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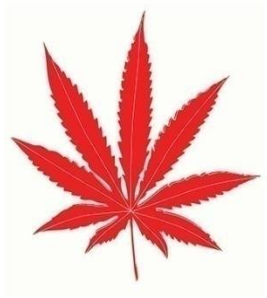

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Original Research Article

SURVIVAL AND PROLIFERATION OF *CAMPYLOBACTER* IN ALBUMEN, YOLK, AND SHELL MEMBRANES OF BROILER BREEDER HATCHING EGGS

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Running title: Survival and growth of campy in hatching eggs

Abstract

Fertile eggs were collected from 47-week-old broiler breeder hens, brought to the laboratory, and separated in 3 segments: albumen, yolk and membranes. Membranes were peeled away from the shell and wiped with 10.0 mL of buffered peptone. Initial pH of albumen and yolk was determined, but no pH was determined for the membranes due to settling of the membrane to the bottom of the tube. Then each segment was stored under refrigeration (4°C), at room temperature (22°C) and in an incubator (37°C) for 2 and 7 d with pH's taken at each time interval. Five replications (individual eggs) were taken. In addition, an inoculum of 3.4 × 10^2/mL of a gentamicin-resistant *Campylobacter coli* was added to each segment of the egg and incubated at 22°C for 3 h; then *C. coli* was enumerated. Three full replications were conducted. Initial pH was 8.7 and 6.1 for the albumen and yolk. After 48 h at 4°C, 22°C and 37°C, the pH of the albumen, yolk and membrane were 9.0, 9.2, 9.4; 6.3, 7.1, 7.2; 8.2, 8.6, 8.4, respectively. When 2.53 $\log_{10}$ of *C. coli* was inoculated into albumen, yolk and membrane, the numbers increased in all 3 and were $\log_{10}$ 3.0, 2.94, and 2.82, respectively. This demonstrates that *Campylobacter* not only survives in the different portions of the fertile egg but multiplies slightly, thereby strengthening the argument that *Campylobacter* may pass from one generation to the next via the fertile egg.

Key words: *Campylobacter*, fertile eggs, broiler breeders, albumen, membranes

Introduction

Campylobacter is now recognized as a cause of human food poisoning as frequently, if not more so than *Salmonella* (Cox et al 2012). *Campylobacter* is a common part of chicken intestinal microflora resulting in a high incidence of *Campylobacter* on carcasses, parts and broiler meat products at retail. The many sources by which broilers may become contaminated with *Campylobacter* is a subject of some debate. In some cases infection may appear within a day or two of hatching which suggests that the transmission could be via the egg. Cox et al (2012) published an extensive review of this subject presenting arguments for both environmental and fertile egg contamination. The idea that *Campylobacter* can pass from one generation of poultry to the next via the fertile egg has not been universally accepted. One reason is the difficulty in routinely culturing this organism from hatchery

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samples such as fluff and egg shell fragments. There is no ideal cultural procedure for the recovery and isolation of *Campylobacter* from these sample types. Numerous plating media, enrichment broths and procedures have been developed for recovery of *Campylobacter*. The majority of *Campylobacter* recovery methods were developed for gut content or fecal samples in which large numbers of healthy *Campylobacter* are likely to exist. These standards methods are not always adequate for recovery of small numbers of sublethally injured or viable nonculturable cells (Cox et al. 2001). Despite the inability to isolate *Campylobacter* from hatchery samples there was evidence for egg transmission. In 1985 (Clark and Bueschkens, 1985) it was demonstrated that when fertile chicken eggs were inoculated with *Campylobacter* by pressure differential, 11% of the resulting chicks had viable cells of the inoculated microorganism in their intestinal tract. In 1986 Lindblom et al raised chickens in isolation units without exposure to any farm environment and found that the growing birds became colonized by *Campylobacter*. Additionally, Maruyama and Katsube (1990) demonstrated that *Campylobacter* could be transmitted to the offspring via the fertile egg after orally inoculating Japanese quail breeder hens. Their findings were further supported by Idris et al (2006) who obtained commercial broiler hatching eggs and incubated them in a research facility and hatched them. Analysis of the ileal, cecal and yolk contents of the day-of-hatch chicks revealed that *Campylobacter* DNA was present before the chicks consumed any food or water. In the same study, in order to determine whether the same strains of bacteria were present in multiple sites associated with the integrated broiler company *Campylobacter* was cultured from a flock of broiler breeders and their 6-week-old progeny residing on a commercial broiler farm. The broiler breeders had been given fluoroquinolone antibiotics, and researchers wanted to determine whether the same fluoroquinolone-resistant strain was present in their progeny. Isolates were typed by pulse-field gel electrophoresis, which confirmed that the parental and progeny flocks contained an indistinguishable strain of fluoroquinolone-resistant *Campylobacter coli*. The objective of the current study was to determine if *Campylobacter* can survive (and possibly proliferate) in the yolk, albumen and shell membranes of a fertile hatching egg.

