Airborne *Campylobacter* in a Poultry Processing Plant

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Abstract

Campylobacter is a foodborne pathogen commonly found in live poultry and raw poultry products. Identifying areas of contamination or modes of transmission during commercial processing can lead to strategies to reduce the level of *Campylobacter* on finished products. Monitoring levels of airborne *Campylobacter* may be useful for identifying the presence or relative concentration of the pathogen in a processing plant environment. In this study, air sampling was used to detect and quantify *Campylobacter* in a commercial chicken processing plant by location within the plant and collection time during the day. Air was sampled from evisceration and post-chill areas in a poultry processing plant on four days and at 4 hour intervals onto Campy-Cefex agar plates or gelatin filters that were subsequently transferred to Campy-Cefex agar plates. Additionally, pre-evisceration and post-chill carcass rinses were analyzed quantitatively for *Campylobacter*. The mean level of airborne *Campylobacter* was 5 CFU/1000L of air sampled (10% samples positive) in comparison with 413 CFU/mL from carcass rinses (70% samples positive). Higher concentrations were found in carcass rinse samples from pre-evisceration. Airborne *Campylobacter* was detected from the evisceration area more

frequently than from the post-chill carcass area of the plant ($P < 0.05$). This study shows that airborne *Campylobacter* can be quantified with a selective agar and with gelatin filter collection. Further research is needed to prove the utility of airborne detection of *Campylobacter* for estimating the relative contamination level of live poultry flocks and the processing plant environment and the potential for cross-contamination.

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Dedication

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Introduction

Campylobacter is a bacterial pathogen that typically causes sporadic cases of foodborne illness. In poultry processing, *Campylobacter* and *Salmonella* are the two most common pathogens associated with foodborne illness. While *Salmonella* has previously been regarded as the largest threat in human foodborne illness related to poultry products, recent studies suggest that *Campylobacter* is far more prevalent. *Campylobacter* causes 1.9 million illnesses and *Salmonella* only 1.3 million foodborne illness cases (Cates et al., 2007). According to the CDC, *Campylobacter* is responsible for 13 cases per 100,000 persons each year, causing 124 deaths per year (CDC, 2005). Despite the sporadic nature of *Campylobacter*, several outbreaks have been identified. In Europe, *Campylobacter* was implicated in 107 foodborne illnesses with poultry accounting for 39 cases. Specifically in the United States, between 1998 and 2004, it was reported that 102 outbreaks and 4,587 outbreak-related cases of foodborne and waterborne illness were linked to *Campylobacter* (Olson et al., 2008). Of these cases, poultry was directly linked to 11 outbreaks and associated with 71 related cases. Since the emergence of FoodNet in 1996, the number of reported foodborne illnesses, particularly related to *Campylobacter*, has increased. Increasing reported cases has also increased identification and surveillance of *Campylobacter* in food products such as poultry.

Recent studies seeking the source of *Campylobacter* contamination in poultry processing have implicated airborne contaminants as a possible factor. In a study by Whyte, 70% of airborne contaminants in the defeathering and evisceration areas of processing were

identified as *Campylobacter* (Whyte et al., 2001). Additionally, Posch found 6 to 40 *Campylobacter* colonies per L of air in the broiler hanging area and evisceration (Posch et al., 2006). Currently there are few studies to support the presence of airborne contaminants in processing and possible effects on product quality. Further research is needed to test this methodology as well as *Campylobacter* prevalence in processing.

The European Food Safety Authority reported an increase in the presence of *Campylobacter* throughout the production process. A study conducted in 2008 found that *Campylobacter* was present in 71.8% of live broilers and was identified in 75.8% of post-processed carcasses, indicating that *Campylobacter* is able to survive and further contaminate the product (EFSA, 2010). The survival of harmful concentrations of *Campylobacter* has been linked to contamination during poultry production. Air sampling is an emerging method for detecting the presence of airborne contaminates, which may contain *Campylobacter*.

The objective of this research is to quantify the level of airborne *Campylobacter* in a poultry processing facility by comparing two air sampling methods. The two air samplers tested were the Sartorius and MicroBio sampler which use gelatin filters or agar plates, respectively. The effectiveness of these air sampling methods was compared with levels of *Campylobacter* quantified from carcass rinse samples taken in evisceration and post chill areas. A final objective of this study was to identify the fluctuation, if any, of airborne *Campylobacter* levels throughout the day of processing as a function of time. This was compared with levels of *Campylobacter* quantified from carcass rinses samples in evisceration and post chill areas.

LITERATURE REVIEW

Campylobacter

Campylobacter spp. are gram negative organisms found in microaerophilic, thermotolerant conditions (Jacobs-Reitsma et al., 2008). The bacteria are commonly identified as a foodborne pathogen and are able to cause foodborne illness, including symptoms such as bloody diarrhea and abdominal cramping (CDC, 2005). More serious illnesses have been linked to *Campylobacter,* such as Guillain-Barré syndrome and Reiter syndrome. Three main serotypes have been found specifically related to foodborne illness, *C. jejuni*, *C. coli* and *C. lari*, with *C. jejuni* as the most common. Most cases have been sporadic incidences, not linked to large outbreaks, but this could be due to the low number of cases that are reported (Stern et al., 2001; Jacobs-Reitsma et al., 2008). Identifying the organism and strain is most commonly done through fecal samples.

Several sources have been implicated in *Campylobacter* cases, such as contaminated water sources, raw milk, pork, and seafood (Jacobs-Reitsma et al., 2008). Primary animal/bird sources can include "hare, waterfowl, wild boar, pheasant, guinea fowl, pigeon, quail, ostrich and domestic duck" (Jacobs-Reitsma et al., 2008; Wagenaar, 2008). *Campylobacter* microorganisms can be found in most animals, especially poultry, as a normal part of their intestinal micro flora. The most common, most abundant source has been found in poultry, with the ceca serving as its primary amplifier (Wagenaar, 2008). Colonization and shedding can occur as early as 2 to 3 weeks of age and can increase exposure to *Campylobacter* depending

on the number of birds being produced. This can lead to an increase in diverse serotypes and genetic variation when sampling for *Campylobacter* (Wagenaar, 2008). After commercial processing of poultry, carcasses have been found to carry 10^2 to 10^6 colony forming units (CFU) per carcass on average. Considering that the infectious dose can be as low as 500 CFU (Jacobs-Reitsma et al., 2008), the level of *Campylobacter* found in live bird flocks and processing should be monitored.

Prevalence in Poultry Processing

In poultry processing, research has been implemented to study how harmful organisms such as *Campylobacter* survive and are transmitted from live bird areas to the end product. In initial studies, it was discovered that microbial load was the highest in areas such as scalding and defeathering (Abu-Ruwaida et al., 1994). These areas are high temperature, high humidity areas and were expected to lead to a reduction in pathogenic organisms. In the Abu-Ruwaida study, pathogens such as *Campylobacter* were found in high concentrations on carcass rinse samples and air samples. Although the test methods used were not as sensitive as current methods, they were able to isolate and enumerate *Campylobacter* et al., gram-negative, harmful organisms throughout processing.

Several plants have been studied to test the prevalence of *Campylobacter* during processing. In one study, 13 plants were sampled with varying results from each. In total, 74% of carcasses produced negative levels of *Campylobacter* (Stern and Pretanik, 2006), but in plants with positive, countable levels, the number of bacteria varied greatly. Levels greater

than 10^5 log CFU per carcass were represented in 3.6% of samples. Similar studies have also found high concentrations of *Campylobacter* present in processing. Studies done in Ireland have shown the incidence of *Campylobacter* as 47-87% of birds, while other studies reported 20 to 40% prevalence in poultry in Northern Europe and 80% in the United Kingdom (Stern and Pretanik, 2006; Wagenaar, 2008). Due to the low infectious dose, 500 colony forming units, leading to human illness, the high prevalence of *Campylobacter* in poultry could lead to frequent cases of foodborne illness.

Contamination in Live Production

Contamination is a key factor in the survival of organisms throughout processing, and preventative measures should be the focus (IFST, 2007). Identifying key areas of contamination for preventing the spread of *Campylobacter* and other harmful organisms throughout poultry production is a continued focus in research. Studies have shown that key areas to control contamination would be poultry farms during grow-out and handling during transport to the slaughterhouse. Two additional areas listed were processing facilities and home kitchens where poultry are prepared and cooked (Wagenaar, 2008). In poultry production an increase in slaughter has occurred from 1.53 billion birds in 1960 to 8.84 billion birds in 2006 (MacDonald, 2008). As an increase in production occurs annually, increasing the number of birds will increase bacterial load carried from grow-out to processing (Bolder, 1998; FSIS, 2008; Wagenaar, 2008).

