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Risk Factor Analysis of Campylobacter Presence within the Broiler Production and Processing Continuum in the Southeastern United States

Kelly Lee Schaf

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By

Kelly Lee Schaf

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By

Kelly Lee Schaf

Approved:

_____
Robert W. Wills
(Co-Major Professor)

_____
R. Hartford Bailey
(Co-Major Professor)

_____
Tung-Lung Wu
(Minor Professor)

_____
Danny Magee
(Committee Member)

_____
Allen Byrd
(Committee Member)

_____
Larry Hanson
(Graduate Coordinator)

_____
Mark Lawrence
Associate Dean of Research and Graduate Studies
College of Veterinary Medicine

Name: Kelly Lee Schaf

Date of Degree: May 4, 2018

Institution: Mississippi State University

Major Field: Veterinary Medical Sciences

Select Appropriate Title: Robert W. Wills and R. Hartford Bailey

Title of Study: Risk factor analysis of *Campylobacter* presence within the broiler production and processing continuum in the southeastern United States

Pages in Study 166

Candidate for Degree of Doctor of Philosophy

The objective of this dissertation was to (1) determine which grow-out and processing sampling points best predicts and causes *Campylobacter* later in production (2) identify risk factors within the hatchery that influenced *Campylobacter* prevalence later in production (3) identify biosecurity risk factors that were associated with *Campylobacter* presence during production and processing (4) identify farm and production characteristics that were associated with *Campylobacter* presence later in production, and (5) to estimate the proportion of variance and the intraclass correlation coefficients within the hierarchical levels (complex, farm, bird) of the data.

The best predictors of post-chill *Campylobacter* carcass status were the exterior whole carcass sample in the grow-out environment and the crop sample upon arrival at the processing plant. The best post-chill causal model contained the grow-out whole carcass sample.

Variables associated with the increased odds of a *Campylobacter* positive sample included controlling the humidity in the hatchery chick room, 2-4 people handling the chicks at the hatchery, washing the setter twice yearly, 2 or more breeder farms providing

eggs for the sampled flock, using low water pressure when washing the hatch trays, having more walk-in doors on the boiler house, the farmer removing the litter from the farm, concrete at most used door of the broiler house, the number of workers that work with the birds during grow-out, having more houses on the farm, standing water on the farm day 1, wood interior walls, a vegetation surface next the house footing, and 6 or less flocks on the litter. Protective factors included the use of footbaths and dedicated shoes, greater frequency of entering the house during brooding, disinfectant added to the drinker lines, having concrete outside the most used door, the cleanliness of the workroom, and harvesting birds at 56-63 days of age.

The highest percent of variance occurred at the farm level meaning intervention efforts should focus on factors at the broiler farm level i.e. factors that are different among farms within a broiler complex.

DEDICATION

I would like to dedicate this work to my family. To my husband, Brian, who has been a source of love, encouragement, and strength in my life. To my children, Kate and Nora, who have made me stronger and more fulfilled than I ever thought possible. Thank you for making me laugh and smile every day and for reminding me to always stop and smell the roses. I love you to the moon and back.

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CHAPTER I

INTRODUCTION

1.1 Poultry production in the United States

Poultry has been an increasing staple of the American diet since the 1950's when small farms began integrating the different stages of production (hatchery, feed-mill, production, and processing) into companies. Poultry used to be a commodity only served on special occasions or holidays due to low availability and thus high costs. In 1980, Americans consumed an average of 47.1 pounds of poultry per person each year (Council 2017). Today, however, that number has almost doubled with the consumption of approximately 91 pounds of poultry per person each year (Council 2017). This demand is fueled by the reliance on the production of safe meat products. Each year, however, the number and cost of illnesses due to foodborne disease increases. In the United States, there are an estimated 48 million illnesses, 127,839 hospitalizations, and 3,037 deaths that are attributed to foodborne diseases each year (Scallan 2011).

Of all of the major foodborne pathogens, *Campylobacter* was among the top 5 in illnesses and hospitalizations (Batz, Hoffmann et al. 2012). Unpasteurized milk, contaminated water, vegetables, and red meat have all been reported sources of human infection (Evans, Ribeiro et al. 2003, Yang, Jiang et al. 2003, Huevelink, Heerwaarden et al. 2009, Vipham, Brooks et al. 2010); however, consumption or handling undercooked poultry has been identified as the major cause of campylobacterosis with approximately

608,231 illnesses, 6,091 hospitalizations, and 55 deaths attributed to poultry products and costs 1,747 million annually (Batz, Hoffmann et al. 2012).

It is estimated that as much as 90% of flocks are *Campylobacter* positive within the United States (Stern, Ladely et al. 2001). In 1996, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) implemented the Pathogen Reduction and Hazard Analysis and Critical Control Point (HACCP) Systems final rule. The goal was to improve food safety by implementing post-harvest performance standards for *Salmonella* in red meat and poultry. The rule also announced plans to work with other government agencies, industry, and academia to take an integrated approach of improving food safety from farm to by looking at hazards before animals reach the plant and after products leave. As a result of these regulations, over the last two decades researchers and industry have identified risk factors within the processing plants that impact the presence of *Salmonella* in broilers. In 2009, FSIS turned regulatory attention towards *Campylobacter* in poultry and announced new pathogen reduction performance standards for *Salmonella* and *Campylobacter* at the processing plant (USDA/FSIS 2010). Research advancements to help processing plants meet these standards have now identified a number of post-harvest risk factors that impact the presence of *Campylobacter* in poultry (Berrang, Buhr et al. 2001, Berrang and Dickens 2004), including many of the processing steps in the evisceration line. Despite these advancements, *Campylobacter* still remains a problem as contaminated poultry continue to exit the processing plant (Berrang, Shaw et al. 2007, Berghaus, Thayer et al. 2013). In order to further reduce the amount of *Campylobacter* entering the poultry plant gate, an

in-depth evaluation at the grow-out (preharvest) level is required to characterize risk factors that influence the *Campylobacter* prevalence at the end of processing.

Herein follows a review of the literature pertaining to risk factors for *Campylobacter* within the poultry production continuum. The focus was on aspects pertaining to the United States poultry industry, however, resources were gathered from worldwide literature.

CHAPTER II

LITERATURE REVIEW

2.1 Campylobacter Characteristics

The *Campylobacter* genus is a member of the family *Campylobacteriacea* and contains 25 species, two provisional species, and 8 subspecies (Man 2011). *Campylobacter*, derived from the Greek word meaning curved rod, is a gram-negative, elongated (0.5 to 5 μm long), slender (0.2 to 0.8 μm wide), and curved, spiral, or rod-shaped bacteria (Holt 1994, Debruyne 2008). It can sometimes appear as an S-shape or gull-winged-shaped when two cells form short chains. They are motile and have a single polar unsheathed flagellum at one or both ends of the cell. The flagellum is long, sometimes several times the length of the cell and gives them their characteristic corkscrew-like darting motility (Holt 1994, Debruyne 2008).

In general, *Campylobacter* spp are regarded as very fragile and cannot grow under normal atmospheric conditions but rather grow best in a microaerophilic environment (5% O_2, 10% CO_2, 85% N_2) (Altekruse, Stern et al. 1999). They have an optimal growth temperature of 37- 42°C (Altekruse, Stern et al. 1999). *Campylobacter jejuni* and *C. coli* are the most important human enteropathogens and will be the focus of this review.

Campylobacter is a ubiquitous organism and can commonly be found in the intestinal tract of beef and dairy cattle (Stanley, Wallace et al. 1998, Wesley, Wells et al. 2000), pigs (Harvey, Young et al. 1999), and chickens (Wallace, Stanley et al. 1997).

Campylobacter jejuni most commonly infects poultry and cattle, whereas *Campylobacter coli* is predominantly found in pigs (Rosef, Underdal et al. 1983, Nielsen, Engberg et al. 1997). Poultry, dairy and beef cattle, and pigs have been recognized as important environmental reservoirs for *Campylobacter* because of the animal gut being the only site for replication (Wesley, Wells et al. 2000, Cardinale, Cisse et al. 2004, Englen, Hill et al. 2007, Fosse, Seegers et al. 2009)

Campylobacter survives best in dark, cool, and moist conditions (Hazeleger, Wouters et al. 1988, Lee, Smith et al. 1998). It is non-spore forming and when exposed to unfavorable conditions such as exposure to air, low or high pH (Chaveerach, ter Huurne et al. 2003), freezing, dehydration, heat, starvation, and prolonged storage can move into a 'viable but non-culturable' (VBNC) state (Altekruse, Stern et al. 1999). The VBNC state cannot be detected by standard culture methods. These cells become predominately coccoid in shape, they lose their motility as their flagellum disappears, and some strains can have decreased pathogenicity (Rollins and Colwell 1986, Cappelier, Magras et al. 1999, Chaveerach, ter Huurne et al. 2003). Albeit, the VBNC form of certain strains of the bacterium plays an important role in the transmission of the disease (Tholozan, Cappelier et al. 1999).

2.2 *Campylobacter* Infection in Humans

Campylobacter is responsible for an estimated 1 million cases of campylobacterosis each year (Scallan 2011). Approximately 99.6% of cases in the U.S. are sporadic in nature with the remaining 0.4% due to outbreaks (Goldstein, Cruz-Cano et al. 2016). Consumption of undercooked poultry is the most common risk factor for sporadic cases of infection (Friedman, Hoekstra et al. 2004) whereas consumption of

unpasteurized milk and contaminated water typically result in large scale outbreaks (Taylor 1982, Vogt, Sours et al. 1982, Hopkins, Olmsted et al. 1984). International travel, contaminated produce, and ingestion of contaminated red meat have also been identified as a source of sporadic cases of campylobacterosis (Friedman, Hoekstra et al. 2004, Taylor, Herman et al. 2013).

Campylobacter infection in humans requires an infectious dose of as little as 500-800 CFUs (Robinson 1981, Black, Levine et al. 1988). The mean incubation period is 3.2 days (Blaser 2008). Onset of symptoms is usually abrupt with the most common symptoms being abdominal cramps, fever, and watery (sometime bloody) diarrhea (Black, Levine et al. 1988, Friedman, Hoekstra et al. 2004). Symptoms typically last a median duration of 6 days (Friedman, Hoekstra et al. 2004). Duration can vary based on dose ingested, virulence of strain, and susceptibility of patient. The bacterium causes a self-limiting illness in most individuals. Although infrequent, the infection can progress and cause severe sequelae such as Inflammatory Bowel Disease (IBD), IrriBowel Syndrome (IBS), Functional Dyspepsia (FD), Celiac Disease, Guillain-Barre Syndrome (Blaser 2008), Miller Fisher Syndrome, bacteremia, septicemia, meningitis, and reactive arthritis (ReA) (Kaldor and Speed 1984, Dhawan 1986, Roberts 1987, Mishu 1993, Ladrón de Guevara C 1994, Allos 1997, Hughes and Res 1997, Lastovica 1997, Saida, Kuroki et al. 1997, Blaser 2008, Nielsen 2009). The economic burden of campylobacteriosis and sequelae has been estimated to be approximately 1,257 million dollars per year (Batz, Hoffmann et al. 2011).

2.3 *Campylobacter* Infection in Broilers

In the United States, approximately 90% of flocks are colonized with *Campylobacter* (Stern, Ladely et al. 2001). Poultry are the most colonized species due to their body temperature (41-42°C) being so close to the temperature requirements for *Campylobacters* (37-42°C) survival and proliferation (Altekruse, Stern et al. 1999). The gastrointestinal tract, especially the ceca and colon, and crop, is known to harbor large amounts of *Campylobacter* (Berrang, Buhr et al. 2000). Although, not just limited to the gastrointestinal tract, *Campylobacter* has also been found in the blood (Richardson, Cox et al. 2011), spleen (Cox, Richardson et al. 2016), thymus (Cox, Bailey et al. 2006), lymphoid organs (Cox, Bailey et al. 2006), liver/gallbladder (Cox, Richardson et al. 2007), and reproductive tract of both hens (Buhr 2002, Cox 2005) and roosters (Hiett, Siragusa et al. 2003).

Under commercial production conditions, *Campylobacter* is rarely isolated from the flocks until the birds are at least 14 days old (Jacobs-Reitsma, van de Giessen et al. 1995, Berndtson, Emanuelson et al. 1996, Achen, Morishita et al. 1998, Newell and Wagenaar 2000, Hiett, Stern et al. 2002, Sahin, Luo et al. 2003, Bouwknegt, van de Giessen et al. 2004, Bull, Allen et al. 2006). Under experimental conditions, however, day of hatch chicks can become *Campylobacter* positive after oral challenging (Young, Ziprin et al. 1999). The reason for this lag phase is thought to be due to protection from maternal antibodies. A chick's maternal antibodies are highest between 1-7 days of age but steadily decrease and disappear by 2 weeks of age (Sahin, Zhang et al. 2001). Once *Campylobacter* has been introduced to the flock, birds quickly become infected through the fecal-oral route. Typically, within 1 week, all birds will be positive for

Campylobacter and at high levels up to 10^9cfu. (Berndtson, Tivemo et al. 1992, Jacobs-Reitsma, van de Giessen et al. 1995, Cawthraw, Wassenaar et al. 1996, Gregory 1997, Evans and Sayers 2000, Shreeve 2000, Bull, Allen et al. 2006). Research has found 3 days of contact with a *Campylobacter* positive bird is enough time for the majority of a flock to become infected (Shanker, Lee et al. 1990). Once positive, the flock remains positive with high levels of *Campylobacter* through the duration of the grow-out cycle (Bull, Allen et al. 2006). The rapid spread of *Campylobacter* through the flock is likely due to the movement of the birds in the house, contaminated litter, communal drinker lines and feed lines, and coprophagy (Shreeve 2000).

2.4 Modes of Transmission

The route of *Campylobacter* contamination of poultry flocks, vertical and/or horizontal transmission, is still debated among researchers. Due to discoveries in the last decade, vertical transmission has been a route of transmission implicated by some researchers. Vertical transmission is usually thought to occur from parent to progeny through the internal contamination of the egg within the reproductive tract before shell deposition (Newell, ELvers et al. 2011). However, Newell and colleagues (2011) suggested extending the definition of vertical transmission to include transmission of organisms from parent to progeny via routes such as fecal contamination on the shell and is termed “Pseudovertical transmission”. After the egg is released it can come into contact with excrement from the hen and thus possibly poses a risk for the unhatched chick and hatchery environment. Laboratory studies have found day-of-hatch chicks capable of being successfully inoculated with doses as low as 35 colony-forming units (Stern, Bailey et al. 1988, Stern 1994, Cappelier, Magras et al. 1999).

In addition, studies have found *Campylobacter* in the reproductive tract of both broiler breeder hens (Buhr 2002, Cox 2005) and roosters (Hiett, Siragusa et al. 2003), semen(Cox 2002a), and in the tray-pads (Byrd, Bailey et al. 2007), fluff, and eggshells (Hiett, Cox et al. 2002) of day-of-hatch chicks suggesting the possibility of transfer to offspring. Furthermore, molecular evidence exists that demonstrates *Campylobacter* isolates from the feces of progeny that are clonal in origin to those of the parent breeder flocks (Cox 2002b). On the other hand, Callicott et al. (2006) used PCR to demonstrate that there was no evidence of transmission from grand-parent flocks through the egg to progeny parent breeders. In addition, eggs collected from broiler-breeder flocks have been found to be negative for the bacterium (Sahin, Kobalka et al. 2003) or if they were positive it was with a different *Campylobacter* type (Bull, Allen et al. 2006). Broilers from *Campylobacter* positive parent flocks have been raised to be *Campylobacter* free at slaughter (Shanker 1986, Annal-Prah 1988, Jacobs-Reitsma 1995, Jacobs-Reitsma, van de Giessen et al. 1995, Callicott, Frioriksdottir et al. 2006), especially when placed under experimental high biosecurity conditions. This compilation of evidence suggests that while vertical transmission could occur, there is still not enough evidence suggesting vertical transmission as a significant source of transmission.

Horizontal transmission is thought to be the main source of transmission of *Campylobacter* to poultry flocks. A well-maintained house should be considered a biosecure environment. Horizontal transmission could be active (flies, beetles, vermin, or humans) or passive (water, feed, or air), as anything entering or exiting the house has the potential of bringing contamination in with it from the external environment (Newell and

Wagenaar 2000, Newell, ELvers et al. 2011). The literature below describes some of the most common risk factors identified in studies worldwide.

2.5 Risk Factors Commonly Associated with *Campylobacter* in Poultry

2.5.1 Season

Many studies worldwide have identified a seasonal trend of *Campylobacter* infection in poultry flocks occurring during warmer months of the year (Kapperud 1993, Jacobs-Reitsma, Bolder et al. 1994, Berndtson, Emanuelson et al. 1996, Wallace, Stanley et al. 1997, Wedderkopp, Rattenborg et al. 2000, Refregier-Petton, Rose et al. 2001, Newell and Fearnley 2003, Bouwknegt, van de Giessen et al. 2004, Patrick, Christiansen et al. 2004, Barrios, Stern et al. 2006, McDowell, Menzies et al. 2008) while other studies have been unable to find an association (Humphrey, Henley et al. 1993, Evans and Sayers 2000). A recent U.S study that looked at the seasonal distribution of *Campylobacter* flocks over a three year period was unable to find a trend for *Campylobacter* prevalence related to month of year, daily maximum temperature, rainfall on day of slaughter, or total rainfall during grow-out (Berrang, Meinersmann et al. 2017). Although, an increase in sporadic cases and outbreaks of human campylobacteriosis during warmer months has been documented within the United States (Taylor, Herman et al. 2013).

2.5.2 Age and Flock Size

There is an association between *Campylobacter* flock contamination and bird age at slaughter (Berndtson, Emanuelson et al. 1996, Evans and Sayers 2000, Bouwknegt, van de Giessen et al. 2004, Barrios, Stern et al. 2006, McDowell, Menzies et al. 2008). The older a flock the more likely it is to be *Campylobacter* positive.

Some studies have identified flock size to be associated with an increased risk of *Campylobacter* infection in larger flocks (Berndtson, Emanuelson et al. 1996, Barrios, Stern et al. 2006, Nather 2009). Other studies, however, have found no link (Humphrey, Henley et al. 1993, Evans and Sayers 2000, Cardinale, Cisse et al. 2004). It has been suggested that larger flocks require more food, water, litter, air, and personnel movement which increases the opportunities for infection (Berndtson, Emanuelson et al. 1996, Nather 2009).

2.5.3 Litter

The most common litter, or bedding material, used today in broiler production is pine shavings and coarse pine sawdust (2005, Ritz, Fairchild et al. 2015). Due to the dry and stressful conditions, litter is an unfavorable environment for *Campylobacter* to survive as one study found survival rates in litter to be 4 hours and survival in poultry feces to be 24 hours due to its preference for a microaerophilic environment (Smith, Meade et al. 2016). Litter samples have been found positive for *Campylobacter* but only after the flock was identified as being positive (Bull, Allen et al. 2006).

Litter is expensive, difficult to dispose, and is not available in the quantities needed to change bedding between each flock. For these reasons, the United States poultry system reuses litter for a few years and removes only the top 'caked layer' between flocks. Used litter is made up of a combination of bedding material, excreta, feathers, wasted feed and wasted water (Ritz, Fairchild et al. 2015). It becomes more and more rich with nutrients after each successive flock that resides on the litter (Chamblee and Todd 2002) which in turn provides the possibility for better viability of bacteria within the litter.

2.5.4 Feed and Water

Feed, due to its dry nature, is an unlikely source for introducing *Campylobacter* into poultry flocks (Stern, Wojton et al. 1992, Humphrey, Henley et al. 1993); however, feed, water and/or water lines (Hiett, Stern et al. 2002, Bull, Allen et al. 2006) have tested positive for *Campylobacter* but only after the organism was detected in the flock (Gregory 1997, Hiett, Stern et al. 2002, Bull, Allen et al. 2006). Feeding broilers non-disinfected well water has been an implicated source for *Campylobacter* flock colonization in some studies (Kapperud 1993, Pearson, Shahamat et al. 1993), while municipally supplied water was implicated in another study (Berndtson, Emanuelson et al. 1996). It is likely that feed and water sources once contaminated are the source of infection for the remainder of the flock (Bull, Allen et al. 2006).

2.5.5 Insects

Field and laboratory studies have found insects, including flies and darkling beetles, to be competent vectors and reservoirs for *Campylobacter* (Shane, Harrington et al. 1985, Jacobs-Reitsma, van de Giessen et al. 1995, Gregory 1997, Strother 2002, Strother, Steelman et al. 2005). Flies and darkling beetles can carry the bacterium on their feet and body surfaces and serve as a mechanical vector or they can pass the bacterium through their alimentary tract and act as a biological vector (Shane, Harrington et al. 1985, Strother, Steelman et al. 2005). Laboratory studies have found flies to carry *Campylobacter* for up to 2 days after initial infection (Berndtson, Danielsson-Tham et al. 1989) while darkling beetles have been found to carry and shed the bacterium for up to 12 hours after initial infection (Strother, Steelman et al. 2005). Poultry can become contaminated with *Campylobacter* through contact with the flies external surface,

consuming the flies, or by ingesting litter, water, food, and/or feces that is contaminated with excreta or ingesta regurgitated by positive flies (Shane, Harrington et al. 1985).

Studies have found flies and darkling beetles located near poultry houses were positive for *Campylobacter* concomitantly or after the flock has been found to be positive (Rosef and Kapperud 1983, Annal-Prah 1988, Jacobs-Reitsma, van de Giessen et al. 1995, Berndtson, Danielsson-Tham et al. 1996, Gregory 1997, van De Giessen, Tilburg et al. 1998, Bates 2004, Skov, Spencer et al. 2004) while other studies found low isolation rates or no positive samples during production or the empty period (Gregory 1997, Strother 2002, Hald, Skovgård et al. 2004, Skov, Spencer et al. 2004, Hansson, Vågsholm et al. 2007). Molecular evidence suggests that genetically distinct isolates have been found to be common to both darkling beetles and poultry; however, the direction of *Campylobacter* infection transfer is unknown (Bates 2004).

Some studies have suggested the seasonal increase of *Campylobacter* infection in poultry and humans during warmer months corresponds to the seasonal presence or activity increase of insects (Jacobs-Reitsma 1997, Nichols 2005) although definitive proof has not been shown. Collectively, this evidence suggests that flies and beetles could serve as a possible source of infection to *Campylobacter* free flocks.

2.5.6 Rodents

Laboratory studies have found mice capable of serving as a reservoir of *Campylobacter* for long periods of time (Berndtson, Danielsson-Tham et al. 1989, Berndtson, Danielsson-Tham et al. 1994). *Campylobacter* has also been isolated from rodents on poultry farms (Annal-Prah 1988). The presence of rodents (visually seen or presence of droppings) has been identified as a risk factor for *Campylobacter* presence in

flocks of some studies (Gregory 1997, McDowell, Menzies et al. 2008), but not in others (Nather 2009).

2.5.7 Wild and Migratory Birds

Wild and migratory birds are also carriers of *Campylobacter* and are a source of environmental contamination on the farm (Pacha, Clark et al. 1988, Hiett, Rothrock et al. 2013) and a source within poultry houses if they are able to gain access (Craven 2000). Wild birds and their droppings have tested positive on poultry farms (Annal-Prah 1988, Craven 2000) for the same *Campylobacter* isolate as the flocks they were located near (Hiett, Stern et al. 2002). The visual presence of wild birds was also associated with *Campylobacter* positive farms (Berndtson, Emanuelson et al. 1996).

2.5.8 Multi-Species Farming

Multi-species farming has been identified by some researchers as a source of broiler flock infection (van de Giessen, Bloemberg et al. 1996, Cardinale, Cisse et al. 2004, McDowell, Menzies et al. 2008). Farm animals including beef cattle, dairy cattle, (Wesley, Wells et al. 2000, Englen, Hill et al. 2007) and pigs (Fosse, Seegers et al. 2009) can be permanent carriers of *Campylobacter* (Jacobs-Reitsma, van de Giessen et al. 1995) and possibly be a source of poultry contamination especially when located on the same farm, or nearby, and tended by the same workers (Kapperud 1993, Gregory 1997, van De Giessen, Tilburg et al. 1998). Some studies identified poultry farms with domesticated animals (dog, cat, sheep, horses) and especially farm animals (cattle, pigs, other poultry) as more likely to have *Campylobacter* positive flocks (Kapperud 1994, van De Giessen, Tilburg et al. 1998, Hald 2000, Bouwknegt, van de Giessen et al. 2004, Cardinale, Cisse

et al. 2004) while other studies found no association (Refregier-Petton, Rose et al. 2001, Bouwknegt, van de Giessen et al. 2004, Bull, Allen et al. 2006)

Tending other livestock including poultry from other farms has been found to be a risk factor in some studies (Kapperud 1993, Berndtson, Emanuelson et al. 1996, Gregory 1997, van De Giessen, Tilburg et al. 1998) and not significant in other studies (Bull, Allen et al. 2006, McDowell, Menzies et al. 2008, Nather 2009). Also, having farm animals on a farm within 1km was also a risk factor (Bouwknegt, van de Giessen et al. 2004). A risk assessment study in the Netherlands found that removing other farm animal species from a farm would only reduce the *Campylobacter* infection from 44% to 41% (Katsma, De Koeijer et al. 2007).

2.5.9 Hygiene barrier presence and hygiene practices

The presence of a hygiene barrier has been found to be an important factor in producing *Campylobacter* free poultry (Berndtson, Emanuelson et al. 1996, Hald 2000, Hansson, Vågsholm et al. 2007, McDowell, Menzies et al. 2008). Keeping the ante-room clean and tidy has also been found to be an important risk factor in some studies (McDowell, Menzies et al. 2008) but not in others (Nather 2009). Studies have found that improving the hygiene barriers has led to a reduced risk of *Campylobacter* flocks (Humphrey, Henley et al. 1993, van De Giessen, Tilburg et al. 1998, Gibbens, Davies et al. 2001).

The hygiene practices of the farm workers are an important factor when trying to prevent or reduce the risk of *Campylobacter* contamination in the house. The proper use of boot dips (Humphrey, Henley et al. 1993, van de Giessen, Bloemberg et al. 1996, Evans and Sayers 2000, Gibbens, Davies et al. 2001, Bouwknegt, van de Giessen et al.

2004, McDowell, Menzies et al. 2008), house specific boots (Hald 2000, Bull, Allen et al. 2006, McDowell, Menzies et al. 2008), dedicated clothes (Gibbens, Davies et al. 2001, Cardinale, Cisse et al. 2004, McDowell, Menzies et al. 2008), and hand washing (McDowell, Menzies et al. 2008) have all been identified as having a protective association against *Campylobacter* flock infection. Clothes, hands, tools, and especially boots can act as mechanical vehicles from the farm surroundings into the poultry house (Jacobs-Reitsma 1997)

Boot dip solutions should be replaced at least once a week and more often if there is a buildup of organic matter or if the solution has been diluted. Changing boot dip solutions more frequently (3-5 days) compared to weekly or less than weekly (McDowell, Menzies et al. 2008) or weekly compared to less than weekly (Evans and Sayers 2000) was found to reduce the risk of *Campylobacter* positive flocks.

It is interesting to note that while studies have found strict adherence to hygiene and biosecurity measures in the house reduces or delays flock *Campylobacter* infection (Humphrey, Henley et al. 1993, van De Giessen, Tilburg et al. 1998, Gibbens, Davies et al. 2001), many studies find that it does not altogether eliminate it (van De Giessen, Tilburg et al. 1998, Shreeve 2000). Farm biosecurity is difficult to maintain through the life of the flock due to the ubiquitous nature of the organism and low infective dose required (Shreeve 2000).

