factor of time. For example, the all-in, all-out method of raising poultry results in the incriminated flock never being available for sampling after the event it is deemed to have caused. Some poultry barns with litter floors will be used for sequential flocks. Those flocks which do become serially infected by \textit{C. jejuni}, have never been tested for a common serotype pattern typical of vertical transfer from flock to flock. Neill et al. (14) found that the occurrence of wet litter was associated with the appearance of \textit{C. jejuni} in flocks, but there is no general agreement on the use of litter analysis as an indicator of bird infection. Doyle and Roman (5) found poor recovery of \textit{C. jejuni} from poultry litter. In five flocks, Pokamunski et al. (16) found only two positive litter samples, although the incidence of \textit{C. jejuni} was 85\% overall in the flocks they examined. Thus the litter environment of the birds has not yet been shown to be a reliable indicator of past or potential infection.
method which is acceptable to the farmer can identify a 10% infection in a flock of 5000 or more birds. The repeated reports of the sudden appearance of \textit{C. jejuni} in flocks at 2-4 weeks is better explained by having taken only 25-30 bird samples from large flocks, than by epidemic spread. The present study examines the effect of one infected chick on horizontal transmission of \textit{C. jejuni} in the incubator and transport box, before the box reaches the farm.

**MATERIALS AND METHODS**

**Cultures**

\textit{C. jejuni} strains INN73-83, 79-102, Case, V48 and C006 were obtained from Dr. F. Klipstein (University of Rochester Medical Center, Rochester, NY, U.S.A.) and represent strains used in references 11 and 12. All were from human patients. Strains from watery (secretory) diarrhea or from bloody (invasive) stools were identified as toxigenic or as invasive by Dr. F. Klipstein (11,12). Strain C006 was neither invasive nor toxigenic. CEPA 3C and CEPA 4C were provided by Dr. J. Penner (Department of Microbiology, University of Toronto) who had received them from Dr. Ruis-Palacios (Instituto Nacional de la Nutricion, Tialpan, Mexico). Strain CN9 was isolated by the present authors from retail poultry and was already known to have no effect on egg hatchability (3). Strain SC138 was from a poultry slaughter house and was also provided by Dr. J. Penner.

**Enrichments and growth media**

Cultures were grown on frozen stocks on blood agar for 48 h at 37°C under a gas mixture of N₂,CO₂, and O₂ in the ratio 85:10:5. Blood agar plates contained Columbia agar base (Gibco Laboratories, Madison, WI) with 5% whole horse blood and the redactants described by George et al. (6) each at 0.25 g/L.

Strains of \textit{C. jejuni} which were to be fed to the chicks were grown at 42°C for 48 h on blood agar without antibiotics. The colonies were harvested into 0.1% peptone water (Oxoid Canada, Nepean, Ontario) to an OD₆₀₀ known to equal about 10⁶ CFU/ml. The precise viable count was made on whole blood agar plates from serial dilutions of this preparation before feeding the chicks.

Enrichment of \textit{C. jejuni} from eggs or tissue was in a lysed blood, Skirrow antibiotic type of Brucella broth (Gibco), followed by plating on a similar agar but without rifampin (2,3). Dead-in-shell (DIS) eggs were surface sterilized with alcohol and broken aseptically. The age of the embryo was noted and if development was less than day 11-12, the whole egg contents were placed into 50 ml of enrichment broth, incubated at 42°C under the gas phase described above, and, after 48 h, 0.1 ml surface plated onto the antibiotic plus rifampin, lysed blood agar medium (2,3). Previous comparisons between media with and without rifampin had shown that there was no difference in the recovery of \textit{C. jejuni} but that rifampin repressed the gram-positive contaminations often found in poultry field samples. Incubation for a further 48 h at 42°C yielded typical \textit{C. jejuni} colonies which, after biochemical verification, were serotyped by the Penner method (8,15). The same procedure was used for the excised intestine of hatched chicks and DIS embryos older than 12 d, except that the entire intestinal tract was cut up into irregular sections in the enrichment broth held in cotton plugged tubes (10 ml of medium in 20 x 160 mm tubes). Egg contents were enriched in urinalysis bottles (2).

**Egg incubation**

Eggs were obtained from registered hatcheries within 48 h of being laid. Incubation has been described previously (2). Each incubator held 70 eggs. In this work DIS eggs included all unhatched eggs. Each was broken and graded as infertile, and either early DIS or late DIS based on whether the embryo had died before or after day 12.

**Chick inoculation**

The first hatched, dried chick to emerge in each incubator was leg banded for identification, and fed 0.5 ml per os of one strain of \textit{C. jejuni} via a Bardic (C.R. Bard (Canada) Mississauga, Ontario) infant feeding tube. When regurgitation was no longer likely, the chick was replaced on the eggs in the incubator. At 21-22 d of incubation all the hatched chicks and the banded bird were taken off the trays. The DIS eggs were set aside for enrichment. The live chicks were placed in standard, cardboard transport boxes designed to hold 100 d-old chicks. The boxes had cardboard dividers to form four sections but these dividers were cut to allow chicks free passage throughout the box. After 24 h at room temperature, all chicks were killed by CO₂ inhalation, and their entire intestinal tract was excised and cultured for \textit{C. jejuni}.