Environmental factors such as air quality, equipment and facility hygiene, as well as carcass to carcass and carcass to machine cross contamination have been identified as

important (Abu-Ruwaida et al., 1994; Jacobs-Reitsma et al., 2008). Studies have found that up to 80% of equipment tested positive for *Campylobacter*, this would include hanging shackles and carrying crates in the plant (Posch et al., 2006). Other factors include employees possibly spreading bacteria through clothing or their movement from one area of a slaughter plant to another (FSIS, 2008).

Before processing can begin, birds must be transported from poultry grow-out houses. Grow-out has an important impact on the level of bacterial contaminants that may be entering the slaughter facility. This can be attributed to infected birds carrying *Campylobacter* and other bacteria in the intestine, crop, or cloaca, which may be shed through the feces (Bolder, 1998; FSIS, 2008). Additional parts of the bird such as skin and feather follicles may collect bacteria from feces and become carriers. Transmission of *Campylobacter* can occur from bird to bird through litter, water or equipment. Feed and water impact the level of cross contamination during grow-out and processing. Water needs to contain "organic acids to reduce cross contamination post harvest" (Bolder, 1998; FSIS, 2008). The acid used in water will decrease bacteria in the crop prior to processing. Water should also be monitored for excess dripping onto litter, which can cause increased moisture where *Campylobacter* and *Salmonella* may thrive. If feed is removed too early or too late, intestines may weaken and rupture during processing exposing carcasses to feces and contaminated food materials. This may lead to the spread of bacteria, including *Campylobacter* and *Salmonella* from infected birds (FSIS, 2008).

Transportation crates and movement of birds by employees can also become factors. The larger number of birds transported to processing and the type of crates have made it more

difficult in some cases to keep carrying crates clean (Bolder, 1998; Cox and Pavic, 2009). Increased stress during transport can increase the amount of feces excreted from healthy and infected birds, increasing the spread of *Campylobacter* amongst flocks (Jacobs-Reitsma et al., 2008). The USDA Food Safety and Inspection Service (FSIS) also found that cases of infected birds worsened after transport, increasing *Salmonella* levels from 5% to 10% and increasing *Campylobacter* levels (FSIS, 2008).

Contamination in Processing

Upon arrival to the processing plant, birds are placed in the live bird loading/receiving area. This area has been considered to contain the highest concentrations of bacterial contamination during processing and can overwhelm processing preventative measures, allowing movement to other areas of the plant (Bolder, 1998; FSIS, 2008). FSIS has noted that movement of bacterial contaminants beyond the receiving area may be due to employee movement between areas and air-contamination (FSIS, 2008). A study by Berrang has suggested that this heavy contamination overload could be avoided by implementing a bird scheduling program. This would allow plants to monitor and schedule bird processing from light to heavier bacterial loads throughout the processing day (Berrang, 2003). This would help to track flocks from load in to end product, and any contaminants could be traced throughout processing.

Load-in/receiving is followed by stunning and bleeding of birds. This can be done in three ways: electrical, mechanical and chemical (FSIS, 2008). Problems in this area that

contribute to contamination can be the release of feces onto other carcasses, which can be carried into subsequent processing steps such as scalding and defeathering. A 1994 study by Abu-Ruwaida found that other areas of high bacterial contamination were scalding, defeathering and evisceration (Abu-Ruwaida et al., 1994).

Scalding is used primarily for loosening feather follicles and can also eliminate bacteria due to the high temperatures used. Several factors can affect the effectiveness of the process including pH of water, scald temperature and build up of organic matter in the scalder (FSIS, 2008). If the pH of scald water remained at 7, *Campylobacter* was found to become heat resistant (FSIS, 2008). The temperature used ranges from 50-60°C, and this does not completely rid the carcass of bacteria (Bolder, 1998; Jacobs-Reitsma et al., 2008). The bacterial load of feces carried on carcass skin or shed through intestinal leakage can enter the scald water, making it another risk factor in cross contamination (Jacobs-Reitsma et al., 2008). The levels detected vary depending on scald temperature, increasing at higher and lower temps such as 52°C and 60°C. The mid temperature of 56°C was linked to the lowest presence of bacterial load, including Salmonella and *Campylobacter* (Bolder 1998). If temperatures are kept too high, *Campylobacter* will be reduced, but it can cause surviving bacteria to physically adhere to the skin of the bird (Jacobs-Reitsma et al., 2008).

In scalding areas, higher bacterial counts were believed to come from “high bacterial load in scalding water and scalding tank” (Abu-Ruwaida et al., 1994) carried by contaminated carcasses. Key areas of the bird such as feathers, feces and skin were implicated in carrying bacteria into scald tanks or spray scalders (Bolder, 1998). Specifically the level of

Campylobacter was increased by "1.5 log cycles after scalding and defeathering, indicating some level of contamination or cross contamination" (Abu-Ruwaida et al., 1994)

Evisceration is the next step, which includes birds from the scald/defeathering area at re-hang and intestine removal until they enter the chill tank. During evisceration there is another opportunity for intestinal rupture or leakage, further exposing carcasses to contaminated feces. Studies acknowledge the benefit from the use of inside-outside washers, which are responsible for spray washing the carcasses clean (Bashor et al., 2004; Cox and Pavic, 2009). Factors such as time and temperature of washers, spray speed and pressure, along with chlorine sanitizers being used have an effect on how well bacteria are eliminated. In some cases the level of *Campylobacter* could be decreased by 1 log cycle by using spray washers, but a measure of end product still showed the existence of high levels of organisms. To a certain level, decreased handling in the evisceration area is believed to cut down on worker contamination (FSIS, 2008). But machine contamination is still viable (Bolder 1998). Abu-Ruwaida reported that evidence in is his study showed that no change during evisceration occurred, but others pointed to an increase of 1 log cycle (Abu-Ruwaida et al., 1994).

Generally, the final step before packaging the end product is chilling. Two chilling methods that have been used are immersion and air chilling. Immersion chilling uses a concentration of chlorine in water between 25 to 50 ppm to decrease microbial load (Bashor et al., 2004). The temperature should be kept at less at 40°C and pH between 6-6.5 (FSIS, 2008; Yang et al., 2001). Microbial load has been shown to decrease with time and exposure to available chlorine. According to Yang, using "10ppm available chlorine will eliminate

Salmonella in 120 minutes and *Campylobacter* in 113 minutes" and if "increased to 50 ppm, available chlorine will eliminate *Salmonella* in 3 minutes and *Campylobacter* in 6 minutes (Yang et al., 2001; FSIS, 2008)." This is the most commonly used method of chilling and works best for antimicrobials.

Studies using air chilling found that it was not as effective as immersion chilling. It does not use chemicals during the process and is affected by environmental sanitation (FSIS, 2008). When using air chilling, less physical contact between birds occurred but drying time was increased. Increasing exposure to air is needed due to the condition of poultry skin, which does not dry as quickly as desired (Jacobs-Reitsma et al., 2008; FSIS, 2008).

Another possible method of *Campylobacter* reduction, listed in a study by Cox and Pavic, was irradiation. This method is not highly accepted in consumer perception but may be effective in eliminating some level of bacteria (Cox and Pavic, 2009). Freezing carcasses and end products that test positive has also been suggested. By freezing contaminated carcasses, it would allow *Campylobacter* to die and be reduced (Posch et al., 2006).

In an article from Institute of Food Science and Technology it was found that 50% of chilled and 30% of frozen meat had *Campylobacter* present on the carcass (IFST, 2007). Jacobs found that in finished raw product, carcasses can have log 2 to log 6 CFU per carcass remaining (Jacobs-Reitsma et al., 2008), which was similar to the amount found by Abu-Ruwaida. In the Abu-Ruwaida study 65% of *Campylobacter* detected was classified as *C. jejuni* (Abu-Ruwaida et al., 1994).

