

FOOD AND ENVIRONMENTAL SAFETY OF PASTURE-RAISED BROILERS  
PROCESSED ON-FARM, IN A MOBILE PROCESSING UNIT AND AT A SMALL USDA-  
INSPECTED FACILITY

By

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(Under the Direction of Walid Alali)

ABSTRACT

The objectives of this study were to quantify the food safety risk represented by pasture-raised broilers processed on-farm, in a Mobile Processing Unit (MPU), and a small USDA-Inspected slaughter facility(U-IF) in addition to providing an assessment of the environmental impact of small-scale poultry production practices on the farm. *Salmonella* and *Campylobacter* levels in carcass rinses, soil, mortality compost, and processing wastewater were determined. *Salmonella* prevalence and concentration on carcasses processed on-farm and at the U-IF were not significantly different. *Salmonella* was not detected on carcasses processed by the MPU. Concentrations of *Campylobacter* were significantly highest on carcasses processed in the MPU and lowest on carcasses processed at the U-IF. Processing wastewater disposal practices and on-farm mortality composting may represent a hazard for dissemination of *Salmonella* and *Campylobacter* into the farm environment. Birds processed in the MPU had the highest risk for *Campylobacter* contamination and the lowest for *Salmonella*.

INDEX WORDS: Pastured poultry, Mobile Processing Unit, Small-scale poultry production, Safety of pasture-raised broilers, On-farm poultry processing, *Salmonella*, *Campylobacter*

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## CHAPTER 1

### LITERATURE REVIEW

#### ***Salmonella* & *Campylobacter*: food safety risk associated with poultry processing**

*Salmonella* and *Campylobacter* are two of the leading causes of foodborne illness in the United States. According to the 2010 data by the Foodborne Diseases Active Surveillance Network (FoodNet), salmonellosis was the most common laboratory-confirmed infection reported (17.6 illnesses per 100,000 persons) (CDC, 2011). It was also associated with the most hospitalizations (28% of 8,256 illnesses) and deaths (0.4% of 8,256 illnesses). *Campylobacteriosis* had the second highest incidence (13.6 illnesses per 100,000 persons) of the six pathogens under surveillance by FoodNet (CDC, 2011). Fifteen percent of *Campylobacter* infections (n=6,365) resulted in hospitalizations and 0.1% resulted in death. According to the World Health Organization (WHO, 2011), *Campylobacter* is the most common bacteria causing gastroenteritis in developed and developing countries (WHO, 2011) and has been associated with the potentially fatal autoimmune condition Guillain-Barre Syndrome (Baker et al., 2012).

Salmonellae are gram-negative, non-spore forming facultative anaerobes that are ubiquitous in nature and are commonly found in the intestinal tracts of humans and animals (Jay, 1992). Certain strains of *Salmonella* have a complex series of stress management response systems to survive harsh conditions encountered inside and outside of the host environment. These include regulatory proteins that respond to conditions such as starvation, osmotic-shock, heat tolerance, acid tolerance and oxidative stress responses (Foster & Spector, 1995). The ability of some strains to readily adapt to extreme environments intensifies the public health concern associated with *Salmonella* contamination (D'Aoust & Maurer, 2007).

The genus *Campylobacter* consists of 17 species of bacteria including *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* that are thermophilic, gram-negative, highly motile microorganisms which colonize the intestines of warm-blooded hosts (Nachamkin, 2007). These bacteria are prevalent in poultry, cattle, pigs, sheep, migratory birds, wild birds and rodents (WHO, 2011; Altekruuse et al., 1999). Optimal growth of *Campylobacter jejuni* occurs at 42°C and growth ceases around 30°C which makes its temperature growth range unique among human pathogens (Hazeleger et al., 1998). Another distinctive characteristic of most campylobacters is their requirement for microaerobic conditions for optimal growth (Nachamkin, 2007). While most cases of campylobacteriosis are foodborne, environmental sources such as animal feces and water associated with outdoor leisure activities have been reported (Brown et al., 2004). Although controversial (Murphy, 2006), researchers have suggested that *Campylobacter* may enter a dormant, non-culturable state that is able to persist at low temperatures outside the host environment (Rollins & Coldwell, 1986; Moore, 2001; Jones et al., 1991), and may have the potential to initiate colonization upon return into the gut of a warm-blooded animal (Jones et al., 1991). Rollins & Coldwell (1986) suggested that this non-culturable state of *Campylobacter* could have important epidemiological implications.

Cross-contamination and consumption of raw or undercooked poultry meat are considered to be a major contributor to the overall disease burden of campylobacteriosis (Luber, et al., 2006; WHO, 2011) and salmonellosis (WHO, 2005). Control of *Salmonella* and *Campylobacter* in poultry production requires a complex, multi-hurdle process (Mohan & Reynolds, 2012). The process includes the application of management practices and control strategies on-farm and during processing. The measures used for *Salmonella* control are often of little assistance in the control of *Campylobacter* in the same environment due to significant differences in the physiology and ecology of these organisms (Newell & Fearnley, 2003). A study conducted by Hue et al. (2011) in French poultry slaughterhouses concluded that the levels of *Campylobacter* and *Salmonella* were not significantly correlated. As a result, risk

management should be practiced through all stages of production as well at the retail and consumer levels (Kijlstra et al., 2009).

Hygiene management practices used during broiler production attempt to minimize the number of carcasses that are positive for human pathogens as well as the concentrations of pathogens (Gast, 2007). An infected flock can potentially contaminate processing equipment resulting in cross-contamination of negative flocks as they move through the processing line. Morris & Wells (1970) reported that carcass washing procedures reduced *Salmonella* contamination, but recontamination occurred in areas of extensive handling or contact with other carcasses. It is well established that potential for contamination can arise at scalding, defeathering, evisceration and chilling of carcasses (Mead et al., 2010; Berrang et al., 2009; Guerin et al., 2010). McCrea et al. (2005) reported that the prevalence of *Salmonella* in free-range broilers increased from 0% upon entering the feather picker to 52% after defeathering. A systematic review of the prevalence of *Campylobacter* during processing reported a trend in the literature of a decrease in concentration during chilling and scalding, and an increase in concentration during evisceration and defeathering (Guerin et al., 2010). The chill tank represents a potential area where cross-contamination of *Campylobacter* (Wempe et al., 1982) and *Salmonella* (Sarlin et al., 1998) can occur. Contaminated water that remains trapped in the feather follicle following immersion chilling may play a role in cross-contamination (Kim et al., 1996). Investigators have also suggested processing may put selective pressures on *Campylobacter* enabling certain subtypes to survive processing better than others (Newell et al., 2001; Hunter et al., 2009).

