Influence of Relative Humidity on Transmission of *Campylobacter jejuni* in Broiler Chickens

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ABSTRACT Horizontal transmission of *Campylobacter jejuni* among broiler chickens has been documented; however, the influence of RH on transmission rates is an important factor that has not been extensively studied. The purpose of our experiments was to determine the rate of *C. jejuni* colonization among groups of broilers raised in microbiological isolation under high (approximately 80%) and low (approximately 30%) RH conditions. Day-of-hatch chicks (n = 100 per group) were placed on wood shavings in high and low humidity-controlled pens and challenged with *C. jejuni* by introducing 2 seeder birds orally inoculated with *C. jejuni* into each group. The rate of colonization was monitored by analyzing ceca from 10 chicks from each group at d 1, 2, 3, 4, and 7. After 3 wk, the remaining chickens were removed, and 100 newly hatched chicks were placed on the contaminated litter. A second trial was conducted with the litter as the only inoculum source. Trials were repeated in this manner with the time between removing birds and placing newly hatched chicks on the litter extended to 6 h, 24 h, and 1 wk. Significant differences in *Campylobacter* colonization rates were observed between chickens raised under the high and low RH conditions. A delay in colonization was observed in birds raised under the low RH conditions, which increased with the increased time between removal of birds and placement of newly hatched chicks. These experiments demonstrate the importance of humidity in the transmission of *Campylobacter* from litter, and they could lead to practical applications to help reduce *Campylobacter* colonization in broilers.

Key words: humidity, *Campylobacter jejuni*, broiler chicken

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INTRODUCTION

Campylobacter continues to be an important human pathogen, and its epidemiological link to poultry as a source is well defined. Although campylobacters are thought to be environmentally fragile and are easily injured or killed by heating, freezing, exposure to oxygen, and drying, they continue to be among the leading causes of human bacterial gastroenteritis in many countries (Altekruse et al., 1999; Corry and Atabay, 2001; Schlundt et al., 2004; Stern et al., 2005). Poultry has been well established as a source of *Campylobacter*, and consumption of undercooked poultry and potential cross-contamination issues in food preparation areas are thought to contribute to many human cases of campylobacteriosis (Skirrow, 1991; Shane, 2000). Improved control measures to prevent contamination of broilers during production are needed to ensure increased safety of raw processed chicken so that food safety risks associated with undercooking or cross-contamination are mitigated.

A potential vector for *Campylobacter* contamination of broiler flocks is the spent litter which remains in poultry production houses between flocks. Although total clean out and disinfection of poultry production facilities is practiced in some European countries (Wegener et al., 2003), it is a relatively common practice in the United States for several flocks of birds to be raised on the same litter prior to cleaning the house about once a year (Axtell, 1999). Some conditioning of the used litter (top dressing) is frequently conducted prior to introduction of the next flock of chicks, and these practices generally result in the accumulation of 15 to 20 cm of litter in the houses. There is generally a lapse in time between removal of the grown birds and introduction of the newly hatched chicks, which can vary depending upon the integrated poultry producer, from about 1 wk to several weeks. Reuse of the litter helps reduce the amount of litter used, and subsequent disposal of the used (spent) litter can be problematic due to environmental concerns in some areas. Indeed, there is research to suggest that spent litter is more bactericidal than fresh litter. Nearly 40 yr ago, Tucker (1967) reported that the persistence of *Salmonella pullorum* and *Salmonella gallinarum* in built-up litter varied from 3 wk in old litter to 11 wk in new litter; *Salmonella thompson* survived 5 to 6 wk in old litter and up to 20 wk in new

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litter. Fanelli et al. (1970) demonstrated *Salmonella infantis* and *Salmonella typhimurium* do not persist as long in built-up poultry litter as in fresh litter. In a study of 20 broiler flocks over 12 mo in Australia, Soerjadi-Liem and Cumming (1984) found that salmonellae were much less frequently isolated from flocks reared on old litter than on new litter. Turnbull and Snoeyenbos (1973) suggested that the salmonellacidal activity of used poultry litter was a result of a water activity ($\mathbf{A_w}$) unfavorable to *Salmonella* cell viability and a high pH from ammonia dissolved in the available moisture of the litter.