Surveillance and Prevention

Prevention through surveillance and sampling have been helpful in identifying areas of contamination and the highest concentrations of *Campylobacter* throughout the plant. Currently there are government guidelines only restricting the level of *Salmonella* present in poultry processing plants. Guidelines have not been implemented in monitoring the levels of *Campylobacter*, but *Salmonella* tests are used as an indicator of the presence of this pathogen (Ernst, 2009; FSIS, 2008). However, there is statistical evidence supporting *Campylobacter* as a viable component in food borne illness and is suggested to be more common than *Salmonella* (Stern et al., 2001). A survey by RTI International found that the incidence of *Salmonella* in food borne illness was implicated in 1.3 million cases while *Campylobacter* was implicated in 1.9 million food borne illnesses (Cates et al. 2007). Based on these reports, the Food Safety and Inspection Service et al., have recognized the need for new food safety guidelines and have begun research in their development.

According to the FSIS, there are general food safety assessments that all plants must follow and implement into plant operation. Hazard Analysis and Critical Control Point program or HACCP is a commonly used method in food processing industries to determine areas and control points that can be hazardous in food production. Specifically in poultry production, FSIS has recommended the "use of HACCP, Sanitation SOP's et al., prerequisite programs"(FSIS, 2008). These plans are evaluated by Enforcement, Investigations, and Analysis Officers (EIAO) under Food Safety Assessments and must maintain information on areas such as "foodborne illness outbreaks related to the product and positive laboratory results related to human

illness" (FSIS, 2008). Primarily these protocols have been set to monitor for *Salmonella,* but USDA is seeking to have *Campylobacter* reported in the same way (FSIS, 2008).

Without set regulations, companies/industry have taken responsibility for surveillance and intervened in decreasing the spread of *Campylobacter* (Cates et al., 2007; USDA et al., 2008). Many companies, in addition to a HACCP plan, have voluntarily implemented and participate in other preventative measures such as private company audits and testing (Viator et al., 2008). An industry survey of 219 companies reported that 75% of plants participated in environmental sampling and 85% in microbiological testing (Cates et al., 2007). This also includes adding measures such as inside-outside washers and chemical spray systems into processing as well as making company laboratories available on site for evaluation (Cates et al., 2007).

The Hazard Analysis and Critical Control Point based Inspection Models project (HIMP) was established as an addition to the 1996 HACCP regulation with the intent to shift the focus of plant inspection from federal inspection agents back to plant employees (Berrang 2008). Development of this program is suggested to allow federal agents in the plant to focus primarily on visual inspection, regulation and implementation of guidelines. In several studies however, including the Berrang study, it was supported that HIMP is equally as effective as HACCP in lowering the bacterial load on end product carcasses (Berrang 2008).

In addition to surveillance efforts, other preventative methods and techniques have been suggested in decreasing the incidence of *Campylobacter*. A suggestion by Jacobs was to use flock surveillance during processing. This would allow plants to monitor and schedule bird

processing from light to heavier bacterial loads throughout the processing day (Berrang et al., 2008; FSIS, 2008). This would help to track flocks from load-in to end product and contaminants could be traced back to specific flocks. A study by Posch also suggested that scheduling negative flocks before positive flocks would be good for processing (Posch et al., 2006). However, studies performed to test this idea have found that flock scheduling may be difficult due to the variations between sample types and consistency in results (Nautu et al., 2009).

An influencing factor in many studies is that results in countable *Campylobacter* levels vary highly among plants (Whyte et al., 2001). Variation factors include number of birds present at slaughter, time of food removal, time of slaughter, number of birds infected with *Campylobacter* prior to slaughter, type of equipment used and sanitation of plant (Abu-Ruwaida et al., 1994). Because there are no set guidelines, each plant may use different techniques and equipment in measuring bacterial load. FSIS also detailed variations in results due to efficiency and quality of implemented HACCP plans and prerequisites. In 2006, it found that several plans were “lacking supporting documentation and records for hazard prevention” along with “inconsistencies in identifying critical control points (CCP) and critical limits” (FSIS, 2008). These findings weaken the effectiveness of their main goals which is to “reduce the level of contamination” and “ensure birds are processed in that manner” (FSIS, 2008) when data is inconsistent.

Airborne Transmission

The role of airborne contamination and airborne transmission of *Campylobacter* in food production and processing has not been researched extensively. Sensitive and specific air sampling methods are essential for this type of research. Currently there is limited research on air sampling (Posch et al., 2006) and no uniform method is recognized. Researchers have found airborne bacteria present throughout processing and have posed the question of whether these contaminants can be contributors to carcass contamination (Berrang et al., 2004). Early studies have shown that the effect of air quality on food products depends on environmental air conditions and time of exposure (Heldman, 1974). Heldman concluded that airborne contamination depended on the viability of microbial aerosols as the influence of air temperature, humidity and particle population. Air flow and rate of ventilation are additional factors in the survival of airborne contaminants (Heldman, 1974). Other research studies referencing Heldman's study proposed that airflow should be directed away from end product areas back toward initial processing areas and out of the facility. Air quality flowing through doorways and pathways should also be monitored throughout the facility since this air could result in product cross-contamination (Burfoot et al., 2005).

In poultry processing the highest prevalence of bacteria has been found in the live bird loading and defeathering areas (Berrang et al., 2004; Whyte et al., 2001). Other studies have widened the location of highest counts to include defeathering, evisceration and post chill (Whyte et al., 2001). In studies of the air quality in poultry processing environments, levels of bacterial contamination found on carcasses could similarly be found in air samples taken in

slaughter, scalding, defeathering and evisceration areas (Abu-Ruwaida et al., 1994). Burfoot identified several other areas including "hanging, stunning/slaughter, inside/outside washers and equipment" areas in airborne contaminants (Burfoot et al., 2005).

A significant decrease in airborne pathogen concentrations was seen from the slaughter area to scalding, but further increased after defeathering (Berrang et al., 2004). Specifically a study in Austria found that airborne *Campylobacter* found in the hanging area ranged from 0 to 8.0×10^3 CFU/m^3 and from 0 to 4.0×10^3 CFU/m^3 in evisceration (Haas et al., 2005). Another study by Whyte found similarly that *Campylobacter* ranged from 6.7 to 70% in evisceration and 46.7 to 70% in defeathering areas (Whyte et al., 2001). In comparison, a study by Burfoot found that carcass contamination was so high, in the defeathering and evisceration areas of the plant, that airborne contamination may not be comparable to surface contaminants (Burfoot et al., 2005).

The source of these contaminants can be attributed to "mist, dust, feather and fecal material (Berrang et al., 2004)" circulated during various parts of processing. In defeathering, for example, feathers removed in the presence of water sprayers have been identified as a method of contamination, where possible airborne mist carrying bacteria may be created. The mist can also be identified as an aerosol or airborne droplets carried throughout processing. High humidity, temperature and moisture in the scalding area contributed to bacteria survival in the air according to Burfoot (Burfoot et al., 2005). Additionally the Austrian study found that bird movement during hanging could increase air contaminants through spreading litter and fecal material (Haas et al., 2005).

According to Heldman, the viability of airborne microorganisms should decrease over time due to “sedimentation to the floor, removal by ventilation and death rate of organisms” (Heldman, 1974). Many of the airborne contaminants noted in the Heldman study have been identified and can include *Campylobacter*. Although these airborne bacteria are believed to decrease over time, there are still high levels reported on end products. It was reported that Live bird fecal samples were contaminated (64%) with *Campylobacter* but end product carcass yielded 85% positive samples at post processing in another study (Posch et al., 2006). Airborne contaminants could be an impacting factor. Burfoot and Whyte both support the idea that end products should be processed and handled in an area that is separate from the rest of processing (Burfoot et al., 2005). Further research and data on the source, transmission method and cause of airborne *Campylobacter* would help to identify preventative steps. Studies supporting a standard method of air sampling and testing would also help provide consistent data as well.

Methodology for Identification of *Campylobacter* in Food Processing Environments

Air Sampling

In early testing of airborne contaminants, basic air sampling methods primarily used petri dishes containing selective and non-selective agar exposed to various atmospheres in the plant for allotted periods of time. These were referred to as ‘settle plates’ in the Burfoot study and could also be linked to carcasses during processing as a collection method (Burfoot et al., 2005). An example of this early study was applied in research by Abu-Rawaida. Plates

containing aerobic plate count-agar were left exposed in the plant for one-minute periods. Slaughter, scalding and evisceration areas contained the highest levels of bacterial contaminants, including *Campylobacter* specifically ranging from 4 CFU/min to over 300 CFU/min in some areas (Abu-Ruwaida et al., 1994). A similar study used plates with Campy-Cefex agar which was exposed for one minute in the chilling and picker (defeathering) area (Berrang et al., 2004). This study found *Campylobacter* only when agar was transferred to enrichment broth. Samples could not be directly enumerated after initial air samples were collected.

