Association of *Campylobacter* spp. Levels between Chicken Grow-Out Environmental

Samples and Processed Carcasses

Matthew W. Schroeder

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Food Science and Technology

Joseph D. Eifert, Chair

Monica A. Ponder

David G. Schmale, III

April 20, 2012

Blacksburg, VA

Keywords: *Campylobacter*, environmental sampling, single flock, poultry

Association of *Campylobacter* spp. Levels between Chicken Grow-Out Environmental Samples
and Processed Carcasses

Matthew W. Schroeder

ABSTRACT

Campylobacter spp. have been isolated from live poultry, production environment, processing facility, and raw poultry products. The detection of *Campylobacter* using both quantitative and qualitative techniques would provide a more accurate assessment of pre- or post harvest contamination. Environmental sampling in a poultry grow-out house, combined with carcass rinse sampling from the same flock may provide a relative assessment of *Campylobacter* contamination and transmission.

Air samples, fecal/litter samples, and feed pan/drink line samples were collected from four commercial chicken grow-out houses. Birds from the sampled house were the first flock slaughtered the following day, and were sampled by post-chill carcass rinses. Quantitative (direct plating) and qualitative (direct plating after enrichment step) detection methods were used to determine *Campylobacter* contamination in each environmental sample and carcass rinse. *Campylobacter*, from post-enrichment samples, was detected from 27% (32/120) of house environmental samples and 37.5% (45/120) of carcass rinse samples. All sample types from each house included at least one positive sample except the house 2 air samples. Samples from house 1 and associated carcass rinses accounted for the highest total of *Campylobacter* positives (29/60). The fewest number of *Campylobact*er positives, based on both house environmental (4/30) and carcass rinse samples (8/30) were detected from flock B. Environmental sampling

techniques provide a non-invasive and efficient way to test for foodborne pathogens. Correlating qualitative or quantitative *Campylobacter* levels from house and plant samples may enable the scheduled processing of flocks with lower pathogen incidence or concentrations, as a way to reduce post-slaughter pathogen transmission.

ACKNOWLEDGMENTS

DEDICATION

I dedicate this body of work to my sister, Emily Schroeder. Thank you for always being there for me. I would not be where I am today without you. I love you!

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Word
CFU	colony forming unit(s)
ft	feet
g	gram(s)
l	liter(s)
lbs	pound(s)
m	meter(s)
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
ppm	parts per million (10^{-6})
µl	microliter(s) (10^{-6} l)

INTRODUCTION

Campylobacter bacteria are Gram negative, slender, spiral-curved rods that cause a majority of the intestinal infectious diseases worldwide (Snelling et al. 2005; Keener et al. 2004). In particular, *Campylobacter jejuni*, one of the "thermophilic" campylobacters, is one of the leading causes of bacterial diarrheal illness reported in the United States (Altekruse et al. 1999; Keener et al. 2004). An estimated 40,000 cases are documented annually in the United States (Keener et al. 2004). In 2011, *Campylobacter* caused 0.8 million illnesses total (Scallan et al. 2011).*Campylobacter jejuni* has a low infectious dose (~500-10,000 organisms), which is normally followed by symptoms including fever, abdominal pain, and diarrhea (Snelling et al. 2005; Kenner et al. 2004). *C. jejuni* grows best between 37°C to 42°C, the approximate body temperature of a chicken, and in a low oxygen (3-15%), or microaerophilic environment (Altekruse et al. 1999).

Reservoirs for *Campylobacter* include wildlife such as ducks and geese, contaminated water, insects, raw milk, and meat; however, 50-70% of *Campylobacter* illness comes from consuming poultry and poultry products (Altekruse et al. 1999; Allos 2001). Chicken has the greatest *Campylobacter* risk, in part, because of the large quantities consumed (Humphrey et al. 2007). Though colonization is not detectable until at least 10 days of age, once infected, *Campylobacter* has been found in up to 100% of birds tested in a given flock (Keener et al. 2004; Moore et al. 2005). This pre-harvest infection, along with the high likelihood of cross contamination inside the poultry processing plant, has caused the United States Department of Agriculture-Food Safety Inspection Service(USDA-FSIS) to revise their regulations to require poultry processors to monitor and control *Campylobacter* (Keener et al. 2004; Moore et al. 2005; USDA 2011b).

Suzuki and Yamamoto (2009) conducted a literature survey of prevalence of *Campylobacter* on retail poultry in the United States. From 1167 samples, *Campylobacter* was found on 71.5% of retail chicken carcasses in the United States (Suzuki and Yamamoto 2009). However, in 2010 less than 10% of U.S. processing facilities were specifically testing for *Campylobacter* in a survey of 167 processors (Alvarado 2011). Because most processors are still concerned about *Salmonella* with less emphasis on *Campylobacter*, the USDA-FSIS has devised new performance standards that focus on both quantitative and qualitative detection methods of *Campylobacter*. These regulations require commercial poultry processors to isolate, identify, and enumerate *Campylobacter* from poultry rinses by direct plating onto selective agars or by enrichment culture (Williams et al. 2009). From post-chill carcass rinses, a direct plating method (1 ml sample split among four plates) will be utilized for enumeration of *Campylobacter*. An additional 30 ml sample from the rinse with an enrichment broth will be used for the qualitative analysis (USDA 2011a). According to the new regulation, acceptable percentages of positive carcasses for the 1 ml samples and combination of the 1 ml and 30 ml samples are 10.4 and 46.4, respectively (Alvarado 2011). That is to say, in a sample set of 51 carcasses, 8 can test positive for *Campylobacter* and still be acceptable (Alvarado 2011; USDA 2011b). If the 1 ml samples are negative, the 30 ml samples are tested with 21 out of 51 samples for the combination considered acceptable (Alvarado 2011). Knowing that 100% of boiler flock chickens entering the processing facility could contain *Campylobacter*, processors now not only need to worry about *Salmonella*, but also *Campylobacter* to ensure the safety of their product (Moore et al. 2005).

In this study, air samples and other environmental swabs in grow-out houses will be tested, while carcasses and chill tank water will be tested before and during slaughter for possible

Campylobacter contamination. The samples taken from the same flock in the grow house, and processing facility may show a relationship between pre- and post harvest contamination. Most previous research has focused on either the grow-out house or the processing facility only. Little research has been done on a single flock continuously from farm through slaughter completion. And, with the adoption of the new government standards concerning *Campylobacter* and the little amount of environmental sampling that has been done in grow-out houses and processing facilities, this project will be valuable to large or small food processors.

The purpose of this study was to 1) determine *Campylobacter* numbers in poultry houses through environmental sampling (i.e.- air samples, shoe bootie samples, and chicken feed pan/drinker samples), 2) determine *Campylobacter* contamination levels in the processing facility after the previously sampled flock is processed, and 3) determine if there is an association between *Campylobacter* levels in commercial chicken production and processing environment through pre- and post- harvest cycles.

Based on the objectives described, the bootie samples will account for the highest amount of *Campylobacter* positives through environmental sampling techniques. The processing facility samples (carcass rinses) will have a lower number of *Campylobacter* positives than the environmental grow-out houses samples; however, there will be an association between these levels.

LITERATURE REVIEW

A. *Campylobacter* in Food Systems

Within the past 25 years, *Campylobacter* has been recognized as a cause for human illness (Keener et al. 2004). In the United States alone, 50-70% of human *Campylobacter* illness is attributed to poultry, with a reported 40,000 cases documented annually (Keener et al. 2004). Though deaths from *Campylobacter* infection are uncommon (680-730 per year), children less than the age of one and young adults aged fifteen to twenty-five more frequently acquire infection (Keener et al., 2004). Most infections are sporadic with clinical symptoms including fever, abdominal pain, and diarrhea (Keener et al. 2004). Though outbreaks with *Campylobacter* are uncommon, *Campylobacter* associated illness and infections still occur. In 2011 in England, the Health Protection Agency (HPA) found that 90% of *Campylobacter* infection associated with catering venues was due to the consumption of chicken liver plate, a popular dish in England and Wales (Health Protection Agency 2011). In 2005, a *Campylobacter* outbreak was also identified in an Australian restaurant where chicken was the source of the problem (Black et al., 2006).

Campylobacter contamination can occur in a variety of areas including production, processing, distribution, retail marketing, and preparation (Zhao et al. 2001). However, *Campylobacter* can also be found in the food supply at retail markets (Altekruse et al. 1999). Retail chicken meat has been isolated with *Campylobacter* at a rate of 98% (Stern and Line 1992). A study was conducted evaluating the prevalence of *Campylobacter* spp., *E. coli*, and *Salmonella* in retail chicken, turkey, pork, and beef in the Washington, D.C. area (Zhao et al. 2001). From the 92 sampling visits from supermarket chains, it was found that 91% of the stores had *Campylobacter* contaminated chicken (Zhao et al. 2001). The potential for transmission of

foodborne pathogens from raw retail meats further emphasizes the importance of consumer and food safety knowledge (Zhao et al. 2001).

The USDA Food Safety and Inspection Service (FSIS) recently changed their regulations regarding the control of *Campylobacter* within processing facilities (USDA 2011b). These new regulations specify that only 8 out of 51 chicken carcasses can test positive for *Campylobacter* and still be considered acceptable (Alvarado 2011; USDA 2011b). If a facility fails to meet these standards, they will be moved to the second highest priority for scheduling to conduct a follow-up (USDA 2011b). This is an important change when considering most processing facilities and growers focus on *Salmonella* and *E. coli* (Northcutt 2003). Most growers use vaccination to control *Salmonella*, but once infected, an entire flock can become infected with *Campylobacter* on the farm (Fielding 2012). Although at processing *Salmonella* has been seen at 13% positive, the majority of carcass contamination is due to *Campylobacter* (Rasschaert 2007). The main focus for controlling *Campylobacter* should be the farm; however, other places such as hatcheries and processing facilities also need to implement control strategies to reduce contamination (Shanker et al. 1990; Bull et al. 2006). These initiatives set forth by the USDA-FSIS hopefully will begin to reduce contamination due to *Campylobacter* to ensure public health safety.

B. Growth and Survival of *Campylobacter*

Campylobacter jejuni, a micro-aerophile, survives best in a low oxygen atmosphere, such as 5% oxygen, 10% carbon dioxide, and 85% nitrogen at a temperature around 42°C (Altekruse et al. 1999; Keener et al. 2004). In the United States, over 99% of the reported infections are with *Campylobacter jejuni*, although 14 species of *Campylobacter* have been identified (Allos

2001). A majority of human infections are from *C. jejuni* and *C. coli*, which is why they are

studied more frequently (Keener et al. 2004). The optimal atmosphere for the growth of various

Campylobacter jejuni and *coli* strains was found to be 5-10% oxygen and 1-10% carbon dioxide

(Bolton and Coates 1983). Rates of inactivation can be influenced by strain, temperature,

humidity, and suspension medium; however, *Campylobacter* cannot survive below an acidic pH

of 4.9 (Keener et al. 2004). The optimal pH range for *Campylobacter* is 6.5-7.5, but at low pH,

survival of the organism is temperature dependent (Curtis 2007). *Campylobacter* has been

shown to grow best at temperatures between 37°C and 42°C (Keener et al. 2004). Minimal

growth temperatures for *Campylobacter jejuni* strains 104 and ATCC 33560 were found to be

32°C and 31°C, respectively (Hazeleger et al. 1998). However, around 30°C, a sudden growth

rate decline was observed (Hazeleger et al. 1998). *Campylobacter* is also sensitive to freezing

and salinity (Altekruse et al. 1999). Doyle and Roman (1982) found that three strains of

Campylobacter jejuni could grow at 1.5% NaCl, but not 2.0% NaCl at 42°C.

C. Infection and Transmission of *Campylobacter* in Poultry Grow-out Houses

      Initial Colonization

Although it is impossible to determine the exact moment a single bird in a flock becomes

contaminated with *Campylobacter* due to flock size and necessary sampling frequency,

understanding the mechanism by which grow-out houses becomes colonized proves important

for controlling the bacteria and establishing appropriate control measures (van Gerwe et al.

