Research Note

Recovery of *Campylobacter* from Commercial Broiler Hatchery Trayliners

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ABSTRACT Previous research has identified *Campylobacter* as one of the leading causes of foodborne illness. Poultry and poultry products have been identified as a major source of *Campylobacter* in human infections. Although many risk factors that contribute to *Campylobacter* levels have been identified, precise identification of the most effective sites for intervention has not been established. Epidemiological studies have identified that *Campylobacter* in the broiler breeder's reproductive tract, fertile eggs, and 2- to 3-wk-old broilers has the potential to contaminate day-of-hatch chicks. Numerous studies have shown that day-of-hatch broilers are *Campylobacter*-negative using conventional culture methods. The purpose of the present study was to demonstrate the prevalence of *Campylobacter* found in day-of-hatch broilers using a peptone water preenrichment followed by conventional *Campylobacter* culture methods. Using conventional tray liner (hatcheries) culture methods, the isolation distribution of *Campylobacter* from 8 commercial broiler hatcheries (n = 2,000) was evaluated. A total of 15 tray liners were positive from 3 different hatcheries. Of the 2,000 chick paper pad tray liners sampled, 0.75% were positive for *Campylobacter*. These data support previous findings indicating the potential for *Campylobacter* to be spread by vertical transmission. This is the first time that *Campylobacter* has been recovered from tray liners collected at commercial broiler hatcheries.

Key words: broiler, *Campylobacter*, tray liner

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INTRODUCTION

Poultry has been identified as a major source of *Campylobacter* infections, which may consist of enteritis and neurological disease in humans (Speed et al., 1987; Lindblom et al., 1989; Gruenewald et al., 1991; Farerell and Harris, 1992). In chickens, *Campylobacter* is a commensal organism and is a common contaminant of raw poultry products (Korolik et al., 1998; Stas, 1999; Young et al., 1999). The entry of *Campylobacter* into broilers before harvesting for slaughter remains unclear. Potential sources of *Campylobacter* include contaminated water (Stern et al., 2002), spread from animal and insect reservoirs (Gregory et al., 1997); vertical transmission through broiler breeder flocks (Cox et al., 2002a,b); contamination within the hatcheries (Hiett et al., 2002); and horizontal transmission from broiler to broiler. Any 1 or a combination of these routes may play a role in the colonization of *Campylobacter* in broilers.

Broiler breeders and hatchery positive samples remain a controversial subject with regard to *Campylobacter* colonization. Numerous studies have suggested that *Campylobacter* is rarely found in broiler chicks until 2 to 4 wk of age (Evans and Sayers, 2000; Shreeve et al., 2000; Stern et al., 2001). One explanation was that *Campylobacter* was present in viable but nonculturable forms in water, which could potentially play a role in the ability to detect *Campylobacter* through traditional culture methods (Stern et al., 1994; Ziprin et al., 1999). Furthermore, the lack of sensitivity and reliability of drag swabs to detect low levels of *Campylobacter* contamination of a flock may explain these results as well. Recent evidence has suggested that broilers may become contaminated with *Campylobacter* through broiler breeders and fertile eggs.

Vertical transmission of *Campylobacter* from breeders to their offspring has not been demonstrated under commercial conditions. Numerous studies have demonstrated that both the hen and roosters have been shown to possess *Campylobacter* in their reproductive tract, which may be passed to the fertile eggs (Buhr et al., 2002; Hiett et al., 2003; Cox et al., 2005). Sahin et al. (2003) found *Campylobacter*-inoculated specific-pathogen-free White Leghorn laying hens produced *Campylobacter*-positive eggs in 3 out of 65 (4.6%) pooled samples by both enrichment and the PCR method. However, *Campylobacter* was not detected in any pooled sample evaluated in *Campylobacter*-positive broiler breeders (Sahin et al., 2003). *Campylobacter* has been shown to survive in the eggs of unhatched broiler chicks after an experimental challenge (Clarke and Bueschkens, 1986). Cox et al. (2002a) demonstrated that the passage of

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Campylobacter through the fertile egg may provide evidence for vertical transmission, horizontal transmission, or both; however, chicks were not found positive for *Campylobacter* after hatch.

Campylobacter can be found throughout the commercial broiler hatcheries, and yet it has not been demonstrated in day-of-hatch chicks under natural conditions. For example, *Campylobacter* can be found on the eggshells, fluff samples, and embryos from commercial hatcheries (Doyle, 1984; Chaudhary et al., 1989; Chuma et al., 1994; Cox et al., 2002a; Hiett et al., 2002). In contrast, several laboratories have not detected *Campylobacter* in day-of-hatch chicks using routine culture methodologies (Pearson et al., 1996; Petersen et al., 2001; Stern et al., 2001; Herman et al., 2003). The present survey was performed to determine if *Campylobacter* could be recovered from chick tray liners collected at commercial hatcheries using traditional culture methods.