Many investigators have studied horizontal (bird to bird) transmission of *Salmonella* and *Campylobacter* from the primary production and processing environment as a potential route of infection in broiler flocks. Modes of transmission which have been suggested include the external environment, shedding by infected chicks in the hatchery, transmission via rodents, humans, insects and other farm animals (Bull et al., 2006; Newell et al., 2011; Shreeve et al., 2000; Byrd et al., 1998; Cason et al., 1993; Meerburg et al., 2006; Van de Geissen et al., 1998). Vertical transmission from parent flocks (Cox et al.,

2002; Toro et al., 2004; Buhr et al., 2002) and combinations of horizontal and vertical transmission have also been studied with regard to broiler colonization (Sahin et al., 2002; Davies & Wray, 1994). Research has also suggested that factors such as flock lineage could be related to the risk of colonization by *Campylobacter* (Stern et al., 1990). In a mini-review of the sources of *Campylobacter* colonization in broilers, Newell & Fearnley (2003) discussed evidence of seasonal variation, dependence on age, flock-size and production system as potential factors that influence flock colonization with *Campylobacter*.

The USDA Food Safety and Inspection Service (FSIS) monitors the levels of *Salmonella* and *Campylobacter* on broilers and turkeys processed in the United States. Current USDA-FSIS standards require an establishment to have less than 8 positive carcasses out of 51 carcasses tested for *Campylobacter* and no more than 5 carcasses that are positive for *Salmonella* out of 51 carcasses tested (USDA-FSIS, 2010). In 2008, the USDA-FSIS conducted a survey to collect microbiological data on young chickens slaughtered in 182 commercial poultry processing establishments to determine the presence and concentration of *Salmonella* and *Campylobacter* during processing (USDA-FSIS, 2008). Carcasses were sampled using the whole carcass rinse method at the re-hanging and post-chilling points in the process. Based on this study, the estimated national prevalence for *Salmonella* and *Campylobacter* in commercially produced broiler carcasses was 7.5% and 46.7% respectively. Recent prevalence estimates for commercially produced broiler carcasses are 3.8% (n=1,688) for *Salmonella* and 8.6% (n=1,633) for *Campylobacter* (USDA-FSIS, 2012).

### **Pasture-raised and Organic poultry farming**

According to USDA economic research data, consumer interest in locally grown, organic and natural foods continues to increase (Greene & Oberholtzer, 2007). In 2006, organic products accounted for around 3% of total national food sales (Oberholtzer et al., 2006). Organic eggs and poultry meat are among the most popular organic products in the United States and organic poultry sales are expected to continue to rise in coming years (Oberholtzer et al., 2006). The USDA National Organic Program

outlines strict requirements which must be met for an operation to obtain organic certification (USDA-AMS, 2012). Specifications to certify organic poultry include compliance with animal health and welfare standards, providing birds with access to the outdoors, the use of organic feed, and restricted use of antibiotics during the production process. Voluntary labels such as “free-range” are also regulated by the USDA; but producers are only required to demonstrate that birds are provided with access to the outdoors. A specific stocking density of birds during production is not required for the “free-range” label (Oberholtzer et al., 2006). Poultry products bearing the “Natural” label are required to demonstrate that the meat is minimally processed with no artificial ingredients; however the requirements are only applicable to processing and do not include farming practices (Post, 2007).

Surveys conducted by Van Loo et al. (2010) showed that consumers in the United States often assume that organic meats contain fewer pesticide residues, are more nutritious and are produced in a more environmentally friendly manner than conventionally produced meats. Brennan et al. (2003) suggested that the lack of harmonization of international regulations for organic food production may have misled consumers to believe that organic standards are based on the analysis and quality of the final product rather than the production and processing of the product. A lack of published microbiological data for organic and free-range chickens could be partially responsible for the common perception that natural growth conditions result in safer products (Bailey & Cosby, 2005).

Nevertheless, small-scale specialty market poultry rearing operations continue to grow in popularity in the United States. One such operation is the pastured poultry production system developed over the last 20 years by Joel Salatin (Salatin, 1993). This production model is a variation of the free-range production system in which producers only have to demonstrate that animals have access to the outside (Post, 2007). The USDA does not currently have a policy for pasture-raised labeling due to variations in pasture-raised agricultural practices (USDA-AMS, 2012).

The pastured poultry production model involves batches of 50-90 chicks introduced into floorless pens that are rotated to fresh pasture on a daily basis to encourage forage intake. Producers typically raise and process an average of 1,500 broilers per year. Foraging constitutes about 20-30% of the birds diet, which is supplemented with organic or natural feed and does not require the use of antibiotics (Glass, 2002). Carcasses are typically elongated, with smaller breasts and larger legs than conventionally produced broilers (Fanatico, 2010). Consumers and poultry growers are drawn to this type of production based on the expectation of improved flavor and nutrition of the meat, animal welfare, soil fertility, sustainability of the farm environment and community involvement (Hilmire, 2011; Fanatico, 2012).

In the United States, some pastured poultry producers use the same fast-growing, high yielding genotypes like the Cornish Cross used in commercial broiler production. These broilers reach a market weight of 4-5 pounds in 5-6 weeks (Jacob et al., 2008). Other small-scale producers use medium to slow-growing breeds such as the Freedom Ranger as an alternative to the Cornish Cross. These broilers are active foragers on pasture and reach a market weight of 4-5 pounds at 8-12 weeks of age (Ussery, 2009). Lewis et al. (1997) suggested that slow-to medium-growing, low feed conversion genotypes adapt to alternative production environments more successfully than the fast-growing genotypes. It has also been reported that meat from slow-to-medium growing birds has gained greater acceptance from a sensory panel than fast-growing birds (Castellini et al., 2002). A series of studies have concluded that in pasture-raised poultry, outdoor access itself does not impact flavor, water-holding capacity or carcass yield of the meat (Fanatico et al., 2005 & 2006). The authors suggest that the genotype of the bird had a greater effect on these qualities. On the other hand, Ponte et al. (2008) reported that pasture intake promotes growth, increases broiler performance and improves meat sensory quality.

### **Pastured poultry processing**

The Poultry Products Inspection Act ensures that poultry products are wholesome and that misbranded or adulterated products do not enter foreign or interstate commerce (USDA-FSIS, 2006). A

1968 amendment to the Act requires that poultry processed for human consumption must be done so under federal or state inspection, unless the processing operation meets certain exemption criteria. Birds labeled with a USDA Seal of Inspection can be sold anywhere in the United States.