2009). Estimation of transmission and dynamics of colonization of *Campylobacter* proves very

difficult and is not well understood (Conlan et al. 2007). However, initial flock colonization

with *Campylobacter* is age dependent with normally no detection when birds are less than two

weeks old (Conlan et al. 2007). A dose as low as 40 CFUs have been shown experimentally to

colonize 1-day-old chicks, although this is dependent on the strain of *Campylobacter jejuni* and

breed of the bird (Conlan et al. 2007).

Campylobacter colonizes the intestinal mucus layer in the epithelium of chickens

(Keener et al. 2004). Once infected, the *Campylobacter* numbers will remain high in the

intestine (Potturi-Venkata et al. 2007). Horizontal transmission seems to be the most likely

mechanism by which *Campylobacter* is introduced into a flock, with rapid colonization of a

flock (3-7 days) once colonization occurs (van Gerwe et al. 2009; Shanker et al. 1990). Possible

sources of initial colonization include: wild birds, other farm animals, rodents, insects,

contaminated groundwater, carryover from previous flock and farm workers (Conlan et al. 2007;

Jacobs-Reitsma et al. 1995; Humphrey et al. 2007).

Age Dependence

Contamination of birds, on the farm, by *Campylobacter* is normally not detectable until at

least 10 days of age (Newall et al. 2000; Byrd et al. 1998). Chickens less than two weeks of age

normally are not colonized due to a "lag phase" derived from maternal antibodies that are

prevalent in young chicks (Conlan et al. 2007). Colonization in broiler chickens is the highest in

the mucosal crypts of the caeca, but also invades the small intestine (Conlan et al. 2007).

Normally the transmission of *Campylobacter* results from fecal-oral transmission and can often

contaminate the entire flock within 5 weeks (Keener et al. 2004; Jacobs-Reitsma et al. 1995). In

2009, van Gerwe et al. estimated that one colonized bird could infect 2.37 birds per day on

average. Theoretically, at this rate, 95% of the flock could be contaminated in one week. Age

and bioaerosol concentration also play an important role in *Campylobacter* contamination

(Northcutt et al. 2003). Birds aged 42 days tested 100% positive for *Campylobacter*, while birds aged 56 days showed a 90% infection rate (Northcutt et al. 2003). Berndtson et al. (1996) found that the rate of positive flocks increased with age when following flocks from week one to week five. Since bioaerosol concentration increases with bird age and air hygiene is considered an important factor for animal health, *Campylobacter* infection in the flock may be spread through the air (Vucemilo et al. 2007; Saleh et al. 2005).

Housing Environmental Factors

Broiler chickens are raised in grow-out houses with dimensions between 40' and 60' (12-18 m) wide and 300' to 600' (90-180 m) long. Each house holds approximately 12,000 to 40,000 birds (Kuntz 2009). Initially, one-day-old chicks are placed in the house where the temperature is heated to around $32^{o}C$ ($90^{o}F$), but as the birds grow and begin to produce their own heat, the temperature of the house decreases, eventually reaching ambient temperatures (70-$75^{o}F$) (Kuntz 2009; Donald et al. 2005).

Farms widely differ in their infection rates (Humphrey et al. 2007). Though these differences could be due to hygiene within the house, birds raised in a poor environment are more susceptible to campylobacters (Humphrey et al. 2007). Environmental factors such as humidity, ventilation, temperature, and bioaerosols can play an important role in the transmission of *Campylobacter*.

Campylobacter are susceptible to dry conditions. Thus, in a grow-out house with higher humidity, *Campylobacter* could survive (Ishihara et al. 2012). In some instances, relative humidity within a grow-out house can reach 75 to 80% (Line 2006; Choct 2010). Line (2006) studied *C. jejuni* colonization under high (80%) and low (30%) relative humidity conditions. He

found that there were differences in rates of colonization of *Campylobacter* on litter held under

high and low relative humidity. The artificially dry, low humidity pen showed a colonization

delay in comparison to the high relative humidity pen (Line 2006). In Japan, Ishihara et al.

(2012) found that locations with higher humidity and shorter periods of sunshine were associated

with increased colonization of broiler flocks. All *Campylobacter* positive locations had a higher

mean humidity than the *Campylobacter* negative locations (Ishihara et al. 2012).

Many grow-out houses in the United States today utilize tunnel ventilation

(Chinivasagam et al. 2009). In this system, large volumes of air are circulated throughout the

house. This mass movement of air is beneficial for maintaining proper temperature and humidity

for the birds inside the house; however, the moving air can also contain pathogens such as

Campylobacter and/or *Salmonella* (Chinivasagam et al. 2009). Air-quality related illnesses in

humans such as cough, runny nose, sore throat and eye irritation have been shown to decrease

when ventilation is effective (Shale and Lues 2007). Air circulation within a grow-out house

increases over the chicken cycle, which also increases the rate at which microbes are distributed

(Shale and Lues 2007; Chinivasagam et al. 2009). This could contribute to the spread of

Campylobacter contamination in a grow-out house.

Seasonality and temperature also have been reported to affect *Campylobacter* incidence

(Ishihara et al. 2012; Humphrey et al. 2007). Aside from temperature contributing to the eating

habits of the birds, the temperature outside as well as inside the house have shown a correlation

to *Campylobacter* colonization (Donald et al. 2005; Humphrey et al. 2007). Ishihara et al.

(2012) found that higher air temperatures during chicken rearing was associated with increased

Campylobacter contamination in the house as well as during processing. In the United States,

increases in the level of human Campylobacteriosis have been seen during the summer months (Sopwith et al. 2003).

With increased bird densities within grow-out houses due to large scale production, exposure to organic dust from chicken feces, litter, feed, and feather formation are leading to human health problems (Oppliger et al. 2008). Air hygiene and exposure to these bioaerosols, which increase during the fattening period in birds, impacts both the health of the birds as well as the humans working in the industry (Oppliger et al. 2008; Vucemilo et al. 2007). Vucemilo et al. (2006 and 2007) found that all pollutants within the housing environment increased with increasing poultry age and body weight, ranging from 3.22×10^3 CFU/m^3 in the first week to 6.40×10^7 CFU/m^3 in the fifth week. Airborne dust, which is influenced by poultry age, litter, and poultry activity, also increased with age, reaching 4.8 mg/m^3 air (Vucemilo et al. 2007).

Chicken bedding is normally comprised of peanut hulls, sawdust, and wood shavings. In the United States, typically dozens of flocks will be raised on a single bed of layered litter (Dumas et al. 2011). Thus, bedding is a possible reservoir of disease-causing bacteria (Dumas et al. 2011). Montrose et al. (1985) studied the role of litter in the transmission of *Campylobacter*. They found that uninfected chickens placed on infected autoclaved and nonautoclaved litter shed *Campylobacter jejuni* after only several days (Montrose et al. 1985). This research indicates the possible transmission of *Campylobacter* from flock to flock from litter.

Campylobacter Detection in the Pre-Harvest Environment

Most studies of the chicken grow-out houses analyze the chicken's surroundings, such as its feed, water, or litter (Shanker et al. 1990). Studies have implicated these items for possible sources of horizontal transmission, but the full epidemiology is still not fully understood (Jacobs-

Reitsma et al. 1995). *Campylobacter* in the environment is detected intermittently; typically, detection doesn't occur until it the flock has been colonized, but the direction of the spread is unclear (Bull et al. 2006). Research has begun focusing on environmental sampling methods inside and around grow-out houses as opposed to live bird sampling due to speed and efficiency.

Campylobacter has even been detected outside of chicken houses, in some cases up to 30 m downwind of the broiler house (Bull et al. 2006; Hansson et al. 2007). *Campylobacter* has also been detected from the air exiting the broiler sheds, which could be important for the transmission of infection (Bull et al. 2006). Chickens attain harvest weight around 40 days of age (Humphrey et al. 2007). Within the last week of fattening, bioaerosols are at their highest concentration and prevalence of contamination is highest (Saleh et al. 2005). Since bioaersol concentration includes poultry feces, litter, and feathers, which are all known sources of *Campylobacter* contamination, air could affect contamination levels inside the house (Vucemilo et al. 2007). As chicks mature, their size increases, subsequently increasing the amount of bioaerosols that may serve as a vehicle for infection in other birds. Aside from the normal fecal, intestinal, and swabbing techniques used to isolate *Campylobacter*, environmental studies including air sampling have become popular to detect presence in grow-out houses (Keener et al. 2004; Berndtson et al. 1996). Olsen et al. (2009) determined that *Campylobacter* colonization could be detected prior to detection in the traditional sock (bootie) sampling techniques and only required 1800ml of air. In 2009, Kuntz et al. found that 28% of air samples within a chicken grow-out house tested positive for *Campylobacter*.

The transmission of *Campylobacter* is normally fecal-oral. Once a flock has become *Campylobacter*-positive, 100% of fecal samples tested positive (Potturi-Venkata et al. 2007). Environmental sampling of poultry litter or feces is common, but a number of different

techniques exist (Keener et al. 2004). Eifert et al. (2003) compared various sampling techniques (fecal swabs and environmental surface "drag" samples) and found that environmental swabs of the litter yielded the highest percentage recovery. Bull et al. (2006) collected fresh fecal samples and found that 83% (189/229) tested positive for *Campylobacter*. A similar technique was used by Stern and Robach (2003) and found that 96.4% of fecal samples tested positive. Williams et al. (2009) and Hansson et al. (2007) placed a bandage or sock over a normal shoe and walked around the house for fecal sampling. Using this method, 23 out of 131 flocks (18%) tested positive for *Campylobacter*.

Campylobacter is most often transmitted by the fecal-oral route and can be spread by direct contact on food and water. Adult chickens consume about 0.05 to 0.16 gallons of water per day depending on temperature and eat about 2.5 lbs of food/lb of weight gain (Frame 2008). Chickens have free access to their feed trays and water lines. Chickens can defecate on their feed and water lines; therefore swab samples of these can be taken to test for the presence of *Campylobacter* (Berndtson et al. 1996). Berndtson et al. (1996) found 90 out of 300 pooled swab samples from water lines (30%) tested positive for *Campylobacter*. Sampling of feed devices in a study conducted by Johnsen et al. (2006) found 25% (4/16) tested positive for *Campylobacter*.

Flying insects, such as flies, have also been implicated as a vector for *Campylobacter* transmission, especially during the summer months (Hansson et al. 2007). Insects are able to carry *Campylobacter* on their exoskeleton (Altekruse et al. 1999). A study showed that flies caught in *Campylobacter*-negative chickens were also negative, but with chickens that were *Campylobacter*-positive, flies were positive as well (Berndtson et al. 1996). Though insects may pose a risk of introduction of *Campylobacter* into a flock based on amount and ventilation

patterns within the house, 1% (3/291) of insects were *Campylobacter* positive (Hansson et al. 2007). Water and various other environmental samples (feces from cows, straw, mud) from surrounding farms were found to contain *Campylobacter* around six of the seven colonized flocks (Bull et al. 2006).

D. Transmission of *Campylobacter* during Transport

When animals are transported from farm to processing facility, the animals are under a tremendous amount of stress (Keener et al. 2004). This stress may increase spreading of intestinal bacteria such as *Campylobacter* (Keener et al. 2004). Bacterial counts on carcasses have shown 1,000-fold increase during transportation (Altekruse et al. 1999). During crating or transport, any pathogen, such as *Campylobacter*, could still colonize the ceca of birds, which would be retained during processing (Keener et al. 2004). Aside from the birds themselves, crates that are not properly cleaned could increase contamination levels (Stern et al. 2001). A survey of over 10,000 poultry companies of varying sizes conducted by Auburn University discovered that 80% of poultry growers don't sanitize their crates and only 18.3% sanitize their trucks and trailers (Fielding 2012). One study found 53% of batches of crates tested positive for *Campylobacter* spp.; the same subtype that was found on the farm and before slaughter (Hansson et al. 2007). In addition to the possible contamination from crates during transport, over 50% of bird catchers' boots, drivers' boots, and truck wheels have tested positive for *Campylobacter* (Ramabu et al. 2004). Following the same flock from farm to the processing plant can provide a more accurate assessment of cross contamination that can occur during transportation. Contamination will likely increase once birds are in transit from farm to processing plant.