Although much has been published on survival of salmonellae in poultry litter, less information is available on survival of campylobacters. The physiological differences between campylobacters and salmonellae suggest that campylobacters might be less able to survive in litter due to their susceptibility to drying and their microaerophilic nature. Nevertheless, Montrose et al. (1985) demonstrated that used broiler litter experimentally contaminated with *Campylobacter jejuni* is capable of infecting specific pathogen-free chicks maintained on the contaminated litter. Clear quantitative information on the transmission of *Campylobacter* is lacking, although some transmission experiments have been conducted (Van Gerwe et al., 2005). Horizontal transmission of *C. jejuni* among broiler chickens has been well documented (Montrose et al., 1985; Shanker et al., 1990; Jacobs-Reitsma et al., 1995; Willis et al., 2000; Heyndrickx et al., 2002); however, the influence of RH on transmission rates is an important factor that has not been extensively studied.

Knowledge of the water content alone is insufficient to predict survivability of microorganisms in litter. A proportion of the total water is bound and unavailable for use by microorganisms (Farkas, 2001). Total moisture content of a food does not determine shelf life; similarly, it is the availability of water that determines survival of microorganisms in the poultry litter environment. Levels of water vapor molecules importantly affect a variety of chemical and biological processes (Mallinson et al., 1998). The availability of water is measured by the A_w of litter. Litter equilibrium RH measures the level of gaseous water molecules emerging from a sample of litter when measured in a closed test chamber after the level of water vapor has stabilized (Mallinson et al., 1998). The percentage of RH is reported as 100 times the A_w (Prescott et al., 1990). This study was conducted to determine the effect of controlled RH on the horizontal transmission of *C. jejuni* among broiler chickens raised on pine-shavings litter. Quantitative knowledge of the transmission of *Campylobacter* is important for the development of control programs (Van Gerwe et al., 2005), and the role of RH is a key factor that has been overlooked thus far.

MATERIALS AND METHODS

Microbiological Isolation Floor Pens

The isolation floor pens utilized in this study were large (approximately 2 m^3) insulated rooms resembling walk-in coolers. The walls and ceilings were made of industrial-grade plastic panels, which could be easily cleaned, and the floors were made of stainless steel. Fresh pine wood shavings were placed on the floor to a depth of about 10 cm. Temperature was maintained by using commercial-style brooder lamps (250-W, model 9440, Brower Mgf. Co., Houghton, IA) in each pen and controlling the incoming air temperature via a digital thermostat (model EWPC 905/T/S, Eliwell, Belluno, Italy) and electric heater strip (duct heater, model DHB2027, Tutco, Inc., Cookeville, TN) in the incoming airflow. The air entering the positive-pressure units was HEPA filtered (Abatement Technologies, Suwanee, GA) as it was forced into the individual floor pens, and it was also filtered as it exited to maintain microbiological isolation. Disinfectant foot baths were maintained at the door to each pen, and animal caretakers and technical personnel were required to wear protective coveralls, boots, and gloves when entering and changing between units to avoid cross-contamination. Control of the RH in the pens was done in 2 ways. To dry the incoming air and achieve low RH conditions, the air was passed through the condensation coils of a standard 5,200 BTU air conditioner before it was then warmed and forced through the filters and into the floor pens. Humidity was then increased inside the pen as required using a computer-controlled, high-pressure water-spray system (MicroMist Systems, Sorenson Engineering, Inc., Yucaipa, CA).

Campylobacter Challenge Strains

Increased colonization potential has been demonstrated for *C. jejuni* after passage through chickens (Stern et al., 1988; Cawthraw et al., 1996; Jones et al., 2004) Therefore, the *C. jejuni* strains (poultry isolates; laboratory designations B5CD47, CE1-5, and D1) to be used in this study were retrieved from frozen storage (–80°C) and passaged through chickens to ensure maximum colonizing ability would be achieved. Individual chickens were orally dosed with an individual strain, and then the ceca was isolated 7 d later. The strains were recovered by direct plating on Campy-Line agar (**CLA**; Line, 2001) and transferred one time to ensure pure cultures of organisms were achieved before they were propagated on *Brucella* agar plates (Accumedia Manufacturers, Baltimore, MD) for creating a challenge dose for the seeder chicks. Cells were harvested from the *Brucella* agar plates after 24 h at 42°C under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) and suspended in phosphate-buffered saline at 4°C. The inoculum was compared with a spectrophotometric standard curve to achieve solutions containing about $\log_{10}$ 7.0 of *C. jejuni*·mL^{-1}.