In order to qualify for federal inspection, a producer must process a minimum of 20,000 birds per year. Regulations vary by state, and many states have a state-run inspection program in place for growers who process less than 20,000 birds per year (USDA-FSIS, 2006). However, the current regulations have resulted in regulatory loopholes in some states such as Georgia, which does not have a state-run poultry inspection program in place. Small-scale poultry growers in Georgia are not eligible for federal inspection if they process less than 20,000 birds per year, yet state law requires an inspected status in order to have access to retail markets. In November of 2011, the Georgia Department of Agriculture emphasized that poultry growers are only legally permitted to process 1,000 birds or less per year on-farm for direct sale to consumers (Georgia Organics, 2011). As a result of this established limit on production volume, the economic feasibility of small-scale poultry production in Georgia is severely limited despite the rising demand for locally grown poultry meat. Many pasture-raised poultry growers have had to postpone production until more affordable processing options are available (personal communication with farmers).

Access to processing is a critical issue for small-scale pastured poultry growers and in most cases the grower has limited options for processing methods. The processing method used depends on the availability of suitable equipment, facilities, labor, and compliance with regulations (Fanatico, 2003). Some pasture-raised poultry producers choose to process their birds manually at the site of production (on-farm). The setup can be open-air or in an enclosed shed that contains kill cones, a single-stage static scalding tank, a mechanical batch or drum picker, stainless steel tables for evisceration, a water hose for spray washing carcasses and a large container filled with ice water as a chill tank. Birds are not stunned and are slaughtered with a sharp knife while inside the kill cones. Since proper temperature of the scald water is difficult to maintain, particularly after the addition of fresh water, the same scald water is often used for an entire batch of birds (personal communication with farmers). These producers typically allow

processing wastewater from the picker, scalding and evisceration table to empty directly into the surrounding soil. The wastewater runoff may empty into the same area after each processing run or the farmer may rotate areas of runoff disposal into the soil.

Processing offal is collected and applied to an on-site mortality composting pile. Composting practices such as aeration, temperature monitoring and pile design vary from farm to farm. In some cases, the compost pile is housed in a wooden bin which is located at a distance from the production and processing site. Alternatively, the compost pile may be on the ground uncovered or covered with straw within view of the production and processing site. Compost ingredients are a mixture of manure from various farm animals, processing offal, wood chips or straw, and dead birds or other small animals.

The type and quantity of antimicrobial interventions used to control pathogens on chicken carcasses also varies from farm to farm. Many producers use ice in the chill tank for pathogen control. Some producers include dilute solutions of vinegar or apple cider vinegar in the chill tank. Depending on available resources and the number of birds that are being processed, a producer may process birds alone or with the assistance of friends or relatives. Workers may or may not wear gloves or protective clothing. Farm animals such as pigs, ducks, cows, goats, feral cats and herding dogs may also be present on these pastured poultry farms.

Another processing method for small-scale pastured poultry producers involves transportation of birds to a small USDA-inspected slaughter facility that is willing to process a limited number of custom batches of birds. Commercial processors generally will not handle birds from independent producers (SARE, 2006). The small slaughter facilities have between 10 and 499 employees and are usually located in rural areas (Johnson et al., 2012). Batches of birds are slaughtered, processed and packaged for a fee based on the number of birds to be processed. An advantage of this processing method is the USDA-inspected label and the resulting access to the favorable market for locally grown broilers. On the other hand, this method can prove to be costly since the number of these small facilities has declined over the

years (Johnson et al., 2012) and farmers may be required to transport their birds to a neighboring state for processing. Furthermore, processing appointments typically have to be scheduled 3-4 months in advance with limited flexibility for rescheduling (personal communication with farmers and small processing facilities). Antimicrobial interventions used in these operations are often those considered to be Processing Aids by the USDA-FSIS (USDA-FSIS, 2008) or the interventions themselves remain undisclosed to the producer by the processing establishment (personal communication with farmers).

Mobile Processing Units (MPUs) may be an alternative method for small-scale growers who are hindered by regulation loopholes or high transportation costs pertaining to processing. An MPU is a self-contained slaughter facility that can travel from farm to farm (Johnson et al., 2012). Although MPUs are often used for game animals and large livestock animals, interest in using such units for poultry processing is growing as small-scale growers search for suitable processing methods (Fanatico, 2003). Units are equipped with kill cones, a scalding, picker, chill tanks and eviscerating stations in an enclosed trailer or truck bed. Models range from very basic to high-end units that cost thousands of dollars. Farmers can buy or lease a unit individually or form a co-op to share the unit between farms. Individual farmers are responsible for all training, labor, electricity, water, proper waste disposal, propane hookups, and packaging supplies used to process their birds (McDonald, 2012).

Disadvantages of processing with the enclosed MPU include expensive maintenance costs and greater difficulty in cleaning and sanitizing the unit. If the unit is shared between farmers, development of shared use management policies and potential cross-contamination from other farms may be an issue (McDonald, 2012). Availability of an appropriate volume of clean water for processing and disposal of processing wastewater may also be a challenge when using an MPU due to costs and regulatory obstacles. Wastewater disposal options include discharge into publicly owned treatment works or discharge through land application on-site or at another authorized location. Both types of disposal may require municipal authorization (McDonald, 2012).

Advantages of processing with an enclosed MPU include the potential to obtain USDA inspection status, decreased dependence on the weather and the potential to increase production output (McDonald, 2012). An MPU with USDA-inspected status must adhere to the same regulatory requirements as fixed USDA-inspected slaughter facilities (USDA-FSIS, 2012). The USDA-FSIS “Mobile Processing Compliance Guide” outlines the procedures necessary to obtain a grant of federal inspection and addresses concerns unique to mobile processing. While several established poultry MPUs are currently in operation in the United States, (Johnson et al., 2012; McDonald, 2012) very few research studies have focused on the safety of chicken meat processed at MPU poultry slaughter facilities (Killinger, 2010).