E. Transmission of *Campylobacter* during Processing

Cross contamination is highly likely to occur within poultry processing facilities (Moore et al. 2005). The status of *Campylobacter* infection at the conclusion of processing is related to the *Campylobacter-* status of the arriving flock (Berrang et al. 2007). Birds entering the facility normally already have high rates of infection around 93% (Jozwiak et al. 2006). By the end of processing, the number of positive carcasses has been shown to increase on occasion, but most often bacterial populations on carcasses have been shown to decrease as processing progresses (Jozwiak et al. 2006; Berrang and Dickens 2000). However, more data is needed to understand the mechanism by which *Campylobacter* contamination changes during processing (Guerin et al. 2010). Poultry processors, now given the current regulations, are required to monitor the presence of *Campylobacter* in their facilities.

Carcass Sampling and Detection

Most previous research has focused on examining whole carcasses or parts of carcasses during different periods of processing (Berrang and Dickens 2000; Berrang et al. 2007). Rasschaert et al. (2007) conducted a study to examine the best carcass sampling site (duodenum, ceca, and crop) for detection of *Campylobacter* and *Salmonella*. He found that only sampling the duodena of the chicken at the slaughterhouse level was sufficient to determine *Campylobacter* infection (Rasschaert et al. 2007). Other studies have sampled the respiratory tract, cloacae, and neck skin of chickens (Berrang et al. 2003; Hansson et al. 2007). Berrang et al. (2003), who examined the respiratory tracts of chickens, found *Campylobacter* in about half the thoraco-abdominal cavities before and after scald. *Campylobacter* was also detected in the

cloacae at slaughter in 23% (30/131) of flocks and 30% (39/131) of neck skins (Hansson et al. 2007).

Post-chill carcass rinses have also been studied. This method of carcass sampling involves removing the entire carcass from the processing line, placing the carcass in a sterile plastic bag with a sterile solution, massaging, and placing the liquid back into a sterile cup (Berrang et al. 2007). Berrang et al. (2000 and 2007) used whole carcass rinses to examine the effectiveness of different processing stages in reducing *Campylobacter* populations. Cason et al (1997) evaluated *Campylobacter* contamination postpick, pre-chill, and post-chill using whole carcass rinses. *Campylobacter* was identified in 94% (198/210) of all carcasses sampled (Cason et al. 1997).

Environmental Sampling and Detection

In processing facilities, much *Campylobacter* research has focused on detection and prevalence on the carcass with less emphasis on the processing environment (Bashor et al. 2004). The water used in the process and the chill tank is also a possible location for cross contamination due to the extensive use of potable water as well as the high speed of processing (Wempe et al. 1983; Peyrat et al. 2008). Processing facilities can use two different methods to chill carcasses to reduce carcass temperature: immersion chilling or air chilling (Berrang et al. 2008). Immersion-chilled carcasses were found to have significantly lower bacterial (*E. coli* and *Campylobacter*) numbers per milliliter than air chilled carcasses (Berrang et al. 2008). Some immersion-chill tanks use sanitizers such as chlorine (50 ppm maximum) to reduce other contaminants such as blood and tissue fragments (Jakarta 2006; Keener et al. 2004). The use of chlorine in the chill tank significantly reduced *Campylobacter* numbers, but does not completely

eliminate bacteria (Berrang et al. 2007). In 1995, the USDA required the addition of 20 to 50 ppm chlorine to prevent cross contamination (Keener et al. 2004). Carcasses that entered the chill tank without *Campylobacter* may become contaminated, whereas carcasses that were heavily contaminated with *Campylobacter* may show a reduced concentration of organisms upon exiting the chill tank (Wempe et al. 1983).

Peyrat et al. (2008) conducted a study that analyzed cleaning and disinfection practices within a poultry slaughterhouse specifically targeting *Campylobacter jejuni*. Among the numerous places they tested, they determined that 60% of the scald tank water tested positive for *Campylobacter* before cleaning. Scalding water is used to ease the plucking procedure and is controlled based on a time/temperature relationship (Jakarta 2006). Scald water temperatures of 49^oC, 53^oC, and 60^oC did not contribute to a lower prevalence of *Campylobacter* (Wempe et al. 1983). Other environmental sampling locations such as the processing equipment and workers are also a likely source of cross contamination, testing 100% positive for *Campylobacter* on staff's hands, slaughtering equipment, and transport boxes (Jozwiak et al. 2006; Berndtson et al. 1996).

F. Control of *Campylobacter* during Grow-out

Due to mass processing, there is near-universal contamination and bacterial burden of *Campylobacter* in flocks. This makes the elimination of *Campylobacter* near impossible (Allos 2001). Several simple strategies have been employed in the grow-out house as well as the processing facility to help prevent the spread of *Campylobacter*.

It seems farmers may be less concerned about flock contamination with foodborne pathogens due to control measures set forth by slaughter or further processing facilities (Kuntz

2009). However, strategies have evolved in order to aid this burden. These include: limiting

animals' consumption of antibiotics, disinfection of their food and water, chlorination of

drinking water, vaccination, or selective breeding (Allos 2001; Keener et al. 2004). Treatment of

chicks with commensal bacteria and immunization of older birds has been shown experimentally

to reduce *Campylobacter* colonization on the farm (Altekruse et al. 1999). Irradiation of food for

animals has also been suggested as a possible strategy; however, it has not been accepted by the

public as of yet (Allos 2001).

G. Control of *Campylobacter* during Processing

Processing Effectiveness

Berrang and Dickens (2000), Berrang et al. (2007) and Guerin et al. (2010) have studied

and reviewed the effectiveness of different stages of processing in reducing the prevalence of

Campylobacter. The sites sampled included pre-scald, post-scald/pre-pick, post-pick, post-

evisceration, pre-chill/post-final washer, and post-chill. Post-pick steps such as evisceration,

final wash, and chilling were found to decrease *Campylobacter* counts (Berrang and Dickens

2000). As a general rule, processing reduces the bacterial population numbers, except for the

defeathering step due to the rubber fingers being contaminated (Berrang et al. 2007; Berrang and

Dickens 2000; Wempe et al. 1983). The prevalence of *Campylobacter* after scalding (20.0-

40.0% decrease) and chilling (26.6-100.0% decrease) decreased in addition to the concentrations

of *Campylobacter* (Guerin et al. 2010). Berrang et al. (2007) found a mean concentration

decrease after chill of 0.43 log CFU/ml. Guerin et al. (2010) saw a maximum concentration

decrease after scalding of 2.9 CFU/ml and after chilling of 1.7 CFU/carcass. Once a carcass has

been chilled, the concentration and prevalence of *Campylobacter* has been considerably reduced (Berrang et al. 2007).

Antimicrobial Rinses

Processors implore several treatments to reduce microbial contamination. These methods include physical methods, chemical methods, and irradiation (Keener et al. 2004). One research project reported that 100% of processing equipment tested positive for *Campylobacter*, therefore, using an antimicrobial agent would most likely reduce this number to some degree (Jozwiak et al. 2006). Contamination can be reduced by 90-99% by washing with potable water, an example of a physical method (SCVMRPH 1998). Using a chemical agent, such as chlorine, acidified sodium chlorite (ASC), trisodium phosphate (TSP), or cetylpyridinium chloride (CPC) has been shown to reduce the level of *Campylobacter* contamination on poultry carcasses, but will not completely eliminate the bacteria (Bashor et al. 2004; Arritt et al. 2002; Berrang et al. 2007). TSP rinse is more active on Gram-negative pathogens such as *Campylobacter*, eliminating the need for off-line reprocessing (Keener et al. 2004). TSP has several modes of action including surfactant properties, removal of bacteria not firmly attached to skin, and disrupting bacterial cell membrane (Keener et al. 2004). TSP has a maximum dosage of 41.5 mg/l for use in drinking water (Keener et al. 2004). Bashor et al. (2004) reported a 1.03 log CFU/ml reduction of *Campylobacter* using a concentration of 12% TSP rinse at pH 11.0. Waldroup et al. (2010) looked at the effects of different application techniques and concentrations of CPC on the reduction of *Campylobacter*. The 0.5% CPC 10 second immersion dip was found to completely eliminate *Campylobacter*, while 1.7-2.2 logs/ml reductions were determined for the other application techniques (0.2 or 0.5% as a mist or 0.2% as a 10-sec spray) (Waldroup et al. 2010).

Logistic Scheduling

Logistic scheduling can also be used to reduce contamination in the poultry processing plant (Potturi-Venkata et al. 2007). Logistic scheduling involves the slaughter of negative flocks before positive flocks to avoid cross contamination (Clements 2011). However, sampling to test for possible bacteria needs to be done as close to slaughter as possible to avoid flocks becoming positive after testing (Clements 2011). Normally, scheduling has been based on *Salmonella* samples because contamination can be determined weeks in advance (Clements 2011). With *Campylobacter*, determination of flock contamination only could come a few days before slaughter, making it much harder to schedule flocks with low prevalence (Clements 2011). This is especially true given the possibility of flocks becoming more contaminated as they are transported to the processing facility. However, logistic scheduling offers a simple system to preserve the negative status of a flock (Potturi-Venkata et al. 2007).

Additional Processing Strategies

In addition to antimicrobial rinse steps and logistic scheduling of flocks, other sanitation and food safety practices can be used to reduce pathogens. Viator et al. (2008) reported a survey of meat and poultry processors that looked at very basic practices, which would undoubtedly aid in the control of bacterial contamination. The survey found 74.5% of processors wash their hands after contact with meat or poultry, while only 45.5% use antimicrobials on food contact equipment on not ready-to-eat products (Viator et al. 2008). The hygienic design of equipment, especially during the evisceration step, could also reduce contamination (Clements 2011). The viscera could rupture if the evisceration machine is not suited to variation in carcass sizes, which

would release intestinal fluids and contaminate remaining carcasses to be processed (Clements 2011). Freezing to -20°C has been used in some countries, but requires a tremendous amount of space and cost of frozen storage (Clements 2011).

H. Detection Methods for *Campylobacter* from Foods

Cultural Methods

Most scientific research regarding microbiology has focused on qualitative analysis. Recently, because of quantitative microbial risk assessments, quantitative data based on direct enumeration of *Campylobacter* without an enrichment step have been reported (Habib et al. 2008; Oyarzabal et al. 2005). Direct plating may be a faster and more cost-effective isolation method for *Campylobacter*, especially with fecal sampling (Potturi-Venkata et al. 2007; Altekruse et al. 1999) and for enumerating postchill carcass rinses (Oyarzabal et al. 2005). Selective media with antimicrobials, oxygen quenching agents, and a low oxygen atmosphere can be used to isolate the microorganism (Altekruse et al. 1999). Numerous selective agar media, including Campy-Cefex, modified Campy-Cefex, mCCDA, Karmali, CAMPY, and Campy-Line agars have been developed to isolate and enumerate *Campylobacter* (Oyarzabal et al. 2005). Studies have shown that Campy-Cefex and modified Campy-Cefex produced the best results for isolation and enumeration of *Campylobacter* (Oyarzabal et al. 2005; Potturi-Venkata, et al. 2007). Stern and Line (1992) found that recovery of *Campylobacter* spp. was most successful with Campy-Cefex agar without enrichment. Plates with either blood or charcoal had better recovery rates in inoculation studies and Campy-Cefex was more efficient than Campy-Line when culturing *Campylobacter* spp. from the cecum and colon (Potturi-Venkata et al. 2007). Since the supplements drive the price, the modified Campy-Cefex employs lysed horse

blood, which drastically reduces cost without altering recovery (Oyarzabal et al. 2005). The

Campy-Cefex agar is also used in the new USDA-FSIS Laboratory Manual for both qualitative

and quantitative isolation, identification, and enumeration of *Campylobacter* in poultry rinses

and sponge samples (USDA 2011a).

Using an enrichment broth prior to plating usually provides better recovery when target

cells are either low in number, injured, or stressed (Williams et al. 2009; Richardson et al. 2009).

Several selective enrichment broths have been used for *Campylobacter* detection including:

Preston broth, Bolton broth, *Campylobacter* enrichment broth, blood-free enrichment broth,

buffered peptone water, Hunt enrichment broth, TECRA broth, Park and Sanders, and Doyle and

Roman enrichment broth (Richardson et al. 2009; Stern and Line 1992). These enrichment

broths may also increase detection sensitivity compared to direct plating (Richardson et al.