In addition to basic methods of exposing plates to air, air sampling machines have been designed for environmental air sampling. There have been several types and brands that have been identified and used in poultry production. Studies by Posch and several others used two separate air samplers, an Anderson Six Stage Viable Cascade Impactor and impingement SKC BioSampler. The Anderson six stage impactor has been used with modified charcoal cefoperazone deoxycholate agar (mCCDA) for sampling and culturing bacteria. Samples were collected at a rate of 28-31 particles per minute, running for 1 to 2 minute cycles. Six plates on the sampler are set in cascading order to allow particles and organisms to pass through based on their decreasing size, as air flow moved further down into the sampler (Whyte et al., 2001; Posch et al., 2006). These air samples were also found to be sensitive to oxygen levels and dehydration. The SKC BioSampler has also been used and collects samples at a rate of 101 particles per minute in 10 minute cycles. The impingement method of the BioSampler used liquid saline solution which was diluted after collection and cultured onto mCCDA agar plates.

Applying these air sampling methods, researchers in the Posch experiment were not able to find airborne *Campylobacter* using the Anderson sampler and had better results using the liquid based BioSampler. *Campylobacter* was detecting in evisceration at 1.2×10^4 CFU/m^3 and 3.9×10^3 CFU/m^3 in the hanging area of load-in (Posch et al., 2006). In a similar study done in Ireland however, the Anderson sampler detected *Campylobacter* levels of 162 CFU/m^3 (Posch et al. 2006). These levels suggest that airborne *Campylobacter* can be detected and may be significant, but further research is needed.

An ilochip A/S sampler was used to specifically capture one organism using an electrostatic method (Diagnostic, 2009; Olsen et al., 2009). This sampler was able to capture particles at a rate of 120 ml of air per minute. Slaughterhouse samples were collected a total of 1800 ml of air on mCCDA agar. Results primarily supported the presence of *Campylobacter* in the hanging area but did not find evidence in the evisceration, defeathering or scalding areas. This sampling method was useful when combined with real-time PCR which could readily identify *Campylobacter* bacteria (Olsen et al. 2009). Also, the Ilochip A/S sampler was used as a part of an intergrated laboratory on a chip (ILOC) technology to include all information gathered from air sampling and PCR results. Other samplers that have been used in air sampling include the BIAP Slitsampler in a Swedish study and the Biotest Dutch air sampler (Posch et al., 2006).

Isolation and Enumeration Media

Detecting these organisms in the lab can be very difficult. Once samples are collected, if

Campylobacter is present, they are very sensitive and “can be injured or destroyed easily by heating, drying, salting, freezing or cooling” (Jacobs-Reitsma et al., 2008). Damage to cells in the sample can often affect the qualitative or quantitative results. For this reason, it is important that samples are plated as soon as possible after collection and dilution, if performed (USDA et al., 2008). The steps following sample collection can be important to the quality of the data reported. In the US, the government has published several suggested, but not mandated, methods for sample collection and analysis. In many cases, samples can be placed on ice packs and shipped to outside laboratories to be plated or analyzed (Berrang et al., 2008). Other samples are held in coolers between 4 to 30°C before plating and use enrichment broths to help ensure quality and survival of viable samples.

In 2008, the Food Safety and Inspection Service recommended the direct plating method for enumerating *Campylobacter* from carcass rinse samples after they were collected and shipped to analytical laboratories. Only samples received in the temperature range 0 to 15°C were used (USDA et al., 2008). In some cases, only samples under 10°C could be plated (Berrang 2008). This method was found to be “rapid and effective” (USDA et al., 2008) and was expected to give the highest viable count (Stern et al., 2001). In comparison, an earlier study by Stern found that direct plating of fresh carcass rinse samples onto Campy-Cefex agar did not strongly affect enumeration of *Campylobacter* colonies on selective media but “reduced the time needed when compared with other methods such as MPN most probable number” (Stern et al., 2001). The alternative most probable number (MPN) method was found to have no affect or benefit in enumeration or analysis time when compared to direct plating (USDA et al.,

2008; Stern et al., 2001).

USDA suggested that when plating the samples directly, no dilutions were needed (10^0). A study by Pearce on *Campylobacter* in swine used serial dilutions in duplicate (Line 2005). One milliliter was spread plated onto four plates with 0.25 mL each and another two plates containing 0.1 mL each. From this research it was reported that spread plated and spiral plated samples were both found to be successful for plating carcass rinse samples. For plating fresh poultry samples, Jacobs found that overgrowth of other microbial contaminants may occur, with some media types, causing difficulty in reading or counting plates (Oyarzabal et al., 2005; Pearce et al., 2003).

The media and methods used when plating carcass rinse samples are important in detecting *Campylobacter*. In using selective media, the addition of supplements is an important part in effectiveness of media and is generally required. Depending on the type of media, supplements that are commonly added include "cefoperazone, amphotericin B, trimethoprim and vancomycin" (Jacobs-Reitsma et al., 2008). Additionally, a substance such as charcoal, lysed or laked horse or sheep blood may be added to help decrease the detrimental effects of exposure to light and oxygen (Jacobs-Reitsma et al., 2008).

The Jacobs-Reitsma study suggested using AHB (Abeyta-Hunt-Bark) agar or mCCDA (modified charcoal cefoperazone desoxycholate) agar. The mCCDA media incorporates charcoal while AHB agar uses horse blood. Campy-Cefex agar is another newer and commonly used agar for *Campylobacter* isolation. It requires a supplement of cefoperazone and sterile laked horse blood. A modified version is also made using sterile lysed horse blood instead of

laked (Oyarzabal et al., 2005).

In a study conducted by Oyarzabal several selective media such as Campy-Line agar, mCCDA, CAMPY agar, Karmali agar and modified Campy-Cefex were compared to test for enumeration quality (Oyarzabal et al, 2005). In total, 63% of these plates contained *Campylobacter*. Results showed that modified Campy-Cefex and Campy-Cefex agar performed the same and had the highest mean colony counts at 0.66 CFU/mL and 0.65 CFU/mL, respectively . Modified charcoal cefoperazone desoxycholate agar had similar mean counts at 0.63 CFU/mL (mean value), followed by Karmali, CAMPY, and Campy-Line agars with 0.53 CFU/mL, 0.51 CFU/mL, and 0.24 CFU/mL, respectively (Oyarzabal et al, 2005).

A similar study conducted by Line reported that Campy-Cefex agar produced a higher count of *Campylobacter* (16.8 CFU/mL) but produced a higher number of contaminants as well (14.1 CFU/mL) (Line and Berrang, 2005). This was compared to Campy-Line agar which had 11.4 CFU/mL *Campylobacter* and 0 CFU/mL contaminants (Bolder, 1998). A benefit to using Campy-Line agar is the color differentiation between colonies and agar while Campy-Cefex allows for “good qualitative recovery”(Stern et al., 2001). It was concluded, however, that the difference between agars may not be statistically significant. The majority of other studies suggest that Campy-Cefex is still the more selective, easier to use media, yielding better results (Oyarzabal et al., 2005).

Enrichment broth such as Preston, Bolton and Hunts method were recommended as equally effective in qualitative data but were not good for quantitative methods (Bolder, 1998; Jacobs-Reitsma et al., 2008). It was suggested that use of enrichment broth would help to

repair any damaged or injured colonies (Jacobs-Reitsma et al., 2008). The 2008 USDA detection study similarly suggested Blood-Free Bolton's broth for increasing detection of weakened or injured cells for direct plating (USDA et al., 2008). Several studies have shown that use of enrichment broth either had slight to no effect on enumeration of *Campylobacter*. In comparison with direct plating onto Campy-Line agar and Campy-Cefex agar, using Bolton enrichment broth provided less significant results ($P \leq .05$) (Pearce et al., 2003). Other studies have found that using enrichment broth may enhance growth of non-*Campylobacter* organisms making it more difficult to enumerate on selective media (Line 2005).