Materials and Methods

In each of five replications 30 hatching eggs were collected from a 47-week-old broiler breeder flock, transported to the laboratory and separated into three portions: albumen, yolk, and egg shell membrane. The membranes were peeled away from the inner portion of the shell and rinsed with 10 ml of 1% buffered peptone to remove egg contents. Initial pH of the albumen and yolk was measured. Next, five replicate samples of each egg component (3ml) were stored at 4^{o}C, 22^{o}C, or 37^{o}C. Samples were removed from storage at 2 and 7 days for pH measurement. In five separate trials each component (yolk, albumen and membrane) was inoculated with approximately 350 CFU of a gentamicin resistant marker strain of *Campylobacter coli* (Cox et al 2009). Inoculum was verified by plating on Campy Cefex plates with 200 ppm gentamicin. The three segments of the egg and was stored at 22^{o}C for 3hrs and then the *C. coli* was enumerated from each sample.

Enumeration involved serial dilutions of *C. coli* and plating in duplicate on Campy Cefex plates containing 200 ppm gentamicin. Plates were inverted and incubated 48h at 42C in resealable bags flushed and filled with microaerobic gas mixture (5% O_2, 10% CO_2 and 85% N_2). Resulting colonies with characteristic morphology were counted. Mean number of CFU per sample were calculated and log transformed for analysis and reported as log CFU per

sample. Mean pH values and colony counts were compared by Student's T test and significance is assigned at $p<0.05$.

Results

Mean pH values of yolk and albumen at various temperature and time points are presented in Table 1. The average initial pH of the albumen was 8.7 and the yolk was 6.1. For three replications the pH of the albumen was 8.91, 8.35 and 8.80 and for the yolk it was 6.11, 6.08, and 6.12. The pH of albumen samples increased in all instances after 2 days of storage; at 7 days the pH also increased at 4°C and 22°C inoculation.

Table 1. Albumen pH

	Days of storage	Temp of Storage		
Rep		4°C	22°C	37°C
1	2	8.99	9.18	9.47
	7	9.05	9.36	9.34
2	2	9.02	9.20	9.32
	7	9.13	9.36	7.47a
3	2	9.09	9.20	9.31
	7	9.06	9.37	7.55a
4	2	8.99	9.28	9.39
	7	9.07	9.39	7.00a
5	2	9.01	9.20	9.41
	7	9.1	9.40	7.29a

a) Chicks had begun to develop.

Table 2. Yolk pH

	Days of storage	Temp of Storage		
Rep		4°C	22°C	37°C
1	2	6.16	7.82	7.51
	7	6.24	6.32	a
2	2	6.2	6.30	7.32
	7	6.2	6.30	a
3	2	6.94	7.32	7.5
	7	6.16	6.32	a
4	2	6.18	7.69	7.05
	7	6.38	6.26	a
5	2	5.93	6.60	6.44
	7	6.04	6.13	a

a) Chicks had begun to develop.

However, after 7 days at 37°C the chicks had begun developing therefore the yolk and albumen samples could not be separated. For the yolk samples there were slight increases at 2 days incubation at 4°C and slightly greater increases at 22°C (Table 2). For 37°C the same situation existed as was observed in albumen samples, the inability to separate the

yolk and albumen samples.

Table 3. Fate of 340 cells of *C. coli* inoculated into albumen, yolk and membranes after 3 hours at 22°C

Rep	Albumen	Yolk	Membrane
1	600a	100	360
2	730	3,000	1,100
3	100	760	600
4	1,100	1,100	660
5	13,000	1,700	800

a) Number of cells or cfi's

The change in *C. coli* numbers in yolk and albumen and on egg shell membranes during 3-hour incubation at 22°C are presented in Table 3. The results 3 hrs post inoculation when 340 cells of a marker *C. coli* were introduced into samples of albumen, yolk and membrane segments and sampled again after 3 hours are shown in Table 3. With albumen, the *C. coli* decreased to 100 cells in one rep, but increased to 600, 730, 1100 and 13,000 in the other four reps. For the yolk samples, one rep decreased to 100 cells, but the other 4 reps ranged from 760 to 3000 cells. Membrane samples showed an increase in every rep ranging from 360-1100.