### **Microbial contamination of organic and pasture-raised broilers**

A number of research studies have been conducted to address concerns regarding the food safety risk associated with specialty market poultry products. Many investigators have compared the prevalence of pathogens in organic and conventionally raised broilers (Heuer et al., 2001; Rodenburg et al., 2004; Luangtongkum et al., 2006; Van Overbeke et al., 2006; Alali et al., 2010). Others have compared the prevalence of pathogens in free-range and pasture-raised birds (Lund et al., 2003) or in pasture-raised and conventionally-raised birds (Siemon et al., 2007). The results of such studies are difficult to compare because of differences in sampling techniques and isolation methods (Humphrey & Jorgensen, 2006). Esteban et al. (2007) reported a 1.7% prevalence (n=60 flocks) of *Salmonella* and a 76.7% prevalence of *Campylobacter* (n=60 flocks) on 34 free-range poultry farms. Bailey & Cosby (2005) reported a 25% prevalence (n=53) of *Salmonella* in commercial free-range broilers and Hanning et al. (2010) reported a 75% prevalence (n=48) of *Campylobacter* on retail pasture-raised broilers. In a review of the safety of organic meats, Van Loo et al. (2012) discussed a general trend in the literature showing that the prevalence of pathogens on organic meats was roughly equal to or higher than those in conventionally raised meats in most of the studies reviewed, with a few studies showing the opposite trend. The current data on specialty market poultry focuses on the prevalence of pathogens such as *Salmonella* and *Campylobacter*, but data on the estimated microbial loads of such pathogens does not exist. To the best of

our knowledge, there is no published data comparing the microbial loads of *Salmonella* and *Campylobacter* in pasture-raised broilers processed on-farm, in an MPU or at a small USDA-Inspected facility.

### **Pasture-raised poultry processing and the farm environment**

Broiler production generates an enormous amount of waste in the form of poultry litter, dead birds, manure, processing offal and wastewater which pose a risk to the environment without proper waste management practices (Edwards & Daniel, 1992). A poultry processing plant may serve as a reservoir of Salmonellae which can exert an influence on the environment at a distance of at least 5 km from the plant (Hoadley et al., 1974). Outdoor poultry rearing systems may heighten this influence since they provide the potential for a continuous dissemination of enteric organisms into the environment surrounding the poultry house (Huneau-Salaun et al., 2007).

On-farm mortality composting is a natural decomposition process used by small-scale poultry producers to manage animal mortality and related agricultural waste on the farm. The purpose of this type of composting is to minimize the transmission of pathogenic bacteria into surrounding soil, air and water, prevent the spread of infection from diseased animals, and convert the carcass into a constructive, nutrient rich material (Kalbasi et al., 2005). Agricultural waste that has been subjected to an effective composting process can be used as a nutrient-rich soil amendment and crop fertilizer on the farm (Singh et. al, 2012).

Kalbasi et al. (2005) described carcass composting as the temporary, above-ground burial of dead animals in a heap of added carbon in which thermophilic microorganisms heat up the pile, digest tissues and kill most of the pathogens under primarily aerobic conditions. Although composting is an established method for pathogen reduction, validation of the microbial safety of mortality composting can be challenging because of diverse pile conditions and process management (Wilkinson, 2007). A study by Shepherd et al. (2010) concluded that the conditions on the surface (< 5cm) of poultry mortality compost heaps are suitable for pathogen survival during the first phase of composting. Furthermore, the authors

reported that the temperature and moisture contents of the compost heaps at participating small and medium-sized poultry farms were often below the recommended level for pathogen inactivation. Enteric pathogens such as *Salmonella* and *Campylobacter* have demonstrated survival in agricultural waste compost (Heringa et al., 2010; Inglis et al., 2010; Singh et al., 2012; Gong et al., 2005) and domestic waste compost containing green waste, fruits and vegetables (Lemunier et al., 2005). Pathogen regrowth has also been demonstrated in finished compost and compost extracts (Kim & Shepherd, 2009; Wilkinson, 2007).

Poultry Science Extension services recommend construction of a proper composting bin that includes a roof, foundation and floor in addition to a nitrogen source, a carbon source and aerobic microorganisms to ensure an effective composting process (Carter et al., 1996). Field trials reported by Shepherd et al. (2011) validated the recommendation for a cover on fresh compost; *E. coli* O157:H7 reduction occurred more rapidly at the surface of heaps covered with finished compost versus fresh straw or uncovered compost heaps.

Land application of poultry production waste and mortality compost can be used as a waste management strategy as well as a soil amendment in the small-scale production environment (Edwards & Daniel, 1992). In each case, the rate of application depends on the primary reason for waste disposal. If the objective is to increase the nutrient content of the soil, waste application is based on the nitrogen requirements of the crop and waste characteristics such as nitrogen availability and the rate at which organic nitrogen is converted into plant-available forms (Edwards & Daniel, 1992). If the farmer is simply trying to avoid the buildup of litter, dead birds and mortality compost, waste is usually applied at a much higher rate than when it is used as a fertilizer (Edwards & Daniel, 1992). However, research has shown that repeated animal waste disposal (Dazzo et al., 1973; Weil & Kroontje, 1979) and poor waste management practices can lead to decreased yield of crops (Shortall & Liebhardt, 1975), soil toxicity (Weil & Kroontje, 1979), and contamination of crops, surface waters and groundwater through the transport of surviving bacteria (Edwards & Daniel, 1992).

Bacterial survival in soil is influenced by the soil texture, pH, moisture content, nutrient availability, temperature, and competing microorganisms (Alexander, 1977; Jamieson, 2002). High moisture content, low UV radiation, low temperatures above freezing, and a neutral pH generally favor survival (Ross & Donnison, 2006). Textural classes of soil have been established based on the soil's content of sand, clay, and silt. A single farm may be composed of several different soil types (Alexander, 1977). Among these soil components, clay has the greatest amount of exposed surface area and the most individual structural units per gram; therefore the clay fraction of soil is the most susceptible to microbiological activity (Alexander, 1977).

Survival of *Salmonella* and *Campylobacter* has been demonstrated in soil where agricultural waste has been applied (Hutchison et al., 2004; Hutchison et al., 2005; Ross & Donnison, 2006; Holley et al., 2006; You et al., 2006). Researchers have reported that the survival of *Salmonella* (Holley et al., 2006) and *Campylobacter* (Ross & Donnison, 2006) was enhanced in manure-amended soils. Ross & Donnison (2006) found that *Campylobacter* survived in the top 5 cm of soil for 25 days at 10°C. Although pathogenic bacteria may only survive 2-3 months or less in soil (Gerba et al., 1975), You et al. (2006) reported survival of *Salmonella* for 332 days in laboratory simulated manure-amended soil.

Poultry processing wastewater (PPW) is generated from scalding, defeathering, washing carcasses, chilling, and rinsing equipment (USEPA, 2002). Raw PPW consists of fats and proteins in both particulate and dissolved forms as well as blood, feathers, viscera, urine and feces (USEPA, 2002). This results in high concentrations of biological oxygen demand (BOD), chemical oxygen demand, (COD), total suspended solids (TSS), nitrogen, and phosphorous. When compared to domestic wastewater concentrations, PPW is considered to be a more concentrated waste (USEPA, 2002). PPW from a commercial plant contains millions of CFU/ml of nonpathogenic indicator bacteria, which may signify the presence of enteric pathogens such as *Salmonella* and *Campylobacter* (USEPA, 2002).