In studies that used enrichment broth or an enrichment step, several combinations were followed. Jacobs suggested using Bolton broth and incubating samples for 24-44 hours at 42°C before plating. A pre-enrichment method was also suggested which involved two different incubation periods of 2 or 4 hours at 37°C (Jacobs-Reitsma et al., 2008). The USDA recommended that enrichment broth samples be incubated for 24 to 48 hours at 42°C before being plated (USDA et al., 2008; Berrang et al., 2008). If there is slow or no growth on plates, an additional 24 hours may be added to incubation time (Oyarzabal et al., 2005). As research has continued in this area, Jacobs recommends that the current effective incubation temperature has been lowered to 41.5°C (Jacobs-Reitsma et al., 2008).

When using enrichment or culturing samples, a modified atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen for samples incubated at 42°C was found to be the most effective (Oyarzabal et al., 2005; Stern et al., 2001). Jacobs suggested that in using modified atmospheres, a range of 5 to 7% oxygen and 5 to 15% carbon dioxide were acceptable along

with replacing nitrogen with hydrogen (Jacobs-Reitsma et al., 2008). Equipment for modified atmosphere incubation may include plastic bags that can be sealed, anaerobic jars and gas cylinders.

Upon incubation of plates, moist media was found to form “spread colonies”(Stern et al., 2001) that were not beneficial to quantitative studies but qualitative growth methods. This spreading is generally called ‘swarming’ of the plate, keeping samples from being easily enumerated (Stern et al., 2001). If cells were older, they were found to be cocciodal and non motile opposed to normal S-shape and darting (Stern et al., 2001). The active darting movement of *Campylobacter* from fresh samples makes it better to identify morphologically, using a phase contrast microscope (Berrang 2008).

Culture Confirmation Test Methods

For sample and culture confirmations, there has been a lack of biochemical identification tests. *Campylobacter* identification and confirmation are frequently performed with morphological exams, using a microscope, or gene-specific tests, using latex agglutination tests for confirmation during enumeration (Stern et al., 2001). Using wet mount slides, organisms are checked for darting motility and unique corkscrew shape. Other methods such as enzyme linked fluorescents (VIDAS system) and PCR are being researched as detection and identification methods. PCR is among many tests, including “immunoassays, enzyme linked immunosorbent assays and immunomagnetic beads, that are considered rapid and antibody based methods” (Jacobs-Reitsma et al., 2008). These methods are suggested to be an

improvement and help to increase cell concentration, DNA purification and inhibit contaminants such as feces or media in testing (Jacobs-Reitsma et al., 2008).

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MATERIALS AND METHODS

Sample Collection

Three sample types were collected: carcass rinses, air, and water samples. Samples were selected from four areas: (1) scalding and defeathering (air and scald tank water), (2) evisceration re-hang (air and carcass rinse), (3) carcass chill tank (air and chill water), and (4) post chill re-hang (air and carcass rinse). A diagram of poultry processing, including selected collection areas can be found in Figure 1.

Samples were collected at a commercial broiler slaughter facility in Virginia. Chickens were processed from 5:30AM until 11:30PM producing an average 250,000 birds slaughtered per day. The slaughter facility was visited once per month over a four month period between September and December 2009. Negative control samples were collected at 5 a.m. and samples were collected from Time 0 (6 a.m.) until Time 12 (6 p.m.) in four hour intervals. Four negative control water samples and two air samples were collected at 5 a.m. (Pre-Operational) before the first chickens were processed. Six carcass rinse and four air samples were collected every four hours during each visit to the facility. Samples were separated between the warmer scalding, defeathering and evisceration areas and the other half from the cooler post chill area. The facility contained an on-site laboratory and samples were plated and incubated immediately.

Water samples:

Water samples were collected from the scald tank and chill tank at 5 a.m. (Pre-

operational) prior to any birds being processed and again at Time 12. Two samples were taken from scald tank water at each sampling time. Additionally, two samples of the carcass chill tank water (negative control) were collected at Time 0 prior to carcasses entering the chill tank. Water was collected from near the carcass entry end of each of the two chiller tanks. All water samples were collected using 250 mL Bibby Sterile "DIPPA" plastic cups (Bibby Sterilin Ltd., Staffordshire, UK) attached to a dipping rod. Within one hour of collection, water samples (1 mL each) were spread plated onto four Campy-Cefex agar plates (~0.25 mL each)

Air samples:

Two types of air samplers were used, a MicroBio MB2 Air Sampler (Bromley, Kent, UK) and a Sartorius AirPort MD8 Air Sampler (Microbiology International, Edgewood, NY). The MicroBio MB2 Air Sampler was used to collect 1000 L of air (100L/min) per sample onto a Campy-Cefex agar plate (55 mm dia., 10 mL). The Sartorius AirPort MD8 was used to collect 500 or 1000L of air (50L/min) onto either a plastic encased disposable gelatin filter (Sartorius Edgewood, NY) or a gelatin membrane filter unit (Sartorius Edgewood, NY). Individually wrapped disposable gelatin filters were placed on a filter adapter (Sartorius Edgewood, NY) to attach to the sampler. Gelatin membrane unit filters were packaged together and were placed on an aluminum filter holder (Sartorius Edgewood, NY) attached to sampler. All gelatin filters were placed onto prepared Campy-Cefex agar plates (90 mm dia., 20 mL) using sterile forceps.

Pre-operational air samples were taken in both the evisceration and post chill area. At 5AM, an evisceration control was collected with the MicroBio sampler at 1000L, running for ten minutes. Another sample (500 or 1000L) was collected at the same time with the Sartorius air

sampler onto a gelatin filter. During the first facility visit, the Sartorius sampler was run for 20 minutes, collecting 1000 L of air, but later was shortened to 10 minute cycles collecting 500 L. Both samplers were placed near the evisceration re-hang and intestine removal area. The same pre-operational samples were taken in post chill, placing samplers closest to the chill tank and re-hang area.

Additional air samples were collected at Time 0, Time 4, Time 8 and Time 12 using two 1000L samples from the MicroBio sampler and two 500 or 1000L samples with the Sartorius AirPort MD8 air sampler. During the first collection date, using the Sartorius sampler, two 20 minutes cycles were run collecting 1000L of air each. On remaining collection dates, 500L of air per sample was collecting in two 10 minutes cycles (50L/min). The MicroBio Sampler could be run for 10 minutes and collect 1000L per sample.

In the evisceration area of the plant, the two Sartorius samples were collected using individually wrapped disposable gelatin filters placed on an adapter attachment. No changes were made to the MicroBio sampler which was used to collect samples directly onto 10 mL agar plates through the stainless steel adapter. Air samplers were placed on shelves or trays located close to evisceration re-hang and processing line or carried throughout evisceration operation area.

Post chill air samples were taken near the chill tank and carcass re-hang area. Two samples were taken with the MicroBio air sampler, collecting 1000L per sample directly onto agar plates. Two 500-1000L samples were collected using the Sartorius sampler. In post chill gelatin membrane unit filters were used and added to the aluminum filter holder using sterile

forceps then attached to Sartorius sampler.

When samples were completed, the disposable gelatin filter from Sartorius sampler was removed from the adapter. The filter case was removed and the filter was pressed against the 20 mL agar plate. This allowed the filter to attach to the agar and begin to dissolve. Plates were covered with petri lid and allowed to sit until completely dissolved. MicroBio 10 mL agar plates could be removed and the lid replaced before placing a new agar plate in sampler.

These steps were repeated for each sampling time. Completed samples were removed from sampler, inverted and labeled according sample location, time of day and sample number. Labels appeared as EV (Evisceration) or PC (Post Chill), control, A (Time 0), B (Time 4), C (Time 8) or D (Time 12), and 1 or 2 (sample number).

Carcass rinse samples:

Whole carcass rinses were obtained at 4 times during processing (Time 0, Time 4, Time 8 and Time 12). At each time, three carcasses were randomly selected in both the evisceration and post chill areas, and placed individually in a 3500 mL BioPro Bird Rinse Bags (International Bioproducts, Bothel, WA) and rinsed with 400 mL of Buffered Peptone Water (Buffered Peptone Bird Rinse; 3M, St. Paul, MN). Evisceration area carcasses were taken from the re-hang line after leaving the scalding and defeathering area. Post chill carcasses were taken from the re-hang table after they exited the chill tank.

Each carcass was handled with sterile disposable gloves and was placed into a sterile bird rinse bag. Buffered peptone water was added, and the bagged carcass was gently shaken for 1-2 minutes. A small portion of the peptone rinse water was returned to peptone bottle

and resealed. The carcass and remaining peptone water were placed back on re-hang line/table. Rinse bags and gloves were discarded after each carcass.