2.5.10 Empty period and house disinfection

In general, research has shown that the *Campylobacter* status of a flock cannot be predicted based on the *Campylobacter* status of a previous flock, although, having a positive flock does increase the risk of a subsequent flock having *Campylobacter*

(Berndtson, Emanuelson et al. 1996). Many studies have found *Campylobacter* positive flocks to be followed by negative flocks and vice versa (Berndtson, Emanuelson et al. 1996). In cases where subsequent flocks were positive they sometimes were the same serotype and other times were different serotypes (Berndtson, Emanuelson et al. 1996). Furthermore, while an association between *Campylobacter* status of flocks and the final depopulation result in the previous cycle existed in a univariable analysis, it falls out in a multivariable analysis (McDowell, Menzies et al. 2008).

The empty period between flocks can vary. One study found farms with a down period of less than 14 days were 5 times more likely to have *Campylobacter* positive flocks (Hald 2000) while another study found an empty period less than 21 days was a risk factor for *Campylobacter* positive flocks (Berndtson, Emanuelson et al. 1996).

House disinfection between flocks is associated with a decreased risk of *Campylobacter* infection (Cardinale, Cisse et al. 2004). The flooring of most U.S poultry houses is dirt making disinfection between flocks difficult. Other countries have found houses without cement floors to be associated with an increased risk of *Campylobacter* (Cardinale, Cisse et al. 2004).

2.5.11 Exterior house environment

Campylobacter is commonly found in the environment surrounding poultry houses (Bull, Allen et al. 2006). Puddles exterior to the house have been found to be positive for *Campylobacter* just prior to chick placement (Hiett, Stern et al. 2002, Bull, Allen et al. 2006) and the *Campylobacter* types were indistinguishable from isolates found in flocks later in their life cycle (Bull, Allen et al. 2006). Exterior contamination may be a mechanism by which flock-to-flock carryover occurs (Bull, Allen et al. 2006).

One study found cleaning and disinfecting the poultry house surroundings to reduce the risk of *Campylobacter* being introduced into the flock (Kazwala, Collins et al. 1990)

Other risk factors on the farm include on site disposal of dead birds and used broiler litter (Evans and Sayers 2000, Cardinale, Cisse et al. 2004). One study found on site disposal of dead birds instead of removal from the farm, increased the risk of infection of the farm by contaminating the environment (Evans and Sayers 2000); although, other studies found no difference (Gibbens, Davies et al. 2001). On site disposal of manure was also a risk factor for *Campylobacter* (Cardinale, Cisse et al. 2004).

2.5.12 Feed withdrawal

Approximately 8-12 hours prior to transport, birds are prevented access to feed. The purpose of feed withdrawal is to allow the partial evacuation of the gastrointestinal tract of broilers and thus reduce the chance of fecal contamination during processing. Byrd et al. (1998) found feed withdrawal to increase the frequency of *Campylobacter* crop contamination. The feed withdrawal process causes an increase in the broiler crop pH and a decrease in crop lactic acid concentration which could allow for the growth of *Campylobacter* (Corrier, Byrd et al. 1999). In addition, the broilers litter pecking activity of possibly contaminated litter increases two-fold two hours after feed withdrawal (Corrier, Byrd et al. 1999).

2.5.13 Transportation and dump cages

After 49-62 days in the grow-out environment birds are ready for transport to the processing plant. Birds are caught, mechanically or by hand, and put into dump cages to

be transported on semi-trucks to the processing plant. The stress of transportation can cause the birds to defecate which coats the feathers, feet, and cloaca with freshly excreted feces (Stern, Clavero et al. 1995, Whyte, Collins et al. 2001). If a flock is positive for *Campylobacter*, transportation has been shown to increase *Campylobacter* shedding within and on the exterior of positive birds leading to cage contamination (Stern, Clavero et al. 1995, Whyte, Collins et al. 2001) and further contamination within the processing plant (Newell, Shreeve et al. 2001). Even if a flock tests negative for *Campylobacter* at the end of grow-out, they can still become positive during transportation if dump cages arrive positive for *Campylobacter* (Newell, Shreeve et al. 2001, Slader, Domingue et al. 2002). Furthermore, catching crews and their equipment, which sometimes visits multiple farms in a day have been found to be a source of *Campylobacter* contamination (Ridley, Morris et al. 2011) .

Transportation crates frequently arrive at a farm positive for *Campylobacter* (Stern, Ladely et al. 2001, Bull, Allen et al. 2006, Ridley, Morris et al. 2011). *Campylobacter* negative birds placed inside contaminated coops have been shown to become *Campylobacter* positive following catching and transport (Slader, Domingue et al. 2002, Berrang, Northcutt et al. 2003, Bull, Allen et al. 2006). In the U.S. crates are used then put right back into circulation without proper cleaning. Only 28% of the U.S. poultry industry washes transport crates regularly before reuse (Northcutt and Jones 2004); however, dried feces is difficult to remove and even the strictest washing programs have been unable to completely remove pathogenic bacteria including *Campylobacter* (Slader, Domingue et al. 2002, Allen, Burton et al. 2008, Hastings, Colles et al. 2010). Dump cages are typically used multiple times during the day and washing in

between uses creates a wet environment for bacteria survival. In addition, washing programs are time consuming, costly, require a lot of water (Berrang, Northcutt et al. 2004) and usually require multiple washings to reduce, not eliminate, pathogens (Slader, Domingue et al. 2002). Furthermore, Berrang and Northcutt (2005) found cages that were allowed 24-48 hours to sit unused and allowed to dry resulted in decreased *Campylobacter* numbers, although, keeping additional cages on hand for use while other cages are in downtime is expensive and impractical (Berrang, Northcutt et al. 2004).

In addition, *Campylobacter* positive catching crates also serve as a source for environmental contamination as fresh litter has been found to be positive after contact with the crates (Ridley, Morris et al. 2011).

2.5.14 Catching crews and equipment

Catching crews used to manually catch the birds and place them into crates have been shown to increase the likelihood of *Campylobacter* positive birds due to possible contaminated gloves of the workers (Slader, Domingue et al. 2002).

Catching crew personnel, vehicles (inside and outside), and equipment have all been found to be positive for *Campylobacter* prior to entering the farm (Allen, Weaver et al. 2008, Ridley, Morris et al. 2011). Personal items, such as lunch boxes, have also been found to be positive for *Campylobacter* that matched the genotype of the subsequent flock (Ridley, Morris et al. 2011). A study by Ridley (2011) tried increasing biosecurity of the vehicles, equipment and catching crews prior to entering the farms in an attempt to prevent contamination from the catching crews to the birds. Biosecurity improvements included washing the vehicles, a dedicated changing room used to wash hands and change into fresh clothing, and use of dedicated footwear that was disinfected prior to

work. The increased biosecurity practices reduced positive samples found on the vehicles, equipment, and crew, however, birds that were negative at thinning (common UK practice, but not in the US) were positive at final clearance. Molecular strain typing was used in another study to track the source of *Campylobacter* contamination and found that *Campylobacter* contamination had spread from one farm to another by use of the same vehicles and/or catching crew (Allen, Weaver et al. 2008). Berndtson et. al. (1996) found that in flocks where staff loading birds to slaughter from several farms were 7.8 times more likely to have *Campylobacter* than if the staff never loaded at other farms. These findings indicate that while increased biosecurity practices can help reduce positive samples of the farm, catching crews and their equipment can still serve as a source of contamination for birds that are about to go to slaughter, the farm environment, as well as farms they go to in the future.

2.5.15 Processing

In the processing plant the birds are dumped from the transport cages, hung on shackles, stunned, killed, scalded, defeathered, eviscerated, washed, cooled, and packaged. *Campylobacter* enters the processing plants in large numbers, on and within live birds (Oosterom, Engels et al. 1983, Berrang, Buhr et al. 2000), and is disseminated through the plant at the different processing steps. Processing plants must work to eliminate pathogens before delivery of the product to retail. Some studies have found *Campylobacter* free flocks that become positive through processing (Stern, Ladely et al. 2001). Overall processors have been successful at reducing the contamination (Berrang, Shaw et al. 2007); however, some steps in the process can be a source of cross-contamination including scalding, defeathering, evisceration, and chilling.

The scalding process is used to open the feather follicles and allow for the feathers to be easily removed during defeathering (Keener, Bashor et al. 2004). During this process a large portion of the dirt, litter, and feces is removed from the carcass. As a result, the water in the scald tank has been found to be highly contaminated with *Campylobacter* and to be a source of cross-contamination between carcasses (Stern, Ladely et al. 2001); however, when properly maintained, it can also be used as an important step to reduce pathogens (Berrang and Dickens 2000). Berrang et al. (2000) found the scald tank to significantly reduce the levels of *Campylobacter* contamination on carcasses from mean 4.7 $\log_{10}$ to 1.8 $\log_{10}$ CFU/ml rinse. The scald tank uses a counter current water flow system which allows the carcasses to move from a dirty to clean gradient. Through the use of high flow rates, regulated time and temperatures (hard scald: 30-75 sec @ 59-64°C or soft scald: 90-120 sec @ 51-54°C), adequate agitation, chlorination, and proper pH the scald tank should reduce the *Campylobacter* levels on the carcasses (Bennett 2006).

Defeathering, or picking, is the process of removing feathers and the upper most layer of skin from the birds using a series of automated defeathering machines containing fingerlike projections (Bennett 2006). This step in processing has been found to increase *Campylobacter* on carcasses by up to 2.0 $\log_{10}$ cfu/mL (Berrang, Buhr et al. 2000). The source of this increase has been attributed to the escape of contaminated feces from the lower GI tract of the birds onto their external surface (Berrang, Buhr et al. 2001). Contact between the pickers and the birds can sometimes put pressure on the carcasses as they go through the line and can result in the release of feces that can contaminate the external surface of the birds and the picking machines (Berrang, Buhr et al. 2001). Soiled pickers,

however, are not thought to be the primary source but rather contaminated fecal leakage (Berrang and Dickens 2004). Post defeathering chlorinated rinses are used to try and decrease contamination (Bennett 2006).

Evisceration is the process of removing the internal organs and any defective trim or pieces and is thought to be a major source of equipment and carcass contamination due to the high levels of *Campylobacter* carried in the crop, ceca, and colon (Oosterom, Engels et al. 1983, Genigeorgis, Hassuneh et al. 1986, Berndtson, Tivemo et al. 1992, Berrang, Buhr et al. 2000). Improper removal of the crop, gastrointestinal tract, and viscera can cause the organs to rupture and lead to the machinery becoming contaminated and causing carcasses that follow to be positive. Berrang et al. (2000) did not find a significant decrease (3.7 $\log_{10}$ to 3.4 $\log_{10}$) of *Campylobacter* contamination during this step.

The chill tank is a step used to reduce the poultry carcass temperature by means of chlorinated (20-50 ppm chlorine) cold water tanks or cold air (Bennett 2006). Both methods have been found to adequately reduce the temperature to the Food Safety Inspection Service standard of 4.4°C in 4 hours following evisceration; however, immersion chilling is most common (Bennett 2006). While research has shown *Campylobacter* counts on a broiler carcass decrease as it proceeds through processing (Izat, Gardner et al. 1988, Berrang and Dickens 2000, Bilgili, Waldroup et al. 2002, Northcutt, Berrang et al. 2003), the number of positive carcasses following chilling have sometimes been shown to increase due to possible cross-contamination in the chiller (Jones, Axtell et al. 1991, Smith, Cason et al. 2005). Monitoring the pH of the chill water

can give an indication of the effectiveness of the free available chlorine in the water and can thus prevent buildup and cross contamination in the chill tank (Bennett 2006).

Within the United States, *Campylobacter* contamination rates on packaged chicken still remain high with 26% of fully processed chicken *Campylobacter* positive (Stern and Pretanik 2006). Approximately 3.6% of all commercially processed broiler carcasses are *Campylobacter* positive with counts as high at 10^5 CFU per carcass (Stern and Pretanik 2006).

2.6 Conclusion

Poultry have been identified as the main source for *Campylobacter* infection among humans. Horizontal transmission has been identified as the main mode of transmission of *Campylobacter* to broiler flocks. Studies worldwide have identified risk factors that are associated with the presence of *Campylobacter* in broiler flocks. The most commonly reported risk factors for *Campylobacter* broiler contamination include season, age and flock size, litter, feed, water, insects (darkling beetles and flies), rodents, wild birds, multi-species farming, hygiene barrier presence and hygiene practices, exterior house environment, feed-withdrawal, transportation and dump cages, catching crews and equipment, and steps (scalding, defeathering, evisceration, and chilling) within the processing plant. When operating correctly, it is estimated that the slaughtering process could reduce Campylobacter contamination levels up to 100 to 1,000 times (Rosenquist, Sommer et al. 2006); however, with birds capable of entering poultry plants with such large numbers (10^9cfu/g) of Campylobacter (Berndtson, Tivemo et al. 1992, Berrang, Buhr et al. 2000, Berrang and Dickens 2000, Smith and Berrang 2006), it is apparent that

processing alone cannot completely eliminate *Campylobacter* contamination on packaged poultry.

CHAPTER III

MULTILEVEL ANALYSIS OF *CAMPYLOBACTER* FLOCK PREVALENCE AND THE RELATIONSHIP WITH SEQUENTIOAL SAMPLING POINTS IN BROILER PRODUCTION AND PROCESSING IN THE SOUTHEASTERN UNITED STATES

Abstract

Campylobacter remains a leading food borne pathogen in the United States and poultry has been identified as a major reservoir. The objective of this observational study was to assess the relationship between the occurrence of *Campylobacter* in different samples and sampling points along the broiler production and processing continuum. Sampling was conducted in two broiler companies located in three states within the Southern United States, which encompassed 10 complexes, 32 farms, and 64 flocks. On day 1 when chicks were placed into the grow-out house, the gastrointestinal tracts of 30 chicks were aseptically collected from each test flock. At the end of grow-out (approximately one week before harvest) and upon arrival at the processing plant, 30 each of ceca, crop, and whole bird carcass rinses were aseptically collected from each flock. During processing, 30 broiler carcasses rinse samples were collected prior to entering and again after exiting the immersion chill tank. Multilevel logistic regression was used to assess relationships between likelihood of *Campylobacter* at post-chill and at plant-arrival samples (ceca, crop, and whole carcass) with preceding plant-arrival and

grow-out samples. Estimates for the proportion of variance residing at the complex, farm, and bird levels were also determined. Results of this work indicated that the best predictors of post-chill *Campylobacter* carcass status were the exterior whole carcass sample in the grow-out environment and the crop sample upon arrival at the processing plant. The best post-chill causal model contained the grow-out whole carcass sample. In the post-chill model, the percentage of variability in *Campylobacter* prevalence occurring at the complex, farm, and bird level were 12%, 63%, and 25%, respectively. The intra-class correlation for birds within the same farm, birds within the same complex but different farms, and farms within the same complex were 0.75, 0.12, and 0.16, respectively.

Keywords

Campylobacter; Broiler; Poultry; Food Safety; Epidemiology; Risk factor analysis;

Public Health Forum

- Contamination on the exterior of the bird in the grow-out environment was an important source for carcass contamination post-chill.
- Cross-contamination between positive and negative flocks occurred in the chill-tank and some of the relationships between the processing plant and pre-harvest samples were disrupted.
- The interclass correlation for samples collected from the same farm (0.57-0.91) was high indicating farm level clustering is important with *Campylobacter* presence and interventions would be most impactful at the farm level.

3.1 Introduction

Campylobacter, a zoonotic pathogen, is a commensal organism within poultry and a common cause of human gastroenteritis worldwide. Consumption, cross-contamination, and the handling of undercooked poultry has been identified as the major cause of this condition. In the United States, 608,231 illnesses, 6,091 hospitalizations, and 55 deaths have been attributed to poultry products and costs 1,747 million dollars, annually (Batz, Hoffmann et al. 2012). The United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) responded by implementing post-harvest *Campylobacter* performance standards for poultry production. For processing plants to meet current performance standards, all poultry sampled at processing plants must be below 10.4% positive (USDA/FSIS 2010). As processing plants work to meet these performance standards, it is estimated that as many as 5,000 fewer illnesses due to *Campylobacter* might occur annually (USDA/FSIS 2010).

Campylobacter causes a mild to severe infection of the gastrointestinal system known as campylobacteriosis (CDC 2013). Symptoms of the disease typically include headache, fever, severe abdominal cramps, watery or bloody diarrhea, and sometimes nausea and vomiting (CDC 2013). Infections are typically self-limiting and clear after a week, however, in some cases more severe sequelae have been reported, such as reactive arthritis, Guillian-Barŕe syndrome, Miller-Fisher syndrome, meningitis, bacteremia, and septicemia.(Kaldor and Speed 1984, Dhawan 1986, Roberts 1987, Mishu 1993, Ladrón de Guevara C 1994, Allos 1997, Hughes and Res 1997, Lastovica 1997, Saida, Kuroki et al. 1997, Nielsen 2009, CDC 2013).

Poultry flocks typically become infected through horizontal transmission with *Campylobacter* infection occurring at 2-3 weeks of age (Jacobs-Reitsma, van de Giessen et al. 1995, Hiett, Stern et al. 2002, Bouwknegt, van de Giessen et al. 2004, Bull, Allen et al. 2006). Once *Campylobacter* is first detected in the flock, birds quickly (within 1 week) become infected and remain positive with high prevalence until slaughter (Bull, Allen et al. 2006). Despite high flock prevalence, broilers do not show signs of illness from infection (Dhillon, Shivaprasad et al. 2006). The ceca (Oosterom, Engels et al. 1983, Stern, Clavero et al. 1995), crop (Byrd, Corrier et al. 1998, Smith and Berrang 2006), and the skin and exterior feathers (Kotula and Pandya 1995, Stern, Clavero et al. 1995, Berrang and Dickens 2000) of the birds, when positive, are known to harbor large numbers of *Campylobacter* (Berrang, Buhr et al. 2000). The crop is frequently damaged during processing and can contaminate the broiler carcass or other flocks during processing (Hargis, Caldwell et al. 1995, Buhr and Dickens 2002, Buhr and Dickens 2002).

Jeffrey et al. (2001) looked at the skin, crop, and intestine samples at post-scald and determined the intestine was the sample that would most likely reflect the *Campylobacter* prevalence within a flock.

The broiler production continuum as characterized in this work, is a sequential process that broilers progress through as they make their way from the hatchery to the processing plant. The segments are: 1) breeder-hatchery, 2) transport from hatchery to grow-out farm, 3) grow-out farm, 4) transport from grow-out farm to processing plant, 5) processing. There have been many studies world-wide that have identified a number of risk factors for *Campylobacter* within the breeder-hatchery (Buhr 2002, Hiett, Cox et al.

2002, Cox 2002a, Cox 2002b, Hiett, Siragusa et al. 2003, Byrd, Bailey et al. 2007), grow-out farm (Kapperud 1993, Evans and Sayers 2000, Hald 2000, Bouwknegt, van de Giessen et al. 2004, Bull, Allen et al. 2006), transportation from farm to processing (Stern, Clavero et al. 1995, Whyte, Collins et al. 2001, Slader, Domingue et al. 2002, Berrang, Northcutt et al. 2003, Ridley, Morris et al. 2011), and in the processing plant (Berrang, Buhr et al. 2000, Berrang and Dickens 2000, Berrang, Buhr et al. 2001, Berrang and Dickens 2004). However, these studies have not provided conclusive evidence of one main source. Many of the studies have been performed in different countries and thus under different settings (i.e. climate, production size, and production practices) than those in the United States. Until more information is known about the true source of *Campylobacter* entering into a flock, it would be helpful to know which sample(s) are best for predicting post-chill flock status early in production (pre-harvest). This information could assist the poultry industry in scheduling flocks into the processing plants based on contamination level of *Campylobacter* to reduce the risk of cross contamination between flocks. Furthermore, establishing which sample points are the best indicators of the probability of *Campylobacter* within flocks would allow for the more strategic placement and evaluation of pathogen mitigation procedures within both the pre- and post-harvest settings.

In order to further reduce the amount of *Campylobacter* entering the poultry plant, an in-depth evaluation at the grow-out (preharvest) level is required to characterize risk factors that influence the *Campylobacter* prevalence at plant arrival and the end of processing. Thus, the objective of this project was to both predict and establish a causal relationship between the most likely grow-out and/or plant arrival sample(s) and the

Campylobacter status of a flock at plant arrival and post-chill. This information may be useful to commercial processors as they work to implement an effective HACCP program that will lower the *Campylobacter* contamination on poultry, thus lowering the number of human illnesses.

3.2 Materials and Methods

3.2.1 Sampling strategy

This prospective observational study was conducted in 3 states (Alabama, Mississippi, and Louisiana) within the southeastern United States from 2003-2006. Two companies that were thought to be representative of the regional poultry industry participated in the study. A complex was defined as having its own hatchery, feed mill, and processing plant. Company A was comprised of 4 complexes while Company B was comprised of 5 complexes. In Company A, 4 grow-out farms from each of 2 complexes and 3 farms from each of the other 2 complexes were selected for a total of 14 farms. Company B was comprised of 5 farms from each of 2 complexes and 4 farms from each of 3 complexes for a total of 22 farms. Two houses from each of the 36 farms were selected for a total of 72 houses. The 2 houses that were selected from each farm for sampling were usually a house on the end of the row and the adjacent house. In total, there were 72 flocks sampled from 36 farms which were sampled from 9 complexes which were selected from 2 companies. The companies selected the farms to be sampled prior to placement so flocks could be processed on Monday or Tuesday to allow for ease of transport and processing of samples.

The sampling strategy was to follow each flock through the production and processing continuum taking samples from each flock at 4 points: (1) 1 week prior to the

end of grow-out and before transportation, (2) after transportation at plant arrival, (3) prior to chilling, and (4) at post-chill.

3.2.1.1 End of grow-out whole carcass rinse, ceca and crop samples

The first sampling point was approximately one week before harvest. The ages of the individual flocks ranged from 48-61 days old. A convenience sample was taken by catching 30 birds at the cool-cell end of the house. The birds were humanely euthanized by cervical dislocation. A whole carcass rinse sample was taken for each of the 30 birds by placing the carcass into a sterile biohazard bag with 250ml of 1% buffered peptone water (BPW) (Difco, Sparks, MD). The carcasses were vigorously shaken for 1 minute and the rinsate was aseptically transferred into a sterile plastic bottle. Following the collection of the whole carcasses rinses the crop and ceca were aseptically removed from each carcass. Each cecum was placed into a sterile Whirl-Pak® Bag (NASCO, Fort Atkinson, WI) and each crop was placed into a Whirl-Pak® Filter Bag (NASCO, Fort Atkinson, WI). BPW was added to each crop sample to make a 1:10 dilution by weight. Samples were placed on wet ice (18 h) and shipped overnight to the Food and Feed Safety Research Unit at College Station, Texas. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

3.2.1.2 Plant arrival whole carcass rinse, ceca and crop samples

The second sampling point was upon arrival at the processing plant. Three trucks were used to transport the flocks to the processing plant. A convenience sample of 2 birds from each of 5 cages was taken from each of the 3 trucks for sampling, totaling 30 birds per flock. A whole carcass rinse sample (described above) was taken for each of the 30

birds and tested for *Campylobacter*. The crop and ceca were removed aseptically from each of the same 30 birds (as descried above), packed on ice and transported to the laboratory. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

3.2.1.3 Pre-chill and post-chill rinse samples

The third and fourth sampling points were taken within the processing plant before the carcasses entered the immersion chill tank and upon exiting the chill tank. Carcass rinse samples were taken from 30 birds before entering the immersion chill tank and upon exiting the immersion chill tank. The carcass rinse samples were collected as described above, except 100ml BPW was added to the bag. The samples for each flock were taken at a repeating time interval so that the entire flock was sampled. Thus, 30 carcass rinses were sampled before the birds entered the chill tank and 30 carcass rinses were sampled upon exiting the chill tank for each of the flocks.

3.2.2 *Campylobacter* isolation and identification

Upon arrival at the Food and Feed Safety Research Unit at College Station, TX, the samples were incubated at 42°C for 24 hours. Selective enrichment was then performed for all samples except for the ceca by transfer of 10ml of the sample to 10 ml of 2x Bolton broth (Lab M, Bury, Lancashire, UK) and allowed to incubate for 24 hours at 42°C in a microaerobic environment (5% O_2, 10% CO_2, and 85% N_2). Each crop and ceca sample was then streaked onto Campy-Cefex agar (Becton, Dickinson and Co., Baltimore, MD) and allowed to incubate for 48 hours 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).

3.2.3 Sample size calculations

3.2.3.1 Number of flocks

The number of flocks used in this study was determined by a rule of thumb of 10 subjects, in this case flocks, per explanatory variable (Petrie and Watson 1999). Therefore, 72 flocks were used which would allow for 7 explanatory variables to be put into each final model.

3.2.3.2 Number of samples per flock

This study was conducted in conjunction with another study that looked at the presence of *Salmonella* in broiler production and processing. The USDA-FSIS reported the national prevalence of *Salmonella* was 10.2% (Progress Report on *Salmonella* Testing of Raw Meat and Poultry Products, 1998-2000, http://www.fsis.usda.gov/ophs/haccp/salmdata2.htm) and that of *Campylobacter* was higher with a prevalence of 21-41% post-chill (Stern, Ladely et al. 2001). The goal was to be able to detect both *Campylobacter* and *Salmonella*. A sample size of 30 birds per flock was adopted which would detect at least a within-flock prevalence of ≥9.5% with 95% confidence (Cannon and Roe 1982), which would ensure detection of *Salmonella* and *Campylobacter* in all flocks where the prevalence was greater than the national *Salmonella* average (the lower of the two prevalences).

3.2.4 Statistical procedures

Flock level prevalence was determined for each of the sample types using Microsoft Excel for Windows 2007. The data was then imported into STATA software version 12.1 (StataCorp LP, College Station, TX, USA) for further analysis.

The following sample points were used in this analysis: grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinse (GOWC), plant-arrival ceca (PACA), plant-arrival crop (PACP), plant-arrival whole carcass rinse (PAWC), and post-chill whole carcass rinse (PPPO).

Multilevel mixed-effects logistic regression (XTMELOGIT) was used to develop causal and predictive models for the presence of *Campylobacter* as well as to estimate the percentage of variance in *Campylobacter* prevalence at each level of the hierarchical structure.

The sampling hierarchy was birds nested within flocks, flocks nested within farms, farms nested within complexes, and complexes nested within company. Company was the highest level of the hierarchy and was not included as a random effect because two companies were too few to accurately estimate the amount of variance at that level. Instead, company was included as a fixed effect to account for any variation between companies; however, company was not found to be significant and was dropped from all models. Flock was also not included as a random effect due to convergence issues, which was attributed to nearly identical prevalence of *Campylobacter* in the two flocks on each of the farms. All of the models used accounted for the random effects of complex, farm, and bird.

The relationship between the occurrence of *Campylobacter* at a sample point and the prevalence of *Campylobacter* at prior sampling points were also assessed. Flock level prevalence at GOCP, GOCA, GOWC, PACP, PACA, PAWC, PPPR were assessed for their relationship with the PPPO outcome. The grow-out (GOCA, GOCP, GOWC) and plant arrival (PACA, PACP, PAWC) flock level prevalences were assessed for their relationship with the PPPR outcome. The grow-out (GOCA, GOCP, GOWC) flock level prevalences were assessed for their relationships with each of the plant arrival outcomes (PACA, PACP, PAWC).

Intraclass correlation and the proportion of total variance attributed to each of the random effects were estimated. The latent variable approach was used which assumes a logistic distribution and a level-one (i.e. birds) variance of $\pi^2/3= 3.29$ (Dohoo, Martin et al. 2009).

A univariable analysis was performed for each of the outcome variables as described above and only those variables with a p-value less than 0.15 were considered as candidates for the multivariable analyses. Continuous variables were checked for collinearity and linearity prior to the multivariable analyses.

