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Association of *Campylobacter jejuni* with Laying Hens and Eggs

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Laying hens were individually caged at 20 weeks of age and tested for fecal excretion of *Campylobacter jejuni* (minimum level of detection was 100 CFU/g) during a 42-week period. Peak rates of *C. jejuni* isolation (~25% of hens positive) occurred at two different times, in October and in late April to early May. Communal raising conditions were likely responsible for the high percentage of positive hens in October. Before being segregated in late September, birds were allowed to consume fecal matter, litter, and communal drinking water, all likely sources of *C. jejuni*. The increased excretion rate in late April may have been due to a climatic change. A small portion (8.1%) of the hens chronically excreted (positive >30% of the sampling times) the organism, whereas *C. jejuni* was not detected in 33% of the hens, even though birds were likely exposed to the organism before being segregated. No correlation could be made between rates of *C. jejuni* excretion and egg production. Of 226 eggs from hens fecally excreting *C. jejuni*, the organism was isolated from two shell surfaces but no egg contents. Egg penetration studies revealed that the organism would not penetrate into the contents of the eggs but could be isolated occasionally from the inner shell and membranes of refrigerated eggs.

Campylobacter jejuni has been commonly associated with live, freshly slaughtered, and retail-ready poultry (2, 5, 6, 10, 13, 14, 16–21, 25, 28–31). Studies indicate that prevalence varies, but often 45 to 85% of retail-ready carcasses, parts, and giblets are *C. jejuni* positive (2, 6, 13, 18–20, 24, 25, 30). Reports indicate that this contamination has resulted in cases and outbreaks of campylobacter enteritis as a consequence of cross-contamination or improper cooking (4, 11, 15, 22, 27). It is beneficial to develop a basic understanding of the association of *C. jejuni* with poultry and eggs so that means can be developed to reduce the prevalence and disease potential of the organism.

The purpose of this study was to determine: (i) the fecal excretion patterns of *C. jejuni* of individually caged laying hens; (ii) the occurrence of *C. jejuni* in and on eggs produced by *C. jejuni*-excreting hens; and (iii) the likelihood of egg penetration by *C. jejuni* under different storage conditions.

MATERIALS AND METHODS

Laying hen studies. Fresh fecal specimens from inbred White Leghorn laying hens (University of Wisconsin Poultry Research Laboratory, Madison, Wis.) were screened for *C. jejuni* every 2 to 3 weeks during a 42-week period. Hens (ca. 20 weeks old) were individually caged (40-cm-height by 46-cm-depth by 30-cm-width cages) in late September 1981 after rearing in group housing. There was no change in rations for or maintenance of the hens during the entire study. Fecal specimens were sampled beginning 15 October 1981 through 6 August 1982, by inserting a moistened, sterile cotton-tipped swab into freshly deposited feces and delivering ca. 0.3 g into 3 ml of cold 0.1% peptone maintained in ice water. Specimens were returned to the laboratory within 1 h and immediately mixed (Vortex mixer) and plated (0.1 ml onto CampyBAP [3] with 50 μg of cycloheximide per ml; minimum level of sensitivity of 110 CFU/g). Cycloheximide was added to CampyBAP to suppress the growth of molds that developed from similar specimens during preliminary tests. Duplicate plates were inoculated with each specimen. Plates were held in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) at 42°C and examined at 48 and 72 h. Colonies with typical *C. jejuni* morphology were examined microscopically and confirmed by biochemical tests (12, 26). In addition, drinking water and feed samples (two each at 25 g) were taken at each testing period from different locations among the birds studied and were assayed for *C. jejuni* by selective enrichment (25 g of specimen in 100 ml of enrichment broth; level of sensitivity of <1 cell per g) (8).

The number of eggs produced by each hen was determined for the entire 10-month period.