Chick Placement and Campylobacter Challenge

Day-of-hatch chicks (n = 100 per group) were placed on fresh wood shavings in the temperature and humidity-controlled, microbiological isolation pens under high

(80% ± 10%) and low (30% ± 10%) RH conditions at 30°C. The chicks in the initial trial were challenged with a 3-strain mixture of *C. jejuni* by introducing 2 seeder birds orally inoculated (0.1 mL) with an equal mixture of about $\log_{10}$ 6.0 of the 3 *C. jejuni* strains into each group. The inoculated seeder birds were never sampled and were marked with a black spot on the head to distinguish them from the other birds in the group. A nonchallenged negative control group was also maintained. The birds in all groups were provided with access ad libitum to water from commercial-type nipple drinkers (Ziggity Systems, Inc., Middlebury, IN). Feed consisted of a standard nonmedicated broiler starter feed, and it was also available ad libitum to the chicks. The rate of Campylobacter colonization was monitored by humanely euthanizing 10 chicks from each group by cervical dislocation at d 1, 2, 3, 4, and 7 and aseptically removing the ceca for microbiological analysis. After 3 wk, all remaining chickens were removed and 100 newly hatched chicks were placed immediately on the contaminated litter in each pen, and a second trial was conducted with the litter as the only inoculum source. Trials were repeated in this manner with the time between removing birds and placing newly hatched chicks extended to 6 h, 24 h, and 1 wk. The study was repeated in its entirety beginning with fresh litter, and the results from the 2 trials were combined. Means and SE of treatment groups were determined using SigmaStat statistical software (Jandel Scientific, San Rafael, CA). All trials were approved by the institutional animal care and use committee.

Microbiological Analysis

Campylobacter populations in the ceca were determined by diluting the ceca 1:3 by weight in phosphate buffer and homogenizing in a Stomacher 80 laboratory blender (Seward Ltd., London, UK) followed by direct plating of serially diluted samples on CLA. Plates were incubated at 42°C for 36 to 48 h under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). An enrichment method was also used by adding 2 mL of diluted ceca suspension to 2 mL of 2× Bolton's enrichment broth and incubating for 48 h under similar conditions. Enriched samples were then plated on CLA for isolation of *C. jejuni*. Following incubation, typical *Campylobacter* colonies were enumerated and confirmed by microscopic wet mount and latex agglutination using a Microscreen *Campylobacter* Agglutination Kit (Microgen Bioproducts, Camberley, Surrey, UK) for confirmation as necessary. Litter samples (approximately 50 g from 5 random locations within each pen) were collected periodically and pooled for each individual pen. Approximately 10 g of the pooled litter sample was analyzed for determination of A_w using an AwQuick water activity meter (Rotronic Instrument Corp., Huntington, NY).

RESULTS AND DISCUSSION

There was no apparent difference in the colonization rate observed between birds held under high or low RH conditions when seeder birds were introduced as the only source of *Campylobacter* (Figure 1, panel A). Chickens are naturally coprophagic; they injest each other's fecal and cecal droppings (Montrose et al., 1985; Stern et al., 2001; Newell and Fearnley, 2003). Perhaps the lack of difference in colonization rate between birds held under high or low RH conditions in this situation can be explained because of the coprophagic nature of the chickens. The fresh cecal or fecal droppings of the seeder birds were consumed rapidly by the pen mates before sufficient drying and subsequent decline in *Campylobacter* populations in the droppings could occur. Lower *Campylobacter* populations might have been expected in the droppings in the low RH pen as they would dry out and die off faster had they remained uneaten. Once colonized, chickens typically excrete as much as $\log_{10}$ 8 cfu of *C. jejuni* per gram of fecal matter, resulting in a high inoculum dose for birds consuming the droppings. The birds consuming the contaminated droppings are then frequently colonized themselves, perpetuating the spread of *Campylobacter* through the flock. Studies have shown that chickens may be infected by as few as 35 to 40 cfu of *C. jejuni* cells (Stern et al., 1988; Cawthraw et al., 1996).

Differences in *Campylobacter* colonization rates were observed between chickens raised on contaminated litter held under the high and low RH conditions. A delay in *Campylobacter* colonization was observed in birds raised under the low RH conditions which increased with the increased time between removal of birds and placement of newly hatched chicks (Figure 1, panels B, C, and D). This observation probably reflects the decreased survival of *Campylobacter* in the litter in the drier pen as compared with the wetter pen. There was a direct relationship between the RH maintained in the pens in this study and the resulting A_w of the litter. In the low (approximately 30%) humidity-controlled pen, the mean A_w of the litter was 0.500, whereas the high (approximately 80%) humidity-controlled pen contained litter with a mean A_w of 0.795. This result was confirmed by preliminary experiments (data not shown) in which a direct relationship was observed between RH of the environmentally controlled floor pens and A_w of litter in the pens. The average A_w in the high humidity pen was more suited for increased survival of campylobacters than the average A_w in the low RH pen. The negative control pen remained free of campylobacters throughout all trials.