Direct application of untreated PPW to the soil in addition to composting solid waste in the on-farm processing environment is easy and economically feasible for the small-scale pastured poultry producer. However, both practices have the potential to increase the flow of *Salmonella* and *Campylobacter* into the farming environment and may result in the dissemination of these pathogens to other food animals and agricultural commodities on the farm.

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CHAPTER 2

FOOD AND ENVIRONMENTAL SAFETY OF PASTURE-RAISED BROILERS  
PROCESSED ON-FARM, IN A MOBILE PROCESSING UNIT AND AT A SMALL USDA-  
INSPECTED FACILITY<sup>1</sup>

<sup>1</sup>Trimble, L.M., and Alali W.Q. To be submitted to *Food Control*.

## ABSTRACT

The objectives of this study were to quantify the food safety risk represented by pasture-raised broilers processed on-farm, in a Mobile Processing Unit (MPU), and a small USDA-Inspected slaughter facility (U-IF) in addition to providing an assessment of the environmental impact of small-scale poultry production practices on the farm. *Salmonella* and *Campylobacter* levels in carcass rinses, soil, mortality compost, and processing wastewater were determined. *Salmonella* prevalence and concentration of birds processed on-farm and at the U-IF were not significantly different. *Salmonella* was not detected in birds processed in the MPU. Concentrations of *Campylobacter* were significantly highest on birds processed in the MPU and lowest on birds processed at the U-IF. Processing wastewater disposal practices and on-farm mortality composting may represent a hazard for dissemination of *Salmonella* and *Campylobacter* into the farm environment. Birds processed in the MPU had the highest risk for *Campylobacter* contamination and the lowest for *Salmonella*.

## Introduction

Salmonellosis and campylobacteriosis are foodborne illnesses that constitute major public health burdens worldwide (WHO 2005; 2011). Foods of animal origin such as meat, poultry, milk and eggs have been implicated in the transmission of these enteric bacteria (WHO 2005; 2011). It is well established that poultry is one of the main sources of *Salmonella* and *Campylobacter* infection (Poppe, 2000; CDC, 2007). The estimated national prevalence of *Salmonella* and *Campylobacter* on commercially produced broiler carcasses is 3.8 % and 8.6% respectively (USDA, 2012).

The farm-to-fork approach to food safety in poultry production includes farmers, processing establishments and consumers in efforts to prevent foodborne illness caused by *Salmonella* and *Campylobacter*. Numerous research studies have focused on the control and prevention of *Salmonella* and *Campylobacter* transmission in conventional poultry production systems with a focus on areas such as preharvest control measures (Bull et al., 2006; Davies & Wray, 1994; Dorea et al., 2010; Gibbens et al., 2001), sources of colonization (Newell & Fearnley, 2003 ; Sahin et al., 2002), the processing environment (Berrang et al., 2008; Morris & Wells, 1970; Newell et al., 2001; Stern et al., 2001; Wempe et al., 1982) and the farm environment (Hansson et al., 2007; Vandeplas et al., 2009). However, there is limited research that focuses on the food and environmental safety of small-scale pasture-raised broiler production systems. The current available data on specialty market poultry (i.e. non-conventionally raised birds) focused on the prevalence of pathogens such as *Salmonella* and *Campylobacter* at the farm, processing, or retail level (Esteban et al., 2008; Siemon et al., 2007; Lund et al., 2003; Heuer et al., 2001; Van Overbeke et al., 2006; Alali et al., 2010; Van Loo et al., 2012; McCrea et al., 2006; Hanning et al., 2010), but data on the microbial loads of such pathogens on broiler carcasses and in the farm environment does not exist.

Consumer interest in sustainable agriculture has resulted in an increasing demand for locally produced products (Johnson et al., 2012). A growing niche in the locally grown food movement is the pastured poultry production model. Batches of 50-90 chicks are introduced into floorless pens that are

rotated to fresh pasture on a daily basis to encourage forage intake. Consumers and producers are drawn to this production model based on the expectation of improved flavor and nutrition of the meat, animal welfare, soil fertility, sustainability of the farm environment and community involvement (Fanatico, 2012; Hilmire, 2011).

Access to the profitable retail market for locally raised poultry meat requires a USDA-inspected status for which many small-scale producers are ineligible. Therefore, these producers face substantial barriers to economic feasibility of their operations. Farmers often process their birds at the site of production (on-farm), in a mobile processing unit (MPU) or birds are transported to a small USDA-inspected facility that will process a limited number of custom batches of birds. The absence of regulatory guidance along with the relative scarcity of studies on pastured poultry processing methods has failed to yield a record of the data that is necessary to validate the safety of these methods. The objectives of this study were to: 1) quantify and compare the prevalence and concentrations of *Salmonella* and *Campylobacter* on pasture-raised broiler carcasses processed on-farm, at a small USDA-Inspected facility and at an MPU Pilot Plant and 2) quantify *Salmonella* and *Campylobacter* concentrations and prevalence in processing wastewater and areas of wastewater disposal on small-scale broiler farms.

## **Materials and Methods**

### *Study Design and Sampling Scheme*

Over a one year period, this study was conducted at pasture-raised broiler farms that processed birds at the site of production (on-farm), a small USDA-inspected facility and a Mobile Processing Unit (MPU) Pilot plant in the southeastern United States. Samples were collected during 12 on-farm visits in accordance with the farmers' broiler processing schedules. Five sampling visits were conducted at the small USDA-inspected facility and 5 processing runs were conducted at the MPU Pilot plant.

The participating small scale pastured-poultry producers processed their birds manually on the farm in an open-air setup or at a processing station in an enclosed shed. Birds were slaughtered using a sharp knife at 9-10 weeks of age. Processing stations included kill cones, a single-stage static scalding, a mechanical batch picker, stainless steel tables for evisceration, a water hose for spray washing of carcasses, and large containers filled with ice as a chill tank. Processing wastewater was emptied directly into the surrounding soil and solid wastes were added to an on-site mortality composting pile. Antimicrobial interventions for control of pathogens, cleaning and sanitizing practices varied from farm to farm. All of the farmers used ice in the chill tank for control of *Salmonella* and *Campylobacter*. One producer raised and processed the Cornish Cross breed, while the other three producers used slower-growing breeds (i.e. K-22 and Freedom Rangers). Workers included family and friends of varying ages and levels of experience with processing broilers. In most cases, farm animals such as pigs, horses, goats, cows and herding dogs were also present on the farms.

