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Broiler Chickens as Potential Source of *Campylobacter* Infections in Humans

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Of 46 broiler chickens from a live poultry market in New York City, 38 (83%) harbored *Campylobacter fetus* subsp. *jejuni* in their rectal flora. The observed mean number of *C. fetus* per g of feces was 4.4×10^6. The organisms survived in the feces for at least 96 h at 4°C whether stored in the gut or transferred to a vial. The best survival medium for pure cultures of *C. fetus* subsp. *jejuni* was heart infusion broth supplemented with sterile blood and kept in a microaerophilic atmosphere.

In recent years much evidence has incriminated *Campylobacter fetus* subsp. *jejuni* as a causative agent of gastroenteritis in children (2, 4-9). The natural source of the infection has not been clearly established.

C. fetus subsp. *jejuni* is present in the gut of many lower animals and birds. Circumstantial evidence suggests that the infection may be transmitted from cows through fecally contaminated, unpasteurized milk (3, 11, 13). Puppies may also be a very real source of human infections. Allegedly, *C. fetus* subsp. *jejuni* causes vibrionic hepatitis in chickens (1, 14). Bertschinger (1) recovered the organism from the bile of 21% of chickens with necrotizing hepatitis and from 2% of normal controls. That chickens could be a source of human infection was further substantiated by Smith and Muldoon (12) who recovered three strains of *C. fetus* subsp. *jejuni* from 165 samples of chicken meat purchased in a retail outlet.

The purpose of the present study was to determine whether the popular broiler chicken presents a potential source of infection with *Campylobacter*.

MATERIALS AND METHODS

Source and treatment of chicken offals. Intestines from freshly slaughtered broiler chickens were obtained from a New York City poultry market. Guts were collected separately, placed in individual plastic bags, transported to the laboratory and processed within 2 h. Colon and rectum were swabbed externally with 70% ethyl alcohol. With sterile instruments, an incision was made just below the junction of the coecae.

Isolation of *C. fetus*. Approximately 250 mg of rectal content was transferred by sterile swab to 20 ml of brain heart infusion broth (purchased from BBL Microbiology Systems, Cockeysville, Md.); the suspension (blended in a Vortex mixer) was centrifuged at $600 \times g$ for 10 min. The supernatant fluid was passed through two 25-mm chambers (Millipore Corp.), the upper of which was equipped with 8.0- and 1.2-μm nonsterile membranes; the lower chamber contained a steam-sterilized 0.65-μm filter. Four to six drops of filtrate were seeded on chocolate agar plates and incubated at 37°C for 48 to 72 h in a microaerophilic atmosphere, i.e., 5% O_2, 10% CO_2, and 85% N_2. Colonies morphologically resembling *C. fetus* were selected and subcultured for further examination.

Identification. An organism was identified as *C. fetus* on the following criteria: (i) small gram-negative curved rod with characteristic darting motility (dark field); (ii) good growth microaerophilically, but not aerobically, at 37°C on chocolate agar; (iii) inability to ferment or oxidize dextrose; (iv) catalase and oxidase positive. *C. fetus* subsp. *jejuni* was differentiated from *C. fetus* subsp. *intestinalis* by its ability to form colonies on chocolate agar incubated microaerophilically at 43°C. J. H. Bryner, National Animal Disease Center, Ames, Iowa, confirmed the identity of 10 isolates according to criteria previously described (5).

Enumeration of *C. fetus* in rectal content. The content was transferred to a vial, weighed, suspended in 39 volumes of brain heart infusion broth, and centrifuged at $600 \times g$ for 10 min. Ten milliliters of the supernatant fluid was filtered as described above, stirred to obtain a homogeneous suspension, and diluted 10-, 100-, and 1,000-fold in brain heart infusion broth. Of each dilution 0.1 ml was seeded on chocolate agar. The plates were incubated microaerophilically at 37°C. Colonies were counted, and representative types were subcultured for bacterial identification.

Survival of *C. fetus* subsp. *jejuni* in vitro. Recent isolates from chicken offals were seeded in 8 ml each of the following freshly prepared media: thioglycolate broth (Difco Laboratories, Detroit, Mich.), heart infusion broth (Difco Laboratories), and heart infusion broth with 0.3% brucella agar. The brucella medium was obtained from Pfizer Diagnostics, New York, N.Y. A 1-ml amount of sterile, undiluted, citrated horse blood was added to one set of heart infusion tubes, and another set was kept as controls. The cultures were incubated as shown in Table 1.