Much is known about the influence of A_w on survival of *Salmonella* in poultry litter, but less information is available regarding the fate of *Campylobacter* in similar environments. Low litter or manure surface humidity can lead to low litter or manure surface *Salmonella* contamination, and this subsequently can lead to low contamination on carcasses (Mallinson et al., 1998). Opara et al. (1992) and Carr et al. (1995) both observed a significant positive correlation between *Salmonella*-positive drag swabs and elevated values of A_w for samples of broiler house litter. Himathongkham et al. (1999) found that at A_w levels of 0.93 and greater, a moderate increase in *Salmonella* populations in chicken manure occurs. Eriksson de Re-

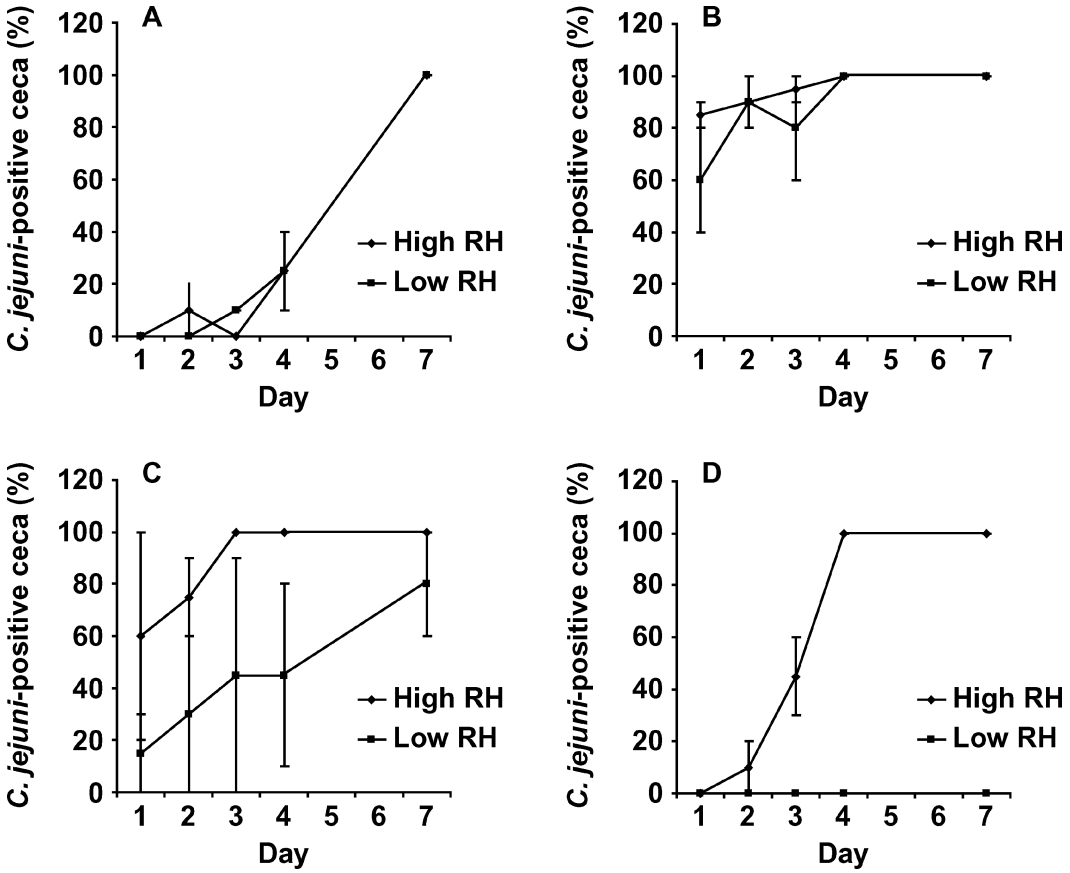

Figure 1. Horizontal transmission of *Campylobacter jejuni* in broiler chickens held under high (80 ± 10%) RH or low (30% ± 10%) RH conditions in microbiological isolation floor pens. Day-of-hatch chicks (n = 100) were placed on fresh pine-shavings litter, and 2 seeder chicks inoculated with *C. jejuni* ($\log_{10}$ 6.0) were introduced as the only inoculum source (A), day-of-hatch chicks (n = 100) were placed on contaminated litter as the only inoculum source immediately after removing the chicks from trial A (B), day-of-hatch chicks were placed on contaminated litter 6 h after removal of birds from trial B (C), and day-of-hatch chicks were placed on the contaminated litter 24 h after removal of the birds from trial C (D).

zende et al., (2001a) conducted a study that indicated that levels of A_w above 0.90 may pose an increased risk for *Salmonella* on farm premises by showing that, in vitro, these organisms multiply under elevated levels of A_w. These researchers and others concluded that maintaining an A_w less than 0.85 in and around litter or manure surfaces in poultry or livestock bedding areas may be a critical factor in safe production of food (Eriksson de Rezende et al., 2001b). Litter A_w less than 0.84 and moisture contents between 20 and 25% are suggested as potential control measures to reduce the load of *Salmonella* and perhaps other foodborne pathogens, including *Campylobacter* in poultry houses (Hayes et al., 2000.)