The small USDA-inspected facility was located in a rural area of the southeastern United States and was equipped to process small batches of pasture-raised broilers. Pasture-raised, slow-growing Red Rangers were processed at this facility. A batch processing system was used and most of the processing was performed manually by employees of the establishment. Visitors were required to sanitize footwear upon entry to the facility and hairnets, aprons, and gloves were required during the sample collection process. Processed birds were inspected by a USDA-FSIS employee. Antimicrobial interventions for pathogen control included treatment of pre-chill carcasses with a citric and lactic-acid based antimicrobial spray.

The Mobile Processing Unit pilot plant was located in a University setting. The batch processing system consisted of a hand-held electric stun knife, a killing tunnel, 5 shackles, a scalding, a spin-picker and a chill tank. Pasture-raised broilers were delivered to the facility by local farmers and were processed the same day. Birds were stunned and killed with a hand-held electric stun knife and were allowed to bleed out for 2-5 minutes. After scalding and defeathering, evisceration was performed manually with

scissors and gloved hands. Breeds included Ross 708, Cobb 700, Freedom Rangers and Naked Necks. Workers were trained poultry scientists, food scientists and graduate students. Antimicrobial interventions were minimal and included ice in the chill tank to control levels of *Salmonella* and *Campylobacter*.

At each visit to the farms that processed birds at the site of production, 10 post-chill broiler carcasses were randomly selected and rinsed using the whole carcass rinse method (USDA, 2011). Additionally, 3 composite processing wastewater samples, soil and compost samples were collected. At the small USDA-inspected facility, 10 post-chill carcasses were selected and rinsed as described previously.

For each MPU processing run, ten post-chill carcasses were selected and rinsed as described previously. Three composite wastewater samples were collected from the scalding and picker during each MPU processing run.

#### *Sample Collection*

-Whole Carcass Rinses. Pasture-raised broiler carcasses were removed from the chill tank after 1 hour of immersion chilling and excess water was drained from the cavity. Each carcass was placed into a sterile poultry rinse bag (Nasco; Fort Atkinson, WI) and 400 mL of sterile water was poured into the cavity. The carcass was rinsed for 1 minute using a rotating arc motion as described in the USDA-FSIS method (USDA 2011). The rinsate was aseptically drained from the rinse bag into a sterile field bottle (Nalgene, Rochester, New York) and was placed on ice for transport to the laboratory.

-Soil Samples. Samples were collected before processing began at each farm visit. Three separate areas of previous wastewater disposal in the soil were chosen using the *Judgmental Sampling approach* (IAEA, 2004). In most cases, previous wastewater disposal occurred at least 1 week prior to sample collection. Sampling points included the areas around the scalding discharge hose, the picker, and the evisceration table. Three 2.5 inch soil cores were collected at each of the 3 disposal areas using a soil auger. Samples were placed in *Whirl-Pak*® bags (Nasco, Fort Atkinson, WI) on ice for transport to the laboratory where they were combined into 3 composite samples (approximately 100 g each).

-Compost samples. Three 100 gram samples in each of 3 areas of the mortality compost pile were collected at each farm visit. A large sterile metal spoon was used to collect the samples at a depth of 2-3 inches. Samples were placed in sterile *Whirl-Pak*® bags on ice for transport to the laboratory where they were combined into 3 composite samples (approximately 100 g each).

-Wastewater Samples. Three composite processing wastewater samples (1000 ml each) were collected into sterile plastic field bottles (Nalgene, Rochester, New York) upon emptying of the scalding and the picker. Runoff was also collected from the evisceration table as well as the offal collection bin. Bottles were placed on ice for transport to the laboratory.

#### *Analysis for Salmonella and Campylobacter*

All samples were processed and assayed on the day of collection. The 3-tube Most Probable Number (MPN) method was used for quantification of *Salmonella* according to USDA-FSIS methods (USDA-FSIS, 2008 a, b). The direct plating and enrichment method was used for detection and enumeration of *Campylobacter* (USDA-FSIS, 2011).

For each carcass rinse sample, nine tubes containing Buffered Peptone Water (BPW; Difco, Sparks, MD) (3 tubes each of 1mL 10X BPW, 9 mL 1X BPW and 9 mL 1X BPW) were used for the pre-enrichment of *Salmonella*. The carcass rinsate was added to the tubes in the amounts of 10 mL, 1 mL and 0.1 mL respectively. Tubes were incubated at 37°C for 24 hours. After incubation, 0.5 mL and 0.1 mL of each BPW pre-enrichment tube were added to 10 mL of Tetrathionate broth (TT broth; Difco) and 10 mL of Rappaport-Vassiliadis (RV broth; Difco) respectively. Enrichment tubes were incubated for 24 hours at 42°C. Tubes were vortexed and a 10 µl loopful from each enrichment broth was streaked onto Brilliant Green Sulfa agar (BGS; Difco) and Xylose Lysine Tergitol-4 Agar (XLT4; Difco) plates and incubated for 24 hours at 37°C. Colonies characteristic of *Salmonella* were inoculated onto Triple Sugar Iron Agar (TSI; Difco) and Lysine Iron Agar (LIA; Difco) slants and were incubated at 37°C for 24 hours. All BGS

and XLT4 plates were incubated for an additional 24 hours and colonies presumed to be *Salmonella* were inoculated onto additional LIA and TSI slants and incubated as previously described.

Slants were examined as sets for reactions typical of *Salmonella* and were further tested for agglutination using *Salmonella* O Poly A-I & Vi antiserum (Difco). Colonies with a presumptive positive reaction on LIA and TSI slants that did not agglutinate were further tested using Real-Time PCR (Stratagene Mx 3005P, Santa Clara CA). Total DNA was extracted from the isolates according to the method described in Anderson et al. (2010). Real-Time PCR detection of *Salmonella* was performed as described in Bohaychuk et al. (2007). Colonies confirmed as *Salmonella* were preserved on Tryptic Soy Agar (TSA; Difco) and stored at 4°C.

Direct plating was performed for the enumeration of *Campylobacter* from the carcass rinse samples. Serial dilutions of the rinsate were prepared in Phosphate Buffered Saline (PBS; Difco) and were spread plated on modified Campy-cefex agar plates (Hardy Diagnostics, Santa Maria, CA). For each sample, 250 µl of undiluted rinse was spread onto 4 plates and subsequent dilutions were achieved by plating 100 µl of the dilution series on duplicate plates. Plates were placed in sealable bags flushed with microaerobic gas (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>) and were incubated at 35°C for 48 hours per the manufacturer's recommendation. After incubation, Campy Cefex plates were examined for typical *Campylobacter* colonies. Confirmation of presumptive positive colonies was based on cellular morphology and motility under a phase contrast microscope (Olympus BX40, Center Valley, PA) and a positive reaction in a latex agglutination immunoassay (Hardy Diagnostics). For each sample, the dilution that contained confirmed colonies within the countable range (15-300 CFU/plate) was used to calculate the CFU/mL of sample according to the method outlined in (USDA-FSIS, 2011).