Isolation and Identification of *Campylobacter*

Samples collected from air, water and carcass rinses were plated onto selective media for isolation of *Campylobacter* spp.; Acumedia Campy-Cefex agar, (Neogen, Lansing, MI, Lot 102021B) and Acumedia Campy-Blood-Free Selective Medium (mCCDA) agar (Acumedia, Lansing, MI, Lot 101733A). The majority of samples were plated onto the Campy-Cefex agar with the exception of the second trip, where half of all samples were plated onto mCCDA agar. Campy-Cefex agar was supplemented with 0.033g/L cefoperazone (Neogen, Lansing, MI) and 5% laked horse blood (Remel, Lenexa, KS). Campy Blood-Free Selective Medium (mCCDA) agar was supplemented with 0.033g/L cefoperazone (Neogen, Lansing, MI). Media was prepared, according to Acumedia specifications, two to three days prior to use to allow plates to dry. Media were evaluated with pure cultures of *Campylobacter* jejuni (082809 AT) and *Campylobacter* coli (082609 ATCC) to determine ability to support growth and to demonstrate the visual appearance of isolated colonies on plates. Inoculated media were incubated at 41C in anaerobic jars under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) using Oxoid CampyGen gas generation sachets (Remel, Lenexa, KS) for a total of 48 hours.

Plated colonies matching the characteristic appearance of *Campylobacter* spp. were streaked onto BD BBL Brucella agar (Franklin Lakes, NJ) plates and re-incubated for 48 hours using microaerophilic conditions as described above. For confirmation of the identity of suspected *Campylobacter* colonies from plated samples, a latex agglutination test kit (Oxoid

Dryspot *Campylobacter* Test, Cambridge, UK) was used to positively identify colonies. Positive test samples showed separation or agglutination, and negative tests were not identified as *Campylobacter*. Gram stains and wet mounts of selected colonies were also prepared to confirm the identifications of *Campylobacter* organisms viewed under the microscope.

Sample Analysis

Water and carcass rinse samples were plated within an hour after collection. Carcass rinse samples (1 mL) were spread plated onto 4 Campy-Cefex agar plates (~0.25 mL each). For rinse samples collected on the second trip, each sample (1 mL) was spread on to two Campy-Cefex plates (~0.25 mL each) and on to two mCCDA agar plates (~0.25 mL each). This was to compare effectiveness of Campy-Cefex agar to mCCDA . The surface of the agar plates were allowed to dry and then inverted. Samples that appeared wetter over a period of time were placed in anaerobic box and placed into ThermoTote 24 portable incubator (Scientific Device Laboratory, Des Plaines, IL) at 40°C ± 1 to help dry the plate before inversion. Wetter samples were generally found on mCCDA plates. On the third trip, carcass rinse samples were spread plated onto four Campy-Cefex agar plates (~0.1 mL each). This lower volume of rinse allowed plates to dry faster.

Water, air and carcass rinse samples were incubated immediately after being plated while in the slaughter facility. Plates were grouped together and set into 7L Remel AeroPak System (Lenexa, KS) anaerobic jars with a Remel Oxoid CampyGen (Lenexa, KS) microaerophilic gas pack. Gas packs were able to modify the atmosphere to one that would support *Campylobacter* growth. The rectangular anaerobic jars were placed into ThermoTote24

portable incubators at 41 ± 1 °C. During return travel to the Virginia Tech Department of Food Science and Technology, the temperature was maintained using portable incubators control device.

Pre-operational (5AM) samples, Time 0 and Time 4 samples were placed in the two incubators and allowed to heat up to 5 hours until the second group of samples, evening Time 8 and Time 12, were collected. After these latter samples were collected, the morning group samples were removed and replaced with the evening group. Evening group samples were also incubated for 4 to 5 hours, at 41 ± 1 °C, prior to arrival at Virginia Tech. Upon return, all samples were placed into a large incubator for 48 ± 2 hr at 41 ± 1 °C in the Virginia Tech lab. Samples were incubated for 48 hours, under modified atmosphere. When incubation was complete, plates were removed from the incubator and stored at 4 °C. Plates were examined and colonies counted within 12 hours of refrigeration.

Data Analysis

Qualitative and quantitative determination of *Campylobacter* colonies was performed for all plates. The proportion of positive samples was determined, and a chi-square analysis was performed to determine if there was a significant difference in *Campylobacter* identification between sample types, across sample locations or across sample times. The chi-squared analysis was prepared using JMP statistical program. Positive *Campylobacter* colonies were enumerated and averaged for each sample category. Water samples and carcass rinses were reported as colony forming units per mL of rinse or water. Air samples were reported as

colony forming units per L of air collected. The total number of countable colonies was averaged for the samples that were *Campylobacter* positive. Further statistical tests on colony concentrations were not performed since a very low concentration of cells was detected, if any, from the samples.

RESULTS

In total, 135 air, 96 carcass rinse and 29 water samples were collected and analyzed. Ten percent of air, 70% of carcass rinse and 28% of water samples tested positive for *Campylobacter.* Data was compared by sample type (air, carcass rinse) and sample location (evisceration, post chill) within the plant. Sample type (air, carcass rinse, water) was also compared by collection time (Pre-operational, Time 0, Time 4, Time 8, and Time 12). The mean concentration in air (CFU/1000L) was calculated by sample location (evisceration, post chill) and media type (gelatin filter, agar plate). Additionally, mean concentration of carcass rinse samples (CFU/mL) was calculated by sample location (evisceration, post chill) and collection time (Time 0, Time 4, Time 8, Time 12). Detection of *Campylobacter* results based on sample type and locations were found to be statistically significant ($P < 0.05$) as described below.

Recovery of *Campylobacter* from air and carcass rinse samples

There was a statistically significant difference ($P < .01$) in both air and carcass rinse (sample type) samples by chi-square analysis. Carcass rinse samples collected yielded the highest percentage of *Campylobacter* positive samples at 70%, which included both evisceration and post-chill samples (Table 1). Positive samples recovered from evisceration were higher at 85% than samples from post chill at 56%. Recovery from air samples was very lower in comparison with carcass rinses, yielding 10% positive for *Campylobacter* in both

evisceration and post-chill. A higher percentage of positive samples of *Campylobacter* were found in evisceration at 16% compared with post-chill at 3%.

Sample concentrations of viable *Campylobacter*

Tables were prepared to compare mean data specifically for carcass rinse (Table 2), by collection time and location, and for air (Table3), by media type and location. Carcass rinse samples yielded the highest positive CFU/mL for *Campylobacter*. Mean concentrations in evisceration were detected at 679 CFU/mL, which was significantly different ($P < 0.05$) than 9 CFU/mL in post chill. The highest concentration of *Campylobacter* was found in evisceration with mean concentrations above 1000 CFU/mL and lowest in post chill with several concentrations below 10 CFU/mL. Carcass rinse concentrations compared by time were not found to be significantly different ($P > 0.05$). The highest concentration for evisceration was at Time 12, while the lowest was at Time 8. In post chill, the highest concentration was at Time 4 and the lowest at Time 0.

The mean concentration of *Campylobacter* positive air was found in evisceration at 11 CFU/1000L and at 2 CFU/1000L in post chill. Air sample concentrations by sample collection location were found to be statistically significant ($P < 0.05$), because of the increased mean concentration of *Campylobacter* in evisceration. Positive samples were detected on both gelatin filters and agar plates. Data for positive *Campylobacter* samples showed 5 CFU/1000L in evisceration and 1 CFU/1000L in post-chill areas using agar plates. Similarly, 6 CFU/1000L in evisceration and 1 CFU/1000L in post chill was detected using gelatin filters. Even though

nearly twice as many positive samples were detected with gelatin filters than with agar plates alone, this difference was not significantly different (P > 0.05).

Recovery of *Campylobacter* across time of day

Carcass rinse samples were found to be the least affected by time, remaining between 75-79% positive from Time 4 samples until Time 12 samples (Table 4). A lower percentage was found at Time 0 but this was not significantly different (P> 0.05) from other times represented. Recovery from air samples, scald and chill tank water was more variable across time. In air samples, Time 12 samples yielded the highest percent of positive samples at 23%. This was found to be statistically different from remaining times (Time 0 to Time 8), which were equal to or less than 10%. For scald and chill tank water samples, only Time 12 samples were positive for *Campylobacter* while pre-operational samples were not positive. As expected, *Campylobacter* was not present in pre-operational samples because samples were collected prior to bird arrival to the plant. One exception to the pre-operational *Campylobacter* negative samples was a *Campylobacter* positive air sample found in the scalding area, but only 1 CFU was found.