2009). Richardson et al. (2009) compared TECRA and Bolton broths on postchill carcasses and

found no statistical difference between the two. Using the TECRA broth, 74% of postchill

carcasses were positive for *Campylobacter*, while 71% were positive using Bolton broth

(Richardson et al. 2009). Research has also been conducted with slight alterations to the

selective enrichment broths. For example, a blood-free Bolton broth as well as a double-strength

Bolton broth has been developed (Williams et al. 2009; Line 2006). In a comparison of Bolton

broth with and without blood, no statistical difference was determined in isolation of

Campylobacter from bootsock and caeca samples, but there was a statistical difference with

chicken carcass rinse samples (Williams et al. 2009). Line (2006) used a double-strength Bolton

broth to examine *Campylobacter jejuni* colonization under high and low relative humidity.

When seeder birds were introduced as the only source of *Campylobacter*, no difference in

colonization rate was observed between the high and low relative humidity conditions (Line 2006).

PCR

Conventional PCR, first developed for *Campylobacter jejuni* and *coli* in 1992, detects chromosomal gene sequences and is able to detect cells at low numbers (Moore et al. 2005). This method detects DNA from live and dead bacteria that are multiplied and then visualized (Humphrey et al. 2007). Real-time PCR (rt-PCR) has also been investigated with *Campylobacter* and is based on mRNA or DNA as a target (Moore et al. 2005). Hunter et al. (2009) analyzed the short variable region (SVR) of the flagellin locus on carcasses at rehang and postchill by PCR. As carcasses moved through processing, genetic diversity of *Campylobacter* decreased; however, 1478 isolates were identified (Hunter et al. 2009). Olsen et al. (2009) used rt-PCR to detect *Campylobacter* from airborne samples in the processing facility.

Confirmation of *Campylobacter*

Culture-based procedures for the confirmation of *Campylobacter* have limitations. Because these procedures are slow and complicated, alternative methods of detection have been developed (Olsen et al. 2009; Moore et al. 2005). Many of these new methods are molecular-based, in particular PCR and rt-PCR. In addition to molecular-based methods, latex agglutination is gradually replacing traditional culture-based methods due to their speed and effectiveness (Moore et al. 2005).

Microscopy

Campylobacter spp. have a unique spiral shape and motility (Ng et al. 1985). Other forms of the organism such as S-shapes, gull shapes, commas, dimpled, and coccoid shapes have also been reported (Ng et al. 1985). Using electron microscopy, these morphological differences can be studied to verify *Campylobacter*. Ng et al. (1985) found that young cells have a spiral shape, but as the cells grow older, they change to a coccoid form. This finding was confirmed by Holler et al. (1998) in a study that analyzed the effect of low temperature on *Campylobacter coli* SP10. Percentages of coccoid cells at day 51 at 37°C, 20°C, 10°C and 4°C were 98%, 94%, 71% and 4%, respectively (Holler et al. 1998). Though this technique is not utilized as often to confirm *Campylobacter*, this is not a practical method of detection directly from food.

Latex Agglutination

Latex agglutination tests, which can provide more rapid species confirmation than conventional phenotypic tests, use polyclonal antibodies to detect antigenic proteins or epitopes from flagella (Moore et al. 2005; Miller et al. 2008). The principle of the latex agglutination test is that the latex particles agglutinate and are easily visible when mixed with *Campylobacter* antigens. Though there are many commercial latex agglutination tests available, the Microgen *Campylobacter* M46 test reacted with all eight *Campylobacter* spp., while the CAMPY (jcl) reacted only with *C. jejuni*, *C. coli,* and *C. lari* (Miller et al. 2008).

REFERENCES

Allos, B.M. 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin. Infect. Dis.* 32:1201-1206.

Altekruse, S. F., N. J. Stern, P.I. Fields, D.L. Swerdlow. 1999. *Campylobacter jejuni* - An emerging foodborne pathogen. *Emerg. Infect. Dis.* 5:28-35.

Alvarado, C. 2011. Dealing with new *Campylobacter* performance standards for poultry. Available at: http://www.meatingplace.com.Accessed 10 July 2011.

Arritt, F.M, J.D. Eifert, M.D. Pierson, and S.S. Sumner. 2002. Efficacy of antimicrobials against *Campylobacter jejuni* on chicken breast skin. *J. Appl. Poult. Res*. 11:358-366.

Bashor, M.P, P.A. Curtis, K.M. Keener, B.W. Sheldon, S. Kathariou, and J.A. Osborne. 2004. Effects of carcass washers on *Campylobacter* contamination in large broiler processing plants. *Poult. Sci.* 83:1232-1239.

Berndtson, E., M. L. Danielsson-Tham, and A. Engvall. 1996. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. *Int. J. Food Microbiol.* 32:35-47.

Berrang, M.E. and J.A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *J. Appl. Poult. Res.* 9:43-47.

Berrang, M.E., R.J. Meinersmann, R.J. Buhr, N.A. Reimer, R.W. Philips, and M.A. Harrison. 2003. Presence of *Campylobacter* in the respiratory tract of broiler carcasses before and after commercial scalding. *Poult. Sci.* 82:1995-1999.

Berrang, M.E., J.S. Bailey, S.F. Altekruse, B. Patel, W.K. Shaw, R.J. Meinersmann, and P.J. Fedorka-Cray. 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 U.S. processing plants. *J. Food Prot*. 70:1556-1560.

Berrang, M.E., R.J. Meinersmann, D.P. Smith, and H. Zhuang. 2008. The effect of chilling in cold air or ice water on the microbiological quality of broiler carcasses and the population of *Campylobacter*. *Poult. Sci.* 87:992-998.

Black, A.P., M.D. Kirk, and G. Millard. 2006. *Campylobacter* outbreak due to chicken consumption at an Australian Capital Territory restaurant. *Commun. Dis. Intell.* 30:373-377.

Bolton, F.J. and D. Coates. 1983. A study of the oxygen and carbon dioxide requirements of the thermophilic *Campylobacters*. *J. Clin. Pathol*. 36:829-834.

Bull, S.A., V.M. Allen, G. Domingue, F. Jorgensen, J.A. Frost, R. Ure, R. Whyte, D. Tinker, J.E. Corry, J. Gillard-King, and T.J. Humphrey. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645-652.

Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, L.H. Stanker, and B.M. Hargis. 1998. Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. *Avian Dis*. 42:802-806.

Cason, J.A., J.S. Bailey, N.J. Stern, A.D. Whittemore, and N.A. Cox. 1997. Relationship between aerobic bacteria, Salmonellae, and *Campylobacter* on broiler carcasses. *Poult. Sci.* 76:1037-1041.

Chinivasagam, H.N., T. Tran, L. Maddock, A. Gale, and P.J. Blackall. 2009. Mechanically ventilated broiler sheds: a possible source of aerosolized *Salmonella, Campylobacter,* and *Escherichia coli. Appl. Environ. Microbiol.* 75:7417-7425.

Choct, M. 2010. Climate in poultry houses. Available at: http://www.poultryhub.org/chicken-meat-industry/climate-in-poultry-houses/. Accessed 10 July 2011.

Clements, M. 2011. *Campylobacter* control during poultry slaughter and processing. Available at: www.WATTAgNet.com.Accessed 14 August 2011.

Conlan, A.J., C. Coward, A.J. Grant, D.J. Maskell, and J.R.Gog. 2007. *Campylobacter jejuni* colonization and transmission in broiler chickens: A modeling perspective. *J.R. Soc. Interface*. 4:819-829.

Curtis, L. 2007. *Campylobacter*. Available at: http://www.foodsafetywatch.com/public/498print.cfm. Accessed 15 February 2012.

Donald, J.O., J. Blakely, J. Garmon, and B. Rochelle. 2005. Environmental management in the broiler breeder laying house. Available at: http://en.aviagen.com/assets/Uploads/EnvMgtLaying.pdf. Accessed 30 January 2012.

Doyle, M.P. and D.J. Roman. 1982. Response of *Campylobacter jejuni* to sodium chloride. *Appl. Environ. Microbiol.*43:561-565.

Dumas M.D., S.W. Polson, D. Ritter, J. Ravel, J. Gelb J, R. Morgan, K.E. Wommack. 2011.Impacts of poultry house environment on poultry litter bacterial community composition. PLoS ONE 6(9): e24785.

Eifert, J.D., R.M. Castle, F.W. Pierson, C.T. Larsen, and C.R. Hackney. 2003. Comparison of sampling techniques for detection of *Arcobacter butzleri* from chickens. *Poult. Sci.* 82:1898-1902.

Frame, D. 2008. Principles of feeding small flocks of chickens at home. Available at:

http://extension.usu.edu/files/publications/publication/AG_Poultry_2008-02pr.pdf.Accessed 9

February 2012.

Fielding, M. 2012. Most poultry farms don't clean crates for pathogens. Available at:

http:www.meatingplace.com. Accessed 30 January 2012.

Guerin, M.T., C. Sir, J.M. Sargeant, L. Waddell, A.M. O'Connor, R.W. Wills, R.H. Bailey, and

J.A. Byrd. 2010. The change in prevalence of *Campylobacter* on chicken carcasses during

processing: A systemic review. *Poult. Sci.* 89:1070-1084.

Habib, I., I. Sampers, M. Uyttendaele, D. Berkvens, and L. De Zutter. 2008. Performance

characteristics and estimation of measurement uncertainty of three plating procedures for

Campylobacter enumeration in chicken meat. *Food Microbiol.* 25:65-74.

Hansson, I., I. Vagsholm, L. Svensson, and E.O. Engvall. 2007. Correlations between

Campylobacter spp. prevalence in the environment and broiler flocks. *J. Appl. Microbiol.*

103:640-649.

Hazeleger, W.C., J.A. Wouters, F.M. Rombouts, and T. Abee. 1998. Physiological activity of

Campylobacter jejuni far below the minimal growth temperature. *Appl. Environ. Microbiol.*

64:3917-3922.

Health Protection Agency. 2011. Over 90 per cent of *Campylobacter* outbreaks at catering

venues in 2011 linked to undercooked chicken liver pate. Available at:

http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1317131748084?p=128

7147958032. Accessed 8 March 2012.

Höller, C., D. Witthuhn, and B. Janzen-Blunck. 1998. Effect of low temperatures on growth, structure, and metabolism of *Campylobacter coli* SP10. *Appl. Environ. Microbiol.*64:581-587.

Humphrey, T., S. O'Brien, and M. Madsen. 2007. Campylobacters as zoonotic pathogens: A food production perspective. *Int. J. Food Microbiol.* 117:237-257.

Hunter, S.M., M.E. Berrang, R.J. Meinersmann, and M.A. Harrison. 2009. Genetic diversity of *Campylobacter* on broiler carcasses collected preevisceration and postchill in 17 U.S. poultry processing plants. *J. Food Prot*. 72:49-54.

Ishihara, K., R. Takahashi, M. Andoh, K. Makita, S. Kamiji, H. Ueno, Y. Muramatsu, and Y. Tamura. 2012. Effects of climatic elements on *Campylobacter*-contaminated chicken products in Japan. *Epidemiol. Infect.*140:991-996.

Jacobs-Reitsma, W.J., A.W van de Giessen, N.M. Bolder, and R.W. Mulder. 1995. Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiol. Infect*. 114:413-421.

Jakarta. 2006. Guidelines on chicken slaughtering and chicken meat handling in small scale chicken slaughterhouses. Available at: http://aitoolkit.org/site/DefaultSite/filesystem/documents/Guidelines_on_Slaughter_in_Small%20Scale%20Chicken%20SH__E.pdf.Accessed 14 February 2012.

Johnsen, G., H. Kruse, and M. Hofshagen. 2006. Genetic diversity and description of transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. *J. Appl. Microbiol.* 101:1130-1139.

Jozwiak, A., O. Reichart, and P. Laczay. 2006. The occurrence of *Campylobacter* species in Hungarian broiler chickens from farm to slaughter. *J. Vet. Med*. 53:291-294.