For enrichment of each carcass rinse, 30 mL of the sample was added 30 mL of Bolton Enrichment Broth (Hardy Diagnostics), and incubated under microaerobic conditions for 48 hours at 35°C. If direct plating of the sample did not display colonies typical of *Campylobacter*, the Bolton Broth enrichment cultures were used to streak for isolation onto Campy-cefex agar and were confirmed as described previously.

For wastewater samples, feathers and debris were removed using a sterile handheld screen. Two 50 mL tubes of wastewater from each composite sample were centrifuged for 20 minutes (9200 rpm at 4°C). The supernatant fluid (45 mL) from each tube was discarded. The pellet and the remaining 5 mL of liquid in each tube were resuspended in 20 mL of room temperature PBS and vortexed. Each tube was used for *Salmonella* and *Campylobacter* assays as described for the carcass rinse samples.

For soil and compost samples, debris (rocks, pebbles and/or bones) were removed using a sterile handheld screen. Two 10-gram samples were each placed in a 50 mL falcon tube and PBS was added to bring up the volume to 50 mL per tube. Each tube was vigorously shaken by hand for 1 minute and placed on a shaker at 200 rpm for 20 minutes at 4°C. Soil tubes were centrifuged at 1200 rpm for 5 minutes and compost samples were centrifuged at 9200 rpm for 15 minutes. The supernatant fluid (45 mL) was poured into a sterile tube and used for *Salmonella* and *Campylobacter* analysis as described for the carcass rinse samples. Enrichment of soil, compost and wastewater samples was performed as described previously using 10 mL of sample and 10 mL of Bolton Enrichment Broth.

## **Data Analysis**

The outcomes of the study were the prevalence and concentrations of *Salmonella* and *Campylobacter* on pasture-raised broiler carcasses and in soil, compost and processing wastewater samples from the pasture-raised broiler farm environment. The concentration data (MPN or CFU/volume or weight) data were adjusted to the original weight or volume collected and were then  $\log_{10}$  transformed to approximate normality. Descriptive statistics of the carcass, soil, compost and processing wastewater were performed. The carcass prevalence data were cross-tabulated and compared by processing method, followed by a comparison of breeds within each processing method using a Fisher's exact test or 2-by-n likelihood ratio chi-square test in STATA software version 10.1 (Stata Corp., College Station, TX).

A p-value of 0.25 was used to select variables to be retained for the Generalized Linear Model (GLM) analyses, with models built in a forward stepping manner. The relationship between the pathogen

prevalence on the carcasses and the pastured broiler processing method was assessed using a generalized linear model, with binomial error distribution, logit link function and adjustment for dependency within farms using generalized estimated equations (GEE) in STATA.

The pathogen concentration data for the carcasses were first compared using one-way ANOVA with post-hoc mean comparison in STATA software. A p-value of 0.25 was used to select variables to be retained for the Generalized Linear Model (GLM) analyses. The relationship between the pathogen concentration and the broiler processing method was assessed using the GEE model, with identity link function to adjust for dependency within farms in STATA.  $P < 0.05$  was considered significant.

## **Results**

A total of 120, 50, and 50 carcass rinse samples were tested from pastured broiler farms, the USDA-inspected facility, and the MPU pilot plant, respectively. Samples collected from the farm environment included a total of 46 wastewater samples, 42 soil samples, and 39 compost samples. Nine wastewater samples were collected from the MPU pilot plant.

### *Salmonella* on Pasture-Raised Broiler Carcasses

The *Salmonella* prevalence and mean log concentration on chicken carcasses by processing method is shown in Table 1. The prevalence of *Salmonella* on broiler carcasses processed at the MPU pilot plant was significantly ( $P < 0.05$ ) lower than chickens processed on-farm or at the USDA-Inspected facility. *Salmonella* prevalence for pasture-raised broilers processed on-farm was not significantly different ( $P > 0.05$ ) between the three breeds. The distribution of the mean log MPN of *Salmonella* on chicken carcasses by processing method is shown in Figure 1. Birds processed at the MPU had significantly lower *Salmonella* concentrations ( $P < 0.05$ ) than those processed on-farm or at the USDA-inspected facility. The breed of the bird did not have a significant effect ( $P > 0.05$ ) on *Salmonella* concentrations on carcasses processed on-farm.

### *Salmonella* in the On-Farm Processing Environment

The prevalence and mean log concentration of *Salmonella* in wastewater, soil, and compost samples is shown in Table 1. Compost samples had a higher prevalence of *Salmonella* than soil and wastewater samples. The distribution of the mean log MPN of *Salmonella* in soil, compost and on-farm processing wastewater is shown in Figure 2. On-farm wastewater samples had higher concentrations of *Salmonella* in the farm environment when compared to soil and compost samples.

### *Campylobacter* on Pasture-raised Broiler Carcasses

The *Campylobacter* prevalence and concentration on pasture-raised broiler carcasses is shown in Table 2. The prevalence of *Campylobacter* on chicken carcasses was not significantly different ( $P>0.05$ ) by processing method. Breed did not have a significant effect ( $P>0.05$ ) on *Campylobacter* prevalence in broilers processed on-farm. The distribution of the mean log concentrations of *Campylobacter* in carcass rinses is shown in Figure 3. Birds processed in the MPU had significantly ( $P<0.05$ ) higher *Campylobacter* concentrations than those processed on-farm and at the USDA-inspected facility. Furthermore, breed had a significant effect on the concentration of *Campylobacter* in birds processed on-farm; Freedom Ranger carcasses had significantly ( $P<0.05$ ) higher concentrations than the Cornish Cross breed.

### *Campylobacter* in the On-Farm Processing Environment

The prevalence and concentration of *Campylobacter* in soil, compost and wastewater is shown in Table 2. *Campylobacter* was more prevalent in MPU processing wastewater and soil samples compared to compost and on-farm wastewater samples. The mean log CFU distribution of *Campylobacter* in soil, compost and wastewater is shown in Figure 4. *Campylobacter* had higher concentrations in compost and MPU wastewater samples compared to soil and on-farm wastewater samples.

## Discussion

The substantial public health burden caused by salmonellosis and campylobacteriosis is often associated with poultry products such as contaminated broiler meat. Control of *Salmonella* and *Campylobacter* is achieved through regulatory oversight, good management practices and the use of interventions at the pre-and post-harvest levels of conventional poultry production systems (Mohan & Reynolds, 2012).