Egg screening. Eggs from hens confirmed 7 to 10 days earlier to contain *C. jejuni* in their feces were aseptically collected within 6 h after being laid. The surface and contents of each egg were assayed for *C. jejuni* by selective enrichment (8). The shell surface was tested by placing an egg in a Whirl-Pak bag (18 oz [510.3 g]) containing ca. 50 ml of enrichment medium. The egg was manually rubbed for 1 min, left undisturbed for 5 min, and rubbed again for 1 additional min. The medium was poured into a flask (100 ml of medium) and treated as described by Doyle and Roman (8) for enrichment (level of sensitivity of <1 cell per g) and isolation of *C. jejuni*. The bottom one-third of the egg (pointed-end side) was swabbed with ethanol (2- to 3-min exposure) and broken open with the blunt end of a sterile forceps. The contents were added to a stomacher bag. The egg was blended for 2 min in a Stomacher 400, and 20 ml of the blended contents were added to 100 ml of medium for enrichment and treated as described above.

Egg penetration studies. *C. jejuni* penetration of eggs was determined by the procedure of Williams and Whittemore (32). Briefly, freshly laid eggs were collected from hens and allowed to equilibrate to the test temperature. An aluminum cylinder (13-mm diameter by 10-mm height) containing sterile feces was attached with paraffin to each egg ca. 8 to 10 mm below the air cell. Eggs were then held for 10 min at the test temperature, 0.1 ml of *C. jejuni* culture was added to feces (60 to 62% moisture content) in each cylinder, and the top of each cylinder was sealed with Parafilm. Three *C. jejuni* strains (FRI-CF74C, 83C, and 115C) isolated from the feces of apparently healthy laying hens were individually tested. Eggs were held at 25, 37, or 37°C for 10 min and then changed to 4°C and assayed periodically. Five eggs were assayed at each sampling interval (20 or 25 eggs per test).

Penetration was assessed at three areas of the egg: (i) the egg contents, (ii) the inner and outer membranes, and (iii) the inner shell. The egg was broken open and contents were sampled and assayed by the procedure described above. Inner and outer membranes were aseptically cut from under the site of *C. jejuni* application and added to 100 ml of medium for enrichment. The inner shell was sampled by rubbing with a moistened (0.1% peptone), cotton-tipped swab the area where the inner and outer membranes were removed. The cotton tip was broken off and added to enrichment medium for culturing and isolation of *C. jejuni*.

C. jejuni survivors in fecal specimens applied to eggs were enumerated at each sampling time on blood agar plates by the procedures of Doyle and Roman (7).

RESULTS

Laying hen studies. Interesting trends were observed in the percentage of hens excreting detectable numbers of *C. jejuni* during the 42-week period (Fig. 1). Sampling began about 3 weeks after the hens were individually caged; before this the birds were raised as a group, being allowed to consume fecal excrement and litter. At the initial sampling (15 October), 25.2% of the hens excreted detectable *C. jejuni* in their feces. The prevalence of the organism declined to 5.8% by mid-November and remained at the 4.2 to 7.8% level until mid-March. The average percentage of positive excreters during these months was 5.8%. In mid- to late April there was a dramatic increase in the percentage of detectable *C. jejuni*-excreting hens, peaking at 24% in early May. By late May the percentage positive declined to 14% and continued to decline to 8.2% in early August.

Results of individual hens indicated that *C. jejuni* was not detected in 33% of the birds throughout the 42-week sampling period (Table 1). Of the 99 (67%) positive birds, 15 hens were detectably positive only before mid-November, within 2 months after they were individually caged. The organism was isolated from only 12 hens more than 30% of the sampling times, indicating that only a small portion (8.1%) chronically excreted *C. jejuni* at detectable levels. There was no apparent correlation between the excretion of *C. jejuni* and egg production, because many hens chronically excreting the organism laid more eggs than hens that were not detectable excreters.

C. jejuni was not isolated from any feed or drinking water samples.

Egg screening. *C. jejuni* was not isolated from the contents but was isolated from the surface of 2 of 226 eggs from hens identified as excreting the organism 7 to 10 days before the eggs were laid. The shell of one of the surface-positive eggs had fecal matter on it, and nine of the *C. jejuni*-negative eggs had cracked shells.