28

Keener, K. M., M. P. Bashor, P.A. Curtis, B.W. Sheldon, and S. Kathariou. 2004. Comprehensive review of *Campylobacter* and poultry processing. *Comp. Rev. Food Sci. Food Saf.* 3:105-116.

Kuntz, T. 2009.*Campylobacter jejuni* and *Salmonella* spp. detection in chicken grow-out houses by environmental sampling methods. M.S. thesis, Virginia Tech.

Line, J.E. 2006. Influence of relative humidity on transmission of *Campylobacter jejuni* in broiler chickens. *Poult. Sci.* 85:1145-1150.

Miller, R.S., L. Speegle, O.A. Oyarzabal, A.J. Lastovica. 2008. Evaluation of three commercial latex agglutination tests for identification of *Campylobacter* spp. *J. Clin. Microbiol*. 46:3546-3547.

Montrose, M.S., S.M. Shane, and K.S. Harrington. 1985. Role of litter in the transmission of *Campylobacter jejuni*. *Avian Diseases*. 29:392-399.

Moore, J.E., D. Corcoran, J.S. Dooley, S. Fanning, B. Lucey, M. Matsuda, D.A. McDowell, F. Megraud, B.C. Millar, R. O'Mahony, L. O'Riordan, M. O'Rourke, J.R. Rao, P.J. Rooney, A. Sails, and P. Whyte. 2005. *Campylobacter*: Review Article. *Vet. Res*. 36:351-382.

Newall, D.G., J.A. Wagenaar, et al. 2000. Poultry infections and their control at the farm level. In: Nachamkin I, Blaser MJ, editors. *Campylobacter*. 2nd ed. Washington, D.C.: ASM Press. 497-510.

Ng, L.K., R. Sherburne, D.E. Taylor, M.E. Stiles. 1985. Morphological forms and viability of *Campylobacter* species studied by electron microscopy. *J. Bacteriol.* 164:338-343.

Northcutt, J.K., M.E. Berrang, J.A. Dickens, D.L. Fletcher, and N.A. Cox. 2003. Effect of broiler age, feed withdrawal and transportation on the levels of *Campylobacter, Escherichia coli,* and *Salmonella* on carcasses before and after immersion chilling. *Poult. Sci.* 82:169-172.

Olsen, K.N., M. Lund, J. Skov, L.S. Christensen, and J. Hoorfar. 2009. Detection of *Campylobacter* bacteria in air samples for continuous real-time monitoring of *Campylobacter* colonization in broiler flocks. *Appl. Environ. Microbiol.* 75:2074-2078.

Oppliger, A., N. Charrière, P.O. Droz, and T. Rinsoz. 2008. Exposure to bioaerosols in poultry houses at different stages of fattening; use of real-time PCR for airborne bacterial quantification. *Ann. Occup. Hyg.* 52:405-412.

Oyarzabal, O.A., K.S. Macklin, J.M. Barbaree, and R.S. Miller. 2005. Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. *Appl. Environ. Microbiol.* 71:3351-3354.

Peyrat, M.B., C. Soumet, P. Maris, and P. Sanders. 2008. Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *Int. J. Food Microbiol.* 124:188-194.

Potturi-Venkata, L.P., S. Backert, A.J. Lastovica, S.L. Vieira, R.A. Norton, R.S. Miller, S. Pierce, and O.A. Oyarzabal. 2007. Evaluation of different plate media for direct cultivation of *Campylobacter* species from live broilers. *Poult. Sci.* 86:1304-1311.

Potturi-Venkata, L.P., S. Backert, S.L. Vieira, and O.A. Oyarzabal. 2007. Evaluation of logistic processing to reduce cross-contamination of commercial broiler carcasses with *Campylobacter* spp. *J. Food Prot*. 70:2549-2554.

Ramabu, S.S., N.S. Boxall P. Madie, and S.G. Fenwick. 2004. Some potential sources for transmission of *Campylobacter jejuni* to broiler chickens. *Lett. Appl. Microbiol.* 39:252-256.

Rasschaert, G., K. Houf, J. van Hende, and L. De Zutter. 2007. Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter. *Vet. Microbiol*. 123:104-109.

Richardson, L.J., N.A. Cox, J.S. Bailey, M.E. Berrang, J.M. Cox, R.J. Buhr, P.J. Fedorka-Cray, and M.A. Harrrison. 2009. Evaluation of TECRA broth, Bolton broth, and direct plating for recovery of *Campylobacter* spp. from broiler carcass rinsates from commercial processing plants. *J. Food Prot.* 72:972-977.

Saleh, M., J. Seedorf, and J. Hartung. 2005. Influence of animal age and season on bio-aerosol concentrations in a broiler house. *Int. Soc. Anim. Hyg.* 2:37-40.

Scallan, E., R. Hoekstra, F.J. Angulo, R.V. Tauxe, M.A. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Fooborne illness acquired in the United States- Major pathogens. *Emerg. Infect. Dis.* 17:7-15.

SCVMRPH. Scientific Committee on Veterinary Measures Relating to Public Health. 1998. Benefits and limitations of antimicrobial treatments for poultry carcasses. Available at: http://europa.eu.int/comm/food/fs/sc/scv/out14_en.html. Brussels, Belgium: European Commission. Accessed 14 July 2011.

Shale, K. and J.F.R. Lues. 2007. The etiology of bioaersols in food environments. *Food Rev. Intern*. 23:73-90.

Shanker, S., A. Lee, and T.C. Sorrell. 1990. Horizontal transmission of *Campylobacter jejuni* amongst broiler chicks: experimental studies. *Epidemiol. Infect.* 104:101-110.

Snelling, W.J., M. Matsuda, J.E. Moore, and J. Dooley. 2005. Under the microscope: *Campylobacter jejuni*. *Lett. Appl. Microbiol.* 41:297-302.

Sopwith, W., M. Ashton, J.A. Frost, K. Tocque, S. O'Brien, M. Regan, Q. Syed. 2003. Enhanced surveillance of *Campylobacter* infection in the North West of England 1997-1999. *J. Infect.* 46:35-45.

Stern, N.J., P.J. Fedorka-Cray, J.S. Bailey, N.A. Cox, S.E. Craven, K.L. Hiett, M.T. Musgrove, S. Ladely, D. Cosby, and G.C. Mead. 2001. Distribution of *Campylobacter* spp. in selected U.S. poultry production and processing operations. *J. Food Prot.* 64:1705-1710.

Stern, N.J. and J.E. Line. 1992. Comparison of three methods of recovery of *Campylobacter* spp. from broiler carcasses. *J. Food Prot.* 55:663-666.

Stern, N.J., M.C. Robach. 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. *J. Food Prot.* 66:1557-1563.

Suzuki, H. and S. Yamamoto. 2009. *Campylobacter* Contamination in Retail Poultry Meats and By-Products in the World: A Literature Survey. *J. Vet. Med. Sci.* 71:255–261.

USDA.2011a. Isolation, identification, and enumeration of *Campylobacter jejuni/coli/lari* from poultry rinse and sponge samples. Available at: http://www.fsis.usda.gov/PDF/MLG_41_00.pdf. Accessed 10 July 2011.

USDA. 2011b. New performance standards for *Salmonella* and *Campylobacter* in chilled carcasses at young chicken and turkey slaughter establishments. Available at: www.fsis.usda.gov/OPPDE/rdad/FSISNotices/31-11.pdf. Accessed 11 July 2011.

van Gerwe, T., J.K. Miflin, J.M. Templeton, A. Bouma, J.A. Wagenaar, W.F. Jacobs-Reitsma, A. Stegeman, and D. Klinkenberg. 2009. Quantifying transmission of *Campylobacter jejuni* in commercial broiler flocks. *Appl. Environ. Microbiol.*75:625-628.

Viator, C.L., S.C. Cates, S.A. Karns, and M.K. Muth. 2008. Adoption of interventions to improve food safety at meat and poultry processing plants in the United States. *Food Prot. Trends* 28:917-927.

Vucemilo, M., B. Vinkovic, and K. Matkovic. 2006. Influence of broiler age on airborne pollutant content in poultry house. *Krmiva* 48:3-6.

Vucemilo, M., K. Matkovic, B. Vinkovic, S. Jaksic, K. Granic, and N. Mas. 2007. The effect of animal age on air pollutant concentration in a broiler house. *Czech J. Anim. Sci.* 6:170-174.

Waldroup, A.L., K.L Beers, P.E. Cook, E.A. Dell, R. Odglen, R.A. Baker, C.W. Coleman, B.A. Smith, and B.W. Maingi. 2010. The effects of cetylpyridinium chloride (Cecure CPC antimicrobial) on *Campylobacter* spp. on raw poultry: a review. *Int. J. Poult. Sci.* 9:305-308.

Wempe, J.M., C.A. Genigeorgis, T.B. Farver, and H.I. Yusufu. 1983. Prevalence of *Campylobacter jejuni* in two California chicken processing plants. *Appl. Environ. Microbiol.* 45:355-359.

Williams, L.K., F. Jørgensen, R. Grogono-Thomas, and T.J. Humphrey. 2009. Enrichment culture for the isolation of *Campylobacter* spp: Effects of incubation conditions and the inclusion of blood in selective broths. *Int. J. Food Microbiol.* 130:131-134.

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D.G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp, *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C. area. *Appl. Environ. Microbiol.*67:5431-5436.

MATERIALS AND METHODS

Overview

       One commercial chicken grow out house from four farms in western Virginia were sampled the day before the chickens were slaughtered (35-38 days of age). Houses averaged ~37,500 birds and humidity ranged from 39-81%, depending on house and time of year sampled. House samples were collected between October, 2011 and January, 2012, so outside temperature averaged 14°C (Table 2). Inside house temperatures averaged 23°C. Houses or flocks were selected as the first flock of the day to be slaughtered at the associated processing plant. Ten environmental swabs of feed pans or water lines, ten fecal/litter samples, and ten air samples were collected each time from each house, producing a total of 30 samples per house per visit. All samples utilized Campy-Cefex agar for both quantitative and qualitative analysis. Qualitative analysis included an enrichment step prior to spread plating on the selective agar. Air samples were collected initially onto gelatin filters, dissolved in a buffer solution, and then spread plated for quantitative analysis.

       The same flock sampled in the grow-out house was also sampled while en route to processing and during processing. Several environmental samples (~4-5 samples) from the transportation vehicle crates used to carry the birds from the grow house to the processing facility were analyzed while the birds were waiting to be processed. At the processing facility, thirty post chill carcasses were analyzed both quantitatively and qualitatively for *Campylobacter* using Campy-Cefex agar. Environmental samples of chill and scald tank water before and during the flock being processed were also collected and analyzed for *Campylobacter* as a possible source of cross contamination.

Grow-out House Sample Collection and Analysis

1. Air Sampling

Ten air samples were collected onto Sartorius Stedim gelatin disposable filters using a

Sartorius AirPort MD8 Air Sampler (Microbiology International, Edgewood, NY). Each 1,000

liter sample was collected over a 10 minute span (100 liters of air per minute). Samples were

collected by hanging air sampler from a wall in the house (~1.5 meters high). Once collected,

sample gelatin filters were dissolved in 99 ml buffered peptone water (BPW) and stored in a

cooler with ice packs until further analysis the following day. For quantitative analysis, 0.1 ml

of solution was pippetted onto two Campy-Cefex plates (~100 μl per plate) and incubated for 48

hours at $42^{o}C$ in an anaerobe jar under 5% O_2 using OxoidCampyGen modified atmosphere

packs (USDA, 2011a) prior to enumeration. Campy-Cefex agar was supplemented with

0.033g/L cefoperazone (Neogen, Lansing, MI) and 5% laked horse blood (Remel, Lenexa, KS).

For qualitative analysis, 25 ml of sample was added to 25 ml of double strength blood-free

Bolton's enrichment broth (2X BF-BEB) and incubated for 48 hours at $42^{o}C$ (USDA, 2011a) in

an anaerobe jar under 5% O_2 using OxoidCampyGen modified atmosphere packs. 2X BF-BEB

was supplemented with cefoperazone, vancomycin, trimethoprim, and cycloheximide (Oxoid

supplement SR0183E). After incubation, samples were spread plated onto a single Campy-

Cefex agar plate and incubated as above (USDA 2011a).