Collinearity was assessed between the continuous variables using Spearman's rank correlation. If the coefficient was greater than 0.8, then one or the other explanatory variable was included in a multivariable model, but not both (Dohoo 2009). When collinearity did exist and there was no biological plausibility for selecting any one variable over another, the two variables were entered into separate models and the model with the smallest Akaike Information Criterion (AIC) was selected.

The assumption of a linear relationship between each continuous predictor variable and the relevant outcome variable was evaluated by generating a lowess plot of the logit vs. the predictor values and evaluated visually. If the lowess curve looked to be non-linear then basic transformations were used to see if linearity could be achieved. If linearity could not be achieved, then variables were categorized and reassessed in the univariable model.

Non-significant ($p > 0.05$) predictor variables were removed from the multivariable models using a manual backward selection process. Each variable that had been eliminated during the model selection process was reintroduced in the final reduced model to determine significance in the absence of non-significant variables. Furthermore, each eliminated variable was assessed for confounding as each non-significant variable was removed from the model. A variable was deemed a confounder and forced into the final model if the coefficient of a significant variable changed by more than 20 percent (Dohoo 2009). When necessary, models were compared using AIC and the model with the smallest value was chosen as the final model. Interactions between predictor variables were not explored because this was an exploratory analysis as well as due to difficulties with interpretation.

Both causal models and predictive models were constructed for each of the outcome variable with the difference being that the causal models contained no intervening variables (Dohoo, Martin et al. 2009).

3.3 Results

3.3.1 Descriptive summary

A summary of *Campylobacter* prevalence by sample type and company are listed in Table 3.1. Due to Hurricane Katrina, company schedule changes, disease outbreaks, and shipping delays data was not available for all of the original 72 flocks. Data was available for 66 of the flocks for the variables PACP, PACA, PAWC, PPPR, and PPPO. Grow-out crop and GOWC contained data from 67 flocks and GOCA contained 68 flocks. Due to the few flocks found to be positive at PPPR this variable was not used as an outcome in the model building process.

The grow-out and plant arrival mean flock prevalence in the ceca were 0.43 and 0.41, respectively. The total mean flock prevalence within the crop increased from 0.11 during grow-out to 0.33 at plant arrival. The grow-out whole carcass mean flock prevalence (0.22) and the plant arrival whole carcass mean flock prevalence (0.21) remained relatively the same. The pre-chill whole carcass mean flock prevalence (0.09) and the post-chill whole carcass rinse sample (0.10) remained relatively the same.

3.3.2 Univariable analysis

In the univariable analysis (Table 3.2), all explanatory variables were found to meet the screening criteria ($p \leq 0.15$) for each of the outcome variables except for the outcome PACA. The variable GOWC did not converge when entered into a model with PACA as the outcome. The variables that met the screening criteria were considered for inclusion in the multivariable analysis.

3.3.3 Multivariable analysis

There was high correlation (above 0.8) between a few of the explanatory variables which eliminated them both from being in the same model (Table 3.3). Variables that were highly correlated included GOWC and GOCA (0.928), GOWC and PPCA (0.806), GOCA and PPCA (0.813), PPCA and PPCP (0.830), and PPCP and PPWC (0.815), Consequently, multiple models were developed for some outcomes then compared using AIC.

The final multivariable models are listed in Table 3.4. Six models were investigated for the predictive model selection with the PPPO outcome, which after elimination of repetitive models, reduced to four distinct models. The predictive model with the lowest AIC for the PPPO outcome included GOWC and PACP. The causal model selection contained no intervening variables and was reduced to two competing models. One model contained the GOWC (AIC=661) and the other contained PPCA (AIC=664). Both models were retained due to the similarity in AIC values and biological plausibility. Since the plant-arrival outcomes contained no intervening variables, the model selection was both predictive and causal. The PAWC outcome produced a multivariable model containing GOWC and GOCA. The PACP outcome produced two competing models and included GOCA (AIC= 1156) and GOWC (AIC=1154). Both were retained due to the similarity in AIC values and biological plausibility. Lastly, the PACA outcome reduced to a univariable model and contained GOCA. The odds ratios for the univariable and multivariable models were reported for a 10%-unit increase in prevalence of each explanatory variable.

3.3.4 Intraclass correlation and percentage of variance

A null multilevel logistic regression model containing only the random effects for complex, farm, and bird was fitted to the data to determine the percentage of variance in *Campylobacter* prevalence that resided at each level. The variance (percent variance) occurring at the complex, farm, and bird level and the total variance at each outcome is listed in Table 3.5. The PPPO outcome was the only outcome that showed variance occurring at the complex level. The farm level variance ranged from 57%-91% variance while the bird level variance ranged from 9%-43%. The intra-class correlations for birds within the same farm, birds within the same complex but different farms, and farms within the same complex are listed for each outcome in Table 3.6. The intra-class correlation for birds within the same farm ranged from 0.57-0.91. Birds within the same complex but different farms and farms within the same complex had zero intra-class correlation except with the PPPO outcome. For this outcome, the intra-class correlation for birds within the same complex but different farms and farms within the same complex were 0.12 and 0.16, respectively.

3.4 Discussion

In general, the mean flock prevalence of *Campylobacter* decreased as birds proceeded through production and processing. This finding is in accordance with other researchers (Berrang and Dickens 2000, Berrang, Bailey et al. 2007, Berghaus, Thayer et al. 2013).

The increase in the mean flock prevalence in the crop from grow-out (0.11) to plant-arrival (0.33) was not unexpected. In this study, the grow-out crop sample was taken 1 week prior to feed withdrawal and transport. Research has shown that an entire flock can become positive within one week (Evans and Sayers 2000, Shreeve 2000, Bull, Allen et

al. 2006). By nature, poultry are coprophagic and will ingest fecal contaminated liter and feces in the absence of feed (Corrier, Byrd et al. 1999). Researchers have previously shown that feed withdrawal can increase the number of *Campylobacter* positive crop samples (Byrd, Corrier et al. 1998). Researchers have also shown no change in the prevalence of *Campylobacter* positive ceca samples following feed withdrawal (Byrd, Corrier et al. 1998) and transportation (Stern, Clavero et al. 1995). This was true for our study as well.

The unchanged grow-out whole carcass prevalence (0.22) and plant arrival whole carcass (0.21) was unexpected as we had predicted the prevalence of whole carcass samples to increase. Stress on the birds from catching and transportation has been shown to increase *Campylobacter* shedding within the birds (Stern, Clavero et al. 1995, Whyte, Collins et al. 2001). Furthermore, reuse of transportation crates for multiple flocks is a common industry practice that frequently results in crates arriving at the farm positive for *Campylobacter* (Stern, Ladely et al. 2001, Bull, Allen et al. 2006, Ridley, Morris et al. 2011). Although researchers have shown the level of *Campylobacter* on the exterior of the birds to increase during transportation (Stern, Clavero et al. 1995) and birds feathers to become dirtier (Buhr, Cason et al. 2000), no one has shown if the flock prevalence increases or decreases during transport. One would expect more whole carcass bird samples to be positive following transport due to increased defecation from stress and the reuse of possibly contaminated transport crates. It is possible that the prevalence does increase but it is below the detectable level of culturing or of the sample size chosen for this study. It is also possible that the process of feed withdrawal is preventing further

increase of *Campylobacter* prevalence on the exterior of the birds. Further research with a larger sample size is needed to draw deeper conclusions.

While the mean *Campylobacter* flock prevalence remained relatively the same at pre-chill (0.09) and post-chill (0.10) the total number of flocks that were positive coming out of the chill tank (11/66) increased (26/66) indicating that the chill tank served as a source of cross-contamination between contaminated flocks and *Campylobacter* free flocks or between positive and negative birds within the same flock. In this study, flocks were typically processed at the start of the day following the sanitation shift to try and avoid any issues of cross-contamination, although due to some scheduling conflicts that was not always the case. Previous researchers have shown that while the *Campylobacter* counts on a broiler carcass decrease as it proceeds through processing (Izat, Gardner et al. 1988, Berrang and Dickens 2000, Bilgili, Waldroup et al. 2002, Northcutt, Berrang et al. 2003), the number of positive carcasses following chilling have sometimes been shown to increase due to possible cross-contamination in the chiller (Jones, Axtell et al. 1991, Smith, Cason et al. 2005). Furthermore, laboratory studies have also shown that contaminated carcasses entering the chill tank can cause other birds to become contaminated. (Smith, Cason et al. 2005).

Our study determined the best predictor and cause of the plant arrival whole carcass being *Campylobacter* positive was the grow-out crop (OR=1.23(1.04-1.45)) and the grow-out ceca (OR=1.27 (1.15-1.41)). The exterior whole carcass rinse is a sample that is representative of the environment. Feed withdrawal causes the contents of the crop and ceca to be expelled and the coprophagic nature of the birds to result in consumption of the litter and its bacterial contamination.

The plant arrival crop outcome had two competing models with similar AIC values, grow-out ceca (OR=1.2 (1.11, 1.32), AIC=1156) and grow-out whole carcass (OR=1.30 (1.16, 1.45), AIC=1154). The model with the lower AIC value, and the model that makes the most biological sense, was the grow-out whole carcass. For reasons explained in the previous model, the grow-out whole carcass sample is the closest representative of the environment. Following feed withdrawal, the litter will be representative of the *Campylobacter* presence of the plant arrival crop.

The best predictor and cause for the plant arrival ceca being positive was the grow-out ceca. As discussed previously, this relationship is likely due to the lack of change in prevalence and level of *Campylobacter* in the ceca in this study and in others.

The post-chill outcome contained 3 models, 1 predictive model and 2 competing causal models. The predictive model contained the two variables that were representative of the house environment, grow-out whole carcass and plant-arrival crop. The first of two competing causal models for the post-chill outcome contained the grow-out whole carcass (OR=1.43 (1.20-1.71), AIC=661.02). The second model contained the plant-arrival ceca (OR=1.40 (1.18-1.67), AIC=664.27). During processing intestinal colonization has been identified as an important factor contributing to carcass contamination, especially during defeathering where the pickers fingers exert pressure on the lower abdomen causing feces to escape onto the exterior of the bird (Berrang, Buhr et al. 2001) and during evisceration where improper removal of the GI tract can cause breakage and spillage and lead to carcass contamination (Oosterom, Engels et al. 1983, Genigeorgis, Hassuneh et al. 1986, Berndtson, Tivemo et al. 1992). The AIC indicates

the model with grow-out whole carcass is the best causal model for the post-chill outcome.

This information indicates that the exterior contamination of the bird is important in predicting the *Campylobacter* status post-chill and causes the birds to be positive post-chill. Previous research has indicated the source of broiler *Campylobacter* contamination in the plant is from intestinal leakage, cut, and/or tears (Oosterom, Engels et al. 1983, Genigeorgis, Hassuneh et al. 1986, Berndtson, Tivemo et al. 1992). Our research is in agreeance with Musgrove et al. (1997) and Northcutt et al. (2003) who reported that the majority of bacteria recovered from carcasses following processing came from the birds' exteriors.

Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. This information indicates that intervention efforts should focus on factors at the farm level i.e. factors that vary among farms within a complex. The intra-class correlations for each of the outcomes indicates that there is high correlation among birds within the same farm and no correlation, with the exception of the PPPO outcome, among birds within the same complex but different farms and farms within the same complex. It is reasonable to think that there is increased correlation among birds within the same complex and among farms within the same complex for the PPPO outcome since complexes are defined by a shared processing plant. The increased correlations that become evident at post-chill are likely due to the cross-contamination and decontamination that can occur within a processing plant.

3.5 Conclusion

In summary, this study shows the *Campylobacter* status of broiler carcasses in the processing plant is related to broiler samples collected pre-harvest. Thus, interventions to further reduce *Campylobacter* prevalence within a flock must be applied pre-harvest at the farm level. By reducing the *Campylobacter* loads entering the processing plants on the exterior bird carcasses, the chill tanks can further reduce *Campylobacter* levels being sent out the door to retail stores.

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Table 3.1 Summary of *Campylobacter* prevalence by company and sample type

	D1GI	GOCA	GOCP	GOWC	PACA	PACP	PAWC	PPPR	PPPO
Company A									
Positive Flocks/Flocks (n)	0/28	17/28	16/27	16/27	16/26	16/26	17/26	8/26	13/26
Mean Flock Prevalence	0	0.54	0.11	0.34	0.46	0.39	0.31	0.23	0.18
Positive Samples/Sample (n)	0/840	454/840	89/810	277/810	359/779	307/779	241/780	177/773	138/779
Company B									
Positive Flocks/Flock (n)	11/44	20/40	19/40	19/40	20/40	23/40	18/40	3/40	13/40
Mean Flock Prevalence	0.01	0.37	0.12	0.15	0.39	0.30	0.16	0.01	0.06
Positive Samples/Samples (n)	19/1312	441/1200	144/1197	183/1200	469/1200	356/1197	186/1195	9/1198	71/1192
Totals									
Positive Flocks/Flocks (n)	11/72	37/68	35/67	35/67	36/66	39/66	35/66	11/66	26/66
Mean Flock Prevalence	0.01	0.43	0.11	0.22	0.41	0.33	0.21	0.09	0.10
Positive Samples/Sample (n)	19/2152	895/2040	233/2007	460/2010	828/1979	663/1976	427/1975	186/1971	209/1971

Table 3.2 Results of univariable logistic regression analysis of association between prevalence of *Campylobacter* at prior sampling points and occurrence of *Campylobacter* in post-chill carcass rinses (PPPO), plant arrival whole carcass rinses (PAWC), plant arrival crop (PACP), and plant arrival ceca (PACA), after accounting for the variability of the random effects farm and complex. The odds ratios are reported for a 10%-unit increase in prevalence of each explanatory variable.

Outcome	Risk Factor	Prevalence	OR (95% CI)	SE	*p*-value
PPPO	Pre-chill Whole Carcass Rinse	0.09	1.510 (1.106, 2.062)	0.240	0.009
	Plant Arrival Whole Carcass Rinse	0.22	1.259 (1.034, 1.532)	0.126	0.022
	Plant Arrival Crop	0.34	1.280 (1.079, 1.520)	0.112	0.005
	Plant Arrival Ceca	0.42	1.404 (1.180, 1.672)	0.125	0.000
	Grow-Out Whole Carcass Rinse	0.23	1.433 (1.203, 1.707)	0.128	0.000
	Grow-Out Crop	0.12	1.286 (0.982, 1.683)	0.177	0.068
	Grow-Out Ceca	0.44	1.287 (1.076, 1.539)	0.117	0.006
PAWC	Grow-Out Whole Carcass Rinse	0.23	1.337 (1.204, 1.486)	0.072	0.000
	Grow-Out Crop	0.12	1.417 (1.208, 1.661)	0.115	0.000
	Grow-Out Ceca	0.44	1.304 (1.184, 1.435)	0.064	0.000
PACP	Grow-Out Whole Carcass Rinse	0.23	1.296 (1.161, 1.446)	0.073	0.000
	Grow-Out Crop	0.12	1.318 (1.106, 1.571)	0.118	0.002
	Grow-Out Ceca	0.44	1.207 (1.106, 1.317)	0.054	0.000
PACA	Grow-Out Whole Carcass Rinse	0.23	No convergence		
	Grow-Out Crop	0.12	1.365 (1.066, 1.748)	0.172	0.014
	Grow-Out Ceca	0.44	1.524 (1.338, 1.736)	0.101	0.000

Table 3.3 Spearman correlation analysis of explanatory variables

	GOWC	GOCA	GOCP	PACA	PACP	PAWC	PPPR	PPPO
GOWC	1.000							
GOCA	0.9275*	1.000						
GOCP	0.7642	0.7967	1.000					
PACA	0.8056*	0.8125*	0.6838	1.000				
PACP	0.7466	0.7420	0.6798	0.8295*	1.000			
PAWC	0.7921	0.7713	0.7494	0.7927	0.8151*	1.000		
PPPR	0.5659	0.5654	0.5370	0.5004	0.5652	0.6651	1.000	
PPPO	0.6229	0.5458	0.4800	0.5069	0.4696	0.5370	0.4220	1.000

*Variables with a spearman correlation above 0.8

Table 3.4 Results of multivariable logistic regression analysis for predictive and causal models of association between prevalence of *Campylobacter* at prior sampling points and occurrence of *Campylobacter* in post-chill carcass rinses (PPPO), plant arrival whole carcass rinse (PAWC), plant arrival crop (PACP), and plant arrival ceca (PACA), after accounting for the variability of the random effects farm and complex. The odds ratios are reported for a 10%-unit increase in prevalence of each explanatory variable.

Outcome Variable	Model Number	Risk Factor (s)	OR (95% CI)	SE	*p*-value	AIC
PPPO (Predicted)	1	Grow-out whole carcass	1.349 (1.140, 1.595)	0.116	0.000	
		Plant arrival crop	1.184 (1.009, 1.390)	0.097	0.039	
PPPO (Causal)	1	Grow-out whole carcass	1.433 (1.203, 1.707)	0.128	0.000	661.02
	2	Plant-arrival ceca	1.404 (1.180, 1.672)	0.125	0.000	664.27
PAWC (Predicted/Causal)	1	Grow-out crop	1.225 (1.037, 1.447)	0.104	0.017	
		Grow-out ceca	1.270 (1.148, 1.405)	0.065	0.000	
PACP (Predicted/Causal)	1	Grow-out ceca	1.207 (1.106, 1.317)	0.054	0.000	1156.2
	2	Grow-out whole carcass	1.296 (1.161, 1.446)	0.073	0.000	1154.1
PACA (Predicted/Causal)	1	Grow-out Ceca	1.524 (1.338, 1.736)	0.017	0.000	

* AIC values can only be used to compare models with the same outcome

Table 3.5 The variance (percent variance) occurring at the complex, farm, and bird level and the total variance at each outcome using a null model for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Complex	Farm	Bird	Total
GOCA	0 (0.0)	32.38 (90.8)	3.29 (9.2)	35.67
GOCP	0 (0.0)	4.39 (57.2)	3.29 (42.8)	7.68
GOWC	0 (0.0)	14.10 (81.1)	3.29 (18.9)	17.39
PACA	0 (0.0)	32.56 (90.8)	3.29 (9.2)	35.85
PACP	0 (0.0)	13.84 (80.8)	3.29 (19.2)	17.13
PAWC	0 (0.0)	13.15 (80.0)	3.29 (20.0)	16.44
PPPO	1.6 (12.0)	8.4 (63.2)	3.29 (24.8)	13.29

Table 3.6 Intra-class correlations, using a null model, for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Birds within the same farm	Birds within the same complex but different farms	Farms within the same complex
GOCA	0.91	0	0
GOCP	0.57	0	0
GOWC	0.81	0	0
PACA	0.91	0	0
PACP	0.81	0	0
PAWC	0.80	0	0
PPPO	0.75	0.12	0.16

CHAPTER IV

MULTILEVEL ANALYSIS OF HATCHERY RISK FACTORS AND *CAMPYLOBACTER* PRESENCE AT SEQUENTIAL SAMPLING POINTS IN BROILER PRODUCTION AND PROCESSING IN THE SOUTHEASTERN UNITED STATES

Abstract

The main objective of this study was to identify risk factors within the hatchery that were associated with *Campylobacter* presence in broilers at the following sampling points: grow-out ceca (GOCA), crop (GOCP), and whole carcass (GOWC), plant arrival ceca (PACA), crop (PACP), and whole carcass (PAWC), and post-chill carcass (PPPO). A questionnaire was developed for hatchery managers that acquired information on parameters and protocols within the hatchery. Multilevel mixed-model logistic regression was used to assess the relationships between the hatchery risk factors and each outcome as well as to estimate the proportion of variance that occurred at the hierarchical levels of complex, farm, and bird. The GOWC, PAWC, and PPPO samples were the only outcomes that resulted in multivariable models. Variables associated with increased odds of detecting *Campylobacter* in the GOWC included washing the setter more often and controlling the humidity in the chick room. Two models were adopted for the PAWC outcome. The first model indicated that washing the setter more often, controlling the humidity in the chick room, and more than one breeder farm providing eggs for the

sampled flock were associated with increased odds of detecting *Campylobacter* on PAWC. The second model indicated that washing the setter more times per year, more than one person handling the chicks, and more than one breeder flock providing eggs for the sampled flocks were all associated with increased odds of detecting *Campylobacter* on PAWC. Variables associated with increased odds of detecting *Campylobacter* on PPPO included washing the setter more often per year and controlling the humidity in the chick room. Vaccinating the chicks on day one, compared to in-ovo, was a protective factor. The complex level percent variance of *Campylobacter* ranged from 0-12%, the farm level percent variance ranged from 57-91%, and the bird level percent variance ranged from 9-43%.

Keywords

Campylobacter; Broiler; Hatchery; Food safety; Risk factor analysis; Multilevel analysis

Public Health Forum

- Intervention efforts should focus on factors at the broiler farm level i.e. factors that are different among farms within a broiler complex.
- This study identified risk factors including controlling the humidity in the chick room, 2-4 people handling the chicks, washing the setter twice yearly, 2 or more breeder farms providing eggs for the sampled flock, and using low water pressure when washing the hatch trays may make flocks more susceptible to *Campylobacter* colonization later in production.

4.1 Introduction

Campylobacter continues to be an important human pathogen, as it is currently ranked third in annual food-borne disease burden within the United States (Scallan 2011). Consumption, cross-contamination, and handling of undercooked poultry has been identified as the major sources of campylobacteriosis (Batz, Hoffmann et al. 2012). It is estimated that 608,231 illnesses, 6,091 hospitalizations, and 55 deaths have been attributed to poultry products annually. campylobacteriosis costs 1,747 million dollars, annually (Batz, Hoffmann et al. 2012).

Campylobacter causes a mild to severe gastrointestinal infection with symptoms including headache, fever, severe abdominal cramps, watery or bloody diarrhea, and sometimes nausea and vomiting (CDC 2013). Infections are typically self-limiting and clear after a week, however, in some cases more severe sequelae have been reported, such as reactive arthritis, Guillian-Barŕe syndrome, Miller-Fisher syndrome, meningitis, bacteremia, and septicemia.(Kaldor and Speed 1984, Dhawan 1986, Roberts 1987, Mishu 1993, Ladrón de Guevara C 1994, Allos 1997, Hughes and Res 1997, Lastovica 1997, Saida, Kuroki et al. 1997, Nielsen 2009, CDC 2013).

The poultry intestinal tract, especially the ceca, colon, and crop is known to harbor large amounts of *Campylobacter* (Berrang, Buhr et al. 2000, Smith and Berrang 2006). Birds can carry *Campylobacter* levels as high as 10^9cfu/g of feces within their intestinal tracts (Oosterom, Noternams et al. 1983, Berndtson, Tivemo et al. 1992, Berrang, Buhr et al. 2000, Rosenquist, Sommer et al. 2006). High levels of *Campylobacter* brought into poultry plants introduce the strong possibility of high *Campylobacter* prevalence from cross-contamination due to gut leakage or accidental gut

tearing (Berrang, Buhr et al. 2000). In addition, contamination residing on the exterior of the bird after transportation can introduce high levels of *Campylobacter* into processing plants (Stern, Clavero et al. 1995, Berrang, Buhr et al. 2000). Interventions must begin prior to processing in order to further reduce the contamination on the broilers that enters the processing plant. To accomplish this, the sources and routes of infection must be known (Newell and Fearnley 2003).

Horizontal transmission of *Campylobacter* to broilers has been well established (Montrose, Shane et al. 1984, Shanker, Lee et al. 1990, Jacobs-Reitsma, van de Giessen et al. 1995, Willis, Talbott et al. 2000). Flies (Shane, Harrington et al. 1985, Hald, Skovgård et al. 2004, Hald, Skovgård et al. 2008), beetles (Skov, Spencer et al. 2004), vermin, wild birds, water, feed, air, and humans have all been identified as sources from which *Campylobacter* can enter the poultry house. Once infection within a flock has been detected, the whole flock typically becomes infected within a week (Jacobs-Reitsma, van de Giessen et al. 1995).Vertical transmission, from parent to progeny, is a route of transmission that is still debated among researchers. Callicott et al. (2006) used PCR to demonstrate that there was no evidence of transmission from grand-parent flocks through the egg to progeny parent breeders. A study conducted by Sahin et al. (2003) observed that eggs collected from *Campylobacter* positive broiler-breeder flocks have been found to be negative for the bacterium (Sahin, Kobalka et al. 2003). Under common sanitary conditions, broilers from infected parents have been raised to be *Campylobacter*-free at slaughter (Annal-Prah 1988). Laboratory research has found day-of-hatch chicks capable of being successfully inoculated with doses as low as 35 colony-forming units (Stern, Bailey et al. 1988, Stern 1994, Cappelier, Magras et al. 1999) however, studies within the

production environment show chicks are not infected with *Campylobacter* on day of hatch, but become infected at three to four weeks of age due to the protection of maternal antibodies (Jacobs-Reitsma, van de Giessen et al. 1995, Sahin, Luo et al. 2003). *Campylobacter* has also been found in the reproductive tract of both broiler breeder hens and roosters (Buhr 2002, Hiett, Siragusa et al. 2003, Cox 2005) and semen (Cox 2002a). Tray-pads (Byrd, Bailey et al. 2007), fluff, and eggshells (Hiett, Cox et al. 2002) of day-of-hatch chicks have also been found to be *Campylobacter* positive, suggesting hatchery debris could be contaminated by feces from the hen and then consumed by the offspring. Vertical transmission implies transovarian transmission but could also include the pseudovertical transmission from parent to prodigy through the contamination and transmission on the surface of the egg (Newell, ELvers et al. 2011, Cox, Richardson et al. 2012). Molecular evidence exists that demonstrates *Campylobacter* isolates from the feces of progeny that are clonal in origin to those of the parent breeder flocks (Cox 2002b). A recent review article made the point that vertical transmission is easily refuted due to the 2-3 week delay in chick infection; however, this delay in infection could be explained by the existence of low transmission rates and insensitive flock sampling methods (Cox, Richardson et al. 2012). Currently the ideal microbiological cultural procedure for the recovery and isolation of *Campylobacter* is lacking (Cox, Richardson et al. 2012). While horizontal transmission is the main mode of transmission, vertical transmission cannot be completely eliminated as a source.

The purpose of this study was to generate hypotheses about practices in the hatchery associated with *Campylobacter* flock infection later in production. The goal was to identify relationships between risk factors within the hatchery and the *Campylobacter*

status of a bird at various sampling points throughout the production and processing continuum. The information obtained from this study could identify associations between practices in the hatchery that could make chicks more susceptible to *Campylobacter* infection later in the broiler production and processing continuum.

4.2 Materials and methods

4.2.1 Sampling strategy

A prospective observational study was conducted in 3 states (Alabama, Mississippi, and Louisiana) within the southeastern United States from 2003-2006. Complexes from 2 companies that were thought to be representative of the regional poultry industry participated in the study. Each complex had its own hatchery, feed mill, and processing plant. Company A was comprised of 4 complexes while Company B was comprised of 5 complexes. In Company A, 4 grow-out farms from each of 2 complexes and 3 farms from each of the other 2 complexes were selected for a total of 14 farms. Company B was comprised of 5 farms from each of 2 complexes and 4 farms from each of 3 complexes were selected for a total of 22 farms. The companies selected the farms to be sampled before placement so that the flocks could be processed at the beginning of the week for ease of sample processing and transportation. Two houses from each of the 36 farms were selected for a total of 72 houses. The 2 houses that were selected from each farm for sampling were usually a house on the end of the row and the adjacent house. In total, there were 72 flocks sampled from 36 farms which were sampled from 9 complexes which were selected from 2 companies. Due to hurricane Katrina, disease outbreaks, company schedule changes, and some samples being lost in transit to the laboratory, samples for some sample points from 6 flocks were lost from the study.