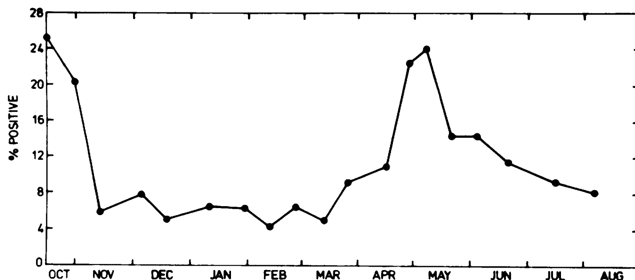

FIG. 1. Percentage of laying hens fecally excreting detectable numbers (>110 CFU/g) of *C. jejuni* during a 42-week period.

TABLE 1. Prevalence of *C. jejuni* in feces of individual laying hens and number of eggs laid during a 42-week period

% Samplings positive for *C. jejuni*	No. of hens positive[a] (%)	No. of eggs laid[b] (avg ± SD)
0	49 (33.1)	159 ± 52
1–10	46 (31.1)	151 ± 61
11–20	30 (20.3)	156 ± 52
21–30	11 (7.4)	149 ± 63
31–40	5 (3.4)	144 ± 60
41–50	3 (2.0)	193 ± 21
51–60	1 (0.7)	
61–70	1 (0.7)	148
80–85	2 (1.3)	167

[a] A total of 148 hens were sampled.

[b] Eggs laid by hens surviving the entire testing period.

Egg penetration studies. Results from the three *C. jejuni* strains tested were similar, hence these data were combined and are presented in Table 2. None of the three areas of the eggs evaluated was positive for *C. jejuni* when the eggs were stored at 25°C, and viable cells were no longer detectable in the inoculated feces at 48 h. The organism was isolated from the outer and inner membranes and inner shell of 4 of 15 eggs after 2 h at 37°C, but not at any of the later sampling times; by 48 h, viable cells were no longer detectable in the inoculated feces. Greatest recovery was from the outer and inner membranes and inner shell of eggs first equilibrated at 37°C and then held at 4°C for the duration of the experiment; however, as sampling time after inoculation increased, the number of eggs indicating penetration by *C. jejuni* decreased. By 72 h only 1 of 15 eggs contained the organism in the membranes and inner shell, and at 10 days neither of these areas had detectable *C. jejuni*. The organism was never isolated from the contents of eggs receiving any of the three temperature treatments.

DISCUSSION

This is the first report to document the fecal excretion rates of *C. jejuni* by individually caged hens during their laying cycle. Peak rates of *C. jejuni* isolation occurred at two different times during this study, i.e., in October and late April to early May. The high percentage of positive birds during October is likely due to the previous raising conditions for the birds. Until late September, the hens were maintained as a group, being allowed to consume fecal excrement, litter, and communal drinking water. Acuff et al. (1) have identified litter and drinking water as likely sources of *C. jejuni* for turkeys raised in commercial brooder facilities. Hence, while hens were raised in this type of communal environment, they were likely to be constantly exposed to the organism through the waste materials and water they consumed. Once individually caged, the hens were no longer continuously exposed to these sources of *C. jejuni*. The relatively low percentage of *C. jejuni*-positive hens from mid-November through mid-March suggests that if birds previously exposed to the organism are maintained in a *C. jejuni*-free environment, only a few will sporadically or chronically excrete detectable numbers of *C. jejuni* in their feces.

The dramatic and unexpected increase in the percentage of *C. jejuni*-positive hens in mid- to late April is not easily

TABLE 2. Penetration of *C. jejuni* into different areas of eggs during exposure to different temperatures

Temp treatment (°C)	Sampling time (h)	No. *C. jejuni* positive/no. assayed			Avg. no. of *C. jejuni* per gram of feces
		Content	Inner and outer membrane	Inner shell	
25	0	—[a]	—	—	8.5×10^8
	2	0/15	0/15	0/15	—
	5	0/15	0/15	0/15	2.6×10^6
	24	0/15	0/15	0/15	2.5×10^3
	48	0/15	0/15	0/15	<300
37	0	—	—	—	5.4×10^8
	2	0/15	4/15	4/15	—
	5	0/15	0/15	0/15	1.5×10^5
	24	0/15	0/15	0/15	3.0×10^2
	48	0/15	0/15	0/15	<300
37 → 4[b]	0	—	—	—	5.0×10^8
	2	0/15	7/15	4/15	1.9×10^8
	5	0/15	7/15	4/15	7.8×10^7
	24	0/15	3/15	2/15	2.2×10^6
	72	0/15	1/15	1/15	8.5×10^4
	240	0/15	0/15	0/15	<300