2. Fecal and Litter Environmental Samples

Ten environmental samples at the chicken grow out house were collected from the litter

using shoe coverings (DuPont Gripper clean room shoe covers; Fisher Scientific). These

coverings were placed over outer protective footwear and worn for about 1 min by the researcher

walking in a 10 ft x 10 ft designated section of the house. Shoe coverings were removed from the outer protective footwear, placed in a large whirl-pack bag with 99 ml buffered peptone water, sealed, and massaged for 1 min to evenly wash the bootie in buffer solution. At least 45 ml of this rinse was transferred to a sterile cup which was then placed in a cooler with ice packs until further analysis. Quantitative and qualitative analysis was performed on the samples the following day. Sample solutions from house 2, 3 and 4 were diluted 1:10 in BPW before spread plating or enrichment due to overgrowth of plates that was observed in house 1. Quantitative analysis was performed in the same manner as described above, with 0.1 ml of solution plated onto two Campy-Cefex agar plates (USDA, 2011a). These plates incubated for 48 hours at 42°C in an anaerobe jar under 5% O_2 using OxoidCampyGen modified atmosphere packs before enumeration. A 25 ml aliquot of the original sample was mixed with 25 ml 2X BF-BEB, incubated for 48 hours at 42°C in an anaerobe jar under 5% O_2, and plated onto Campy-Cefex agar for qualitative analysis. After another 48 hours at 42°C in an anaerobe jar, plate was analyzed for presence/absence of *Campylobacter* (USDA, 2011a).

3. Feeder and Drinker Environmental Samples

Ten sponge samples of feed pans and drink water lines were collected by directly swabbing feed pans or water dispensers using a stick sponge wetted with 10 ml of BPW (3M Food Safety, St. Paul, MN). Samples were designated as either feed or water samples. No single sample had both feed and water lines. Samples were placed in Whirl-pak bags and stored in a cooler with ice packs until further analysis. The following day, 99 ml BPW was added to a Whirl-pak bag before quantitative and qualitative analysis was performed in the same manner as described above. A 0.1 ml aliquot was plated onto two Campy-Cefex plates and allowed to

incubate for 48 hours at 42°C in an anaerobe jar under 5% O_2 using OxoidCampyGen modified atmosphere packs prior to enumeration. For qualitative analysis, 25 ml of the diluted sample was mixed with 25 ml of double strength blood-free Bolton's enrichment broth, incubated for 48 hours at 42°C in an anaerobic jar under 5% O_2, and plated onto a single Campy-Cefex plate. After another 48 hours at 42°C in an aerobic jar, the plate was analyzed for presence/absence of *Campylobacter* (USDA, 2011a).

Transportation Sample Collection and Analysis

Since transport cages or vehicles are a known source of cross contamination of *Campylobacter*, the crates used to carry the birds from grow out house to processing were tested (Keenor et al., 2004; Ramabu, et al., 2004). Each truck that transports birds from the farm to the processing plant carried about 5,000 birds, and there were approximately seven trucks of birds for each flock. While the birds were waiting to be processed, five environmental swabs were taken from the bird crates using a stick sponge wetted with 10 ml of BPW (3M Food Safety, St. Paul, MN). Sponges were used to swab the entire bird crate. Crates on individual trucks were selected at random to produce a total of five samples. Samples were stored in a cooler with ice packs until further analysis the following day. Due to the high bacterial load expected from these samples, only qualitative analysis was performed. After samples were collected, 25 ml of original sample was added to 25 ml of 2X BF-BEB and allowed to incubate for 48 hours at 42°C in an anaerobic jar under 5% O_2. After 48 hours, samples were plated onto a single Campy-Cefex plate, allowed to incubate for 48 hours at 42°C in an anaerobic jar, and examined for the *Campylobacter* colonies.

Processing Plant Sample Collection and Analysis

All processing plant samples were collected from the same plant between October, 2011 and January, 2012. The same flock from the grow-out house was sampled at the processing plant. Because each night the processing facility was thoroughly cleaned, the sampling in the processing facility only took place on the first flock that was on schedule for that particular day.

1. Carcass Rinse

Whole carcass rinses were obtained post chill. Each trip, thirty birds were randomly collected within a 2 to 3 hour period (between 6:30 AM to 8:30 AM) during which only the flock that's grow-out house was sampled were being processed. Once collected, carcasses were placed in a 3500 ml BioPro Bird Rinse bag (3M Food Safety, St. Paul, MN) and rinsed with 400 ml of BPW (3M Food Safety, St. Paul, MN). The bag was massaged for 1-2 minutes and a portion (~100 ml) of the rinse was placed back into the original BPW bottle. For quantitative analysis, 0.1 ml samples were plated within an hour of collection in the processing facility laboratory onto two Campy-Cefex agar plates (~100 μl per plate) and immediately incubated in an anaerobic box with Oxoid CampyGen packs and placed in a portable incubator (Thermotote 24, Scientific Device Laboratory #FS 7146). Another portion (25 ml) of the sample was enriched with 25 ml of 2X BF-BEB and immediately incubated in an anaerobic box under 5% O_2 and placed in the Thermotote 24 portable incubator in the processing facility laboratory (Scientific Device Laboratory #FS7146). Both qualitative and quantitative samples incubated for 48 hours at 42^oC. Quantitative samples were immediately enumerated after incubation, while qualitative samples were plated onto a Campy-Cefex agar plate and presence/absence was determined after 48 hours at 42^oC.

2. Chill Tank and Scald Tank Water

Water samples from the scald tank and each chill tank were collected prior to birds arriving at processing facility (~ 5 A.M.). In addition to these pre-operational samples, three water samples of the chill tank were taken throughout the bird processing cycle near the carcass entry end of the tank. Two additional scald tank samples were also taken while birds were being processed. All water sample types were collected using 250 ml Bibby Sterile plastic cups attached to a dipping rod (BibbySterilin Ltd 193A). Similar to the quantitative analysis for other samples, these water samples (0.1 ml) were plated onto two Campy-Cefex agar plates within one hour of collection in the processing facility laboratory. Samples were incubated for 48 hours at 42°C in an anaerobe jar under 5% O_2 using OxoidCampyGen modified atmosphere packs prior to enumeration. Qualitative analysis was performed in the same manner listed above.

Campylobacter **Confirmation**

After plating on Campy-Cefex selective agar and appropriate incubation periods for both quantitative and qualitative analysis, *Campylobacter* colonies were confirmed in two different ways. Upon visual inspection, if colonies were translucent or mucoid, glistening and pink in color with no particular size requirements, colonies were reported positive for *Campylobacter* (USDA, 2011a). For each sample type, selected colonies were tested using the Microgen M46 *Campylobacter* rapid latex agglutination test (Microbiology International, Frederick, MD). Approximately three samples of each sample type per 10 were tested for *Campylobacter* using latex agglutination.

Statistical Analysis

Qualitative samples where *Campylobacter* was detected were totaled and analyzed using a chi-square analysis. Fisher's exact test was used for analysis that involved a 2x2 table. All tests of difference were at a statistical significance level α= 0.05. Calculations were performed using JMP 9 statistical software (SAS Institute Inc., Cary, N.C.).

RESULTS

Campylobacter was detected across all sample types in each house and plant, except the air samples in house 2. *Campylobacter* was identified in 32% (77/240) of samples collected across each sampling method (air filter, bootie, sponge, and carcass rinse) post-enrichment. *Campylobacter* was discovered in 27% (32/120) of all house samples (air filter, bootie, and sponge) and 37.5% (45/120) of carcass rinse samples after enrichment. Quantitative results frequently found both bootie samples and sponge samples to be too numerous to count; however, air samples showed 1-85 CFU/l. *Campylobacter* enumeration from carcass rinse samples was found to be 10- $3.6x10^3$ CFU/ml. The proportion of *Campylobacter* positive samples in each house and plant, by sample type and location, is shown in Table 1.

With the use of chi-square statistical analysis, no significant difference (α>0.05) was found between each sample type across all four houses. However, the sponge sample method was found to have a significantly higher (α<0.05) proportion of *Campylobacter* positive samples (45%) than the bootie method (20%) and air method (15%) when sample types of all the houses were compared. The highest number of positive sponge samples was found in house 3. When each sample type (air, bootie, sponge, and carcass rinse) was compared separately from each

flock, only the carcass rinse samples showed a statistically significant difference ($\alpha<0.05$) (Table 1).

All house samples (air, bootie, and sponge) were compared to all carcass rinses taken from the same flock. A statistically significant ($\alpha<0.05$) difference was only identified from flock A, indicating a lower association between the corresponding house and carcass rinse samples. No significant difference ($\alpha>0.05$) was found between flocks B, C, and D, indicating an association between house samples and corresponding carcass rinses. Samples from house 1 and associated carcass rinses accounted for the highest total of *Campylobacter* positives (29/60). And, the fewest number of *Campylobacter* positives, from both house environmental (4/30) and carcass rinse samples (8/30) were detected from flock B.

A total of 42 environmental samples (chill tank water, scald/defeathering tank water, and crate) in the processing plant were also tested for *Campylobacter*. For only flocks C and D, crate samples using the same swabbing technique as the feed/water lines in the house, were tested for *Campylobacter*. Quantitative results found 10-100 CFU/ml for chill tank water and 10-700 CFU/ml for scald/defeathering tank water. Quantitative analysis from crate samples was not performed in this experiment. Recovery of *Campylobacter* was found to be 0% (0/20), 25% (3/12), and 50% (5/10) for chill tank water, scald/defeathering tank water, and crate samples, respectively after enrichment.

DISCUSSION

According to several published surveys, *Campylobacter* has been found on 71.5% of retail chicken carcasses in the United States (Suzuki and Yamamoto 2009). The new USDA-FSIS guidelines specify that out of 51 carcasses sampled, no more than 8 can test positive for *Campylobacter* (Alvarado 2011). Thus, no more than 16% of carcasses can be contaminated with *Campylobacter* after the last antimicrobial rinse according to the USDA. In this study, *Campylobacter* was isolated from 32% of all samples tested from both house and plant, which is not a surprising result. Carcasses in this study were tested prior to the final antimicrobial rinse (CPC rinse). However, prior to carcasses entering the chill tank, a trisodium phosphate (TSP) rinse was performed. Assuming this final antimicrobial rinse is effective, the percentage reported in this study would decrease.

During the last week of fattening, bioaersols, which include poultry feces, litter, and feathers, are at their highest concentration (Saleh et al. 2005; Vucemilo et al. 2007). *Campylobacter* has been shown to survive in the air at higher relative humidities, but only in negligible amounts (Vucemilo et al. 2007; Olsen et al. 2009). The concentration of microorganisms in a poultry house varies greatly: ranging from 360 to 5860 CFU/l air (Vucemilo et al. 2007). Kuntz et al. (2009) reported 28% of air samples tested positive for *Campylobacter* in a chicken grow-out house and Johnsen et al (2006) found *Campylobacter* in 31% (5/16) of air samples. This study found that 15% of air samples tested positive post-enrichment with a majority of the positives coming from flock C and D (5 of 6). In addition, quantitative results from direct plating yielded 1-85 CFU/l air. The low incidence of positive air samples from flocks A and B were most likely due the low relative humidities (39-44%) observed in house 1 and 2. Relative humidities in grow-out houses can reach 75-80% and studies have shown a dry,

low humidity pen had a colonization delay in comparison to a high relative humidity pen (Choct 2010; Line 2006). These low humidities coupled with differences in ventilation techniques could explain the low enumeration numbers as well as the low post-enrichment *Campylobacter* positives.

Chickens are known to be coprophagic, meaning they ingest their own feces (Keener et al. 2004). This could be a mode of cross contamination, especially since once a flock has become *Campylobacter*-positive, 80-100% of fecal samples have tested positive (Bull et al. 2006; Potturi-Venkata et al. 2007). Many different fecal sampling methods are used; however, this study placed a "sock" over the shoe and proceeded to walk in a designated area. Williams et al. (2009) and Hansson et al. (2007) utilized this method and found 18% (23/131) of flocks tested positive for *Campylobacter*.

