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

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; Farerre 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
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 2x Bolton broth (Lab M, Bury, Lancashire, UK) and allowed to incubate for 24 h at 42°C in a microaerobic environment (5%, O2, 10% CO2, and 85% N2). 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.

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 non-selective preenrichment to traditional culture methods.

ACKNOWLEDGMENTS

We are grateful for the very capable technical assistance provided by Earl Munson, Clayton Myers, Terry Doler, and Denise Caldwell.

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