[a] —, Not done.
[b] Eggs were equilibrated at 37°C, held 10 min at 37°C after application of *C. jejuni*, and then held at 4°C.

explained. None of the feed or water samples assayed for *C. jejuni* was positive, suggesting that these were not sources of the organism. However, because intensive testing of all water and feed was not done, transmission of the organism by these sources cannot be precluded entirely. There was a notable change in climate in April, with an increase in temperature and relative humidity. Perhaps the organism was present in many hens in an attenuated state and the seasonal change in environment induced a change in the physiology of the hens or produced a stressful event resulting in proliferation of *C. jejuni* in the intestinal tract and subsequently increased excretion in the feces. Because 110 CFU/g was the minimum level of sensitivity of the plating procedure used to detect *C. jejuni*, it cannot be concluded that the organism was not present in feces in which *C. jejuni* was not isolated. Use of a more sensitive procedure, such as selective enrichment (8), would have been beneficial; however, this method was not available at the time this study was initiated.

A relatively small portion of the hens (8.1%) chronically excreted *C. jejuni*, suggesting that a small percentage of chickens would continue to propagate the high prevalence of *C. jejuni* among communally housed poultry should *C. jejuni*-free birds be introduced into the flock.

The lack of correlation between egg production and fecal excretion of *C. jejuni* suggests that the presence of the organism in the intestinal tract of the hens does not adversely affect their egg-laying productivity.

Results of egg screening and penetration studies indicate that *C. jejuni* is not likely to contaminate the contents of sound (uncracked) eggs. This agrees with observations of Acuff et al. (1), who were unable to isolate the organism from 20 fertile turkey eggs or 20 newly hatched turkey poults. Additionally, Shanker et al. (23) were unable to isolate *C. jejuni* from broilers of eight flocks that were stocked with birds hatched from the eggs of a *C. jejuni*-contaminated breeder flock. This observation suggests that vertical transmission of *C. jejuni* by eggs is not likely. Hence, data suggest that the internal contents of eggs from hens fecally excreting *C. jejuni* will be free of the organism. Although nine cracked eggs from this study were *C. jejuni* negative, many more cracked eggs must be screened before this apparent lack of association can be confirmed.

Egg penetration studies revealed that *C. jejuni* could be isolated occasionally from the inner shell and membranes of refrigerated eggs, suggesting that the organism may contaminate liquid eggs which may contain shell membranes after the breaking procedure. Eggs stored at room temperature (25°C) or warmer for more than 48 h are not likely to contain detectable cells of *C. jejuni* because the organism is not hardy (it is particularly sensitive to drying) and will not grow, but rather dies off relatively rapidly at this temperature (present study; 7, 9).

Several noteworthy observations have been made from this study. They include: (i) individually caging hens and providing *C. jejuni*-free drinking water and feed substantially reduced the number of birds previously identified as fecally excreting detectable (>110 CFU/g) numbers of *C. jejuni*; however, a seasonal change in late April coincided with an unexpected increase of *C. jejuni* excretion among caged hens; (ii) some hens did not excrete detectable numbers of the organism even though the birds were communally raised among hens later identified as *C. jejuni* excreters; (iii) a small percentage of hens chronically excreted the organism after being individually caged; (iv) rates of fecal excretion of *C. jejuni* could not be correlated with egg production; (v) *C. jejuni* was present on the shell of about 1% of the eggs from *C. jejuni*-excreting hens but not in any of the contents of the eggs; and (vi) *C. jejuni* did not penetrate into the contents of eggs held at any of three temperatures but did contaminate the inner shell and membranes of refrigerated eggs.

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