In general, within the US poultry industry, a broiler company owns the hatchery segment of production and provides healthcare and feed to the breeder and the grow-out flocks which are grown on privately owned farms. The layout of the hatcheries is similar, however, they may differ in the type of equipment used and procedures for cleaning the equipment. Hatchery management practices including vaccination protocols, temperature, humidity settings for each room, and handling procedures may also differ between hatcheries and within a hatchery over time.

The sampling strategy was to follow each flock through the production and processing continuum taking samples from each flock at 4 points: (1) 1 week prior to the end of grow-out and before transportation, (2) after transportation at plant arrival, (3) prior to chilling, and (4) at post-chill.

4.2.2 Sample collection

4.2.2.1 End of grow-out whole carcass rinse, ceca and crop samples

The first sampling point was approximately one week before harvest. The ages of the individual flocks ranged from 48-61 days old. A convenience sample was taken by catching 30 birds at the cool-cell end of the house. The birds were humanely euthanized by cervical dislocation. A whole carcass rinse sample was taken for each of the 30 birds by placing the carcass into a sterile biohazard bag with 250ml of 1% buffered peptone water (BPW) (Difco, Sparks, MD). The carcasses were vigorously shaken for 1 minute and the rinsate was aseptically transferred into a sterile plastic bottle. Following the collection of the whole carcasses rinses the crop and ceca were aseptically removed from each carcass. Each cecum was placed into a sterile Whirl-Pak® Bag (NASCO, Fort Atkinson, WI) and each crop was placed into a Whirl-Pak® Filter Bag (NASCO, Fort

Atkinson, WI). BPW was added to each crop sample to make a 1:10 dilution by weight. Samples were placed on wet ice (18 h) and shipped overnight to the Food and Feed Safety Research Unit at College Station, Texas. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

4.2.2.2 Plant arrival whole carcass rinse, ceca and crop samples

The second sampling point was upon arrival at the processing plant. Three trucks were used to transport the flocks to the processing plant. A convenience sample of 2 birds from each of 5 cages was taken from each of the 3 trucks for sampling, totaling 30 birds per flock. A whole carcass rinse sample (described above) was taken for each of the 30 birds and tested for *Campylobacter*. The crop and ceca were removed aseptically from each of the same 30 birds (as descried above), packed on ice and transported to the laboratory. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

4.2.2.3 Pre-chill and post-chill carcass rinse samples

The third and fourth sampling points were taken within the processing plant before the carcasses entered the immersion chill tank and upon exiting the chill tank. Carcass rinse samples were taken from 30 birds before entering the immersion chill tank and upon exiting the immersion chill tank. The carcass rinse samples were collected as described above, except 100ml BPW was added to the bag. The samples for each flock were taken at a repeating time interval so that the entire flock was sampled. Thus, 30 carcass rinses were sampled before the birds entered the chill tank and 30 carcass rinses were sampled upon exiting the chill tank for each of the flocks.

4.2.3 *Campylobacter* isolation and identification

Upon arrival at the Food and Feed Safety Research Unit at College Station, TX, the samples were incubated at 42°C for 24 hours. Selective enrichment was then performed for all samples except for the ceca by transfer of 10ml of the sample to 10 ml of 2x Bolton broth (Lab M, Bury, Lancashire, UK) and allowed to incubate for 24 hours at 42°C in a microaerobic environment (5% O_2, 10% CO_2, and 85% N_2). Each crop and ceca sample was then streaked onto Campy-Cefex agar (Becton, Dickinson and Co., Baltimore, MD) and allowed to incubate for 48 hours 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).

4.2.4 Questionnaire

A questionnaire (Volkova 2007) was developed to be filled out by the hatchery managers. The questionnaire was also used in another study that reported on *Salmonella* (Volkova, Bailey et al. 2011). The questionnaire contained 14 sections and a total of 65 questions. The sections contained information on breeder farms that provided eggs to the hatchery, egg collection, setter and incubator parameters, egg candling, hatcher parameters, transport box sanitation, chick processing, chick room parameters, vaccination protocols, chick loading, hatchery premises, transport vehicles, and biosecurity practices.

Pilot testing for the questionnaire was conducted on two occasions. First, the questionnaire was administered to two poultry veterinarians that were actively involved

with the project. Secondly, after editing, the questionnaire was administered to the managers of two broiler complexes in the area of study. Further edits were made before the final instrument was adopted.

4.2.5 Sample size calculation

4.2.5.1 Number of flocks

The number of flocks used in this study was determined by a rule of thumb of 10 subjects, in this case flocks, per explanatory variable (Petrie and Watson 1999). Therefore, 72 flocks were used which would allow for 7 explanatory variables to be put into each final model.

4.2.5.2 Number of samples per flock

This study was conducted in conjunction with another study that looked at the presence of *Salmonella* in broiler production and processing. The USDA-FSIS reported the national prevalence of *Salmonella* was 10.2% (Progress Report on *Salmonella* Testing of Raw Meat and Poultry Products, 1998-2000, http://www.fsis.usda.gov/ophs/haccp/salmdata2.htm) and that of *Campylobacter* was higher with a prevalence of 21-41% post-chill (Stern, Ladely et al. 2001). The goal was to be able to detect both *Campylobacter* and *Salmonella*. A sample size of 30 birds per flock was adopted which would detect at least a within-flock prevalence of ≥9.5% with 95% confidence (Cannon and Roe 1982), which would ensure detection of *Salmonella* and *Campylobacter* in all flocks where the prevalence was greater than the national *Salmonella* average (the lower of the two prevalences).

4.2.6 Statistical procedures

The *Campylobacter* status (positive or negative) was used to model the relationship between risk factors in the grow-out and processing phases and the following sampling points: grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinse (GOWC), plant-arrival ceca (PACA), plant-arrival crop (PACP), plant-arrival whole carcass rinse (PAWC), and post-chill whole carcass rinse (PPPO).

The data was analyzed using STATA software version 15.1 (StataCorp LP, College Station, TX, USA). Multilevel mixed-effects logistic regression (MEQRLOGIT) was used to develop causal models for the presence of *Campylobacter* as well as to estimate the percentage of variance in *Campylobacter* prevalence at each level of the hierarchical structure.

The sampling hierarchy was birds nested within flocks, flocks nested within farms, farms nested within complexes, and complexes nested within company. Company was the highest level of the hierarchy and was not included as a random effect because two companies were too few to accurately estimate the amount of variance at that level. Instead, company was included as a fixed effect to account for any variation between companies; however, company was not found to be significant and was dropped from all models. Flock was also not included as a random effect due to convergence issues, which was attributed to nearly identical prevalence of *Campylobacter* in the two flocks on each of the farms. Consequently, complex, farm and bird were included as random effects in all models. The proportion of total variance attributed to each of the random effects were estimated using the latent variable approach which assumes a logistic distribution and a level-one (i.e. birds) variance of $\pi^2/3= 3.29$ (Dohoo, Martin et al. 2009). The intraclass

correlation coefficient for birds within the same farm was calculated by dividing the variance of the farm plus the complex by the total variance. The intraclass correlation coefficient for birds within the same complex but different farms was calculated by dividing the variance of the complex by the total variance. The interclass correlation coefficient for farms within the same complex was calculated by dividing the variance of the complex by the sum of the farm and complex variance.

A univariable analysis was performed for each of the explanatory variables and only those variables with a p-value less than 0.15 were considered for inclusion in the multivariable analysis.

The assumption of a linear relationship between each continuous predictor variable and the relevant outcome variable was evaluated by generating a lowess plot of the logit vs. the predictor values and evaluated visually. If the lowess curve looked to be non-linear then basic transformations were used to see if linearity could be achieved. If linearity could not be achieved, then variables were categorized and reassessed in the univariable model.

For continuous variables, which were candidates for multivariable models, collinearity was assessed between variables using Spearman's rank correlation. If the coefficient was greater than 0.8, then one or the other explanatory variable was included in a multivariable model, not both (Dohoo 2009). When collinearity did exist, and in some cases there was no biological plausibility for selecting any one variable over another, the two variables were entered into separate models and the final model with the smallest Akaike Information Criterion (AIC) was selected.

Non-significant ($p > 0.05$) predictor variables were removed from the multivariable models using a manual backward selection process. Each variable that had been eliminated during the model selection process was reintroduced in the final reduced model to determine significance in the absence of non-significant variables and to determine if the variable was a confounder. A variable was deemed a confounder and forced into the final model if the coefficient of a significant variable changed by more than 20 percent (Dohoo 2009). Interactions between predictor variables were explored when it made biological sense. Causal models, containing no intervening variables, were constructed for each of the outcome variables (Dohoo, Martin et al. 2009).

4.3 Results

4.3.1 Surveys collected

Of the 72 flocks sampled, the managers completed questionnaires for 59 sampled flocks. The 13 surveys that were not returned were all from the same company. This is likely due to competing time of the managers since Hurricane Katrina occurred in the middle of the sample collection period in 2005. The 59 sampled flocks were hatched from one of seven hatcheries (5-16 flocks per hatchery). The number of chicks the hatcheries hatched per day and per week ranged from 153,300 to 388,300 and 664,900 to 1,540,000, respectively. The number of chicks hatched was not included in the analysis due to the inability to make the fit of the variables in the models linear by transformation or categorizing them and due to the large number of missing observations.

Of the 59 flocks used for the analysis 51.7% of the flocks originated from a single parent breeder flock, while 48.3% came from 2 or more parent flocks. Once the eggs arrived to the hatchery, the number of days the eggs stayed in the egg room prior to

placement in the incubator was only reported for 13 flocks but ranged from 0-7 days. In addition, the temperature of the egg rooms ranged from 17.8°C to 20°C. The humidity of the egg room was controlled in 91.8% of flocks, with levels at 70%, 75% or 80%.

The hatcheries either had air-conditioning (39%) or evaporative cooling (61%) ventilation systems. For incubation, all of the hatcheries used the Jamesway system with removable setter buggies produced by the same company. The incubators were kept at a temperature of 37.1°C and RH range of 74% to 86% with the majority keeping the RH at 84%. While in the incubator, eggs were disinfected in 52.5% of cases with one of two disinfectants (Clinafarm spray or a Quaternary ammonia). Methods for applying these disinfectants varied between hatcheries. The setters were washed once (79.7%) or twice (20.3%) a year with protocols for disinfection varying; every set (10%), twice a week (29%), weekly (17%), once a year (44%).

Eggs were candled in 76.3% of the flocks on days 17(35.6%) and 18 (64.4%) before going into the hatcher. In the hatcher, the temperature ranged from 36.4°C to 37.1°C and RH ranged from 84% to 86% during the hatches for the sampled flocks. The eggs were fumigated with formaldehyde in 62.7% of the flocks. The hatcher and hatch trays were disinfected in all cases between each hatch. The hatch trays were washed with either using high pressure (66.1%) or not using high pressure (33.9%).

Separation of the chicks from the eggshells was done by a separator in 83% of the flocks and was done manually in the remainder of the flocks. Following separation, a chick handler would manually remove any dead birds from the processing line. Anywhere from 0-4 personnel handled the chicks. This variable was dichotomized to include 0-1 (39%) chick handlers and 2-4 (61%). Chick handlers were required to wash

their hands or change gloves prior to contact with the chicks in 74.6% of flocks. Chicks were then counted and loaded into chick loading boxes by machine (81.4%) or manually (18.6%). The chick line conveyor belts were washed and disinfected daily. All of the hatcheries used chick tray pads to line the chick boxes and the tray pads were not reused with subsequent flocks. The chick boxes were washed and disinfected between flocks in 10% of flocks with most hatcheries disinfecting the chick boxes weekly. The chick boxes were washed either using high pressure (38.8%) or not utilizing high pressure (61.2%).

Following processing, the birds were kept in the chick room for 1-12 hours before being loaded for transportation to farms. Evaporative cooling was used in all of the reported chick rooms with temperatures ranging from 22.8°C to 26.7°C. In 50.8% of flocks, the humidity was controlled in the chick room. For those hatcheries that did control the humidity, the humidity was kept at either 70 or 80 RH. The chick room was washed daily in 93% of flocks with 72.7% of the cases also disinfecting daily. No fly control was reported in the chick room.

Rodent control was reported being used both inside (100%) and outside (89.8%) of the hatcheries. Seventy-one percent of the cases reported using a professional rodent control service while the other 28.8% used bait boxes.

A Marek's disease live virus vaccine was delivered in-ovo when the eggs were transferred from the incubator to the hatcher in 74.6% of the sampled flocks and 18.6% of the flocks by injection on the day of hatch. Infectious bursal disease virus vaccine was given in-ovo in 42.9% of cases. In addition, the chicks received Newcastle disease vaccine (100%) and infectious bronchitis live virus vaccine (96.2%) on day of hatch via spray.

Personnel wore over-clothes and over-shoes prior to entering the premises for 100% of the flocks. Some hatcheries required personnel to change shoes (27%) and change clothes (10%). Footbaths were reported being used by personnel in 83% of reported flocks.

4.3.2 Univariable and multivariable analysis

The GOCA outcome univariable analysis contained 6 variables that met the screening criteria ($p < 0.15$) to be considered as candidates for development of a multivariable model and are listed in Table 4.1. There were three pairs of variables that were correlated with $r > 0.8$. There was no biological reason for choosing any one of the variables over the other when correlation existed so they were entered into separate models for the multivariable analysis. Eight models consisting of combinations of the 6 variables were constructed. Two models did not converge while the other 6 models successfully converged. The result of the multivariable analysis was 6 univariable models. There was not a multivariable model that contained variables with $p \leq 0.05$. The 3 univariable models that had significant fixed effects were 2 or more people handled the chicks (OR=124.6, CI=1.38, 11214.27), humidity controlled in the chick room (OR=84.7, CI=1.08, 6629.16), and low-pressure water used for washing the hatching trays (OR=104.03, CI=1.00, 10824.99). All had very similar AIC values.

The univariable analysis for the GOCP response variable contained 3 variables with $p \leq 0.15$ and are displayed in Table 1. Two of the variables were highly correlated ($r > 0.8$) and were put into separate models. Thus, two models were created for comparison in the multivariable selection process. The models, after the multivariable selection process, each contained only one variable and those variables were not significant ($p >$

0.05); however, the model for humidity controlled in the chick room approached the cutoff with p=0.056 (OR=4.52).

The GOWC response univariable analysis contained 6 variables with $p \leq 0.15$ and are displayed in Table 4.1. Two pairs of variables were correlated ($r > 0.80$) and were placed into separate models. Four models were created from combinations of the 6 variables without including correlated variables in the same model. Two of the four models did not converge. The final grow-out whole carcass rinse model (Table 4.2) with the lowest AIC and $p \leq 0.05$ included washing the setter twice a year (OR=39.4, CI=1.22, 1264.48) and the humidity controlled in the chick room (32.6, CI=1.83, 582.85).

The univariable analysis for the PACA outcome contained only 1 variable with $p \leq 0.15$ (Table 1). Since 2 or more breeder flocks provided eggs for the sampled flock was the only significant variable (OR=2.3, CI=1.01, 5.13), a multivariable model was not constructed.

The univariable analysis for the PACP contained two variables with $p \leq 0.15$ and are displayed in Table 4.1. These variables were not correlated and were analyzed together in a multivariable model. The model resulted in a univariable model in which 2 or more people handled the chicks was not significant ($p = 0.080$).

The response variable PAWC contained 5 variables with $p \leq 0.15$ and are displayed in Table 4.1. Two or more people handled the chicks and the humidity controlled in the chick room were two variables that were correlated ($r > 0.82$). There was no biological reason for choosing one variable over the other so two multivariable models were constructed and compared. The final Model 1 included 3 variables (2 or more breeder farms provided eggs for the sampled flock (OR=1.67, CI=1.04, 2.69), setter

washed twice per year (OR=56.9, CI=2.45, 1318.1), and controlling the humidity in the chick room (OR=12.5, CI=1.11, 140.9) and is displayed in Table 4.2. The AIC for Model 1 was 1041.2. The final Model 2 included 3 variables (2 or more breeder farms provided eggs for the sampled flock (OR=1.69, CI=1.05, 2.73), setter washed twice per year (OR=47.2, CI=1.92, 1159.1), and 2-4 workers handle the chicks (OR=12.7, CI=0.90, 178.8) and is displayed in Table 4.2. The AIC for Model 2 was 1041.6. Both models were adopted since the AIC values were so similar.

The post-chill outcome contained 9 variables that were associated below the $p \leq 0.15$ cut-off and are displayed in Table 4.1. Two pairs of variables were correlated with $r > 0.8$ and were included in different models. There were four models constructed from the 9 variables containing combinations of non-correlated variables. The final model that was the most biologically plausible and with the lowest AIC contained 4 variables chicks vaccinated Day 1 (OR=0.03, CI=0.00, 0.56), humidity controlled in the chick room (OR=294.3, CI=17.04, 5083.6), and the setter washed twice a year (OR=309.9, CI=19.24, 4990.8) with $p \leq 0.05$ and is displayed in Table 4.2. The fourth variable was the procedure for washing the hatch trays and was a confounder.

An overview of the univariable associations ($p \leq 0.15$) between *Campylobacter* presence and risk factors throughout all samples of the production and processing continuum are presented in Table 4.3. Table 4.3 shows these relationships across the continuum and demonstrates that some variables were associated with a number of outcomes. The variables humidity controlled in the chick room status and the total number of people that handled the chicks shows the most consistency in relationships with all of the outcomes over time although not all of the relationships have p-values ≤

0.05. The number of times the setter was washed per year is a variable that occurs more consistently through the end of production and processing continuum.

4.3.3 Intraclass correlation and percentage of variance

The variance and percent of total variance occurring at the complex, farm, and bird level and the total variance at each outcome is displayed in Table 4.4. The PPPO outcome was the only outcome that showed variance occurring at the complex level. The farm level variance ranged from 57.2%-90.8% variance while the bird level variance ranged from 9.2%-42.8%. The intraclass correlation coefficients for birds within the same farm, birds within the same complex but different farms, and farms within the same complex are listed for each outcome in Table 4.5. The intraclass correlation coefficients for birds within the same farm ranged from 0.57-0.91. Birds within the same complex but different farms and farms within the same complex had zero intraclass correlation except with the PPPO outcome. The intraclass correlation for birds within the same complex but different farms and farms within the same complex were 0.12 and 0.16, respectively.

4.4 Discussion

For the outcomes that produced a multivariable model, and for some outcomes that did not, there were consistencies in some of the variables that were found to be associated with the occurrence of *Campylobacter* and include if the humidity was controlled in the chick-room, washing the setter twice a year, 2-3 people handling the chicks, washing the hatch trays with low pressured water, and 2 or more breeder farms providing eggs for a flock.

Flocks that came from hatcheries that controlled the humidity in the chick room were more likely to be *Campylobacter* positive at GOCA, GOCP, GOWC, and PPPO outcomes in the univariable analysis than flocks that came from hatcheries that did not control the humidity. This variable remained in the models of the multivariable analysis in the GOCA, GOWC, PAWC, and PPPO outcomes. The hatcheries that controlled the humidity either kept the humidity at 70 or 80 RH. *Campylobacter* is sensitive to desiccation and thrives in moisture rich environment (Hazeleger, Wouters et al. 1988, Lee, Smith et al. 1998, Altekruse, Stern et al. 1999). Controlling the humidity within the chick room at these levels could provide a more suitable environment for the organism's survival. Although much has been published on the survivability of *Salmonella* at different levels of humidity within poultry samples, few studies have looked at the relationship between *Campylobacter* and humidity. In a study conducted in Japan, Ishihara et al. (2012) demonstrated that grow-out flocks raised in areas of higher humidity were more likely to be colonized with *Campylobacter*. A study by Line et al. (2006) demonstrated differences in rates of *Campylobacter* colonization on litter held under high (80%) and low (30%) humidity. In the current study, the flocks from the hatcheries that did not control the humidity could have experienced more variable humidity which would have been less conducive to the organism's survival.

In this study, the odds of a *Campylobacter* positive sample increased when the setter was washed twice a year compared to once a year in the PAWC and PPPO outcomes of the univariable analysis and the GOWC, PAWC, and PPPO outcomes of the multivariable analysis. The idea that washing a setter more frequently increases the odds of *Campylobacter* positive flocks is counterintuitive. However, research has shown that

Campylobacter may enter a viable but non-cultural state when in the presence of environmental stresses (Rollins and Colwell 1986) and may integrate and prolong survival in pre-established water system biofilms (Buswell, Herlihy et al. 1998, Trachoo, Frank et al. 2002). Biofilms can provide protection to *Campylobacter* and an environment suitable for its survival due to the moisture, decreased dissolved oxygen, and sometimes a concentration of nutrients (Buswell, Herlihy et al. 1998, Trachoo, Frank et al. 2002). It is possible that washing the setter more frequently disrupts the biofilms causing the dispersal of large number of cells that can further spread within the environment.

The odds of a *Campylobacter* positive sample increased at the GOCA outcome for the univariable analysis and GOCA and PAWC outcome for the multivariable analysis when 2-4 people handled the chicks while in the hatchery. One explanation is that each additional person that handles the chicks could be an additional source of stress on the birds while in the hatchery. This in turn could cause higher defecation rates among chicks within the hatchery and be a source of spreading *Campylobacter* due to the coprophagy nature of the birds. Another explanation is that each additional person handling the chicks could be transferring the organism on their gloves and contaminating the bird's exterior. While few positive samples have been found in hatchery samples, including the fluff (Hiett, Cox et al. 2002), egg shell (Hiett, Cox et al. 2002), and tray pads (Byrd, Bailey et al. 2007), there is still a possibility that the VBNC state of the organism allows it to survive on the exterior of the egg and could be transferred by more people putting stress on the chicks or more people handling the chicks.

Washing the hatching trays with low pressure increased the odds of a sample being *Campylobacter* positive at the GOCA and GOWC outcomes of the univariable

analysis and was a confounder in the PPPO outcome of the multivariable analysis. Dried feces is difficult to remove from surfaces and even the strictest washing programs have been unable to completely remove *Campylobacter* from transportation crates following the grow-out period (Slader, Domingue et al. 2002, Allen, Burton et al. 2008, Hastings, Colles et al. 2010). Low pressure washing is not likely to remove as much of the feces buildup compared to high pressure and the added moisture could prolong the survival of *Campylobacter* on the trays.

The odds of a *Campylobacter* positive sample increased for sample flocks that were made up of eggs from 2 or more breeder flocks in the PACA and PAWC of the univariable analysis and the PAWC of the multivariable analysis. Breeder flocks have been found to be *Campylobacter* positive (Jacobs-Reitsma 1995); however, serotyping and PCR results do not support vertical transmission (Jacobs-Reitsma 1995, Callicott, Frioriksdottir et al. 2006). This relationship was not present in the grow-out environment but rather appeared at plant arrival. The difference between these two sampling points included a 1-week time difference, feed withdrawal, and transportation. Once the first bird in a flock becomes *Campylobacter* positive, all birds become positive within 1 week. Thus, the 1-week difference is enough time for the *Campylobacter* status within a flock to change. In addition, due to the coprophagic nature of broilers, feed withdrawal can cause the birds to consume litter and contaminated feces within the environment which has been shown to increase contamination within the crop. Finally, the stress of transportation can increase fecal shedding within the birds and can cause *Campylobacter* negative birds within a flock to become positive. Transportation of *Campylobacter* negative flocks in previously contaminated cages could also change the *Campylobacter*

status of a flock. All of these steps that occur between the grow-out sampling point and plant-arrival sampling point could contribute to the disruption of the relationship and could be why broiler flocks made up of 2 breeder flocks compared to one was a significant variable at plant-arrival. It is likely this variable is a proxy variable for some unmeasured variable or a spurious association.

Vaccinating the chicks on day 1 (compared in in-ovo) was a protective factor for *Campylobacter* in the post-chill sample. Vaccinating chicks in-ovo could offer *Campylobacter* entrance from the exterior shell into the egg during development. Laboratory studies, however, have shown *Campylobacter* inoculated into eggs has limited survivability (Sahin, Kobalka et al. 2003). This variable was significantly associated with *Campylobacter* presence in the multivariable analysis of the PPPO outcome. It was not significant (p=0.159) at the univariable level. It is likely vaccinating chicks on day1 and the PPPO outcome was a spurious association.

In this study, many hatchery variables were analyzed to determine if associations existed with the presence of *Campylobacter* later in production. Relatively few statistically significant associations were found and some of these are not easily explained or are counterintuitive. Due to the nature of this investigation considering a large number of variables and the timing of the samples taken, detecting spurious associations is certainly one possibility. Another possibility is that the variables found to be significantly associated with an outcome may actually be a confounder for another unmeasured variable. For example, although one would have expected the control of humidity in the chick room to be associated with decreased occurrence of

Campylobacter, this is not what was found. Either another, confounding, variable was responsible or perhaps a more direct measure of humidity was needed.

Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. This information indicates that intervention efforts should focus on factors at the farm level i.e. factors that vary among farms within a complex. The intraclass correlation coefficients for each of the outcomes indicates that there is high correlation among birds within the same farm and no correlation, with the exception of the PPPO outcome, among birds within the same complex but different farms and farms within the same complex. It is reasonable to think that there is increased correlation, for PPPO, among birds within the same complex and among farms within the same complex since complexes are defined by a shared processing plant. The increased correlations that become evident at post-chill are likely due to the cross-contamination and decontamination that can occur within a processing plant.

4.5 Conclusion

The route of *Campylobacter* contamination of poultry flocks, vertical and/or horizontal transmission, is still debated among researchers. The evidence suggests horizontal transmission is the main mode of transmission of *Campylobacter* in broilers while vertical transmission occurs infrequently. The ability of *Campylobacter* to enter the VBNC form and insensitive culturing methods has made identifying the source difficult. This study identified risk factors in the hatchery including controlling the humidity in the chick room, 2-4 people handling the chicks, washing the setter twice yearly, 2 or more breeder farms providing eggs for the sampled flock, and the procedure for washing the

hatch trays that may make flocks more susceptible to *Campylobacter* colonization later in production. The highest proportion of variance for all the outcomes was at the farm level suggesting there are farm level risk factors that should be considered. However, it also found a number of significant associations between hatchery level factors and the occurrence of *Campylobacter* at multiple sampling points in later production and processing suggesting that hatchery factors may contribute to vulnerability of broilers to *Campylobacter* infection or persistence of *Campylobacter* within the flock.

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Table 4.1 Results of univariable logistic regression analysis of association between hatchery risk factors and occurrence of *Campylobacter* in grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinse (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO) after accounting for the variability of the random effects farm and complex.