**RESULTS AND DISCUSSION**

When single birds, leg-banded for identification, were fed serotyped strains of \textit{C. jejuni}, the same serotype as had been fed was later recovered from the intestinal tract of each banded bird. Direct culture of the incubator humidifying water rarely grew \textit{C. jejuni}, but enrichment of 5 ml did in all but two instances (Table 1). The serotype isolated corresponded to that fed the banded chick. The doses fed were estimated to be 2 to 4 x 10⁷ CFU to avoid the effects of dose variation upon carriage. The size of the dose given to the banded chick was chosen because of the report of Sanyal et al. (21) that 10⁷-10⁸ CFU were necessary to establish intestinal infection with ensuing diarrhea. Ruis-Palacios (20) also reported oral infective doses of 7 x 10⁶ CFU. Table 1 shows the actual CFU received per banded bird. Serotype 3 occurred in five different strains fed to the banded chicks but the degree of spread of \textit{C. jejuni} between the chicks in the incubator and transport box was typical of the strain rather than of the serotype. Whether spread is independent of serotype is not known, but this fact could be established by repeating these feeding experiments with multiple isolates of a variety of serotypes.

The method of spread could not have been via the humidity water because this water was separated from the turning grid in these incubators by a 1/4-in. mesh and was beyond the reach of the chicks. Further, the water was static and was not sprayed, so this route of infection can be excluded in the results. Incubators were swabbed and cultured before use. No control incubator for each of the experiments yielded \textit{C. jejuni} and the humidity water in the bottom of the incubators was originally from sterilized reverse osmosis.
TABLE 1. Recovery of C. jejuni from chicks exposed in the incubator and transport box to a carrier chick.

<table>
<thead>
<tr>
<th>Strain carried by banded chick</th>
<th>Penner serotype</th>
<th>CFU per bird</th>
<th>Recovery of C. jejuni from Incubator water</th>
<th>Hatched chicks</th>
<th>DIS eggs</th>
<th>% hatched chicks with Distended intestine</th>
<th>Mottled liver</th>
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<tr>
<td>INN 73-83</td>
<td>3</td>
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<td></td>
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<td>24</td>
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<tr>
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<td>2</td>
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<td>6</td>
<td>2</td>
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<td></td>
<td></td>
<td>9.5x10⁶</td>
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<td>36</td>
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<td>50</td>
<td>14</td>
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<td>62</td>
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<td>CN 9</td>
<td>8:23:36 N</td>
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<td>70</td>
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</table>

aData from Klipstein and Engert (11,12): I=Invasive; T=Toxigenic; N=Neither I or T; ND=Not Determined.

b0.1 ml plated. Data in parenthesis is for 5.0 ml water enriched before plating.

cPercentage recovery from hatched chicks excluding the banded chick.

dExcluding banded birds.

water which was replaced from bottles of similar sterilized water. The organisms in the water therefore came from the chicks but were not the cause of the spread of C. jejuni among the chicks in the incubators. This is supported by the three cases where no C. jejuni could be enriched from 5 ml of water, yet the chicks which had been in these incubators showed between 15% and 33% carriage of C. jejuni.

Of the 960 eggs used in the experiments recorded in Table 1, only two DIS eggs yielded C. jejuni. The contamination either occurred due to a hairline crack in the shell or from incomplete sterilization of the shell at the time of sampling because death had occurred before the banded bird had hatched.

The condition of the intestinal tract and liver of the hatched chicks was influenced by the strain of C. jejuni carried by the banded chick. The two criteria of distended intestinal tract, often with foamy intestinal contents, and red or mottled yellow liver surface, corresponded to exposure to toxigenic or invasive C. jejuni. It was particularly of note that not all chicks with these symptoms yielded C. jejuni from the intestinal tract (Table 1), and the symptoms were more widely distributed than were culturable C. jejuni. This is emphasized by the results for one test with C. jejuni strain Case: no C. jejuni were culturable from the chicks, yet 57% of the hatched chicks had intestinal abnormalities. No chick from non-inoculated control incubators showed these symptoms. Viable, but non-culturable, C. jejuni have been described recently by Rollins and Colwell (17), while the disease of avian vibrionic hepatitis was routinely described as yielding coccoid masses with very little possibility of positive culture (9). It is at present not clear why C. jejuni was not culturable, but subsequent work has shown that chicks are highly sensitive to the lipopolysaccharide of certain strains of C. jejuni suggesting that live campylobacters are not necessarily essential for pathogenic effects.

Chicks from this study were never grown on and we have no data for the effect of the internal symptoms on the subsequent health of the birds. At the time of sampling all chicks were outwardly healthy and no feces in the transport boxes contained overt blood although some of the strains fed invasive. A farm receiving such birds would have no reason to question their health. The extent of the spread of C. jejuni from the one inoculated bird in each group was probably by the fecal/oral route. Alternatively, down could have been contaminated by infected feces in the incubators. As no water was available to the chicks for the 24 h they were in the shipping boxes, this common source is eliminated. In practice, the originally infected bird would have to have been infected from a contaminated egg or from misted humidity water. In the laboratory-induced egg infection by temperature differential, it is possible to obtain 100% infection by day 2 but the DIS eggs rarely yield C. jejuni. Using this technique the present authors have shown that there exist strains of C. jejuni which greatly decrease the hatchability of fertile eggs, while poultry-derived strains such as CN 9 have no such effect. The data in Table 1 support this finding that there exist strains which have marked effects in poultry, and that some of the most virulent strains isolated from humans not only influence hatchability, but spread within 48 h of hatching among the outwardly healthy birds. The data provide evidence that there is the possibility for one infected bird to cause rapid horizontal spread of C. jejuni before the birds reach the grower.

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REFERENCES