Quantitative results from the bootie samples were inconclusive. Many of the plates were overgrown, or had growth of other microorganisms. Though *Campylobacter* was found after enrichment, dilutions will be necessary for future use in direct plating. A 1:10 dilution slightly helped the direct plating method, but further dilutions are necessary. *Campylobacter* positive samples were found in 20% (8/40) of bootie samples post- enrichment. This result does seem slightly lower than normal, but can most likely be explained by the dry bedding conditions, sampling techniques, and low humidities. House 1, which had the highest number of positives (3/10) had unusually wet conditions in the house. The other houses showed drier conditions, and since *Campylobacter* can survive 2-4 weeks in wet conditions, this could explain the low number of positives (Hunt et al. 2001). In addition, feces were not directly sampled.

Chicken feed and water lines run the entire length of the house. Because chickens have free access to their feed and water lines, cross contamination from feces on chicken feed is another possible mode of transmittance (Berndtson et al. 1996). Feces were observed in some of the chicken feed pans, so environmental sampling of feed and water lines is plausible method to access *Campylobacter* contamination. Berndtson et al. (1996) found 30% (90/300) of water lines tested positive for *Campylobacter*, while Johnsen et al. (2006) found 25% (4/16) of feed lines and 63% (10/16) of water lines tested positive for *Campylobacter*. In this study, feed pans and water lines were sampled separately. *Campylobacter* was isolated from 45% of feed pans or water lines, which is not surprising based on previous research. Similar to the bootie samples, quantitative results were found to be inconclusive. This could be explained by the tremendous amount of dust on the feed pans and drink lines. The amount of airborne particles coupled with the contamination from the transport of microorganisms from the chickens themselves, make quantitative analysis difficult to execute. Dilution schemes would most likely aid in this type of analysis, but were not done in this experiment.

Most research has focused on examining whole birds or part of birds to check for *Campylobacter* contamination during processing. Carcass rinse samples, similar to the sampling method used in this study, have been used by Berrang et al. (2007) and Johnson (2010) during different stages of processing. Johnson (2010) found that 56.3% of post-chill carcasses were contaminated with *Campylobacter*. Quantitative results from carcass rinses yielded 10- 3.6×10^3 CFU/ml. Carcass rinses showed 37.5% (45/120) post-enrichment for *Campylobacter* positives in this study. *Campylobacter* was isolated from 23% (30/131) of flocks when the cloacae was studied at slaughter (Hansson et al. 2007).

Environmental sampling in the processing facility included water samples from the chill and scald/defeathering tank as well as truck samples from flock C and D. The first water samples were taken before any birds entered the processing line. Interestingly, 2 positives out of 12 samples observed from the scald and defeathering tanks were before the birds entered. Both water (chill and scald/defeathering) tanks are thoroughly cleaned each night after the final flock has been processed. However, Peyrat et al. (2008) found 60% of scald tank water tested positive for *Campylobacter* before cleaning. Therefore, it is certainly possible that some *Campylobacter* remained in the tank after cleaning. The final positive came from the defeathering water. Bacterial counts of *Campylobacter* have been shown to increase following defeathering (Berrang et al. 2000). No *Campylobacter* was found in any of the chill tank water samples across the four flocks. This particular facility used chlorine in their chill tank, which is known to reduce *Campylobacter* numbers (Berrang et al. 2007). This study found 50% (5/10) of crate samples tested positive post-enrichment. Transportation from the farm to processing facility is certainly a possible source of cross contamination as one study found 53% of batches of crates tested positive for *Campylobacter* (Hansson et al. 2007). Because of the amount of stress during transportation, increase spreading of intestinal material can occur, which could lead to an increase in bacterial contamination upon arrival at processing (Keener et al. 2004). This is especially a problem with a *Campylobacter*-heavy flock since a survey found only 18.3% of processors sanitize their trucks and trailers (Fielding 2012). This indicates the possibility for cross contamination that can occur while birds are in transport from grow-out house to processing facility.

Continuing Research

      Potential continuation of this research may include dilution schemes to better quantify *Campylobacter* within the grow-out house. Direct plating of environmental samples in the house appears feasible, but more efficient dilutions would make this process easier. An additional study could look at carcass rinses pre-chill and/or before antimicrobial rinse to better understand the effectiveness of the chill tank in reducing bacterial loads. More environmental sampling such as air and equipment sampling in the plant could also provide more information about possible sources of cross contamination within a flock. In the house, environmental sampling such as bedding sampling could offer another possible source of *Campylobacter* transmission. An additional study could compare different heights of air sampling as it relates to humidity and *Campylobacter* contamination. *Campylobacter* levels may differ between carcass rinses taken from the first birds of a flock in comparison to carcasses at the end of the flock. This comparison may provide valuable information possible cross contamination within a flock. Hopefully this research coupled with future studies can provide more safe poultry products to the general public.

REFERENCES

Altekruse, S. F., N. J. Stern, P.I. Fields, D.L. Swerdlow. 1999. *Campylobacter jejuni* - An emerging foodborne pathogen. *Emerg. Infect. Dis.* 5:28-35.

Alvarado, C. 2011. Dealing with new *Campylobacter* performance standards for poultry. Available at: http://www.meatingplace.com.Accessed 10 July 2011.

Berndtson, E., M. L. Danielsson-Tham, and A. Engvall. 1996. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. *Int. J. Food Microbiol.* 32:35-47.

Berrang, M.E. and J.A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *J. Appl. Poult. Res.* 9:43-47.

Berrang, M.E., J.S. Bailey, S.F. Altekruse, B. Patel, W.K. Shaw, R.J. Meinersmann, and P.J. Fedorka-Cray. 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 U.S. processing plants. *J. Food Prot*. 70:1556-1560.

Bull, S.A., V.M. Allen, G. Domingue, F. Jorgensen, J.A. Frost, R. Ure, R. Whyte, D. Tinker, J.E. Corry, J. Gillard-King, and T.J. Humphrey. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645-652.

Choct, M. 2010. Climate in poultry houses. Available at: http://www.poultryhub.org/chicken-meat-industry/climate-in-poultry-houses/. Accessed 10 July 2011.

Fielding, M. 2012. Most poultry farms don't clean crates for pathogens. Available at: http:www.meatingplace.com. Accessed 30 January 2012.

Hansson, I., I. Vagsholm, L. Svensson, and E.O. Engvall. 2007. Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *J. Appl. Microbiol.* 103:640-649.

Hunt, J.M., C. Abeyta, and T. Tran. 2001. Bacteriological Analytical Manual: Isolation of *Campylobacter* species from food and water. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm072616.htm.Accessed 13 March 2012.

Johnsen, G., H. Kruse, and M. Hofshagen. 2006. Genetic diversity and description of transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. *J. Appl. Microbiol.* 101:1130-1139.

Johnson, A. 2010.Airborne *Campylobacter* in a poultry processing plant. M.S. thesis, Virginia Tech.

Keener, K. M., M. P. Bashor, P.A. Curtis, B.W. Sheldon, and S. Kathariou. 2004. Comprehensive review of *Campylobacter* and poultry processing. *Comp. Rev. Food Sci. Food Saf.* 3:105-116.

Kuntz, T. *Campylobacter jejuni* and *Salmonella* spp. Detection in chicken grow-out houses by environmental sampling methods. M.S. thesis, Virginia Tech, 2009.

Line, J.E. 2006. Influence of relative humidity on transmission of *Campylobacter jejuni* in broiler chickens. *Poult. Sci.* 85:1145-1150.

Olsen, K.N., M. Lund, J. Skov, L.S. Christensen, and J. Hoorfar. 2009. Detection of *Campylobacter* bacteria in air samples for continuous real-time monitoring of *Campylobacter* colonization in broiler flocks. *Appl. Environ. Microbiol.* 75:2074-2078.

Peyrat, M.B., C. Soumet, P. Maris, and P. Sanders. 2008. Recovery of *Campylobacter jejuni* from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *Int. J. Food Microbiol.*124:188-194.

Potturi-Venkata, L.P., S. Backert, S.L. Vieira, and O.A. Oyarzabal. 2007. Evaluation of logistic processing to reduce cross-contamination of commercial broiler carcasses with *Campylobacter* spp. *J. FoodProt*. 70:2549-2554.

Saleh, M., J. Seedorf, and J. Hartung. 2005. Influence of animal age and season on bio-aerosol concentrations in a broiler house. *Int. Soc. Anim. Hyg.* 2:37-40.

Suzuki, H. and S. Yamamoto. 2009. *Campylobacter* contamination in retail poultry meats and by-products in theworld: a literature survey. *J. Vet. Med. Sci.* 71:255–261.

Vucemilo, M., K. Matkovic, B. Vinkovic, S. Jaksic, K. Granic, and N. Mas. 2007. The effect of animal age on air pollutant concentration in a broiler house. *Czech J. Anim. Sci.* 6:170-174.

Williams, L.K., F. Jørgensen, R. Grogono-Thomas, and T.J. Humphrey. 2009. Enrichment culture for the isolation of *Campylobacter* spp: Effects of incubation conditions and the inclusion of blood in selective broths. *Int. J. Food Microbiol.* 130:131-134.

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D.G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C. area. *Appl. Environ. Microbiol.*67:5431-5436.

TABLES AND FIGURES

Table 1.*Campylobacter* samples percent positive (post-enrichment),by sample type, from grow-out houses and processing plant

Location	Sample Type	Flock A	Flock B	Flock C	Flock D	Flock Total
House	Air	10% ^{Ab}	0% ^{Aa}	30% ^{Ab}	20% ^{Aa}	15% ^{a}
House	Bootie	30% ^{Ab}	10% ^{Aa}	20% ^{Ab}	20% ^{Aa}	20% ^{a}
House	Sponge	60% ^{Aa}	30% ^{Ba}	70% ^{Aa}	20% ^{Ba}	45% ^{b}
House	Air, Bootie, Sponge Total	33% ^{ABb}	13% ^{Aa}	40% ^{Bb}	20% ^{ABa}	27% ^{ABa}
Plant	Carcass Rinse	63% ^{Aa}	27% ^{Ba}	33% ^{Bb}	27% ^{Ba}	37.5% ^{Ba}

n=10 for house samples, n=30 for plant samples

Significant differences ($p<0.05$) in chi-square statistic between rows are designated with a lower case superscript letter.

Significant differences ($p<0.05$) in chi-square statistic between columns are designated with an upper case superscript letter.

Table 2. Grow-out house and flock information

	House 1	**House 2**	**House 3**	**House 4**
Date started	9/15/11	9/29/11	11/7/11	12/6/11
Date collected	10/18/11	11/3/11	12/12/11	1/11/12
Age	34 days	35 days	36 days	36 days
Bird count	38,147	31,007	44,974	36,548
Humidity	42-44%	39%	68-84%	70-81%

All information was obtained from farmer.

Humidity measurement with Fisher Scientific Thermo-Hygro (S01560) hygrometer.