Outcome	Risk Factor	Response	Mean (range) or count of flocks	OR (95% CI)	SE	p-value
GOCA	Total number of people that handled the chicks [b]	2-4	35	124.6 (1.38, 11214.3)	286.05	0.036
		0-1	23	Reference		
	Humidity controlled in the chick room status [b]	Yes	29	84.7 (1.08, 6629.5)	188.45	0.046
		No	29	Reference		
	Procedure for washing the hatching trays [c]	Low Pressure	19	104.0 (1.00, 10825.1)	246.54	0.050
		High Pressure	39	Reference		
	How often chick transport vehicles washed [a]	Less Often	24	38.5 (0.43, 3419.6)	88.07	0.111
		Between hatchery days	34	Reference		
	Are chick transport vehicles disinfected [a]	Yes	39	0.02 (0.00, 2.49)	0.055	0.115
		No	19	Reference		
	Were eggs disinfected/fumigated in hatcher [c]	Yes	37	0.03 (0.00, 3.09)	0.07	0.137
		No	21	Reference		
GOCP	Humidity controlled in the chick room status [a]	Yes	29	4.52 (0.963, 21.26)	3.57	0.056
		No	29	Reference		
	Total number of people that handled the chicks[a]	2-4	35	4.39 (0.82, 23.5)	3.75	0.084
		0-1	23	Reference		
	Procedure for washing the chick boxes	Low Pressure	29	3.98 (0.64, 24.7)	3.70	0.138
		High Pressure	19	Reference		

Table 4.1 (Continued)

GOWC	Procedure for washing the hatch trays [b]	Low Pressure	19	23.5 (1.18, 470.0)	35.91	0.039
		High Pressure	39	Reference		
	Humidity controlled in the chick room status [a]	Yes	29	17.8 (0.95, 332.2)	26.57	0.054
		No	29	Reference		
	Total number of people that handled the chicks [a]	2-4	35	14.7 (0.63, 344.8)	23.698	0.094
		0-1	23	Reference		
	Were eggs disinfected/fumigated in the hatcher [b]	Yes	37	0.08 (0.00, 1.76)	0.131	0.110
		No	21	Reference		
	Number of times setter washed per year	Twice	12	19.3 (0.46, 809.6)	36.85	0.120
		Once	46	Reference		
	Type of hatchery ventilation system	Evaporative Cooling	35	11.7 (0.49, 279.64)	18.93	0.129
		Air Conditioning	23	Reference		
PACA	Number of breeder flocks that provided eggs for the sampled flock [a]	≥ 2 farms	27	2.3 (1.01, 5.13)	0.94	0.047
		1 farm	29	Reference		
PACP	Total number of people that handled the chicks	2-4	36	11.8 (0.74-188.8)	16.7	0.080
		0-1	21	Referent		
	Number of times setter washed per year	Twice	10	13.8 (0.41, 468.3)	24.8	0.144
		Once	47	Reference		
PAWC	Number of times setter washed per year	Twice	10	46.2 (1.50, 1419.2)	80.7	0.028
		Once	47	Reference		
	Number of breeder farms provided eggs for sampled flock	≥2 farms	27	1.7 (1.0, 2.7)	0.41	0.033
		1 farm	29	Reference		
	Total number of people that handled the chicks [a]	2-4	36	10.0 (0.55, 181.6)	14.8	0.119
		0-1	21	Reference		
	Humidity controlled in chick room status [a]	Yes	30	8.1 (0.6-118.9)	11.1	0.126
		No	27	Reference		
	Procedure for washing the hatch trays	Low Pressure	20	8.3 (0.5, 139.4)	11.9	0.143
		High Pressure	37	Reference		

Table 4.1 (Continued)

PPPO	Humidity controlled in chick room status [b]	Yes	30	25.8 (2.13-312.8)	32.8	0.011
		No	27	Reference		
	Number of times setter washed per year	Twice	10	33.1 (1.64, 669.46)	50.8	0.022
		Once	47	Reference		
	Are eggs are candled during incubation [a]	Yes	43	0.03 (0.00-0.77)	0.04	0.035
		No	14	Reference		
	Total number of people that handle the chicks [b]	2-4	36	10.9 (0.64-186.6)	15.8	0.099
		0-1	21	Reference		
	Procedure for washing the hatch trays	Low Pressure	20	17.7 (0.51-613.8)	32.0	0.112
		High Pressure	37	Reference		
	Type of ventilation system in the hatchery	Evaporative Cooling	34	18.3 (0.49-680.2)	33.7	0.115
		Air Conditioning	23	Reference		
	Procedure for washing the chick boxes	Low Pressure	30	8.93 (0.47-170.3)	13.4	0.145
		High Pressure	17	Reference		
	Has the egg room been disinfected/fumigated [a]	Yes	47	0.09 (0.00-2.50)	0.15	0.153
		No	10	Reference		
	When the chicks were vaccinated	Day 1	35	7.98 (0.44-144.2)	11.8	0.159
		In Ovo	22	Reference		

[a,b,c] Variables within the same outcome with the same superscript were correlated with r> 0.80

Table 4.2 Results of multivariable logistic regression analysis of association between hatchery risk factors and occurrence of *Campylobacter* in grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinse (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO)

Outcome	Model Number	Fixed Effects (response)	OR (95% CI)	p-value	AIC
GOCA	1	Humidity controlled in the chick room status (Yes/No)	84.72 (1.08, 6629.16)	0.046	793.50
	2	Total number of people that handle the chicks (2-4/0-1)	124.59 (1.38, 11214.27)	0.036	793.01
	3	Procedure for washing the hatch trays (low pressure/high pressure)	104.03 (1.00, 10824.99)	0.050	793.60
GOWC	1	Number of times setter washed per year (Twice/Once)	39.4 (1.22, 1264.48)	0.038	
		Humidity controlled in the chick room status (Yes/No)	32.6 (1.83, 582.85)	0.018	
PAWC	1	Number of times setter washed per year (Twice/Once)	56.9 (2.45, 1318.1)	0.012	1041.2
		Number of breeder farms providing eggs for sampled flock (≥2 farms/1 farm)	1.67 (1.04, 2.69)	0.034	
		Humidity controlled in the chick room status (Yes/No)	12.5 (1.11, 140.9)	0.041	
	2	Number of times setter washed per year (Twice/Once)	47.2 (1.92, 1159.1)	0.018	1041.6
		Number of breeder farms providing eggs for sampled flock (≥2 farms/1 farm)	1.69 (1.05, 2.73)	0.030	
		Total number of people that handle the chicks (2-4/0-1)	12.7 (0.90, 178.8)	0.059	
PPPO	1	Number of times setter washed per year (Twice/Once)	309.9 (19.24, 4990.8)	<0.001	
		Humidity controlled in the chick room status (Yes/No)	294.3 (17.04, 5083.6)	<0.001	
		When the chicks were vaccinated (Day 1/ In-Ovo)	0.03 (0.00, 0.56)	0.020	
		Procedure for washing the hatch trays (low pressure/high pressure)	6.42 (0.55, 75.29)	0.139	

Table 4.3 Results of univariable analyses in which risk factor variables were associated ($p \leq 0.15$) with outcomes at sampling

Risk Factor	Outcomes OR (p-value)						
	GOCA	GOCP	GOWC	PACA	PACP	PAWC	PPPO
Humidity controlled in the chick room status (Yes/No)	84.7 (0.046)	4.52 (0.056)	17.8 (0.054)			8.1 (0.126)	25.8 (0.011)
Total number of people that handled the chicks (2-4/1-0)	124.6 (0.036)	4.39 (0.084)	14.7 (0.094)		11.8 (0.080)	10.0 (0.119)	10.9 (0.099)
Procedure for washing the hatching trays (low pressure/high pressure)	104.0 (0.050)		23.5 (0.039)			8.3 (0.143)	17.7 (0.112)
Procedure for washing the chick boxes (low pressure/high pressure)		3.98 (0.138)					8.93 (0.145)
How often chick transport vehicles washed (less often/between hatchery days)	38.5 (0.111)						
Are chick transport vehicles disinfected (Yes/No)	0.02 (0.115)						
Were eggs disinfected/fumigated in hatcher (Yes/No)	0.03 (0.137)		0.08 (0.110)				0.09 (0.153
Number of times setter washed per year (Twice/Once)			0.05 (0.120)		0.07 (0.144)	0.02 (0.028)	0.03 (0.022)
Type of hatchery ventilation system (Evaporative cooling/Air conditioning)			11.7 (0.129)				18.3 (0.115)
Number of breeder flocks that provided eggs for the sampled flock (≥2 farms/1 farm)				2.3 (0.047)		1.7 (0.033)	
Are eggs candled during incubation (Yes/No)							0.03 (0.035)
When were the chicks vaccinated (Day 1/In-ovo)							7.98 (0.159)

*Shaded boxes indicate a univariable association $p \leq 0.05$

Table 4.4 The variance (percent variance) occurring at the complex, farm, and bird level and the total variance at each outcome using a null model for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Complex	Farm	Bird	Total
GOCA	0 (0.0)	32.38 (90.8)	3.29 (9.2)	35.67
GOCP	0 (0.0)	4.39 (57.2)	3.29 (42.8)	7.68
GOWC	0 (0.0)	14.10 (81.1)	3.29 (18.9)	17.39
PACA	0 (0.0)	32.56 (90.8)	3.29 (9.2)	35.85
PACP	0 (0.0)	13.84 (80.8)	3.29 (19.2)	17.13
PAWC	0 (0.0)	13.15 (80.0)	3.29 (20.0)	16.44
PPPO	1.6 (12.0)	8.4 (63.2)	3.29 (24.8)	13.29

Table 4.5 Intra-class correlations, using a null model, for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Birds within the same farm	Birds within the same complex but different farms	Farms within the same complex
GOCA	0.91	0	0
GOCP	0.57	0	0
GOWC	0.81	0	0
PACA	0.91	0	0
PACP	0.81	0	0
PAWC	0.80	0	0
PPPO	0.75	0.12	0.16

CHAPTER V

BIOSECURITY RISK FACTORS ASSOCIATED WITH *CAMPYLOBACTER* FLOCK STATUS IN THE BROILER PRODUCTION AND PROCESSING CONTINUUM IN THE SOUTHEASTERN UNITED STATES

Summary

Campylobacter remains a leading food borne pathogen in the United States and poultry has been identified as a major reservoir. The main objective of this prospective observational study was to identify biosecurity risk factors throughout the production and processing continuum that were associated with *Campylobacter* presence within the grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass (PAWC), and post-chill (PPPO). Survey instruments were used to gather information on biosecurity practices used on the farm. Multilevel logistic regression was used to evaluate farm biosecurity characteristics as risk factors for *Campylobacter* presence at various sampling outcomes as well as to estimate the proportion of variance and the intraclass correlation coefficients. This study identified protective factors that emphasize the importance of the hygiene of the workers on the farm including the use of footbaths and dedicated shoes, greater frequency of entering the house during brooding, disinfectant added to the drinker lines, having concrete outside the most used door (multivariable analysis), and the cleanliness of the workroom, which is likely a proxy for the overall hygiene habits on the

farm. Having more walk-in doors on the house, the farmer removing the litter, concrete at most used door (univariable analysis), and the number of workers were associated with increased risk of *Campylobacter* positive samples. Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. This information indicates that intervention efforts should focus on factors at the farm level i.e. factors that vary among farms within a complex.

Keywords

Campylobacter; Broiler; Poultry; Food Safety; Biosecurity; Multilevel Analysis

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- Intervention efforts should focus on factors at the broiler farm level i.e. factors that are different among farms within a broiler complex.
- Hygiene of the workers on the farm including the use of footbaths and dedicated shoes and the cleanliness of the workroom as well as other variables were associated with reduced risk of *Campylobacter* in broilers.
- Having more walk-in doors on the house, the farmer removing the litter, and the number of workers on a farm were associated with increased risk of *Campylobacter* positive samples.

5.1 Introduction

Consumption, cross-contamination, and handling of undercooked poultry has been identified as the major sources of human campylobacteriosis (Batz, Hoffmann et al. 2012). In the United States, 608,231 illnesses, 6,091 hospitalizations, and 55 deaths have been attributed to poultry products and costs 1,747 million dollars, annually (Batz, Hoffmann et al. 2012). Symptoms of the disease typically include headache, fever, severe

abdominal cramps, watery or bloody diarrhea, and sometimes nausea and vomiting (CDC 2013). Infections are typically self-limiting and clear after a week, however, in some cases more severe sequelae have been reported, such as reactive arthritis, Guillian-Barŕe syndrome, Miller-Fisher syndrome, meningitis, bacteremia, and septicemia (Kaldor and Speed 1984, Dhawan 1986, Roberts 1987, Mishu 1993, Ladrón de Guevara C 1994, Allos 1997, Hughes and Res 1997, Lastovica 1997, Saida, Kuroki et al. 1997, Nielsen 2009, CDC 2013).

The poultry intestinal tract, especially the ceca (Oosterom, Engels et al. 1983, Stern, Clavero et al. 1995), crop (Byrd, Corrier et al. 1998, Smith and Berrang 2006), and the skin and exterior feathers (Kotula and Pandya 1995, Stern, Clavero et al. 1995, Berrang and Dickens 2000) of the birds, when positive, are known to harbor large numbers (10^9cfu/g) of *Campylobacter* (Berndtson, Tivemo et al. 1992, Berrang, Buhr et al. 2000, Berrang and Dickens 2000, Smith and Berrang 2006). High levels of *Campylobacter* brought into poultry plants due to exterior contamination of the bird after transportation (Stern, Clavero et al. 1995, Berrang, Buhr et al. 2000) and interior contamination (gut leakage or accidental gut tearing) (Berrang, Buhr et al. 2000) introduce the strong possibility of high *Campylobacter* incidence rates from cross-contamination. Thus, control of contamination must begin prior to processing, at the farm, in order to further reduce the contamination on the broilers coming into the processing plant.

Clothes, hands, tools and especially boots can act as mechanical vehicles for transmission of *Campylobacter* from farm surrounding (i.e. puddles, other animals, used litter piles) into the broiler houses (Jacobs-Reitsma 1997, Johnsen, Kruse et al. 2006,

Ridley, Allen et al. 2008). Reducing and/or preventing such transmission begins with proper hygiene practices and research has shown these practices to be important factors when trying to prevent or reduce the risk of *Campylobacter* contamination in the house (Humphrey, Henley et al. 1993, van De Giessen, Tilburg et al. 1998, Shreeve 2000, Gibbens, Davies et al. 2001). House specific boots (Hald 2000, McDowell, Menzies et al. 2008), clothes (Gibbens, Davies et al. 2001, Cardinale, Cisse et al. 2004, McDowell, Menzies et al. 2008), hand washing (McDowell, Menzies et al. 2008) use of overshoes, and the effective use of boot dips (Humphrey, Henley et al. 1993, Evans and Sayers 2000, Gibbens, Davies et al. 2001) have all been associated with a reduced risk of flock infection.

Due to the differences in the size of the poultry production industry between countries, the feasibility and economic ability to impose some biosecurity standards can be difficult. In the United States, biosecurity and hygiene recommendations exist; however, implementation at the company and farm level can vary. The number of quantitative epidemiological investigations to identify risk factors associated with *Campylobacter* positive flocks within the United States poultry industry are lacking. Thus, the objective of this study was to identify biosecurity risk factors that may be associated with the increased presence of *Campylobacter* within a flock.

5.2 Materials and methods

5.2.1 Sampling strategy

This prospective observational study was conducted in 3 states (Alabama, Mississippi, and Louisiana) within the southeastern United States from 2003-2006. Two companies that were thought to be representative of the regional poultry industry

participated in the study. A complex was defined as having its own hatchery, feed mill, and processing plant. Company A was comprised of 4 complexes while Company B was comprised of 5 complexes. In Company A, 4 grow-out farms from each of 2 complexes and 3 farms from each of the other 2 complexes were selected for a total of 14 farms. Company B was comprised of 5 farms from each of 2 complexes and 4 farms from each of 3 complexes for a total of 22 farms. Two houses from each of the 36 farms were selected for a total of 72 houses. The 2 houses that were selected from each farm for sampling were usually a house on the end of the row and the adjacent house. In total, there were 72 flocks sampled from 36 farms which were sampled from 9 complexes which were selected from 2 companies. The companies selected the farms to be sampled prior to placement so flocks could be processed on Monday or Tuesday to allow for ease of transport and processing of samples.

The sampling strategy was to follow each flock through the production and processing continuum taking samples from each flock at 4 points: (1) 1 week prior to the end of grow-out and before transportation, (2) after transportation at plant arrival, (3) prior to chilling, and (4) at post-chill.

5.2.2 Sample Collection

5.2.2.1 End of grow-out whole carcass rinse, ceca and crop samples

The first sampling point was approximately one week before harvest. The ages of the individual flocks ranged from 48-61 days old. A convenience sample was taken by catching 30 birds at the cool-cell end of the house. The birds were humanely euthanized by cervical dislocation. A whole carcass rinse sample was taken for each of the 30 birds by placing the carcass into a sterile biohazard bag with 250ml of 1% buffered peptone

water (BPW) (Difco, Sparks, MD). The carcasses were vigorously shaken for 1 minute and the rinsate was aseptically transferred into a sterile plastic bottle. Following the collection of the whole carcasses rinses the crop and ceca were aseptically removed from each carcass. Each cecum was placed into a sterile Whirl-Pak® Bag (NASCO, Fort Atkinson, WI) and each crop was placed into a Whirl-Pak® Filter Bag (NASCO, Fort Atkinson, WI). BPW was added to each crop sample to make a 1:10 dilution by weight. Samples were placed on wet ice (18 h) and shipped overnight to the Food and Feed Safety Research Unit at College Station, Texas. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

5.2.2.2 Plant arrival whole carcass rinse, ceca and crop samples

The second sampling point was upon arrival at the processing plant. Three trucks were used to transport the flocks to the processing plant. A convenience sample of 2 birds from each of 5 cages was taken from each of the 3 trucks for sampling, totaling 30 birds per flock. A whole carcass rinse sample (described above) was taken for each of the 30 birds and tested for *Campylobacter*. The crop and ceca were removed aseptically from each of the same 30 birds (as descried above), packed on ice and transported to the laboratory. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

5.2.2.3 Pre-chill and post-chill carcass rinse samples

The third and fourth sampling points were taken within the processing plant before the carcasses entered the immersion chill tank and upon exiting the chill tank. Carcass rinse samples were taken from 30 birds before entering the immersion chill tank

and upon exiting the immersion chill tank. The carcass rinse samples were collected as described above, except 100ml BPW was added to the bag. The samples for each flock were taken at a repeating time interval so that the entire flock was sampled. Thus, 30 carcass rinses were sampled before the birds entered the chill tank and 30 carcass rinses were sampled upon exiting the chill tank for each of the flocks.

5.2.3 *Campylobacter* isolation and identification

Upon arrival at the Food and Feed Safety Research Unit at College Station, TX, the samples were incubated at 42°C for 24 hours. Selective enrichment was then performed for all samples except for the ceca by transfer of 10ml of the sample to 10 ml of 2x Bolton broth (Lab M, Bury, Lancashire, UK) and allowed to incubate for 24 hours at 42°C in a microaerobic environment (5% O_2, 10% CO_2, and 85% N_2). Each crop and ceca sample was then streaked onto Campy-Cefex agar (Becton, Dickinson and Co., Baltimore, MD) and allowed to incubate for 48 hours 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).

5.2.4 Questionnaire

Three different evaluation instruments were developed to collect information concerning management practices and characteristics of each farm. The first instrument was a questionnaire (Volkova 2007) to be filled out by the farmer and contained 8 sections and a total of 85 questions. The 8 sections contained questions on biosecurity

and sanitary practices, visitor biosecurity practices, litter and house sanitary practices, housing characteristics, housing ventilation and lighting systems, feeding and watering, pest and fauna control, and workroom and instrument sanitation (Volkova, Wills et al. 2011). The research team utilized the other two instruments (checklists). The first checklist (Volkova 2007) was completed on day 1 and collected information on the transportation of chicks from hatchery to farm, unloading the chicks, characteristics on territory around the house, characteristics of the house, litter, brooding, presence of pests and their control, and workroom and equipment characteristics. The team's second checklist (Volkova 2007) was completed in week 7 and addressed some of the same questions from the day 1 check-list, including biosecurity and sanitation conditions, that could be used for comparison (Volkova, Wills et al. 2011)

Pilot testing for the questionnaire was conducted on two occasions. First, the questionnaire was administered to two poultry veterinarians that were actively involved with the project. Secondly, after editing, the questionnaire was administered to the managers of two broiler complexes in the area of study. Further edits were made before the final instrument was adopted.

5.2.5 Sample size calculation

5.2.5.1 Number of flocks

The number of flocks used in this study was determined by a rule of thumb of 10 subjects, in this case flocks, per explanatory variable (Petrie and Watson 1999). Therefore, 72 flocks were used which would allow for 7 explanatory variables to be put into each final model.

5.2.5.2 Number of samples per flock

This study was conducted in conjunction with another study that looked at the presence of *Salmonella* in broiler production and processing. The USDA-FSIS reported the national prevalence of *Salmonella* was 10.2% (Progress Report on *Salmonella* Testing of Raw Meat and Poultry Products, 1998-2000, http://www.fsis.usda.gov/ophs/haccp/salmdata2.htm) and that of *Campylobacter* was higher with a prevalence of 21-41% post-chill (Stern, Ladely et al. 2001). The goal was to be able to detect both *Campylobacter* and *Salmonella*. A sample size of 30 birds per flock was adopted which would detect at least a within-flock prevalence of ≥9.5% with 95% confidence (Cannon and Roe 1982), which would ensure detection of *Salmonella* and *Campylobacter* in all flocks where the prevalence was greater than the national *Salmonella* average (the lower of the two prevalences).

5.2.6 Statistical procedures

The *Campylobacter* status (positive or negative) was used to model the relationship between risk factors in the grow-out and processing phases and the following sampling points: grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinse (GOWC), plant-arrival ceca (PACA), plant-arrival crop (PACP), plant-arrival whole carcass rinse (PAWC), and post-chill whole carcass rinse (PPPO).

The data was analyzed using STATA software version 15.1 (StataCorp LP, College Station, TX, USA). Multilevel mixed-effects logistic regression (MEQRLOGIT) was used to develop causal models for the presence of *Campylobacter* as well as to estimate the percentage of variance in *Campylobacter* prevalence at each level of the hierarchical structure.

The sampling hierarchy was birds nested within flocks, flocks nested within farms, farms nested within complexes, and complexes nested within company. Company was the highest level of the hierarchy and was not included as a random effect because two companies were too few to accurately estimate the amount of variance at that level. Instead, company was included as a fixed effect to account for any variation between companies; however, company was not found to be significant and was dropped from all models. Flock was also not included as a random effect due to convergence issues, which was attributed to nearly identical prevalence of *Campylobacter* in the two flocks on each of the farms. Complex was also removed from the models of all outcomes except PPPO, due to lack of variance present at that level and to convergence issues. Consequently, farm and bird were included as random effects in GOCA, GOCP, GOWC, PPCA, PPCP, and PPWC outcome models. The PPPO outcome contained the random effects farm, bird, and complex. The proportion of total variance attributed to each of the random effects were estimated using the latent variable approach which assumes a logistic distribution and a level-one (i.e. birds) variance of $\pi^2/3= 3.29$ (Dohoo, Martin et al. 2009). The intraclass correlation coefficient for birds within the same farm was calculated by dividing the variance of the farm plus the complex by the total variance. The intraclass correlation coefficient for birds within the same complex but different farms was calculated by dividing the variance of the complex by the total variance. The interclass correlation coefficient for farms within the same complex was calculated by dividing the variance of the complex by the sum of the farm and complex variance.

Explanatory variables were included in the analysis if the categories within that variable contained a frequency of >10%. If the variables contained categories with a

frequency of ≤ 10%, that category would be combined with another category if there was biological plausibility to do so. If not, that variable was not used in the analysis.

A univariable analysis was performed for each of the outcomes variables as described above and only those variables with a p-value less than 0.15 were considered as candidates for the multivariable analyses.

All variables were checked for collinearity prior to the multivariable analyses. Categorical variables were first coded as zero and one and then collinearity was assessed between all variables using Spearman's rank correlation. If the coefficient was greater than 0.8, then one or the other explanatory variable was included in a multivariable model, but not both (Dohoo 2009). When collinearity did exist, and in some cases there was no biological plausibility for selecting any one variable over another, the two variables were entered into separate models and the final model with the smallest Akaike Information Criterion (AIC) was selected.

The assumption of a linear relationship between each continuous predictor variable and the relevant outcome variable was evaluated by generating a lowess plot of the logit vs. the predictor values and evaluated visually. If the lowess curve looked to be non-linear then basic transformations were used to see if linearity could be achieved. If linearity could not be achieved, then variables were categorized and reassessed in the univariable model.

Non-significant ($p > 0.05$) predictor variables were removed from the multivariable models using a manual backward selection process. Each variable that had been eliminated during the model selection process was reintroduced in the final reduced model to determine significance in the absence of non-significant variables. Furthermore,

each eliminated variable was assessed for confounding as each non-significant variable was removed from the model. A variable was deemed a confounder and forced into the final model if the coefficient of a significant variable changed by more than 20 percent (Dohoo 2009). Interactions between predictor variables were explored when it made biological sense. Causal models, containing no intervening variables, were constructed for each of the outcome variables (Dohoo, Martin et al. 2009).

5.3 Results

5.3.1 Surveys Collected

Of the 68 flocks sampled, the farmers completed questionnaires for 64 sampled flocks. The 4 surveys that were not returned were all from the same company. This is likely due to competing time of the managers since Hurricane Katrina occurred in the middle of the sample collection period in 2005.

The number of daily workers included workers that worked with chickens on the farm on a daily basis. Farms had 1 daily worker (31%) or 2-3 daily workers (69%). The number of times per day that workers entered the house during brooding ranged from 1.5-11 entrances with a mean of 4.8. The number of times per day that workers entered the house during the rest of production ranged from 1-7 entrances with a mean of 4.

Biosecurity practices utilizes by farm personnel varied among farms. Footbaths were reported by farmers as being utilized by workers before entering the house in 66% of the flocks. Of those farms that used footbaths, they were changed weekly (52%) or more than weekly (48%). The farmers reported the footbaths contained disinfectant, although, the footbath disinfectant concentration was monitored in only 2 of the 42 houses with footbaths. Footbaths were located near the most frequently used door at all

houses. Footbaths were reported to have been changed either as needed (16/42), weekly (18/42), or less than weekly (8/42) although we could not analyze this variable further due to the limited number of houses with footbaths. Dedicated shoes were reported as being used before entering a house in 63% of houses. Workers had dedicated clothes they used before entering the house in 19% of flocks. Workers washed their hands prior to entering the house in only 13% of flocks. Workers wore disposable boots in 6% of flocks and washed and disinfected boots in 9% of flocks, but these variables could not be considered for inclusion in the study due to the few responses. Routine practices of company and non-company personnel included use of disposable boots in 88% of the flocks. The chick unloading team used a footwear biosecurity practice (disposable footwear, footbaths, or disinfected boots) before placing the chicks in the house in 46% of flocks. The other 54% did not use any foot protection.

Fifty-three percent of the flocks were on farms that also farmed cattle. Workers that worked on the farm were reported to also work with the cattle in 49% of the flocks and with cattle and other animals in 53% of the flocks.

The water for the birds came from a treated community water source (23%) or from a well (77%). The well water was nontreated water at all farms except for 1. Before flock placement into the house, the drinker lines were flushed in 94% of the houses. In 66% of houses, disinfectant was added in the water for flushing the drinker line. Disinfectant used (Bleach, DAC 20, Saniclean, Iodine, Proxyclean, and PWT) varied between farms and could not be analyzed further due to low responses for some disinfectants.

Tractors and implements were dedicated for use in the chicken houses in 50% of sampled flocks. In 59% of flocks, the tractors and implements were washed prior to the sampled flock. The tractors and implements that were used away from the chicken houses were washed in 43.5% of the sampled flocks. When the litter was completely changed in a house, the farmer removed the litter in 55% of the flocks whereas a contractor removed the litter in 45% of flocks.

The surface outside of the most used door into the house was concrete in 75% of the houses and was another material (gravel, dirt, wood, or vegetation) in the other 25% of houses. The number of walk-in doors to the houses ranged from 2-6 with the mean being 4.5 and a mode of 5. Eighty percent of houses have between 4-6 doors.

The cleanliness of the workroom was assessed by the research team on day 1 when chicks were placed and at the end of grow-out (1 week before harvest). Fifty eight percent of the flocks sampled were in houses with clean workrooms on day 1. At end of grow-out, 41% of flocks were in houses with clean workrooms.

5.3.2 Univariable analysis

The results of the univariable analysis are listed in 5.1. Variables that met the screening criteria ($p \leq 0.15$) for any outcome were considered in the corresponding multivariable analysis. There was no correlation (above 0.8) between the explanatory variables.