Discussion

Although there was considerable variation, data showed that at room temperature numbers increase, in some cases rapidly in just 3 hours. It remains to be seen whether a prolonged incubation at higher temperatures (such as that of egg incubation in the fertile egg setter) would allow for survival and/or proliferation. Clark and Bueschkens (1985) showed that prolonged exposure to a higher temperature using *C. jejuni* did produce some form of injury to the bacteria. Although there was some cellular injury it was demonstrated that 11% of the newly hatched chicks carried the inoculated *C. jejuni* in their intestinal tract. The present study is the first step in showing *Campylobacter* can conceivably survive in various portions of the egg and proliferate at room temperature. A freshly laid fertile egg will be at room temperature for some time, then placed in a cooler before being placed in the incubator (setter). Although transovarian transmission can occur it is not the only means of transmission via the fertile egg. Feces can easily contaminate the surface of a fertile egg with bacteria, including *Campylobacter* and be drawn through the shell by temperature differential, aided by the presence of moisture (the sweating of an egg). *Campylobacter* may have a difficult time surviving on a dry shell or in the hostile albumen but could survive in the moist egg shell membranes. Upon hatching chicks emerging from the egg, can ingest the *Campylobacter* entrapped in the shell membranes and become colonized. Birds can then spread this contamination to flock mates, possibly through ingestion of cecal droppings. This egg passage can certainly occur without involving the reproductive tract or follicles of the hen. This occurs in a relatively small number of birds placed in a grow house, making it unlikely for an insensitive sampling method such as a drag swab to reveal the presence of *Campylobacter*. The present study clearly demonstrated that inoculated *C. coli* could survive and frequently multiply after 2 and 7 days of storage at several temperatures. During the

next phase of this study varying levels of *C. coli* will be inoculated into freshly laid fertile eggs and after 7, 14, and 21 days in the incubator and hatchery, there will be attempts to detect and enumerate the marker strain in the membranes, hatching debris and new hatchlings during grow out.

Conclusion

An assortment of published studies has documented the presence of *Campylobacter* in breeder flocks, fertile eggs and chicks. In fact, chicks have been shown to be contaminated with *Campylobacter* prior to being exposed to any possible environmental source. As improvements in the cultural laboratory methods are made, our understanding of the ecology of *Campylobacter* in poultry will also improve. At some point, egg passage of *Campylobacter* will no longer be a subject of controversy. This will allow the development and implementation of more effective intervention strategies.

References

1. Cox NA, Richardson LJ, Maurer JJ, Berrang ME, Fedorka-Cray PJ, Buhr RJ, Byrd JA, Lee MD, Hofacre CL, O'Kane PM, Lammerding AM, Clark AG, Thayer SG, Doyle MP. Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. J Food Prot 2012; 75(10): 1896-1902.
2. Cox NA, Berrang ME, Stern NJ, Musgrove MT. Difficulty in recovering inoculated *Campylobacter jejuni* from dry poultry-associated samples. J Food Prot 2001; 64(2): 252-254.
3. Clark AG, Bueschkens DH. Laboratory infection of chicken eggs with *Campylobacter* jejuni by using temperature or pressure differentials. Appl Environ Microbiol 1985; 49(6): 1467-1471.
4. Lindblom GB, Sjorgren E, Kaijser B. Natural *Campylobacter* colonization in chickens raised under different environmental conditions. J Hyg (London) 1986; 96(3): 385-391.
5. Maruyama S, Katsube Y. Isolation of *Campylobacter jejuni* from the eggs and organs in experimentally infected laying Japanese quails (*Coturnix coturnix japonica*). Jpn J Vet Med Sci 1990; 52(3): 671-674.
6. Idris U, Lu J, Maier M, Sanchez S, Hofacre CL, Harmon BG, Maurer JJ, Lee MD. Dissemination of fluoroquinolone-resistant *Campylobacter* spp. within an integrated commercial poultry production system. Appl Environ Microbiol 2006; 72(5): 3441-3447.