Knowledge of the parameters underlying *Campylobacter* transmission in broiler flocks is important for the development of control strategies (Van Gerwe et al., 2005). For each of the trials in which day-of-hatch chicks were placed with contaminated seeder chicks or placed on contaminated litter and held under high humidity conditions, all the chicks in the pen were colonized with *Campylobacter* within 3 to 7 d. This result is in agreement with observations made by Shanker et al. (1990), who showed that transmission of *Campylobacter* among birds exposed to *C. jejuni*-contaminated water occurred within 2 to 7 d. Likewise, Smitherman et al. (1984) reported that in commercial flocks, once *Campylobacter*-positive samples began to be detected, all samples from the chicken flock (10,000 to 20,000 birds) became positive within 7 d. It may be assumed in these studies that RH was not controlled as it was not reported. Van Gerwe et al. (2005) developed a mathematical model to quantify the *Campylobacter* transmission rate among broilers, which was determined to be 1.04 new cases per colonized chick per day; however, RH was not considered as a variable in the model. Under commercial conditions, the RH in a poultry house will vary widely throughout the day, depending upon temperature, ventilation practices, and weather conditions. It is not uncommon for the RH to approach 75 to 80% in a commercial house, especially if ventilation and heating systems are not managed properly (Donald and Campbell, 2004). One of the most basic psychrometric principles is that warm air can hold more moisture than cold air (Czarick and Lacy, 1997); thus, as the temperature decreases, the RH increases. It is interesting that in the artificially dry pen (~30% RH), the colonization of the chicks was delayed compared within the high (~80% RH) pen. After a 24-h delay between removal of the previous contaminated flock and placement of the newly hatched chicks under the dry conditions, there was insufficient *Campylobacter* surviving to serve as a colonization source

for the birds. In the high RH pen all the birds experienced the same 24-h delay in placement but were again positive for *Campylobacter* within 6 d (Figure 1, panel D). Only when the litter had been held for a full week between removal of contaminated birds and placement of newly hatched chicks were the *Campylobacter* populations in the high RH pen insufficient to cause colonization of the chicks (data not shown).

These experiments demonstrate the importance of RH in horizontal transmission of *Campylobacter* and could lead to practical applications to help reduce *Campylobacter* colonization in broilers. Jacobs-Reitsma (1997) and coworkers (1995) reported no evidence for vertical transmission of *Campylobacter* from breeder flocks via the hatchery nor horizontal transmission from one broiler flock to the next via persistent contamination of broiler houses in the Netherlands. The major route of *Campylobacter* colonization of poultry was thought to be horizontal transmission from the environment. Our study suggested that lag times among flocks of at least 1 wk are not very likely to result in *Campylobacter* contamination of subsequent flocks of birds from previously contaminated litter. These observations emphasized the importance of maintaining a minimum lag time of at least 1 wk for sufficient reduction of campylobacters in commercial houses (with occasionally high RH) to prevent horizontal contamination among flocks, and they demonstrated the potential value in drying the litter to help prevent cross-contamination among flocks. Maintaining lower RH during production has been shown to offer additional benefits. In a study conducted by Weaver and Meijerhof (1991) a higher mean body weight was observed in birds exposed to 45% RH during production as opposed to higher regimens of 75 and 80% RH. The incidence and severity of ammonia burns on the breast and infected foot pads was also reduced at the lower RH level, likely due to reduced ammonia present at the lower RH. Van Gerwe et al. (2005) state that quantitative knowledge of the transmission of *Campylobacter* is important for the development of control programs for several reasons: 1) it enables researchers to determine measures which can reduce transmission and to what extent; 2) the transmission rate affects the prevalence of an infection in a population in time, which helps determine the probability of detection; and 3) it may help to determine the moment of introduction of *Campylobacter* in commercial broiler flocks under field conditions. Van Gerwe et al. (2005) conclude that with this knowledge, control measures could focus more on high-risk periods and perhaps be better targeted. Reduction of RH in poultry rearing houses through ventilation and water handling practices that promote dryer litter have been suggested as primary considerations for *Salmonella* reduction during production (Mallinson et al., 1998), and the results of our studies suggest that such practices should also help reduce exposure of chicks to viable *Campylobacter* in the litter environment.

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