5.3.3 Multivariable analysis

The final multivariable models are listed in 5.2. The final model for the GOCA outcome included the workroom clean on day 1 (OR=0.13, CI= 0.05-0.31) and workroom

clean at the end of grow-out (OR=0.02, CI=0.000-1.48). Concrete at the most used door was included as a confounding variable. Workroom clean at the end of grow-out was not significant (p=0.074) but was included in the model because it was significant(p=0.039) in the absence of the confounding variable. The final model for the GOCP outcome included number of walk-in doors (OR=4.00, CI=1.99-8.08). The workroom clean at the end of grow-out was also included as a confounding variable. The GOWC outcome contained 2 models. The first multivariable model contained 2 significant variables which were the number of daily workers (OR=206.56, CI=2.76-6316.75) and concrete at the most used door (OR=0.04, CI=0.002-0.95). It also contained workroom clean at the end of grow-out and the farmer removes the litter as confounding variables. The AIC for model 1 was 823.6. The 2nd multivariable model contained the number of daily workers (OR=44.27, CI=2.04, 961.34) and the workroom clean at the end of grow-out (OR=0.03, CI=0.00, 0.39) but without any confounding variables forced into the model during the model selection process.

The PACA multivariable outcome contained the workroom clean at day 1 (OR= 0.26 (0.13, 0.49). It contained concrete at the most used door as a confounding variable. The PACP model contained workers use footbaths before entering the house (OR=0.04, CI=0.002, 0.57) and the number of walk-in doors (OR=2.05, CI=1.41, 2.97). The PAWC model contained the number of times workers entered the house during brooding (OR=0.53, CI=0.32, 0.90) and workroom clean at the end of grow-out (OR=0.11, CI=0.01, 0.97). The PPPO model analysis resulted in a single univariable model containing workroom clean at the end of grow-out (OR=0.10, CI=0.01, 0.75).

The variance and percent of total variance occurring at the complex, farm, and bird level and the total variance at each outcome is displayed in 5.3. The PPPO outcome was the only outcome that showed variance occurring at the complex level. The farm level variance ranged from 57.2%-90.8% variance while the bird level variance ranged from 9.2%-42.8%. The intraclass correlation coefficients for birds within the same farm, birds within the same complex but different farms, and farms within the same complex are listed for each outcome in 5.4. The intraclass correlation coefficients for birds within the same farm ranged from 0.57-0.91. Birds within the same complex but different farms and farms within the same complex had zero intraclass correlation except with the PPPO outcome. For this variable, the intraclass correlation for birds within the same complex but different farms and farms within the same complex were 0.12 and 0.16, respectively.

5.4 Discussion

5.4.1 Number of daily workers/number of visits to the house

The number of daily workers was found to be a significant ($p \leq 0.15$) variable with 2 of the univariable models (GOWC, PACA). It stayed significant through the multivariable model selection process in only the GOWC outcome. The odds of a bird having *Campylobacter* was 206 times greater in a farm that had 2-3 daily workers compared to 1 worker. An odds ratio this large may indicate excessive variation in the data or that there is indeed a large effect. Based on the *Campylobacter* literature and that the source of *Campylobacter* is likely from multiple sources, it is unlikely that this one variable has this large of an impact on *Campylobacte*r presence. However, we do believe there to be a relationship between *Campylobacter* presence and the number of workers

that enter the house daily. A second model was constructed for the GOWC to further explore the large odds ratio. The second model did not contain the confounding factors and the odds of a bird having *Campylobacter* was 44 times greater in a farm that had 2-3 daily workers compared to 1 worker. Our research is in agreement with other studies that identified the odds of a flock being *Campylobacter* positive to be 2-3 times greater in farms that had 2 or more people taking care of the flock (Refregier-Petton, Rose et al. 2001, Chowdhury, Sandberg et al. 2012)

For the PAWC multivariable model, the risk of a flock being *Campylobacter* positive increased the less the workers entered the house during brooding. This was a surprising finding as it is intuitive to think that the more often a worker enters a biosecurity area, the more opportunity of introducing *Campylobacter* into a flock. A French study that looked at risk factors for *Campylobacter* in free-range broilers found that flocks were more likely to be *Campylobacter* positive when the farmer inspected the flock twice (compared to 3 times) during the indoor rearing period (Huneau-Salaun, Denis et al. 2007). They found that this was more common on farms where poultry farming was a secondary production and likely spent less time with broilers and thus led to less rigorous flock management. The majority of the flocks in this study had workers that entered the house 3-6 times daily during brooding and we found no relationship with farms that farmed other animals. The number of visits inside the house during production was not a significant variable in this analysis. Collectively, this suggests an apparent association between PAWC and the number of visits inside the house during brooding is a spurious finding.

5.4.2 Workers tending other animals

Workers tending other livestock has been found to be a risk factor for the presence of *Campylobacter* in some studies (Kapperud 1993, Gregory 1997, van De Giessen, Tilburg et al. 1998) and not significant in other studies (McDowell, Menzies et al. 2008, Nather 2009). Our research found that workers that handled cattle and also worked with other animals was not associated with an increased risk of *Campylobacter* positive flocks.

5.4.3 Workers hygiene practices

On the farm, the lack of hygiene practices of the workers has been identified as an important risk factors when trying to prevent or reduce the occurrence of *Campylobacter* contamination in the house. The proper use of boot dips (Humphrey, Henley et al. 1993, van de Giessen, Bloemberg et al. 1996, Evans and Sayers 2000, Gibbens, Davies et al. 2001, Bouwknegt, van de Giessen et al. 2004, McDowell, Menzies et al. 2008), house specific boots (van de Giessen, Bloemberg et al. 1996, Hald 2000, Bull, Allen et al. 2006, McDowell, Menzies et al. 2008), dedicated clothes (Gibbens, Davies et al. 2001, Cardinale, Cisse et al. 2004, McDowell, Menzies et al. 2008), and hand washing (van de Giessen, Bloemberg et al. 1996, McDowell, Menzies et al. 2008) have all been identified as having a protective association against *Campylobacter* flock infection.

Our study is in agreement with those studies mentioned above that in houses where workers used footbaths, *Campylobacter* was less likely to occur in all of the outcome variables analyzed in the univariable analysis except for GOCP. This variable was also in the final multivariable model of the PPCP outcome. The odds of *Campylobacter* positive PACP samples were 3.9 times greater for flocks whose workers

did not use footbaths prior to entering the house. Dedicated shoes in this study meant farmers wore boots dedicated for use in tending all poultry on the farm and were not house specific shoes. Use of dedicated shoes was significantly associated in the univariable analysis with GOCA, GOWC, PACA, PPPO; however, the variable fell out of all multivariable models. Clothes, hands, tools, and especially boots can act as mechanical vehicles from the farm surroundings into the poultry house (Jacobs-Reitsma 1997, van De Giessen, Tilburg et al. 1998). It has been previously reported that it is easy to be carless during the hurry of a daily routine and just dip toes, heels, or quickly pass through the boot dip and sometimes still have clumps of mud on the boots (Berndtson, Emanuelson et al. 1996). Farm biosecurity is difficult to maintain through the life of the flock due to the ubiquitous nature of the organism and low infective dose (Shreeve 2000). However, enhanced biosecurity has resulted in reduction in *Campylobacter* although not complete elimination (van De Giessen, Tilburg et al. 1998, Shreeve 2000).

5.4.4 Workroom Presence and Cleanliness / Presence of concrete stoop

In the U.S. poultry houses typically have workrooms (in Europe known as ante-room or changing room) located inside one of the entrances to the house. Ideally, the workroom is located at the main entrance and acts as a hygiene barrier where footwear can be changed or disinfected, hands can be disinfected, and clothes can be changed before entering the house. Research has shown the presence of a hygiene barrier to be an important factor in producing *Campylobacter* free poultry (Berndtson, Emanuelson et al. 1996, Hald 2000, Hansson, Vågsholm et al. 2007, McDowell, Menzies et al. 2008).

Our study showed that just the presence of a workroom did not reduce the likelihood of producing *Campylobacter* positive flocks at the univariable level. It is possible that in the farms sampled that the workroom is not treated as a strict hygiene barrier.

In this study workroom clean on day 1 was a significantly protective in the univariable and multivariable models for GOCA and PACA. Workroom clean at the end of grow-out was also significantly protective at the univariable level in GOCA, GOWC, and PPPO. It was also significant in the multivariable models for GOCA, GOCP, GOWC, PAWC, and PPPO. Research has shown that the cleanliness of the workroom is an important biosecurity practice in preventing *Campylobacter* positive flocks (Humphrey, Henley et al. 1993, Kapperud 1993, McDowell, Menzies et al. 2008). This study is in agreement with these other studies; although, the cleanliness of the workroom variable is likely a proxy for the cleanliness habits of the workers. Keeping only the workroom clean is unlikely to result in *Campylobacter* free flocks. A clean workroom may indicate stricter adherence to biosecurity rules on the farm including proper use of footbaths, etc.

In this study, farms that have concrete (compared to wood, vegetation, dirt, or gravel) in front of the most used door to a house are more likely to have *Campylobacter* positive GOWC samples in the univariable analysis. Paradoxically, in the multivariable models, concrete at the most used door had a significant protective effect on GOWC. It was a confounding variable in the GOCA and PACA models. Further analysis indicated houses that have concrete outside of the main door to the house are more likely to have a clean workroom on day 1. Concrete may facilitate better cleaning of footwear before entering the workroom. It may also be less likely to harbor *Campylobacter* compared to

other substrates. We speculate the increased risk of positive GOWC seen in the univariable analysis is spurious or due to unidentified confounders.

5.4.5 Poultry water source and drinker line disinfection

In this study, the use of well water (compared to treated community water) was not found to be significantly associated with any of the outcomes. In water, *Campylobacter* can be found in water in the viable but non-culturable form (VBNC) (Pearson, Shahamat et al. 1993) especially when in a biofilm (Trachoo and Frank 2002). Water samples taken from broiler houses have been mostly negative (Hansson, Vågsholm et al. 2007) due to the difficulty of isolating *Campylobacter* from the small samples and the difficultly culturing the VBNC *Campylobacter* cells (Kapperud 1993). Other studies have also found the water source to be of little risk (Humphrey, Henley et al. 1993, Berndtson, Danielsson-Tham et al. 1996). Some studies have reported positive water samples but they have always occurred after the flock was positive (Gregory 1997, Herman, Heyndrickx et al. 2003, Bull, Allen et al. 2006). One risk factor study indicated providing broilers undisinfected water increased the risk of flock colonization (Kapperud 1993).

Disinfectant added to the drinker lines before placement of the flocks was significantly protective in GOCP, GOWC, PACP, PPPO outcomes of the univariable analysis; however, it also fell out of the multivariable models for all outcomes. Campylobacter has been found to survive in water and biofilms (Buswell, Herlihy et al. 1998, Trachoo, Frank et al. 2002) and biofilms are often present in water supply and plumbing systems. In this study the source (community or well) of the water did not have an influence on the *Campylobacter* flock status, however disinfecting the line prior to the

placement of each flock did have a protective effect at the univariable level but not at the multivariable level. Other researchers have found water lines in poultry houses to be positive for *Campylobacter* (Berndtson, Danielsson-Tham et al. 1996, Johnsen, Kruse et al. 2006, Schroeder, Eifert et al. 2014). In one study the water lines were positive for identical *Campylobacter* subtypes as the present flock but only after the flock was positive, suggesting the flock shedding *Campylobacter* was responsible for contaminating the environment (Hiett, Stern et al. 2002). Disinfection of the water source and lines has been found to be associated with fewer *Campylobacter* positive flocks (Evans and Sayers 2000). While our research does not add to the knowledge on which direction transmission occurs between water sources and poultry houses, it does suggest disinfecting the lines is associated with reducing *Campylobacter* presence.

5.4.6 Number of walk-in doors

The number of walk-in doors was significant in both the univariable and multivariable models for GOCP and PACP. Houses that have more doors are more likely to have positive flocks. The odds of a flock having *Campylobacter* was 1.93-4.00 times greater for each additional door a broiler house had above 2. This is likely due to workers using doors other than the main door and not going through the hygiene barrier that is typically located at the main door.

5.4.7 Farm Equipment

Vehicles and equipment can be a source of contamination if contractors are used on multiple farms to remove birds (Ridley, Morris et al. 2011). Molecular strain typing was used in another study to track the source of *Campylobacter* contamination and found

that *Campylobacter* contamination had spread from one farm to another by use of the same vehicles and/or catching crew (Allen, Weaver et al. 2008). In the current study the farmer removing the litter from the farm was associated with a higher risk of *Campylobacter* compared to the contractor in the PACP of the univariable analysis and was a confounder in the GOWC multivariable model. One possible explanation may be that contractors use a higher level of sanitation than farmers do between flocks. Alternatively, stock piling or composting the litter on the farm allows *Campylobacter*, if present in the litter, to remain a source of contamination on the farm. Further analysis identified that of the 16 farms that had litter removed by the farmer, 13 farms stockpiled the litter somewhere on the farm. Having tractors dedicated to the house, washing the tractors, or using the tractors away from the house were not significantly associated with any of the outcomes in this study.

5.4.8 Proportion of variance and interclass correlation

Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. This information indicates that intervention efforts should focus on factors at the farm level i.e. factors that vary among farms within a complex. The intraclass correlations for each of the outcomes indicates that there is high correlation among birds within the same farm and no correlation, with the exception of the PPPO outcome, among birds within the same complex but different farms and farms within the same complex. It is reasonable to think that there is increased correlation, for PPPO, among birds within the same complex and among farms within the same complex since complexes are defined by a shared processing plant. The increased

correlations that become evident at post-chill are likely due to the cross-contamination and decontamination that can occur within a processing plant.

5.5 Conclusion

Although epidemiological studies have identified many risk factors for *Campylobacter* broiler colonization, our research evaluated those risk factors under the poultry production conditions within the south-eastern United States. This study identified protective factors that emphasize the importance of the hygiene of the workers on the farm including the use of footbaths and dedicated shoes, greater frequency of entering the house during brooding, disinfectant added to the drinker lines, having concrete outside the most used door (multivariable analysis), and the cleanliness of the workroom, which is likely a proxy for the overall hygiene habits on the farm. Having more walk-in doors on the house, the farmer removing the litter, concrete at most used door (univariable analysis), and the number of workers were associated with increased risk of *Campylobacter* positive samples. The highest proportion of variance occurred at the farm level indicating intervention efforts should focus on factors at the farm level.

5.6 Acknowledgements

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Table 5.1 Results of univariable logistic regression analysis of association between biosecurity factors within the grow-out environment and the occurrence of *Campylobacter* in grow-out ceca samples (GOCA), grow-out crop samples (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca samples (PACA), plant arrival crop samples (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO), after accounting for the variability of the random effects of farm in all outcomes and complex in the PPPO outcome.

Variable Name	**Response**	**Mean (range) or count of flocks**	**GOCA**	**GOCP**	**GOWC**	**PACA**	**PACP**	**PAWC**	**PPPO**
			Odds Ratio (CI) p-value						
Number of daily workers	2 or 3	44	35.54 (0.233, 5424.24) *p*=0.164	2.20 (0.341,14.19) *p*=0.407	23.11 (0.729, 732.35) *p*=0.075	47.63 (0.511, 4443.40) *p*=0.095	6.030 (0.315, 115.37) *p*=0.233	6.012 (0.276, 130.99) *p*=0.254	3.326 (0.168, 65.77) *p*=0.430
	1	20	Referent						
Number of times enter house during brooding	Continuous	4.8 (1.5-11)	0.60 (0.21-1.74) *p*=0.35	0.97 (0.65, 1.45) *p*=0.88	1.01 (0.59, 1.73) *p*=0.96	1.13 (0.49, 2.59) *p*=0.78	0.85 (0.44, 1.62) *p*=0.62	0.66 (0.39, 1.14) *p*=0.14	0.72 (0.40, 1.31) *p*=0.29
Number of times enter house during production	Continuous	4.0 (1-7)	0.60 (0.15, 2.49) *p*=0.49	1.18 (0.65, 2.14) *p*=0.58	1.18 (0.63, 2.21) *p*=0.60	1.67 (0.59, 4.76) *p*=0.34	0.97 (0.39, 2.41) *p*=0.95	0.63 (0.32, 1.23) *p*=0.18	0.81 (0.38, 1.72) *p*=0.58
Workers use footbaths	Yes	42	0.02 (0.00, 1.79) *p*=0.09	0.35 (0.06, 2.01) *p*=0.237	0.04 (0.00, 0.74) *p*=0.031	0.03 (0.00, 1.90) *p*=0.10	0.05 (0.00, 0.72) *p*=0.03	0.04 (0.00, 0.64) *p*=0.02	0.09 (0.01, 1.10) *p*=0.06
	No	22	Referent						
Workers use dedicated shoes	Yes	40	0.01 (0.00, 0.91) *p*=0.05	0.29 (0.05, 1.68) *p*=0.17	0.03 (0.00, 0.73) *p*=0.03	0.01 (0.00, 0.48) *p*=0.02	0.14 (0.01, 2.14) *p*=0.16	0.09 (0.01, 1.42) *p*=0.09	0.06 (0.01, 0.83) *p*=0.04
	No	24	Referent						

5.1 Continued

Workers use dedicated clothes	Yes	12	0.03 (0.00, 15.86) *p*=0.271	0.25 (0.03, 2.35) *p*=0.23	0.04 (0.00, 2.90) *p*=0.14	0.03 (0.00, 7.89) *p*=0.21	0.58 (0.02, 18.05) *p*=0.75	0.08 (0.00, 2.90) *p*=0.17	0.14 (0.01, 4.34) *p*=0.37
	No	52	Referent						
Workers wash hands	Yes	8	0.17 (0.00, 179.55) *p*=0.62	0.36 (0.03, 4.55) *p*=0.43	0.38 (0.00, 39.64) *p*=0.68	0.61 (0.00, 347.07) *p*=0.88	0.93 (0.02, 56.00) *p*=0.97	0.35 (0.01, 20.33) *p*=0.61	0.00 (0.00, 0.00) *p*=0.97
	No	56	Referent						
Routine company & Non-company personnel use disposable boots	Yes	56	0.27 (0.00,217.76) *p*=0.70	0.81 (0.07, 9.96) *p*=0.87	0.76 (0.01, 75.95) *p*=0.91	0.30 (0.00, 138.37) *p*=0.70	0.61 (0.01, 37.55) *p*=0.81	0.68 (0.01, 43.70) *p*=0.85	0.19 (0.00, 6.89) *p*=0.36
	No	8	Referent						
Chick unloading team wear footwear or use boot disinfection	Yes	32	0.67 (0.01, 39.14) *p*=0.85	0.94 (0.20, 4.38) *p*=0.94	0.66 (0.04, 10.22) *p*=0.77	1.33 (0.02, 84.24) *p*=0.89	0.40 (0.03, 5.43) *p*=0.49	0.29 (0.02, 4.05) *p*=0.36	0.55 (0.04, 7.46) p=0.66
	No	38	Referent						
Workers also work with cattle	Yes	30	2.53 (0.03, 244.20) *p*=0.69	2.25 (0.43, 11.85) *p*=0.34	1.69 (0.08, 35.06) *p*=0.73	0.81 (0.01, 55.53) *p*=0.92	2.37 (0.15, 38.81) *p*=0.55	1.97 (0.12, 31.71) *p*=0.63	4.04 (0.34, 47.56) *p*=0.27
	No	34	Referent						
Workers also work with other animals	Yes	34	1.43 (0.01,140.33) *p*=0.88	1.59 (0.30, 8.57) *p*=0.59	0.93 (0.04, 19.35) *p*=0.96	0.71 (0.01, 48.23) *p*=0.88	2.02 (0.12, 32.83) *p*=0.621	1.56 (0.10, 24.89) *p*=0.753	2.20 (0.12, 39.44) *p*=0.59
	No		Referent						

5.1 (Continued)

Drinking water origin	Well water	46	9.02 (0.02,5342.5) *p*=0.50	2.71 (0.31, 23.72) p=0.37	4.90 (0.10, 430.94) p=0.42	74.77 (0.18, 30404.28) *p*=0.16	14.82 (0.44, 500.13) *p*=0.13	4.52 (0.12, 166.06) *p*=0.41	2.99 (0.12, 76.03) *p*=0.51
	Community	14	Referent						
Disinfectant added to drinker line between flocks	Yes	42	0.01 (0.00, 1.30) *p*=0.07	0.13 (0.03, 0.60) *p*=0.01	0.04 (0.00, 0.72) *p*=0.03	0.03 (0.00, 2.25) *p*=0.11	0.07 (0.00, 1.03) *p*=0.05	0.08 (0.00, 1.23) *p*=0.07	0.05 (0.00, 0.56) *p*=0.02
	No	22	Referent						
Farmer removes the litter from the farm	Farmer	32	42.51 (0.45, 3979.9) p=0.11	2.25 (0.43, 11.76) p=0.34	12.23 (0.57, 260.42) p=0.109	30.87 (0.55, 1734.42) p=0.10	17.91 (1.24,258.00) p=0.03	3.69 (0.20, 68.57) p=0.38	5.93 (0.62, 56.31) p=0.12
	Contractor	26	Referent						
Tractors dedicated to poultry houses	Yes	32	0.14 (0.00, 13.42) p=0.40	0.57 (0.10, 3.09) p=0.51	0.54 (0.03, 11.16) p=0.69	0.38 (0.01, 24.56) p=0.65	0.44 (0.03, 6.98) p=0.56	0.72 (0.04, 11.49) p=0.82	0.72 (0.06, 8.13) p=0.79
	No	32	Referent						
Tractors washed before sampled flock	Yes	38	2.44 (0.02, 258.33) p=0.71	1.78 (0.32, 9.81) p=0.51	1.04 (0.05, 23.00) p=0.98	9.66 (0.13, 729.04) p=0.30	3.68 (0.22, 61.21) p=0.36	0.99 (0.06, 17.00) p=0.99	0.95 (0.06, 15.06) p=0.97
	No	26	Referent						
Tractors used away washed	Yes	27	0.29 (0.06-1.53) p=0.15	0.38 (0.10, 1.48) p=0.17	0.67 (0.24, 1.88) p=0.45	0.71 (0.23, 2.19) p=0.56	2.31 (0.57, 9.36) p=0.24	0.15 (0.01, 2.24) p=0.17	0.56 (0.14, 2.25) p=0.36
	No	35	Referent						
Concrete at most used door	Yes	48	0.08 (0.00, 1.42 *p*=0.09	0.61 (0.24, 1.55) *p*=0.30	11.04 (1.57, 77.34) *p*=0.02	0.09 (0.01, 1.18) *p*=0.07	0.90 (0.13, 6.19) p=0.91	4.94 (0.74, 32.87) p=0.10	2.38 (0.49, 11.59) *p*=0.28
	No	21	Referent						

5.1 (Continued)

Number walk-in doors	Yes	4.5 (2-6)	1.36 (0.76, 2.43) *p*=0.30	2.42 (1.47, 3.99) *p*=0.00	1.36 (0.97, 1.92) *p*=0.08	1.20 (0.69, 2.08) *p*=0.53	1.93 (1.34, 2.79) *p*=0.00	1.11 (0.79, 1.55) *p*=0.55	1.30 (0.76, 2.23) *p*=0.34
	No		Referent						
Workroom presence	Yes	62	0.14 (0.00, 36.16) *p*=0.49	0.91 (0.10, 7.96) *p*=0.93	0.56 (0.01, 25.45) *p*=0.77	0.12 (0.00, 34.17) *p*=0.47	0.13 (0.00, 4.90) *p*=0.27	0.78 (0.02, 32.54) *p*=0.90	0.36 (0.01, 10.46) *p*=0.55
	No	10	Referent						
Workroom clean day 1	Yes	36	0.32 (0.16, 0.64) *p*=0.00	1.96 (0.93, 4.14) *p*=0.08	0.81 (0.47, 1.37) *p*=0.43	0.36 (0.20, 0.64) *p*=0.00	0.86 (0.48, 1.54) *p*=0.61	1.33 (0.71, 2.49) *p*=0.38	0.92 (0.32, 2.64) p=0.87
	No	26	Referent						
Workroom clean at end of grow-out	Yes	23	0.01 (0.00, 0.68) p=0.03	0.21 (0.04, 1.17) p=0.08	0.08 (0.01, 0.91) p=0.04	0.08 (0.01, 0.91) p=0.07	0.20 (0.01, 3.87) p=0.29	0.13 (0.01, 1.16) p=0.07	0.10 (0.01, 0.75) p=0.03
	No	33	Referent						

Light shading indicates p≤ 0.15, dark shading indicated p≤0.0

Table 5.2 Results of multivariable logistic regression analysis of association between biosecurity factors within the grow-out environment and the occurrence of *Campylobacter* in grow-out ceca samples (GOCA), grow-out crop samples (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca samples (PACA), plant arrival crop samples (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO), after accounting for the variability of the random effects of farm in all outcomes and complex in the PPPO outcome.

Outcome Variable	Model #	Explanatory Variable(s)	Response Mean (range)	OR (95% CI)	SE	p-value	AIC
GOCA	1	Workroom clean day 1	Yes	0.13 (0.05, 0.31)	0.06	0.000	
			No	Referent			
		Workroom clean at end of grow-out	Yes	0.02 (0.00, 1.48)	0.04	0.074	
			No	Referent			
		Concrete at most used door[a]	Yes	1.46 (0.01, 183.00)	3.61	0.877	
			No	Referent			
GOCP	1	Number walk-in doors	4.5 (2-6)	4.00 (1.99, 8.08)	1.44	0.000	
		Workroom clean at end of grow-out[a]	Yes	0.18 (0.03, 1.30)	0.18	0.090	
			No	Referent			
GOWC	1	Daily workers	2 or 3	206.56 (6.76, 6316.75)	360.47	0.002	823.6
			1	Referent			
		Concrete at most used door	Yes	0.04 (0.00, 0.95)	0.07	0.046	
			No	Referent			
		Workroom clean at end of grow-out	Yes	0.18 (0.02, 2.23)	0.23	0.182	
			No	Referent			
		Farmer removes litter himself a	Yes	2.22 (0.25, 19.87)	2.48	0.476	
			No, contractor	Referent			
	2	Daily workers	2 or 3	44.27 (2.04, 961.34)	2.41	0.016	824.5
			1	Referent			
		Workroom clean at end of grow-out	Yes	0.03 (0.00, 0.39)	0.04	0.008	
			No				

5.2. (Continued)

PACA	1	Workroom clean day 1	Yes	0.26 (0.13, 0.49)	0.09	0.000
			No	Referent		
		Concrete at most used door[a]	Yes	1.63 (0.01, 248.48)	4.19	0.848
			No	Referent		
PACP	1	Workers use footbaths	Yes	0.04 (0.00, 0.57)	0.05	0.018
			No	Referent		
		Number walk-in doors	4.5 (2-6)	2.05 (1.41, 2.97)	0.39	0.000
PAWC	1	Number of times entering house during brooding	4.8 (1.5-11)	0.53 (0.32, 0.90)	0.14	0.017
		Workroom clean at end of grow-out	Yes	0.11 (0.01, 0.97)	0.12	0.047
			No	Referent		
PPPO	1	Workroom clean at end of grow-out	Yes	0.10 (0.01, 0.75)	0.10	0.025
			No	Referent		

[a] Denotes variables included in the model as confounding factors.