After 48 h at 37°C the temperature was lowered to 22°C, but the respective gaseous environments were

maintained. Viability of the organisms was determined by subculturing with Pasteur pipettes from the bottom and the surface of the tubes to chocolate agar. The plates were examined after 72 h of microaerophilic incubation at 37°C.

RESULTS

Incidence. The rectal content of 38 (83%) of the 46 broiler chickens examined was infected with *C. fetus* subsp. *jejuni*. One of the infected birds yielded growth of both *C. fetus* subsp. *jejuni* and subsp. *intestinalis*.

Enumeration of *C. fetus* in rectal content. The observed number of *C. fetus* subsp. *jejuni* in five chickens ranged from 5.6×10^4 to 1.2×10^7/g of rectal content, with a mean of 4.4×10^6. In view of these high counts, we attempted to recover *C. fetus* from rectal content by plating of progressively diluted, nonfiltered specimens. The experiments were not successful.

Survival of *C. fetus* in the chicken gut stored at 4°C. *C. fetus* subsp. *jejuni* survived at least 96 h in 5 out of 6 offals kept at 4°C. In one case the organisms were no longer detectable after 48 h of storage.

Survival of *C. fetus* in rectal flora stored at 4°C. To determine whether the intestinal tissue is important to the survival of *C. fetus*, the intestinal content was removed from the gut and placed at 4°C. Of the specimens examined, six of six remained positive for 48 h, but only four of six were positive for 96 h.

Survival of *C. fetus* subsp. *jejuni* in culture media. Difficulties in keeping pure cultures of *C. fetus* subsp. *jejuni* alive in the laboratory prompted us to examine the survival of the organisms in a variety of media. Eighteen strains of *C. fetus* subsp. *jejuni* were incubated at 37°C for 48 h and then transferred to room temperature. The survival was invariably shorter in the surface layers of the medium than at the bottom of the tube, a difference which increased with the duration of the incubation period. Table 1, recording the survival of *C. fetus* subsp. *jejuni* in the bottom layers, shows that the poorest results were obtained with heart infusion broth incubated aerobically (medium 3). Addition of brucella agar (medium 2) increased the survival time considerably and became similar to that observed with thioglycolate broth. Microaerophilic incubation of heart infusion broth more than doubled the survival time (medium 5), which was further improved by addition of 11% horse blood (medium 6).

TABLE 1. *Survival of 18 recent isolates of C. fetus subsp. jejuni in culture media*

Surviving cultures (%)	Days survival in culture media[a]					
	Aerobic incubation				Microaerophilic incubation	
	no. 1	no. 2	no. 3	no. 4	no. 5	no. 6
90	3	8	7	9	21	24
50	15	15	9	21	31	39
10	28	21	12	35	42	53
0	30	25	16	38	ND	ND

[a] Culture media: no. 1, thioglycolate broth; no. 2, heart infusion broth with 0.3% brucella agar; no. 3, heart infusion broth; no. 4, heart infusion with 11% horse blood; no. 5, same as no. 3, microaerophilic incubation; no. 6, same as no. 4, microaerophilic incubation.

DISCUSSION

Prevalence of *C. fetus* in feces of broiler chickens. According to unpublished findings of one of us (N.J.R.), approximately 99% of *C. fetus* organisms are retained by the 0.65-μm filter; therefore, the observed 83% infection rate in chickens is a minimum figure. A similar incidence was observed in chickens in Johannesburg (10). The high rate is not necessarily at variance with a prevalence of 21% in the bile of chickens with necrotizing hepatitis, as against 2% in the bile of normal chicken. It is conceivable that the invasion of the biliary system by *C. fetus*, even in chickens with intestinal campylobacteriosis, is a difficult task. To what extent our findings are representative for infection in flocks of chickens elsewhere in the city or in the country is unknown.

For the reasons stated above, the mean number of *C. fetus* subsp. *jejuni* per gram of fecal material is also a minimum figure. It is probably closer to 10^8 than to 4.4×10^6. Infection of this intensity, together with the ability of the organisms to survive at normal refrigeration temperature, virtually guarantees that every home handling raw chicken meat is exposed to the infection. Indeed, unless carefully decontaminated, the organisms may well be found on many cutting boards and numerous butcher knives.

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