Discussion

The design of this study focused on methods for *Campylobacter* detection and quantification in a poultry processing plant. This included the use of modern air samplers, direct agar plating and prompt incubation of samples at 41°C without a cold storage period. Pre-operational and carcass rinse samples were implemented to provide baseline data

comparison to air samples for the presence of *Campylobacter* in scald/defeathering, evisceration and post-chill areas. It was anticipated that samples collected from carcass rinses would confirm the presence of *Campylobacter* on raw chicken carcasses, and serve as comparison for air samples collected in the same areas. Difference in air and carcass rinse (sample type) as well as the low concentration from air samples and frequently high concentration from carcass rinse samples, did not allow for a significant correlation to be made between the two sample types.

The samples in this study were collected over four separate processing days in a four month time frame. Variation occurred over each sample date and was analyzed. Significant differences were in positive sample concentrations and percentages were identified by sample type (air, water, carcass rinse), sample location (evisceration, post chill) and by collection time for air samples. Significant differences were not found in collection times in other sample types, such as carcass rinse or water, or in media type (gelatin filter, agar plate) used for air sampling. Airborne *Campylobacter* was identified, however, and quantified in evisceration and post-chill areas, which may lead to transmission to other parts of the processing plant environment.

In studying the presence of *Campylobacter,* various locations throughout processing were identified for collecting carcass rinse and air samples. These areas included evisceration and post-chill, with the addition of negative control samples such as pre-operational scald and chill water for monitoring the flow of contaminants. According to FSIS guidelines, the evisceration area can range from carcass re-hang, after scalding/defeathtering, until reaching the carcass chill tank of post-chill (FSIS, 2008). Carcasses entering evisceration from the

scalding and defeathering area are intended to have a decrease in microbial load due to the temperature used in scald water. For the current study, evisceration carcasses rinses were taken from the re-hang area immediately following scalding and defeathering, but prior to intestine removal. Selecting carcasses from evisceration re-hang area was expected to provide high concentrations of *Campylobacter* and was similarly studied in another 2008 study by Berrang (Berrang 2008). The high concentration of *Campylobacter* is believed to come from the existence of contaminants, such as intestinal leakage or feces in scald water, on the carcasses which may survive in the evisceration area which was suggested by Abu-Raiwada and Bolder (Abu-Raiwada et al., 1994; Bolder, 1998).

In the current study, evisceration air samples were similarly collected at re-hang or a location near intestine removal. Additional studies performed using air samplers in evisceration collected samples at gall bladder or liver separation, accounting for a *Campylobacter* positive maximum of 4.0×10^4 CFU/1000L of air in the Posch study and a median of 6.2×10^3 CFU/1000L of air in the Haas study (Haas et al., 2005; Posch et al., 2006). In both the Posch and Haas studies, post chill carcass rinse and air samples were additionally collected at re-hang, near the chill tank. This sampling location did not vary compared to the current study and previous studies performed.

For comparing air sampling methods, there are currently few studies that exist using different methods. In some of the previously reported studies testing air, an opened agar plate or 'settle' plate was used for air sampling (Burfoot et al., 2005). This method may have permitted another way to compare the two air samplers in this study. By using open plate with

selective media, it could have been used as a standard for air quality in comparison with samplers and a few carcass rinses. For example, a study by Olsen used an open mCCDA plate located in the same area as an ilochip air sampler to capture active cultures (Olsen et al., 2009). Other studies have similarly started out by using the open plate method in place of modern air samplers to check for airborne *Campylobacter*.

There are advantages to using modern air samplers, however, such as particle and sample size control which can be done with samplers like the ilochip. Certain size particles are able to fit into the filter, which may reduce the collection of other particles in sampling. Samplers such as the Anderson Six Stage Viable sampler are known for these qualities (Posch et al., 2006). The Anderson is able to use six filters, decreasing in size, as air is sampled, only allowing samples to be distinguished by particle size. Sample size and time should be monitored, however, when using modern air samplers. This is due to the level of oxygen exposure during sampling. The Posch study recommends shorter sampling periods to decrease the effects of oxygen on viable *Campylobacter* colonies (Posch et al., 2006).

Air samples for this study were collected at 4 hour intervals at 100L/minute using both the Sartorius and MicroBio sampler. These two samplers were not found to be used in previous studies on airborne *Campylobacter* detection. Inability to increase sample speed and volume was an issue experienced with both samplers. When setting the volume and/or speed on the Sartorius sampler, with gelatin filters, to 100L/minute, an error occurred leading to blocked airflow. This could have been due to the filter thickness or a problem with air pressure on the sampler. The largest sample size that could be collected with the MicroBio air sampler was

100L/minute for 10 minutes per sample. Restrictions in sampling air volume and speed may have affected sampling quality and quantity. If sample collection were increased in volume per sample, it may have allowed for more comparable data to be collected per time and location.

There were other issues involving the use of air samplers, particularly with sample location and atmospheric conditions. For the Sartorius sampler, high humidity from evisceration and post-chill was an issue when using sampler. The evisceration sample location was relatively close to scalding and defeathering, meaning high moisture could be transferred into evisceration. Additionally, dripping water or other forms of condensation from spray cleaning or overhead shackle lines could reach the sampler and moisten the filter. Sample time and/or volume were reduced due to high humidity (~70% RH) and moisture present. This was done to prevent damage to gelatin filters used for sampling. High humidity was not an issue in post-chill (~50% RH), but dripping water from overhead shackles onto filters was a remaining factor.

When gelatin filters became moist or wet it became more difficult to remove from plastic filter case and would not properly lay on the agar plate during transfer. For this reason, samples collected onto agar plates using the MicroBio sampler performed better under high moisture conditions and were not as difficult to remove as gelatin filters when wet. In applying the filter, however, the individually wrapped gelatin filters were the easiest to apply and most convenient. Other studies using air samplers did not note problems with moisture or wetness when sampling in the evisceration area. This could be attributed to the various types of air sampler used.

The use of Campy-Cefex selective agar is recommended by the United States Department of Agriculture study and was used in this experiment (USDA et al., 2008). This is a selective media and was expected to only grow *Campylobacter* colonies. However, contamination and swarming or spreading of colonies was a re-occurring issue when using the Campy-Cefex agar. After incubation, high levels of contamination were seen on most carcass rinse and water plated samples as well as a few air sample plates. This made the plates difficult to observe and count *Campylobacter* colonies in the cases where microscopic identification or latex agglutination testing was not performed.

Similar issues with contamination and overgrowth on Campy-Cefex agar were experienced by Line, Oyarzabal and Pearce. Specific data was supplied by Line and Berrang recovering *Campylobacter* at 16.8 CFU/ml and additional contaminants at 14.1 CFU/ml (Line and Berrang, 2005). The Oyarzabal study identified these contaminants as *Pseudomonas* spp. and *Staphylococcus hominis* (Oyarzabal et al., 2005). In the current study, small round colonies were seen under the microscope when checking for *Campylobacter* colonies and could have possibly been these contaminants. No further identification steps were performed for contaminants. For plates with high concentration of contaminants, further isolation of possible *Campylobacter* colonies onto Brucella agar plates was needed to test for positive colonies. In contrast, plates yielding low to no growth of *Campylobacter* colonies were recommended for further incubation of 24 hours by Oyarzabal and 18-24 hours by Stern (Oyarzabal et al., 2005; Stern, 2001). This would also include the addition of fresh modified atmosphere gas packs after 48 hours.

Modified charcoal cefoperazone deoxycholate agar was also tested on a small portion of samples for use during the experiment. When plates were observed, fewer contaminants were found but concentrations of *Campylobacter* produced were similar to that of Campy-Cefex agar. For this reason, data was not analyzed or included in this study using counts from mCCDA plates. In other studies comparing Campy-Cefex agar and mCCDA, similarly no statistical difference was found between the two media when testing for enumeration of *Campylobacter*. These two media were also found to be comparable with media such as CAMPY agar and Karmali agar (Oyarzabal et al., 2005).