Table 5.3 The variance (percent variance) occurring at the complex, farm, and bird level and the total variance at each outcome using a null model for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Complex	Farm	Bird	Total
GOCA	0 (0.0)	32.38 (90.8)	3.29 (9.2)	35.67
GOCP	0 (0.0)	4.39 (57.2)	3.29 (42.8)	7.68
GOWC	0 (0.0)	14.10 (81.1)	3.29 (18.9)	17.39
PACA	0 (0.0)	32.56 (90.8)	3.29 (9.2)	35.85
PACP	0 (0.0)	13.84 (80.8)	3.29 (19.2)	17.13
PAWC	0 (0.0)	13.15 (80.0)	3.29 (20.0)	16.44
PPPO	1.6 (12.0)	8.4 (63.2)	3.29 (24.8)	13.29

Table 5.4 Intra-class correlations, using a null model, for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Birds within the same farm	Birds within the same complex but different farms	Farms within the same complex
GOCA	0.91	0	0
GOCP	0.57	0	0
GOWC	0.81	0	0
PACA	0.91	0	0
PACP	0.81	0	0
PAWC	0.80	0	0
PPPO	0.75	0.12	0.16

CHAPTER VI

FARM CHARACTERISTICS ASSOCIATED WITH *CAMPYLOBACTER* FLOCK STATUS AT VARIOUS POINTS THROUGHOUT THE BROILER PRODUCTION AND PROCESSING CONTINUUM IN THE SOUTHEAST UNITED STATES

Abstract

Campylobacter remains a leading food borne pathogen in the United States and improper handling, cross contamination, and consumption of poultry products has been identified as a major reservoir. The main objective of this prospective observational study was to identify farm characteristics throughout the production and processing continuum that were associated with *Campylobacter* presence on broilers at grow-out ceca (GOCA), grow-out crop (GOCP), grow-out (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass (PAWC), and post-chill (PPPO). Survey instruments were used to gather information on farm characteristics. Multilevel logistic regression was used to evaluate farm characteristics as risk factors for *Campylobacter* presence at various sampling outcomes as well as to estimate the proportion of variance and the intraclass correlation coefficients. This study identified risk factors including the number of houses on a farm, standing water around house on day 1, wood interior house walls, vegetation adjacent to the exterior house footing, and the number of flocks on the last litter. Standing water around the house at 7 weeks and harvesting birds 56-63 days were

protective factors. Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. This information indicates that intervention efforts should focus on factors at the farm level i.e. factors that vary among farms within a complex.

Keywords

Campylobacter; Broiler; Poultry; Food Safety; Biosecurity; Multilevel Analysis

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- Intervention efforts should focus on factors at the broiler farm level i.e. factors that are different among farms within a broiler complex.
- Having more houses on the farm, standing water on day 1, wood interior walls, a vegetation surface next the house footing, and 6 or less flocks on the litter were associated with increased risk of *Campylobacter* in broilers.
- Harvesting birds at 56-63 days of age and other variables were associated with reduced risk of *Campylobacter* in broilers.

6.1 Introduction

Campylobacter continues to be an important human pathogen, as it is currently ranked third in annual disease burden within the United States (Scallan 2011). Consumption, cross-contamination, and mishandling of undercooked poultry has been identified as the major sources of campylobacteriosis (Batz, Hoffmann et al. 2012). On an annual basis in the United States, 608,231 illnesses, 6,091 hospitalizations, and 55 deaths have been attributed to poultry products at a cost of 1,747 million dollars (Batz, Hoffmann et al. 2012). Symptoms of the disease typically include headache, fever, severe abdominal cramps, watery or bloody diarrhea, and sometimes nausea and vomiting (CDC

2013). Infections are typically self-limiting and clear after a week, however, in some cases more severe sequelae have been reported, such as reactive arthritis, Guillian-Barŕe syndrome, Miller-Fisher syndrome, meningitis, bacteremia, and septicemia (Kaldor and Speed 1984, Dhawan 1986, Roberts 1987, Mishu 1993, Ladrón de Guevara C 1994, Allos 1997, Hughes and Res 1997, Lastovica 1997, Saida, Kuroki et al. 1997, Nielsen 2009, CDC 2013)

The poultry intestinal tract, especially the ceca, colon, and crop is known to harbor large amounts of *Campylobacter* (Berrang, Buhr et al. 2000, Smith and Berrang 2006). It is approximated that birds can carry *Campylobacter* levels as high as 10^9cfu/g of feces within their intestinal tracts (Oosterom, Noternams et al. 1983, Berndtson, Tivemo et al. 1992, Berrang, Buhr et al. 2000). High levels of *Campylobacter* brought into poultry plants introduce the strong possibility of high *Campylobacter* incidence rates from cross-contamination due to gut leakage or accidental gut tearing (Berrang, Buhr et al. 2000). In addition, contamination residing on the exterior of the bird after transportation can introduce high levels of *Campylobacter* into processing plants (Stern, Clavero et al. 1995, Berrang, Buhr et al. 2000). Interventions must begin prior to production in order to further reduce the contamination on the broilers that enters the processing plant.

Studies have identified many risk factors during production for *Campylobacter* flock contamination and include age of birds (Berndtson, Emanuelson et al. 1996, Evans and Sayers 2000, Bouwknegt, van de Giessen et al. 2004, Barrios, Stern et al. 2006, McDowell, Menzies et al. 2008), lack of hygiene practices (Humphrey, Henley et al. 1993, van de Giessen, Bloemberg et al. 1996, Evans and Sayers 2000, Hald 2000,

Gibbens, Davies et al. 2001, Bouwknegt, van de Giessen et al. 2004, Cardinale, Cisse et al. 2004, McDowell, Menzies et al. 2008) or a hygiene barrier (Berndtson, Emanuelson et al. 1996, van de Giessen, Bloemberg et al. 1996, Evans and Sayers 2000), human traffic and equipment (Kapperud 1993, Berndtson, Emanuelson et al. 1996, Evans and Sayers 2000, Shreeve 2000, Cardinale, Cisse et al. 2004), multi-species farming (Hald, 2000, Bouwknegt et al., 2004, Cardinale et al., 2004, Kapperud, 1994, van De Giessen et al., 1998), non-disinfected water sources (Kapperud 1993), litter (Cardinale, Cisse et al. 2004, Arsenault, Letellier et al. 2007), insects (flies and darkling beetles) (Shane, Harrington et al. 1985, Hald, Skovgård et al. 2004, Hald, Skovgård et al. 2008) wild birds (Stern, Myszewski et al. 1997, Hiett, Stern et al. 2002), rodents (Huneau-Salaun, Denis et al. 2007), and catching crews and transportation crates (Stern, Clavero et al. 1995, Hiett, Stern et al. 2002, Slader, Domingue et al. 2002, Hansson, Ederoth et al. 2005, Rasschaert, Houf et al. 2007).

The number of quantitative epidemiological investigations to identify risk factors associated with *Campylobacter* positive flocks within the United States poultry industry are lacking. Thus, the objective of this study was to identify farm characteristics that may be associated with the presence of absence *Campylobacter* within a broiler flock under commercial production conditions within the southeastern United States.

6.2 Materials and Methods

6.2.1 Sampling Strategy

This prospective observational study was conducted in 3 states (Alabama, Mississippi, and Louisiana) within the southeastern United States from 2003-2006. Two companies that were thought to be representative of the regional poultry industry

participated in the study. A broiler complex was defined as having its own hatchery, feed mill, and processing plant. Company A was comprised of 4 complexes while Company B was comprised of 5 complexes. In Company A, 4 grow-out farms from each of 2 complexes and 3 farms from each of the other 2 complexes were selected for a total of 14 farms. Company B was comprised of 5 farms from each of 2 complexes and 4 farms from each of 3 complexes for a total of 22 farms. Two houses from each of the 36 farms were selected for a total of 72 houses. The 2 houses that were selected from each farm for sampling were usually a house on the end of the row and the adjacent house. In total, there were 72 flocks sampled from 36 farms which were sampled from 9 complexes which were selected from 2 companies. The companies selected the farms to be sampled prior to placement so flocks could be processed on Monday or Tuesday to allow for ease of transport and processing of samples.

The sampling strategy was to follow each flock through the production and processing continuum taking samples from each flock at 4 points: (1) 1 week prior to the end of grow-out and before transportation, (2) after transportation at plant arrival, (3) prior to chilling (immersion chill tank), and (4) at post-chill.

6.2.2 Sample Collection

6.2.2.1 End of grow-out whole carcass rinse, ceca and crop samples

The first sampling point was approximately one week before harvest. The ages of the individual flocks ranged from 48-61 days old. A convenience sample was taken by catching 30 birds at the cool-cell end of the house. The birds were humanely euthanized by cervical dislocation. A whole carcass rinse sample was taken for each of the 30 birds by placing the carcass into a sterile biohazard bag with 250ml of 1% buffered peptone

water (BPW) (Difco, Sparks, MD). The carcasses were vigorously shaken for 1 minute and the rinsate was aseptically transferred into a sterile plastic bottle. Following the collection of the whole carcasses rinses the crop and ceca were aseptically removed from each carcass. Each cecum was placed into a sterile Whirl-Pak® Bag (NASCO, Fort Atkinson, WI) and each crop was placed into a Whirl-Pak® Filter Bag (NASCO, Fort Atkinson, WI). BPW was added to each crop sample to make a 1:10 dilution by weight. Samples were placed on wet ice (18 h) and shipped overnight to the Food and Feed Safety Research Unit at College Station, Texas. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

6.2.2.2 Plant arrival whole carcass rinse, ceca and crop samples

The second sampling point was upon arrival at the processing plant. Three trucks were used to transport the flocks to the processing plant. A convenience sample of 2 birds from each of 5 cages was taken from each of the 3 trucks for sampling, totaling 30 birds per flock. A whole carcass rinse sample (described above) was taken for each of the 30 birds and tested for *Campylobacter*. The crop and ceca were removed aseptically from each of the same 30 birds (as descried above), packed on ice and transported to the laboratory. In total, there were 30 crops, 30 ceca, and 30 whole carcass rinses sampled for *Campylobacter* from each flock.

6.2.2.3 Pre-chill and post-chill carcass rinse samples

The third and fourth sampling points were taken within the processing plant before the carcasses entered the immersion chill tank and upon exiting the chill tank. Carcass rinse samples were taken from 30 birds before entering the immersion chill tank

and upon exiting the immersion chill tank. The carcass rinse samples were collected as described above, except 100ml BPW was added to the bag. The samples for each flock were taken at a repeating time interval so that the entire flock was sampled. Thus, 30 carcass rinses were sampled before the birds entered the chill tank and 30 carcass rinses were sampled upon exiting the chill tank for each of the flocks.

6.2.3 *Campylobacter* isolation and identification

Upon arrival at the Food and Feed Safety Research Unit at College Station, TX, the samples were incubated at 42°C for 24 hours. Selective enrichment was then performed for all samples except for the ceca by transfer of 10ml of the sample to 10 ml of 2x Bolton broth (Lab M, Bury, Lancashire, UK) and allowed to incubate for 24 hours at 42°C in a microaerobic environment (5% O_2, 10% CO_2, and 85% N_2). Each crop and ceca sample was then streaked onto Campy-Cefex agar (Becton, Dickinson and Co., Baltimore, MD) and allowed to incubate for 48 hours 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).

6.2.4 Questionnaire

Three different evaluation instruments were developed to collect information concerning management practices and characteristics of each farm. The first instrument was a questionnaire (Volkova 2007) to be filled out by the farmer and contained 8 sections and a total of 85 questions. The 8 sections contained questions on biosecurity

and sanitary practices, visitor biosecurity practices, litter and house sanitary practices, housing characteristics, housing ventilation and lighting systems, feeding and watering, pest and fauna control, and workroom and instrument sanitation (Volkova, Wills et al. 2011). The research team utilized the other two instruments (checklists). The first checklist (Volkova 2007) was completed on day 1 and collected information on the transportation of chicks from hatchery to farm, unloading the chicks, characteristics on territory around the house, characteristics of the house, litter, brooding, presence of pests and their control, and workroom and equipment characteristics. The team's second checklist (Volkova 2007) was completed in week 7 and addressed some of the same questions from the day 1 check-list, including biosecurity and sanitation conditions, that could be used for comparison (Volkova, Wills et al. 2011)

Pilot testing for the questionnaire was conducted on two occasions. First, the questionnaire was administered to two poultry veterinarians that were actively involved with the project. Secondly, after editing, the questionnaire was administered to the managers of two broiler complexes in the area of study. Further edits were made before the final instrument was adopted.

6.2.5 Sample size calculations

6.2.5.1 Number of flocks

The number of flocks used in this study was determined by a rule of thumb of 10 subjects, in this case flocks, per explanatory variable (Petrie and Watson 1999). Therefore, 72 flocks were used which would allow for 7 explanatory variables to be put into each final model.

6.2.5.2 Number of samples of flocks

This study was conducted in conjunction with another study that looked at the presence of *Salmonella* in broiler production and processing. The USDA-FSIS reported the national prevalence of *Salmonella* was 10.2% (Progress Report on *Salmonella* Testing of Raw Meat and Poultry Products, 1998-2000, http://www.fsis.usda.gov/ophs/haccp/salmdata2.htm) and that of *Campylobacter* was higher with a prevalence of 21-41% post-chill (Stern, Ladely et al. 2001). The goal was to be able to detect both *Campylobacter* and *Salmonella*. A sample size of 30 birds per flock was adopted which would detect at least a within-flock prevalence of ≥9.5% with 95% confidence (Cannon and Roe 1982), which would ensure detection of *Salmonella* and *Campylobacter* in all flocks where the prevalence was greater than the national *Salmonella* average (the lower of the two prevalences).

6.2.6 Statistical procedures

The *Campylobacter* status (positive or negative) was used to model the relationship between risk factors in the grow-out and processing phases and the following sampling points: grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinse (GOWC), plant-arrival ceca (PACA), plant-arrival crop (PACP), plant-arrival whole carcass rinse (PAWC), and post-chill whole carcass rinse (PPPO).

The data was analyzed using STATA software version 15.1 (StataCorp LP, College Station, TX, USA). Multilevel mixed-effects logistic regression (MEQRLOGIT) was used to develop causal models for the presence of *Campylobacter* as well as to estimate the percentage of variance in *Campylobacter* prevalence at each level of the hierarchical structure.

The sampling hierarchy was birds nested within flocks, flocks nested within farms, farms nested within complexes, and complexes nested within company. Company was the highest level of the hierarchy and was not included as a random effect because two companies were too few to accurately estimate the amount of variance at that level. Instead, company was included as a fixed effect to account for any variation between companies; however, company was not found to be significant and was dropped from all models. Flock was also not included as a random effect due to convergence issues, which was attributed to nearly identical prevalence of *Campylobacter* in the two flocks on each of the farms. Complex was also removed from the models of all outcomes except PPPO, due to lack of variance present at that level and to convergence issues. Consequently, farm and bird were included as random effects in GOCA, GOCP, GOWC, PPCA, PPCP, and PPWC outcome models. The PPPO outcome contained the random effects farm, bird, and complex. Intraclass correlation and the proportion of total variance attributed to each of the random effects were estimated. The latent variable approach was used which assumes a logistic distribution and a level-one (i.e. birds) variance of $\pi^2/3 = 3.29$ (Dohoo, Martin et al. 2009).

Explanatory variables were included in the analysis if the categories within that variable contained a frequency of >10%. If the variables contained categories with a frequency of ≤ 10%, that category was combined with another category if there was biological plausibility to do so. If not, that variable was not used in the analysis.

A univariable analysis was performed for each of the outcomes variables as described above and only those variables with a p-value less than 0.15 were considered as candidates for the multivariable analyses.

All variables were checked for collinearity prior to the multivariable analyses. Categorical variables were first coded as zero and one and then collinearity was assessed between all variables using Spearman's rank correlation. If the coefficient was greater than 0.8, then one or the other explanatory variable was included in a multivariable model, but not both (Dohoo 2009). When collinearity did exist, and in some cases there was no biological plausibility for selecting any one variable over another, the two variables were entered into separate models and the final model with the smallest Akaike Information Criterion (AIC) was selected.

The assumption of a linear relationship between each continuous predictor variable and the relevant outcome variable was evaluated by generating a lowess plot of the logit vs. the predictor values and evaluated visually. If the lowess curve appeared to be non-linear then basic transformations were used to see if linearity could be achieved. If linearity could not be achieved, then variables were categorized and reassessed in the univariable model.

Non-significant ($p > 0.05$) predictor variables were removed from the multivariable models using a manual backward selection process. Each eliminated variable was assessed for confounding as each non-significant variable was removed from the model. Furthermore, each variable that had been eliminated during the model selection process was reintroduced in the final reduced model to determine significance in the absence of non-significant variables. A variable was deemed a confounder and forced into the final model if the coefficient of a significant variable changed by more than 20 percent (Dohoo 2009). Interactions between predictor variables were explored

when it made biological sense. Causal models, containing no intervening variables, were constructed for each of the outcome variables (Dohoo, Martin et al. 2009).

6.3 Results

6.3.1 Surveys collected

Of the 72 flocks sampled, the managers completed questionnaires for 64 sampled flocks. There were 2 farms (4 flocks) that the managers did not return the surveys and were from the same company. This is likely due to competing time of the managers since Hurricane Katrina occurred in the middle of the sample collection period in 2005. Data was available for 66 of the flocks for the outcome variables PACP, PACA, PAWC, PPPR, and PPPO due to Hurricane Katrina, company schedule changes, disease outbreaks, and shipping delays. Grow-out crop and GOWC contained data from 67 flocks and GOCA contained 68 flocks. Due to the few flocks (11/66) found to be positive and convergence issues at PPPR, this variable was not used as outcomes in the model building process.

The age of the birds at the end of grow-out (approximately 1 week before harvest) when samples were taken ranged from 41-57 days. This variable was not linear and was categorized into 3 groups: 41-43 days (30%), 46-51 days (30%), and 53-57 days (40%). The median age of the birds at the grow-out sampling point was 49.5 days. The age of the birds on the day of harvest ranged from 46-63 days. This variable was not linear and was categorized into 3 groups: 46-51 days (32%), 56-57 days (21%), and 59-63 days (47%). The median age of birds at harvest was 57 days. As would be expected, the age of the birds at the end of grow-out sampling point was highly correlated (0.938) with the age of the birds on the day of harvest.

The season was determined based on the month birds went to harvest; December, January, and February were classified as winter; March, April, and May were classified as spring; June, July, and August were classified as summer; September, October, and November were classified as autumn. There were 24 flocks (33%) harvested in the spring, 18 flocks (25%) harvested in the winter, and 30 flocks (42%) harvested during the summer/autumn season.

All sampled houses used an 'all-in all-out' broiler management system. The houses were constructed on dirt pad foundations and were oriented in an east to west direction. Tunnel ventilation was used in all houses. The median number of houses on a farm was 4 (2-16). This variable was not linear and was categorized into 2 groups: 2-4 houses (51%) and 5-16 houses (49%). The median age of the houses was 10 (0-30) years. This variable was not linear and was categorized into 2 groups: 0-9 years (47%) and 10-30 years (53%). The interior walls were constructed of wood (31%) or were a mix of plastic and wood (69%).

The surface of the roads between houses on a farm were either gravel (74%) or dirt/vegetation (26%). The surface adjacent to the footing of the house was vegetation (74%) or dirt/gravel (26%).

Standing water was present on the exterior of the house on day 1 in 70% of the flocks. Standing water was present at the end of grow-out in 56% of flocks.

Dogs were allowed on the farm in 63% of the flocks studied, while cats were also allowed on the farm in 63% of the flocks. Cattle was also located on the farm in 53% of the flocks studied, while other farm animals (pigs, cattle, goats, horses) besides chickens were located on the farm in 56% of flocks.

In 34% of flocks, a commercial chicken facility was located within 1/4th mile of the farm. Backyard chicken flocks were located within 1/4th mile of the farm in 19% of the flocks.

Used broiler litter was piled and stored on farms in 72% of flocks studied. The median distance to the used litter pile was 100 (20-600) yards. This variable was not linear and was categorized. Studies have shown broiler houses located within 200 yards of used litter piles to be at a higher risk of *Campylobacter* contamination (Arsenault, Letellier et al. 2007). Thus, the variable was categorized into flocks that were less than 200 yards (75%) from the used litter pile and those flocks that were more than 200 yards (26%) from the used litter pile. Due to the limited number (34) of farms that had used litter piles, this variable was not analyzed in the multivariable analysis. Used litter was spread on the farm in 69% of flocks studied.

All litter used for the sampled houses was pine shavings. The litter for the sampled flock had a median age of 12 (0-60) months. This variable was not linear and was categorized into 2 groups: litter less than 12 months (61 %) and greater than 12 months (39%). Age of the previous flock's litter had a median age of 24 (10-84) months. This variable was not linear and was categorized into 3 groups: 12 months or less (24%), 13-24 months (52%), and greater than 24 months (24%). The median number of flocks on the previous litter was 4 (0-30). This variable was not linear and was divided into 0-6 flocks (70%) and 7-30 (30%). The median duration of the empty period between flocks was 11(0-26) days. This variable was not linear and was categorized into 3 groups: 0-7 days (22%), 8-14 days (58%), and 14-26 days (20%).

6.3.2 Univariable analysis

The results of the univariable analysis are listed in Table 6.1. Variables that met the screening criteria ($p \leq 0.15$) for each of the outcomes were considered for inclusion in the corresponding multivariable analysis.

6.3.3 Multivariable analysis

The final multivariable models are listed in Table 6.2. The final multivariable model for the GOCA outcome included the number of houses on the farm (OR=95.63, CI=1.91-4781.17) and standing water outside of the houses on day 1 (OR=33.38, CI=2.08-535.14). The final multivariable model for the GOCP outcome included the presence of standing water around the house at the end of grow-out (OR=0.18, CI=0.04-0.81) and wood as the material of the interior walls (OR=9.88, CI=2.60-37.59). The GOWC multivariable model selection process resulted in a univariable model containing the presence of a backyard flock within 1/4th of a mile from the farm (OR= 0.06, CI=0.00-2.25), but was not significant (p=0.13).

The PACA multivariable model selection process resulted in a univariable model containing the presence of a used litter pile on the farm (OR=34.92, CI=0.29-4143.66), but was not significant (p=0.15). The PACP multivariable model selection process resulted in a univariable model that contained the presence of standing water around the house on day 1 (OR=6.24, CI=2.71-14.39). The PAWC final model contained vegetation as the surface material adjacent to the footing (OR=3.60, CI=1.30-9.98).

The age of the birds at grow-out sampling and the age of the birds at harvest were highly correlated variables and thus were entered into separate models for the PPPO outcome. The model containing the age of the birds at grow-out resulted in a non-

significant (p=0.07) univariable model. The model containing the age of the birds at harvest resulted in a multivariable model that contained the age of the birds at harvest and the number of flocks on the previous litter (OR=14.61, CI=1.31-162.29).

6.3.4 Intraclass correlation and percentage of variance

The variance and percent of total variance occurring at the complex, farm, and bird level and the total variance at each outcome is displayed in Table 6.3. The PPPO outcome was the only outcome that showed variance occurring at the complex level. The farm level variance ranged from 57.2%-90.8% variance while the bird level variance ranged from 9.2%-42.8%. The intraclass correlation coefficients for birds within the same farm, birds within the same complex but different farms, and farms within the same complex are listed for each outcome in Table 6.4. The intraclass correlation coefficients for birds within the same farm ranged from 0.57-0.91. Birds within the same complex but different farms and farms within the same complex had zero intraclass correlation except with the PPPO outcome. For this outcome, the intraclass correlation for birds within the same complex but different farms and farms within the same complex were 0.12 and 0.16, respectively.

6.4 Discussion

6.4.1 Age of birds and flock size

Our research found a significant relationship between the age of the birds on the day of harvest and the presence of *Campylobacter* in the PPPO outcome of the univariable and multivariable analysis. We found that older birds (56-63 days) were less likely to have *Campylobacter* than younger birds (46-51 days). However, other research

has shown an association between *Campylobacter* flock contamination and bird age at slaughter with older birds more likely to be positive (Berndtson, Emanuelson et al. 1996, Evans and Sayers 2000, Bouwknegt, van de Giessen et al. 2004, Barrios, Stern et al. 2006, McDowell, Menzies et al. 2008). Other researchers have also found a decline in the *Campylobacter* prevalence and counts on birds following a peak at 42 days (Northcutt, Fletcher et al. 2003)..

Some studies have identified flock size to be associated with an increased risk of *Campylobacter* infection in larger flocks (Berndtson, Emanuelson et al. 1996, Barrios, Stern et al. 2006, Nather 2009), while others have found no link (Humphrey, Henley et al. 1993, Evans and Sayers 2000, Cardinale, Cisse et al. 2004). It has been suggested that larger flocks require more food, water, litter, air, and personnel movement which increases the opportunities for infection (Berndtson, Emanuelson et al. 1996, Nather 2009). Our research identified the PACP in the univariable analysis as the only outcome associated ($p \leq 0.05$) with the number of birds placed in the house. The odds of a positive *Campylobacter* sample increased 8 times for farms that placed 21046-27500 compared to those that placed 16000-21045. This variable, however, was not significant at the multivariable level.

6.4.2 Season

A seasonality trend in the presence of *Campylobacter* in poultry has been identified in many studies worldwide (Berndtson, Emanuelson et al. 1996, Evans and Sayers 2000, Refregier-Petton, Rose et al. 2001, Barrios, Stern et al. 2006, McDowell, Menzies et al. 2008). Late summer and early autumn is typically when the flock prevalence and risk of *Campylobacter* contamination is at its peak, however, this varies

by country and sometimes by year (Nylen, Dunstan et al. 2002). The only study that looked at the seasonality of *Campylobacter* in poultry within the U.S. did not identify a seasonal trend (Berrang, Meinersmann et al. 2017). Our study looked at the seasonality of *Campylobacter* presence over 4 years and was also unable to find a relationship between season and the presence of *Campylobacter* in broiler flocks within the southeastern United States.

6.4.3 Age and number of houses on farm

House age can be thought of as a proxy variable for the general state of repair of the house and the ability to maintain a bio-secure environment that excludes rodents and wild life (Newell, ELvers et al. 2011). Our research is in agreement with others that found house age was not associated with *Campylobacter* positive flocks (Berndtson, Emanuelson et al. 1996, Messens, Herman et al. 2009). Both newly constructed houses and older houses have been found to be *Campylobacter* positive (Gregory 1997).

Farms that have more houses have been found to be at an increased risk for *Campylobacter* positive flocks (Gibbens, Davies et al. 2001, Refregier-Petton, Rose et al. 2001, Bouwknegt, van de Giessen et al. 2004, McDowell, Menzies et al. 2008, Ridley, Morris et al. 2011). The present study identified an association between farms with a greater number of houses and *Campylobacter* positive samples in the PACP outcome of the univariable analysis. In the multivariable analysis, the odds of *Campylobacter* positive samples of the GOCA outcome increased when a farm had 5-16 houses compared to 2-4 houses. This research is in agreement with a study conducted in the Netherlands that found farms that had 5 or more houses were at an increased risk of *Campylobacter* contamination (Bouwknegt, van de Giessen et al. 2004). Other studies

found 3 or more houses to increase the risk of flock *Campylobacter* presence (Refregier-Petton, Rose et al. 2001, McDowell, Menzies et al. 2008).

6.4.4 Presence of domestic animals and multi-species farming

Farm animals (cattle, pigs, sheep) frequently excrete *Campylobacter* spp. in their feces (Van De Giessen, Mazurier et al. 1992, Kapperud 1993, van De Giessen, Tilburg et al. 1998). Multi-species farming (cattle, pigs, other poultry) and/or tending other livestock has been linked to farms that have *Campylobacter* positive flocks (Kapperud 1993, Kapperud 1994, van de Giessen, Bloemberg et al. 1996, Gregory 1997, van De Giessen, Tilburg et al. 1998, Hald 2000, Bouwknegt, van de Giessen et al. 2004, Cardinale, Cisse et al. 2004, Katsma, De Koeijer et al. 2007, Zweifel, Scheu et al. 2008, Messens, Herman et al. 2009). *Campylobacter* has also been detected in flocks raised closer to other poultry (Berndtson, Emanuelson et al. 1996). However, our study agrees with that of other researchers that did not find an association with the presence of cattle or other animals on the farm (van De Giessen, Tilburg et al. 1998, McDowell, Menzies et al. 2008, Nather 2009). Our research did not find a significant relationship between having a commercial chicken facility or backyard poultry flocks within ¼ of a mile of the farm.