MATERIALS AND METHODS

Experimental Design

The offspring of 98 broiler breeder flocks were evaluated for *Campylobacter* in 8 commercial hatcheries. During the November 1998 through August 2005 sampling times, paper chick tray liners were taken from the chick trays of each breeder flock. Each hatchery tray liner was sampled at the hatchery or upon delivery to the growout farm on the day of hatch. Using disposable gloves, each individual paper tray liner was placed in a gallon-size bag (Pactiv Corp., Lake Forest, IL). Following transport on ice to the laboratory (2 to 3 h), each tray liner was cut in half, and each half was placed in a 3.785 L-size bag with 100 mL of 1% peptone water (Difco, Sparks, MD). One-half of the tray liner was evaluated for *Salmonella* (data not shown), and the other half was evaluated for *Campylobacter*. The samples were shipped overnight to our laboratory on wet ice (18 h). Immediately upon arrival, peptone-tray liner samples were incubated at 42°C for 24 h. Following incubation, 10 mL of the incubated sample was transferred to 10 mL of 2× Bolton broth (Lab M, Bury, Lancashire, UK) and allowed to incubate for 24 h at 42°C in a microaerobic environment (5%, O_2, 10% CO_2, and 85% N_2). Following selective enrichment, each sample was streaked onto Campy-Cefex agar (Becton, Dickinson and Co., Baltimore, MD), and all plates were incubated for 48 h at 42°C, as described by Stern et al. (1992). Suspect colonies were confirmed as *Campylobacter* spp. by examination of cellular morphology and motility on a wet mount under phase-contrast microscopy and by using a latex agglutination test kit, INDEX-Campy (JCL; Integrated Diagnostics Inc., Baltimore MD).

RESULTS AND DISCUSSION

Campylobacter recovery rates by peptone water preenrichment and Bolton broth enrichment are summarized in Table 1. *Campylobacter* was isolated from tray liners from 3 of the 8 hatcheries evaluated. Data were summarized for the 8 hatcheries by taking paper pad tray liners after day-of-hatch chicks were allowed contact for at least 1 h. A total of 15 *Campylobacter*-positive chick paper pad tray liners were detected from 2,000 tray liners sampled (0.75%). Although this study had low numbers of *Campylobacter*-positive tray liners, it was the first time that *Campylobacter* was recovered by traditional culture methods from hatchery samples. A single tray liner represents 100 d-of-hatch chicks; therefore, the present study evaluated 200,000 d-of-hatch chicks. When the data were reexamined with regard to breeder flock (lot), *Campylobacter* was detected in 5 of 63 (7.94%) of the total lots sampled. This is an important point, because broiler chicks from more than 1 breeder source are placed in an individual broiler house. Because 7.94% of the chicks from lots evaluated were actively shedding *Campylobacter*, chicks shedding *Campylobacter* could serve as a source of infection for the remaining chicks.

Table 1. Incidence of *Campylobacter* found on tray liners of 8 hatcheries

Hatchery	Positive tray liners/ total tray liners sampled (%)	Positive breeder lots/ total lots sampled (%)
1	1/330 (0.30%)	1/13 (7.7%)
2	0/420 (0%)	0/14 (0%)
3	0/120 (0%)	0/4 (0%)
4	7/420 (1.67%)	4/14 (28.6%)
5	0/240 (0%)	0/8 (0%)
6	0/180 (0%)	0/6 (0%)
7	0/120 (55%)	0/4 (0%)
8	7/170 (4.11%)	—
Total	15/2,000 (0.75%)	5/63 (7.94%)

Numerous laboratories have demonstrated that breeders have the potential to spread *Campylobacter* to their offspring (Camarda et al., 2000; Buhr et al., 2002, 2005; Hiett et al., 2002; Sahin et al., 2003; Cox et al., 2004, 2005). The reproductive tract of broiler breeders is the most likely source of contamination of the chicks, with *Campylobacter* being recovered from the oviduct, ovarian follicles, and semen of males (Camarda et al., 2000; Buhr et al., 2002, 2005; Cox et al., 2004). In experimental studies, *Campylobacter* survived in inoculated eggs for up to 14 d and was detected by PCR, but could not be recovered using traditional culture methods. (Sahin et al., 2003).

In 2001, Petersen et al. (2001) went a step further and evaluated eggshell, fluff, and dust hatchery samples, as well as parent flocks by PCR. These authors could not recover *Campylobacter* from the hatchery samples, but they recovered *Campylobacter* from the parent flocks and 2- to 3-wk-old chicks. These authors concluded that vertical or horizontal transmissions are not significant routes of *Campylobacter* to broiler chicks. In contrast, Heitt et al. (2002) found *Campylobacter* could be detected in both fluff and eggshell samples by molecular methods but could not determine if the *Campylobacter* was living or dead. This phenomenon may be due to the well-documented genetic instability of *Campylobacter* (Har-

rington et al., 1997; Hänninen et al., 1999; Wassenaar et al., 1998; Wassenaar and Newell, 2000). Information from studies such as these may be considered proof that hatchery samples are not contaminated with *Campylobacter* spp.

Previous large-scale investigation surveyed for *Campylobacter* in hatchery samples using selective enrichment broth and agar plates found no positive samples (Stern et al., 2001). In the current study, the same methodology was used, except that tray liners were preenriched overnight at 42°C in the peptone water before being placed into *Campylobacter* enrichment broth. The use of peptone water as a preenrichment has been commonly used for the detection of *Salmonella*, but has not previously been used to isolate *Campylobacter* from hatchery samples (Cox et al., 1990). Direct enrichment with Bolton broth may provide false negatives for certain types of samples, as seen with the enrichment of cecal samples (Musgrove et al., 2001).

In the present study, the recovery of *Campylobacter* from tray liners from commercial hatcheries (0.75%) and from specific breeder flocks (7.94%) suggests that *Campylobacter* could be spread from the breeders to their offspring. The approach used in this study is the first method to demonstrate that *Campylobacter* can be recovered from commercial hatchery samples by adding nonselective preenrichment to traditional culture methods.

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