Media used, such as Campy-Cefex and Brucella agar, were prepared according to manufacturer directions. In the current study, the addition of laked horse blood in Campy-Cefex was used, which according to Jacobs-Reitsma was reported to be responsible for decreasing the effects of light and oxygen (Jacobs-Reitsma et al., 2008). To ensure Campy-Cefex agar plates were dry for sampling, they were prepared several days prior to use. Decreasing plate wetness prior to inoculation and incubation can limit the spread of possible contaminants. The United States Department of Agriculture recommends keeping the agar dry and away from light exposure to assist in decreasing contamination (USDA et al., 2008). Direct plating of samples using 0.25 mL of carcass rinse onto each of four plates of Campy-Cefex agar was performed within an hour of collection. Plates were allowed to dry for 2 to 4 hours prior to inverting and incubating at 41°C. Several plates that appeared to be excessively damp were placed in a portable incubator to aid in the drying process. It is possible that all plates should have been placed in an incubator or dim area and allowed to dry fully before addition of gas

packs and incubation. This may have decreased the number of contaminants and spread colonies that occurred due to increased moisture. By decreasing spread colonies and contaminants, colonies might have isolated better into smaller, countable colonies.

To decrease plate surface contamination and possibly drying time, a variation in dilutions was attempted. On the third plant visit, carcass rinse samples were plated at 0.1 mL per plate which helped decrease the time needed to dry each plate. This further resulted in a decrease in contaminants but the appearance of smeared or spread colonies still remained on several plates. This could be attributed to additional problems with excess moisture and condensation created in the anaerobic jars once the modified atmosphere gas packs were added. Moisture created in the anaerobic jars occasionally collected in the lid of inverted plates.

On the fourth plant visit, all pre-evisceration carcass rinse samples were diluted by a factor of 10 before plating 0.25 mL onto each plate. These plates had fewer contaminants but *Campylobacter* colonies were still spread out on the plate. This can be attributed to smaller plated concentration and the remainder of moisture in containers. Stern cited that the swarming nature of *Campylobacter* was helpful in finding growth of the organism, but was a hindrance in colony enumeration (Stern, 2001). To decrease this moisture, FSIS recommended the use of filter paper containing glycerol for decreasing moisture and spreading of colonies (USDA, 2008; USDA, 2010). This procedure was published after the current experiment had begun and was not used during this project but should be tested in future experimental designs.

For the current study, direct plating was used to promote the collection of quantitative data. By using direct plating, handling of samples was decreased and was also expected to decrease stress to possible *Campylobacter* colonies. Many studies agreed with this format of direct plating, finding it to be an effective method. But some studies varied, including recommendations from FSIS, which support the use an enrichment media step using Blood-Free Bolton's or Preston's enrichment broth for 24-48 hours prior to plating (USDA, 2008; Jacobs-Reitsma et al., 2008). Use of an enrichment step generally leads to qualitative data, resulting in positive or negative reports for the presence of *Campylobacter*. Jacobs-Reitsma suggested that enrichment of fresh meat/carcass samples created overgrowth of colonies on spread plates (Jacobs-Reitsma et al., 2008). It was also suggested by Jacobs-Reitsma that the use of an enrichment step would help to enhance or repair injured cells that may have been damaged in sample collection or transport. However, in this study, by using the direct plating methods, concentration of *Campylobacter* were able to be identified and calculated without an enrichment step. The use of immediate plating and incubation may be additional factors in finding countable *Campylobacter* colonies and decreasing the number of injured cells.

The use of immediate plating and incubation using portable incubators (within 1-2 hours after collection) was another step that varied widely from previous studies was. No outside data was found under the scope of this study to support immediate incubation. This method was effective in increasing convenience and making incubated samples readily available in a shorter time period. Many outside studies not using an enrichment step would store samples on ice before plating or ship samples to other laboratories for plating and analysis, increasing

the preparation time of incubated samples. Also, by storing samples on ice or using a cold step, there is a possibility that viable *Campylobacter* colonies may incur damage or injury prior to incubation. Incubation times across many studies varied from 24-48 hours. In this study, samples were incubated for 48 hours then placed into a laboratory refrigerator until they could be counted.

Samples placed in the refrigerator remained in the anaerobic container, but the modified gas packs were removed. Contaminants on the plates were observed prior to refrigeration but additional contamination from laboratory refrigerator, if left for an extended period of time, may have added to mold contaminants found on some plates. Additionally, exposure to atmospheric oxygen during counting could have also damaged fragile *Campylobacter* colonies prior to testing and microscopy. If colonies were damaged or injured during handling, this could weaken the response during latex agglutination tests as well as during microscopy.

Overall this study was useful in identifying and quantifying the presence of *Campylobacter* throughout the processing facility. Despite minor setbacks, the use of air samplers was convenient and helpful in collecting data. Further research is needed to support its continued use in poultry processing. Studying *Campylobacter* presence based on time was not supported by this study but could be tested further. In review of results it is evident that *Campylobacter* is present and can pose a possible threat to end product and human consumers. A standard guideline on detection methods should be identified and used to uniformly identify key sources and areas of contamination of *Campylobacter* in poultry processing.

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Table 1. ***Campylobacter*** **detection by sample type and location in a poultry processing plant**

Sample Type / Location	**# Positive Samples**	**# Samples Collected**	**% Positive Samples**
Air			
Evisceration	11	68	16.2
Post-chill	2	67	3.0
Air Total	13	135	9.6^{A}
Carcass Rinse			
Evisceration	41	48	85.4
Post-chill	27	48	56.3
Carcass Rinse Total	68	96	70.8^{B}

Significant difference (P < 0.01), using Chi-square analysis, for sample type indicated by superscript letters.

Table 2. Mean concentration of *Campylobacter* for carcass rinse samples in a poultry processing plant by time of day and location

Sample Location	Evisceration		Post-chill	
Collection Time	Mean CFU/mL	% Samples Positive	Mean CFU/mL	% Samples Positive
Time 0	785.3	58.3	4.4	41.7
Time 4	597.4	91.7	16.9	66.7
Time 8	144.1	100.0	7.5	50.0
Time 12	1275.5	91.7	4.8	66.7
All Samples	678.7	85.4^{A}	8.9	56.3^{B}

n=12 samples collected per time and location

Statistically significant differences (P < 0.05) in percentage positive, using Chi-square analysis, for sample locations are designated with upper case superscript letters.

Table 3. Mean concentration of airborne *Campylobacter* by media type and location in a poultry processing plant[1]

Sample Location	Evisceration		Post-Chill	
Air Sample Media Type	**Mean CFU/ 1000L Air**	**# Samples Positive**	**Mean CFU/ 1000L Air**	**# Samples Positive**
Agar Plate[a]	5	3 of 32	1	1 of 31
Gelatin Filter[a]	6	8 of 36	1	1 of 36
Air Total	11	11 of 68[A]	2	2 of 67[B]

[1] Air sample concentrations compared by media type (gelatin filter or agar plate)

Statistically significant differences ($P < 0.05$) in percentage positive, using Chi-square analysis, for sample locations are designated with upper case superscript letters.

Statistically significant differences ($P < 0.05$) in percentage positive, using Chi-square analysis, for air sample media type are designated with lower case superscript letters.

The difference in mean air concentration CFU/1000L, by sample location, is not statistically significant ($P > 0.05$) by Chi-square analysis.

Table 4. Campylobacter detection by sample type and collection time in a poultry processing plant

Sample Type / Collection Time	# Positive Samples	# Samples Collected	% Positive Samples
Air			
Pre-operational*	1	18	5.6[A]
Time 0	0	27	0.0 [A]
Time 4	2	30	6.7 [A]
Time 8	3	30	10.0 [A]
Time 12	7	30	23.3 [B]
Air Total	13	135	9.6
Carcass Rinse			
Time 0	12	24	50.0[a]
Time 4	19	24	79.2 [a]
Time 8	18	24	75.0 [a]
Time 12	19	24	79.2 [a]
Carcass Rinse Total	68	96	70.8
Scald Tank			
Pre-operational*	0	6	0.0
Time 12	2	7	28.6
Scald Tank Total	2	13	15.4
Chill Tank			
Pre-operational*	0	8	0.0
Time 12	2	8	25.0
Chill Tank Total	2	16	12.5
All samples	85	260	32.7

* Pre-operational samples collected prior to bird arrival in plant

Statistically significant differences ($P < 0.05$) in percentage positive, using Chi-square analysis, for collection times (including pre-operational samples) are designated with upper case superscript letters.

Statistically significant differences ($P < 0.05$) in percentage positive, using Chi-square analysis, for collection times are designated with lower case superscript letters.

Figure 1. **Collection Areas in a Poultry Processing Plant**