Figure 1.Number of *Campylobacter* positive samples (post-enrichment) from houses and plant
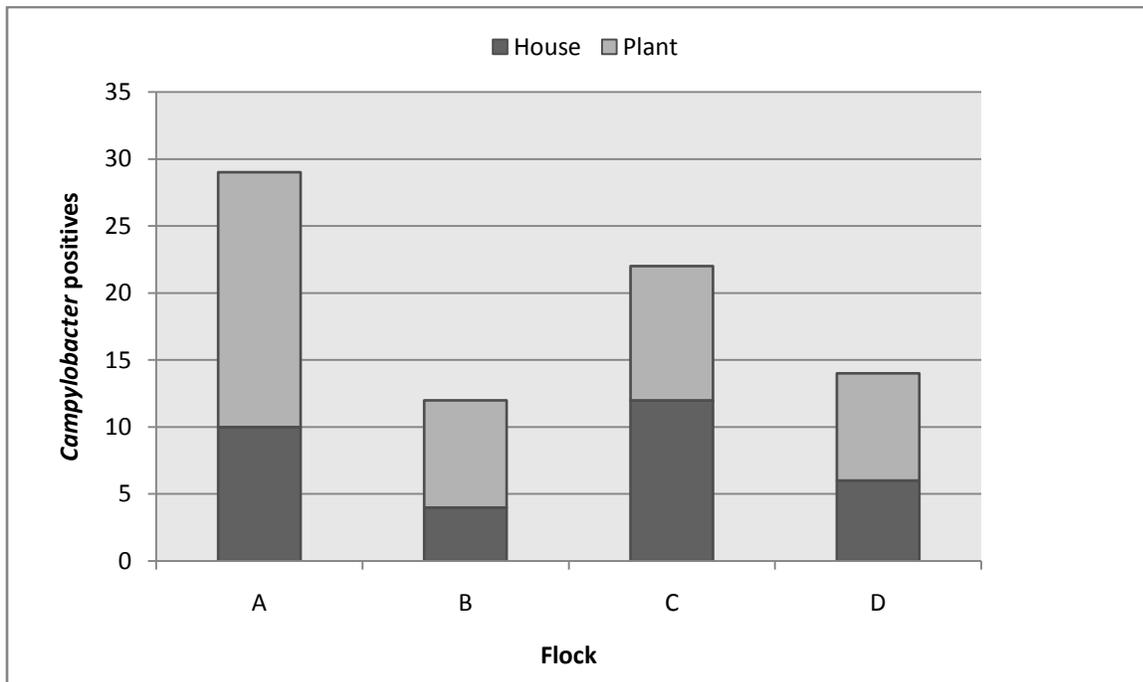
n=30 for both house and plant

Figure 2.Grow-out house sampling flow chart
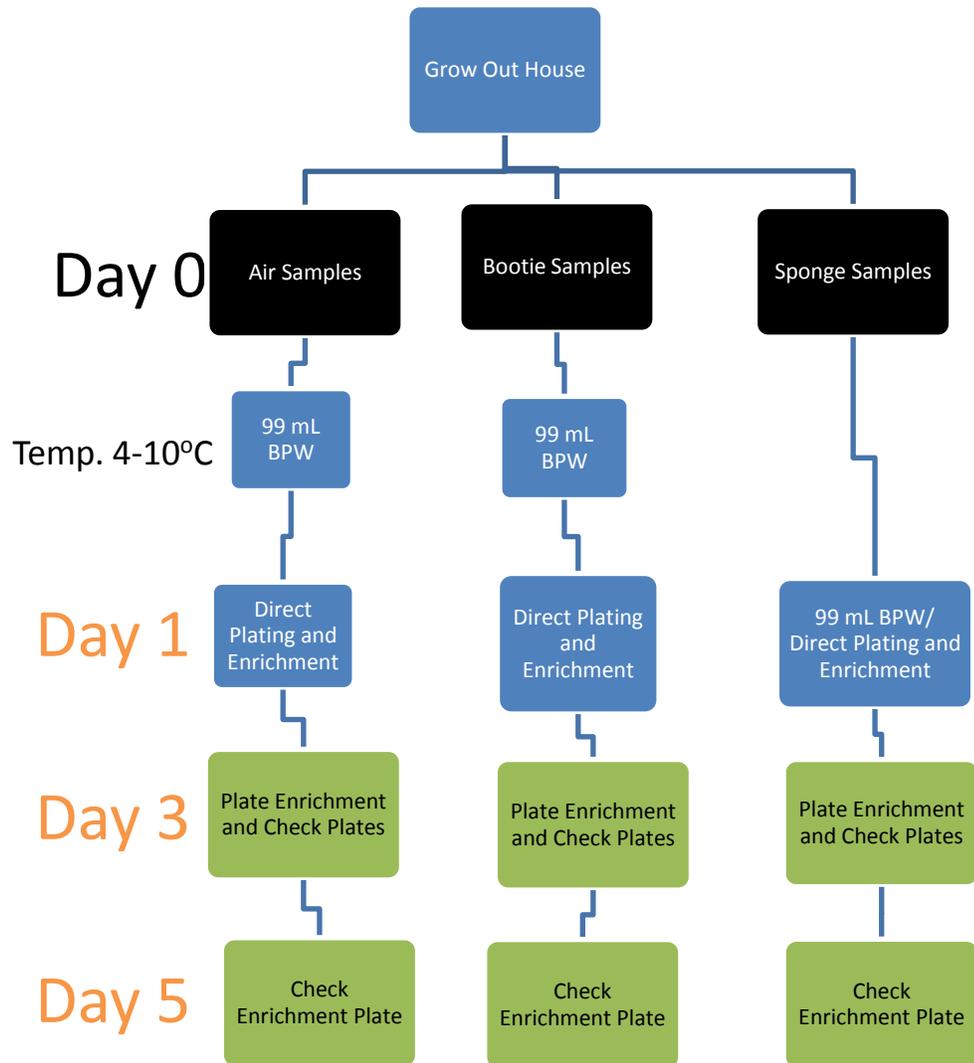
Enrichment Procedure

- 25 ml Bolton's Broth + 25 ml BPW w/ sample

Plating Procedures

- Direct Plating- 2 Campy-Cefex Plates (1/10 ml each plate)
- After Enrichment- 1 Campy-CefexPlate (1/10 ml each Plate)

Figure 3.Flow chart for processing facility analysis
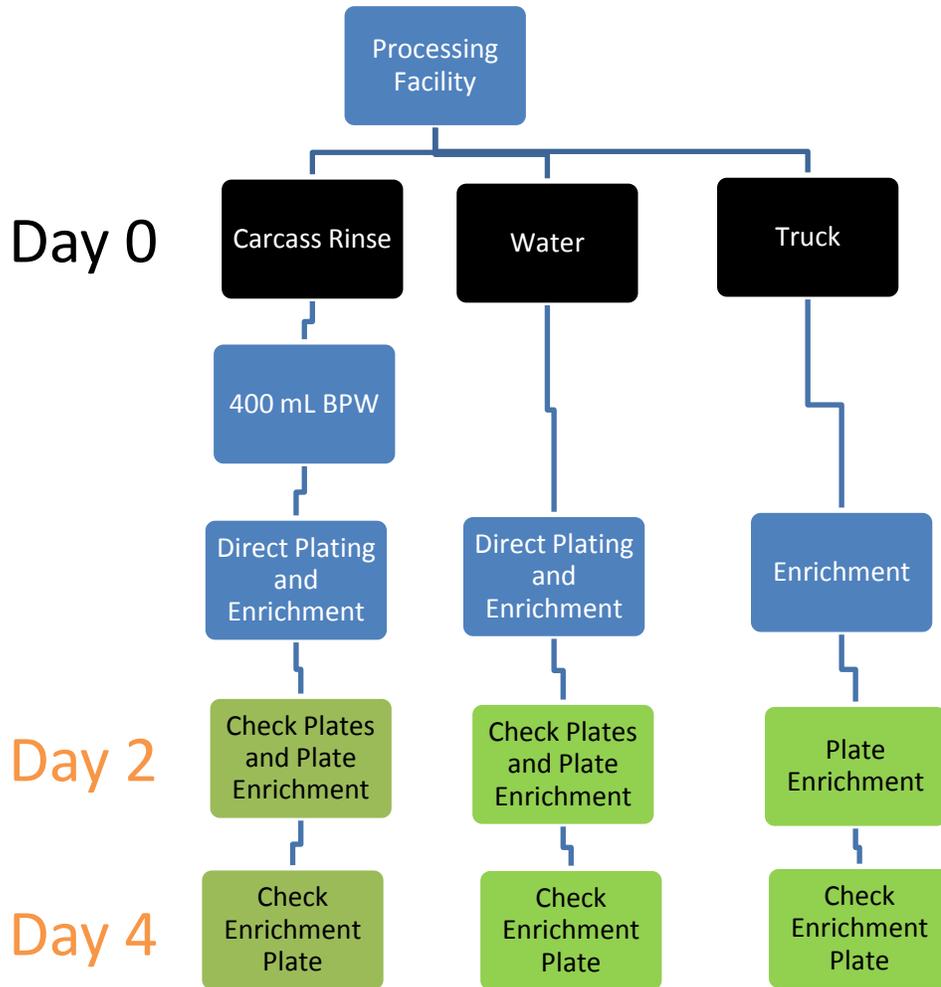
Enrichment Procedure

- 25 ml Bolton's Broth + 25 ml BPW w/ sample

Plating Procedures

- Direct Plating- 2 Campy-Cefex Plates (1/10 ml each plate)
- After Enrichment- 1 Campy-Cefex Plate (1/10 ml each Plate)

Figure 4.*Campylobacter* transolomission through chicken processing
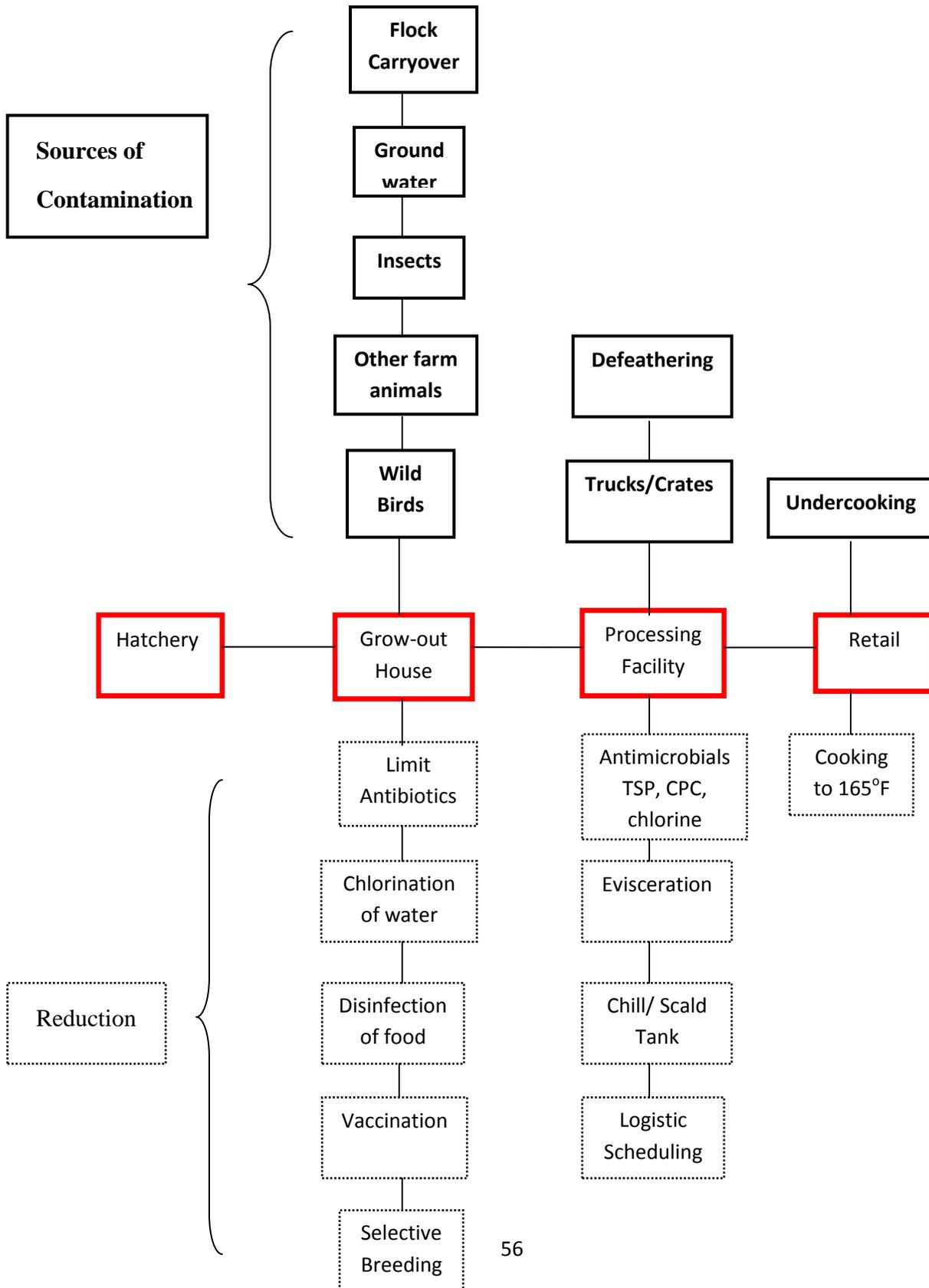
Figure 5.Example of sampling locations within a grow-out house