Domestic animals (dogs and cats) have also been found to be carriers of *Campylobacter* (Hald and Madsen 1997). Dogs and cats can act as a mechanical vector for the bacterium especially on farms where multi-species farming occurs. In this current study, the presence of dogs and/or cats on the farm was not associated with the presence of positive *Campylobacter* samples.

6.4.5 Litter and the downtime between flocks

Due to the dry and stressful conditions, properly maintained litter is an unfavorable environment for *Campylobacter* to survive. As the litter ages, it becomes more and more rich with nutrients after each successive flock (Chamblee and Todd 2002) which in turn provides the possibility for better viability of bacteria within the litter. There is conflicting literature on the duration *Campylobacter* can survive in used poultry litter. A recent controlled experimental study found *Campylobacter* only able to survive for a short time (under 24 hours) in used broiler litter due to its preference for a microaerophilic environment (Smith, Meade et al. 2016). Some, on the farm, studies have been unable to find *Campylobacter* in the litter (Jacobs-Reitsma, van de Giessen et al. 1995, Zweifel, Scheu et al. 2008) while others have found *Campylobacter* able to survive for 4 weeks (Rothrock, Cook et al. 2008). The survivability of *Campylobacter* highly depends on the available moisture in the litter as *Campylobacter* is sensitive to dry environments (Smitherman, Genigeorgis et al. 1984, Berndtson, Emanuelson et al. 1996). Our research was unable to find an association between the age of the current litter or the age of the last litter and the presence of *Campylobacter* in the univariable analysis. In the multivariable analysis, the odds of a *Campylobacter* positive sample increased with the presence of fewer flocks (0-6) on the last litter in the PPPO outcome. This was unexpected because as litter ages it becomes richer with nutrients and possibly more moisture (depending on how well it is maintained) which would support the survival of *Campylobacter*. A spurious association would be another possible explanation as this variable was associated with only one outcome.

Researchers have found that there is a higher risk of producing a *Campylobacter* positive flock with a shorter downtime between flocks. One study found the odds of having a *Campylobacter* positive flock increases by 5 times with a downtime period of less than 14 days (Hald 2000). Another study found having an empty house period less than 21 days was a risk factor for a *Campylobacter* positive flock (Berndtson, Emanuelson et al. 1996). In our study, we did not find an association between the length of the downtime period and the presence of *Campylobacter* in a sample. Carry-over from one flock to the other has been reported to occur infrequently (Van De Giessen, Mazurier et al. 1992, Evans and Sayers 2000, Shreeve, Toszeghy et al. 2002, McDowell, Menzies et al. 2008, Zweifel, Scheu et al. 2008). In general, research has shown that the *Campylobacter* status of a flock cannot be predicted based on the *Campylobacter* status of a previous flock, although, having a positive flock does increase the risk of a subsequent flock having *Campylobacter* (Berndtson, Emanuelson et al. 1996). Studies have found *Campylobacter* positive flocks to be followed by negative flocks and vice versa (Berndtson, Emanuelson et al. 1996, Evans and Sayers 2000). In cases where subsequent flocks were positive they sometimes were the same serotype and other times were different serotypes (Berndtson, Emanuelson et al. 1996).

6.4.6 Litter pile on the farm and/or spread on the farm and distance to litter pile

Disposal of broiler manure on the farm has been identified at a risk factor for *Campylobacter* presence (Herman, Heyndrickx et al. 2003, Cardinale, Cisse et al. 2004). One study traced the source of farms that consistently had *Campylobacter* positive flocks and the dung hill and puddles were environmental sources from which the flock contamination originated from (Herman, Heyndrickx et al. 2003). Spreading used broiler

litter on the farm has also been identified as a risk factor (Guerin, Martin et al. 2007). In this study, the presence of a used litter pile on the farm was not found to be associated with *Campylobacter* positive samples. Spreading the litter on the farm was also not associated with *Campylobacter* presence.

Distance from the house to the used litter pile on a farm has been shown to be a significant risk factor for *Campylobacter* presence (Arsenault, Letellier et al. 2007). In the univariable analysis, our study also found the odds of a *Campylobacter* positive sample to increase on farm when the litter pile was located less than 200 yards from the houses in the GOCA, GOCP, and PAWC outcomes.

6.4.7 Interior wall material

In this study, farms that had interior wood walls were more likely to have *Campylobacter* positive samples in GOCA, GOCP, and PACP outcomes in the univariable analysis. In the multivariable analysis, the odds of having a *Campylobacter* positive sample in the GOCP outcome was 9.88 times greater for farms that had wood interior walls instead of plastic walls or a combination of wood and plastic. Berndtson et al. (1996) found the material (concrete, plywood, wood, or sheet metal) of the interior of the buildings was not a significant risk factor. *Campylobacter* survives best in dark, cool, and moist conditions (Hazeleger, Wouters et al. 1988, Lee, Smith et al. 1998). Wood absorbs moisture and may offer an environment that may prolong the survival of *Campylobacter* in the environment.

6.4.8 Environment around house

Campylobacter has been recovered previously from puddles surrounding poultry houses during grow-out and just prior to chick placement (Hiett, Stern et al. 2002, Herman, Heyndrickx et al. 2003, Rivoal, Ragimbeau et al. 2005, Bull, Allen et al. 2006, Johnsen, Kruse et al. 2006, Hansson, Vågsholm et al. 2007) The organism can survive well in the presence of water (Blaser, Hardesty et al. 1980) and recovery is highest after it rains (Hansson, Vågsholm et al. 2007). *Campylobacter* isolated from puddles surrounding the house have matched the genotype of those found to contaminate the flocks later on (Hiett, Stern et al. 2002, Rivoal, Ragimbeau et al. 2005, Bull, Allen et al. 2006, Johnsen, Kruse et al. 2006, Hansson, Vågsholm et al. 2007). One study determined *Campylobacter* was more frequently isolated from outdoor samples when there was cloudy or rainy weather the two days prior to sampling compared to sunny days (Hansson, Vågsholm et al. 2007). In this study, standing water around houses on the day of chick placement was associated with having a *Campylobacter* positive samples at GOCA, GOCP, PACP, and PAWC in the univariable analysis. Following the multivariable analysis, farms that had standing water outside of the houses on the day of chick placement had 6.24-33.38 greater odds of having a *Campylobacter* positive samples at GOCA and PACP. Having standing water around the houses at the end of grow-out, one week prior to harvest, was a protective factor in the multivariable analysis for the GOCP outcome. This relationship is not understood. However, with the large number of variables analyzed in this study and the variable being associated with *Campylobacter* at only one of the outcomes, a spurious relationship must be considered as a possible explanation.

6.4.9 House surface adjacent to footing/road surfaces

Vegetation adjacent to the footing of the exterior house walls was determined to increase the odds of *Campylobacter* presence at GOCA, GOCP, and PAWC outcomes of the univariable analysis. This variable was the only variable in the final model of PAWC outcome. The odds of *Campylobacter* presence increased 3.6 times or greater on farms where vegetation was the surface adjacent to the footing of the exterior wall compared to dirt or gravel. One possible explanation is that the vegetation holds more moisture close to the house footing and litter allowing *Campylobacter* a better opportunity for survival. The surface of the roads between houses was not found to be associated with *Campylobacter* presence.

6.4.10 Proportion of variance and interclass correlation

Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. This information indicates that intervention efforts should focus on factors at the farm level i.e. factors that vary among farms within a complex. The intraclass correlations for each of the outcomes indicates that there is high correlation among birds within the same farm and no correlation, with the exception of the PPPO outcome, among birds within the same complex but different farms and farms within the same complex. It is reasonable to think that there is increased correlation, for PPPO, among birds within the same complex and among farms within the same complex since complexes are defined by a shared processing plant. The increased correlations that become evident at post-chill are likely due to the cross-contamination and decontamination that can occur within a processing plant.

6.5 Conclusion

Studies worldwide have identified many risk factors for *Campylobacter* broiler colonization, however, our research evaluated those risk factors under the commercial production conditions in the Southeastern United States. This study identified risk factors including the number of houses on a farm, standing water around house on day 1, wood interior house walls, vegetation adjacent to the exterior house footing, and the number of flocks on the last litter. Standing water around the house at 7 weeks and harvesting birds 56-63 days were protective factors. The highest proportion of variance was at the farm level indicating intervention efforts should focus on factors at the farm level.

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Table 6.1 Results of univariable logistic regression analysis of association between farm characteristics within the grow-out environment and the occurrence of *Campylobacter* in grow-out ceca samples (GOCA), grow-out crop samples (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca samples (PACA), plant arrival crop samples (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO), after accounting for the variability of the random effects of farm in all outcomes and complex in the PPPO outcome

Variable Name	Response	Mean (range) or count of flocks	GOCA	GOCP	GOWC	PACA	PACP	PAWC	PPPO
			Odds Ratio (CI) p-value						
Age of birds at grow-out sampling	41-43 days	20	Referent						
	46-51 days	20	2.46 (0.01, 463.43) p=0.74	0.80 (0.10, 6.08) p=0.83	0.99 (0.03, 30.62) p=0.99	1.36 (0.01, 311.50) p=0.91	0.33 (0.01, 10.73) p=0.53	0.37 (0.01, 11.12) p=0.57	0.23 (0.01, 4.14) p=0.32
	53-57 days	28	0.23 (0.00, 29.30) p=0.55	0.89 (0.14, 5.75) p=0.90	0.18 (0.01, 4.64) p=0.30	0.34 (0.00, 53.45) p=0.68	0.17 (0.01, 4.27) p=0.28	0.14 (0.01, 3.43) p=0.23	0.04 (0.00, 0.63) p=0.02
	Chi2 p-value		0.62	0.98	0.48	0.84	0.56	0.48	0.07
Age of birds at harvest/plant arrival sample	46-51 days	22	Referent						
	56-57 days	14	7.45 (0.04, 1553.37) p=0.46	0.80 (0.09, 6.75) p=0.84	1.18 (0.04, 38.83) p=0.93	3.10 (0.01, 859.04) p=0.69	0.33 (0.01, 11.90) p=0.55	0.31 (0.01, 9.54) p=0.50	0.10 (0.01, 1.75) p=0.12
	59-63 days	32	0.07 (0.00, 5.81) p=0.24	0.49 (0.08, 2.81) p=0.42	0.07 (0.00, 1.50) p=0.09	0.10 (0.00, 10.13) p=0.32	0.06 (0.00, 1.22) p=0.07	0.06 (0.00, 1.02) p=0.05	0.02 (0.00, 0.22) p=0.01
	Chi2 p-value		0.17	0.71	0.14	0.37	0.18	0.14	0.01
Number of birds placed	21046-27500	49	0.04 (0.00, 2.16) p=0.11	0.57 (0.12, 2.69) p=0.48	0.52 (0.05, 5.40) p=0.58	0.57 (0.03, 11.51) p=0.71	8.08 (1.95, 33.55) p=0.00	1.24 (0.32, 4.76) p=0.76	4.03 (0.50, 32.73) p=0.19
	16000-21045	23	Referent						

6.1 (Continued)

Season	Spring	24	0.69 (0.01, 80.82) p=0.88	0.95 (0.16, 5.86) p=0.96	0.44 (0.02, 10.70) p=0.61	0.05 (0.00, 5.71) p=0.22	0.08 (0.00, 1.60) p=0.10	0.16 (0.01, 3.38) p=0.24	0.51 (0.03, 8.11) p=0.64
	Winter	18	1.39 (0.01, 265.22) p=0.90	0.97 (0.14, 6.95) p=0.98	0.44 (0.02, 10.70) p=0.61	0.13 (0.00, 25.12) p=0.45	0.46 (0.02, 12.08) p=0.64	0.60 (0.02, 17.89) p=0.77	0.62 (0.03, 13.54) p=0.76
	Summer/Autumn	30	Referent						
	Chi2 p-value		0.97	0.99	0.82	0.45	0.25	0.49	0.89
Number of houses on farm	5-16	35	39.82 (0.78, 2044.8) p=0.07	2.82 (0.62, 12.76) p=0.18	2.33 (0.24, 22.88) p=0.47	2.44 (0.11, 51.90) p=0.57	0.12 (0.03, 0.50) p=0.00	1.06 (0.28, 4.04) p=0.94	0.35 (0.05, 2.40) p=0.29
	2-4	37	Referent						
Age of house	10-30 yrs	34	1.17 (0.01, 114.24) p=0.95	0.69 (0.13, 3.67) p=0.66	0.65 (0.03, 13.65) p=0.78	0.76 (0.01, 50.84) p=0.90	0.57 (0.04, 8.93) p=0.69	0.42 (0.03, 6.75) p=0.54	1.00 (0.08, 12.09) p=0.998
	0-9 yrs	30	Referent						
Dogs allowed on Farm	Yes	40	1.93 (0.02,211.51) p=0.78	1.93 (0.35, 10.76) p=0.45	0.62 (0.03, 13.99) p=0.76	0.62 (0.01, 47.68) p=0.83	0.69 (0.04, 12.37) p=0.80	0.94 (0.05, 16.81) p=0.97	1.69 (0.10, 29.82) p=0.63
	No	24	Referent						
Cats allowed on Farm	Yes	40	1.17 (0.01,112.78) p=0.95	1.82 (0.35, 9.59) p=0.48	1.46 (0.07, 30.74) p=0.81	3.23 (0.05, 211.64) p=0.58	4.36 (0.28, 68.10) p=0.29	3.10 (0.19, 49.36) p=0.42	1.06 (0.07, 15.18) p=0.91
	No	24	Referent						
Cattle on Farm	Yes	34	0.43 (0.00, 41.53) p=0.72	1.16 (0.22, 6.23) p=0.86	0.54 (0.03, 11.48) p=0.70	0.15 (0.00, 10.06) p=0.38	0.66 (0.04, 10.47) p=0.77	0.65 (0.04, 10.34) p=0.76	1.29 (0.07, 24.05) p=0.87
	No	30	Referent						
Other Animals on Farm	Yes	36	0.58 (0.01, 58.07) p=0.82	1.14 (0.21, 6.20) p=0.88	0.52 (0.02, 11.02) p=0.68	0.30 (0.00, 20.99) p=0.58	1.07 (0.07, 17.34) p=0.97	0.90 (0.06, 14.43) p=0.94	1.35 (0.08, 22.31) p=0.84
	No	28	Referent						

6.1 (Continued)

Commercial chicken facility within ¼ mile of farm	Yes	22	2.75 (0.02,336.41) p=0.68	0.52 (0.09, 2.97) p=0.46	5.00 (0.21, 120.07) p=0.32	1.47 (0.02, 125.15) p=0.87	2.02 (0.11, 37.95) p=0.64	4.23 (0.23, 77.94) p=0.33	1.22 (0.08, 17.75) p=0.88
	No	42	Referent						
Backyard flocks within ¼ mile of farm	Yes	12	54.63 (0.19, 15614.4) p=0.17	4.70 (0.71, 31.15) p=0.11	15.92 (0.45, 569.20) p=0.13	30.55 (0.17, 5340.78) p=0.19	4.62 (0.17, 129.42) p=0.37	9.75 (0.36, 252.91) p=0.17	5.22 (0.28, 98.94) p=0.27
	No	50	Referent						
Presence of used litter pile on farm	Yes	46	32.71 (0.14, 7660.52) p=0.21	4.18 (0.60, 29.05) p=0.15	8.02 (0.23, 283.96) p=0.25	34.92 (0.29, 4143.96) p=0.15	11.02 (0.59, 205.26) p=0.11	12.09 (0.56, 261.43) p=0.11	4.90 (0.28, 85.63) p=0.28
	No	18	Referent						
Used litter is spread on farm	Yes	44	1.09 (0.01, 158.68) p=0.97	1.13 (0.18, 7.21) p=0.90	1.15 (0.04, 31.21) p=0.93	1.74 (0.02, 168.18) p=0.81	2.88 (0.14, 57.27) p=0.49	2.77 (0.14, 60.00) p=0.51	2.23 (0.15, 32.51) p=0.56
	No	20	Referent						
Distance to used litter	< 200 yds	34	178.01 (1.08, 29330.1) p=0.05	15.59 (2.22, 109.44) p=0.01	22.5 (0.77, 660.80) p=0.07	65.02 (0.83, 5101.03) p=0.06	39.92 (0.75, 2123.4) p=0.07	57.58 (1.43, 2319.37) p=0.03	2.55 (0.14, 47.41) p=0.53
	≥ 200 yds	12	Referent						
Surface of roads between houses	Dirt/ Vegetation	18	2.20 (0.02, 223.15) p=0.74	1.14 (0.20, 6.38) p=0.88	4.80 (0.25, 93.33) p=0.30	3.89 (0.04, 374.99) p=0.56	9.76 (0.61, 156.80) p=0.11	8.50 (0.53, 135.97) p=0.13	3.00 (0.20, 44.71) p=0.43
	Gravel	52	Referent						
Standing water around house day 1	Yes	46	23.15 (1.74, 308.38) p=0.02	5.19 (1.68,16.06) p=0.00	1.40 (0.54, 3.63) p=0.50	1.69 (0.53, 5.43) p=0.38	6.24 (2.71, 14.39) p=0.00	2.37 (1.04, 5.38) p=0.04	1.64 (0.35, 7.74) p=0.53
	No	20	Referent						

6.1 (Continued)

Standing water around house at end of grow-out	Yes	37	0.96 (0.07, 13.97) p=0.98	0.27 (0.07, 1.05) p=0.06	0.56 (0.20, 1.60) p=0.28	0.20 (0.02, 1.81) p=0.153	0.85 (0.10, 7.57) p=0.89	1.74 (0.52, 5.85) p=0.37	0.48 (0.11, 2.10) p=0.33
	No	29							
Interior house walls material	Wood	22	8.11 (1.05, 62.50) p=0.04	8.09 (2.18, 30.08) p=0.00	0.83 (0.30, 2.25) p=0.71	1.02 (0.29, 3.51) p=0.98	3.39 (1.20, 9.58) p=0.02	2.44 (0.89, 6.73) p=0.08	0.09 (0.01, 1.22) p=0.07
	Plastic or mixed	50	Referent						
House surface adjacent to the footing	Vegetation	52	6.68 (1.68, 26.56) p=0.01	5.59 (1.78, 17.52) p=0.00	1.24 (0.60, 2.60) p=0.56	1.50 (0.64, 3.51) p=0.35	2.24 (0.97, 5.20) p=0.06	3.60 (1.30, 9.98) p=0.01	0.88 (0.25, 3.06) p=0.84
	Dirt/gravel	18	Referent						
Age of current litter	>12 months	25	0.95 (0.01, 68.43) p=0.98	1.38 (0.26, 7.28) p=0.71	0.58 (0.03, 11.08) p=0.72	0.48 (0.01, 24.96) p=0.72	0.27 (0.03, 2.46) p=0.25	0.85 (0.06, 12.24) p=0.90	0.18 (0.02, 1.89) p=0.153
	≤12 months	39	Referent						
Age of last litter	>12 months ≤24 months	26	16.09 (0.02, 12949.2) p=0.42	2.13 (0.22, 20.13) p=0.51	2.03 (0.03,162.57) p=0.75	0.81 (0.01, 127.01) p=0.26	2.29 (0.05,116.01) p=0.68	0.50 (0.01, 35.91) p=0.75	1.32 (0.18, 9.90) p=0.79
	>24 months	12	0.22 (0.00, 721.94) p=0.71	1.31 (0.09, 18.12) p=0.84	0.30 (0.00, 55.44) p=0.65	0.03 (0.00, 12.39) p=0.26	0.67 (0.01, 59.68) p=0.86	0.54 (0.00, 69.82) p=0.81	0.81 (0.07, 9.79) p=0.87
	≤ 12 months	12	Referent						
	Chi2 p-value		0.46	0.78	0.70	0.42	0.80	0.95	0.89
Number of flocks on previous litter	0-6	45	5.15 (0.05, 570.33) p=0.50	0.87 (0.15, 5.12) p=0.88	3.80 (0.16, 91.14) p=0.41	3.67 (0.05, 251.64) p=0.55	3.80 (0.39, 37.31) p=0.25	1.72 (0.10, 29.07) p=0.71	7.43 (0.52, 105.15) p=0.14
	7-30	19	Referent						

6.1 (Continued)

Empty Period Between Flocks	8-14 days	36	7.89 (0.03, 2182.20) p=0.47	2.63 (0.31, 21.97) p=0.37	5.33 (0.12, 242.28) p=0.39	1.45 (0.01, 266.78) p=0.89	3.91 (0.12, 127.84) p=0.44	2.01 (0.07, 57.54) p=0.68	4.61 (0.18, 116.72) p=0.35
	14-26 days	12	1.38 (0.00, 1637.24) p=0.93	1.20 (0.09, 16.65) p=0.89	7.65 (0.07, 869.48) p=0.40	0.43 (0.00, 338.13) p=0.80	3.14 (0.04, 275.02) p=0.62	7.62 (0.10, 610.26) p=0.36	19.84 (0.36, 1094.09) p=0.14
	0-7 days	14	Referent						
	Chi2 p-value		0.71	0.59	0.64	0.91	0.74	0.66	0.34

Light shading indicates p≤ 0.15, dark shading indicates p≤0.05.

Table 6.2 Results of multivariable logistic regression analysis of association between farm characteristic factors within the grow-out environment and the occurrence of *Campylobacter* in grow-out ceca samples (GOCA), grow-out crop samples (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca samples (PACA), plant arrival crop samples (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO), after accounting for the variability of the random effects of farm in all outcomes and complex in the PPPO outcome

Outcome Variable	Explanatory Variable(s)	Response Mean (range)	OR (95% CI)	SE	*p*-value	Chi^2 p-value
GOCA	Number of houses on the farm	5-16	95.63 (1.91, 4781.17)	190.88	0.022	
		2-4	Referent			
	Standing water around house day 1	Yes	33.38 (2.08, 535.14)	47.26	0.013	
		No	Referent			
GOCP	Standing water around house at end of grow-out	Yes	0.18 (0.04, 0.81)	0.14	0.026	
		No	Referent			
	Interior house wall material	Wood	9.88 (2.60, 37.59)	6.74	0.001	
		Plastic or mixed	Referent			
PACP	Standing water around house day 1	Yes	6.24 (2.71, 14.39)	2.66	0.000	
		No	Referent			
PAWC	Surface material adjacent to footing	Vegetation	3.60 (1.30, 9.98)	1.87	0.014	
		Dirt/Gravel	Referent			
PPPO	Age of birds at harvest	56-57 days	0.04 (0.00, 0.65)	0.05	0.025	0.003
		59-63 days	0.02 (0.00, 0.18)	0.02	0.001	
		46-51 days	Referent			
	Number of flocks on last litter	0-6	14.61 (1.31, 162.29)	17.95	0.029	
		7-30	Referent			

Table 6.3 The variance (percent variance) occurring at the complex, farm, and bird level and the total variance at each outcome using a null model for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Complex	Farm	Bird	Total
GOCA	0 (0.0)	32.38 (90.8)	3.29 (9.2)	35.67
GOCP	0 (0.0)	4.39 (57.2)	3.29 (42.8)	7.68
GOWC	0 (0.0)	14.10 (81.1)	3.29 (18.9)	17.39
PACA	0 (0.0)	32.56 (90.8)	3.29 (9.2)	35.85
PACP	0 (0.0)	13.84 (80.8)	3.29 (19.2)	17.13
PAWC	0 (0.0)	13.15 (80.0)	3.29 (20.0)	16.44
PPPO	1.6 (12.0)	8.4 (63.2)	3.29 (24.8)	13.29

Table 6.4 Intra-class correlations, using a null model, for grow-out ceca (GOCA), grow-out crop (GOCP), grow-out whole carcass rinses (GOWC), plant arrival ceca (PACA), plant arrival crop (PACP), plant arrival whole carcass rinses (PAWC), and post-chill carcass rinses (PPPO).

Outcome	Birds within the same farm	Birds within the same complex but different farms	Farms within the same complex
GOCA	0.91	0	0
GOCP	0.57	0	0
GOWC	0.81	0	0
PACA	0.91	0	0
PACP	0.81	0	0
PAWC	0.80	0	0
PPPO	0.75	0.12	0.16

CHAPTER VII

CONCLUSIONS

Campylobacter is one of the most common causes of human food-borne infection within the United States. Consumption, cross-contamination, and mishandling of undercooked poultry has been identified as the major source of campylobacteriosis. High levels (10^9 CFU) of *Campylobacter* enter processing plants on the exterior and interior of birds causing cross-contamination of equipment and contaminating *Campylobacter* free birds. In order to further reduce the amount of *Campylobacter* entering and exiting the processing plant, interventions must be applied during grow-out. Many risk factors for *Campylobacter* flock infection have been identified world-wide including age of birds, lack of hygiene practices or hygiene barrier, human traffic and equipment, multi-species farming, non-disinfected water sources, litter, insects, wild birds, rodents, and catching crews and transportation crates. Hygiene practices such as house specific boots, clothes, hand washing, use of overshoes, and effective use of boot dips have been associated with a decreased risk of *Campylobacter* flock infection. Although many risk factors and protective factors have been identified, there has been limited research on these factors under broiler production practices within the southeastern United States.

The first objective of this study was to both predict and establish a causal relationship between the most likely grow-out and/or plant arrival sample(s) and the *Campylobacter* status of a flock at plant arrival and post-chill. Results of this work

indicated that the best predictors of post-chill *Campylobacter* carcass status were the exterior whole carcass sample in the grow-out environment and the crop upon arrival at the processing plant. The best post-chill causal model contained the grow-out whole carcass.

The second objective of this study was to generate hypotheses about practices in the hatchery associated with *Campylobacter* flock infection later in production. This study identified risk factors in the hatchery including controlling the humidity in the chick room, 2-4 people handling the chicks, washing the setter twice yearly, 2 or more breeder farms providing eggs for the sampled flock, and the procedure for washing the hatch trays that may make flocks more susceptible to *Campylobacter* colonization later in production.

The third objective of this study was to identify biosecurity risk factors on the farm that were associated with increased presence of *Campylobacter* later in production. This study identified protective factors that emphasize the importance of the hygiene of the workers on the farm including the use of footbaths and dedicated shoes, greater frequency of entering the house during brooding, disinfectant added to the drinker lines, having concrete outside the most used door (multivariable analysis), and the cleanliness of the workroom, which is likely a proxy for the overall hygiene habits on the farm. Having more walk-in doors on the house, the farmer removing the litter, concrete at most used door (univariable analysis), and the number of workers were associated with increased risk of *Campylobacter* positive samples.

The fourth objective was to identify farm characteristics that were associated with *Campylobacter* later in production and processing. This study identified risk factors

including the number of houses on a farm, standing water around house on day 1, wood interior house walls, vegetation adjacent to the exterior house footing, and the number of flocks on the last litter. Standing water around the house at 7 weeks and harvesting birds 56-63 days were protective factors.

The final objective was to estimate the proportion of variance at each of the hierarchical levels (complex, farm, and bird) and the intraclass correlation coefficients. Within all of the outcomes, the highest percent of variance occurred at the farm level compared to either complex or bird levels. The intraclass correlation coefficients for each of the outcomes indicates that there is high correlation among birds within the same farm and no correlation, with the exception of the PPPO outcome, among birds within the same complex but different farms and farms within the same complex.

Over all this work identified many risk factors and protective factors in the hatchery, grow-out environment, and processing plant that were associated with *Campylobacter* positive samples later in production. The contamination on the exterior of the bird was identified as the cause of contamination post-chill. This study found that interventions should focus on factors at the farm level.

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