On the other hand, small-scale pastured poultry growers face obstacles as such as limited processing resources as well as ineligibility for regulatory guidance and oversight. These obstacles may in part, impact the safety of the broilers produced in small-scale production systems since many growers continue to raise, process and sell broilers directly to consumers despite the lack of regulatory guidance. Therefore, an understanding of the food safety risk associated with small-scale poultry production systems is essential.

Current data on the safety of specialty market poultry products is limited and often shows conflicting results. The majority of available data focuses on the prevalence of pathogens such as *Salmonella* and *Campylobacter* on specialty market broiler carcasses, while the microbial loads of these pathogens on carcasses remain undetermined. The current study established a record of quantified *Salmonella* and *Campylobacter* populations on pasture-raised broilers processed on-farm, in a Mobile Processing Unit Pilot plant and at a small USDA-inspected facility.

In this study, the prevalence of *Salmonella* and *Campylobacter* on broiler carcasses was not significantly different between the on-farm and USDA-inspected processing methods. *Salmonella* was not detected in birds processed in the MPU, which was significantly lower than the prevalence in birds processed by the other methods. Although *Campylobacter* prevalence was not significantly different when compared to other processing methods, this pathogen was detected on all of the carcasses processed in the MPU.

The undetectable *Salmonella* occurrence and high *Campylobacter* prevalence in MPU processed birds may be due to farm management practices aimed to control *Salmonella* in addition to a seasonal effect on the pasture-raised broiler farms. In a six year study of raw retail broilers (n=1127), Wilson et al. (2002) reported a significant seasonal trend of increased *Salmonella* prevalence during the first quarter of each year. In the current study, sampling at the farms located on the eastern end of the southeast region of the United States occurred during all four seasons from the fall of 2011 through the summer of 2012. Sample collection for birds processed in the MPU occurred during the summer of 2012. In a one-year study of brand-named retail market broilers, Willis & Murray (1997) reported that the highest recovery percentage of *Campylobacter* occurred during June and July of that year, and both months had a 96.7% (n=30) *Campylobacter*-positive percentage. Additionally, Stern et al. (2001) reported that the highest prevalence of *Campylobacter* in fecal samples of 32 broiler flocks was detected during the summer months.

The prevalence of *Salmonella* in MPU-processed birds is in agreement with Killinger et al. (2010) which reported a zero prevalence of *Salmonella* in post-wash, pasture-raised carcasses used as untreated controls (n=60) during MPU processing. Heuer et al. (2001) detected *Campylobacter* in 100% (n=22) of organic flocks taken from pre-slaughter cloacal swabs. Griggs et al. (2006) reported a 96% (n=299) prevalence of *Campylobacter* in pre-chill, antibiotic free broilers raised in small-scale production systems. The *Campylobacter* prevalence data for carcasses processed on-farm and at the small USDA-inspected facility shown in Table 2 are in agreement with data reported by Hanning et al. (2010). These authors reported 75% (n=48) of pasture-raised retail carcasses were positive for *Campylobacter*. However, Wempe et al. (1982) and Berrang & Dickens (2000) have reported a flock effect associated with the incidence and levels of *Campylobacter* contamination in broiler carcasses.

The prevalence of *Salmonella* in birds processed on-farm and the small USDA-inspected facility is relatively greater than data reported in previous studies. Lestari et al. (2009) reported 20.8% of national-brand organic broiler carcasses (n=53) examined from 7 chain grocery stores in Louisiana were *Salmonella*-positive. Harrison et al. (2001) reported 30% of fresh whole carcasses sold at a Welsh butcher

shop were *Salmonella*-positive and 69% of fresh carcasses sold at supermarkets were *Salmonella*-positive. The authors suggested the higher prevalence in supermarket carcasses was due to processing practices such as poor moisture control in packaging. Along the same line, Bailey & Cosby (2005) reported a 60% prevalence (n=25) of *Salmonella* in certified organic free-range broiler carcasses at the retail level and a 25% (n=53) prevalence in retail broilers with the all-natural and antibiotic-free label.

The lack of regulatory guidance regarding controlled rearing and processing practices on-farm combined with an emphasis on minimal antimicrobial interventions may play a role in the high prevalence of *Salmonella* and *Campylobacter* on small-scale broiler farms. During on-farm processing, the use of single-stage, static scalders without replacing the water may increase the potential for cross contamination of carcasses. Hard scalding temperatures (approximately 58°C-63°C) may not significantly reduce *Salmonella* contamination on carcasses (Slaviket et al., 1995). Feather picking in a batch picker is an abrasive process that may result in the transfer of bacteria between birds by the rubber projections. McCrea et al. (2005) reported that the prevalence of *Salmonella* in free-range broilers increased from 0% upon entering the feather picker to 52% after defeathering. Moreover, Wempe et al. (1982) reported a 94.4% prevalence of *Campylobacter* in commercial feather picker drip samples.

Since the evisceration process has been associated with increased levels of *Campylobacter* (Izat et al., 1988) and *Salmonella* (Morris & Wells 1970; Sarlin et al., 1998), the practice of manual evisceration on a flat surface instead of using shackles or a stand may present a potential route for cross contamination if the surface is not properly cleaned and sanitized between birds. Additionally, immersion chilling has been named as a potential site for cross-contamination since multiple carcasses share the same water bath (Morris & Wells 1970; Sarlin, 1998). Commercial processing operations use chlorinated water with agitators to move carcasses through one or more chill tanks (Mead et al., 2010). Most of the participating small-scale processors used a single, static chill tank which may have resulted in cross-contamination of carcasses over a period of time due to an accumulation of bacteria. Fanatico (2003) suggests that small-scale producers use one chill tank to lower the body temperature for broilers for 15

minutes followed by a second chill tank for the remainder of the immersion chilling process. Only one of the participating pasture-raised broiler producers in this study used 2 successive chill tanks.

Enumeration of pathogens in poultry production systems can provide data which may be used to assess risk and progress with regard to food safety interventions. To the best of the authors' knowledge, current data on the quantification of *Salmonella* and *Campylobacter* for broilers raised and processed in small-scale poultry production systems are not available for comparison.

The 2008 USDA-FSIS baseline survey of *Salmonella* and *Campylobacter* in commercially processed, post-chill broilers reported a mean log concentration of 1.75 MPN/carcass of *Salmonella* (USDA-FSIS, 2008c), which seems to correspond with the mean log concentrations of *Salmonella* in birds processed on-farm (1.78 MPN/carcass) and at the USDA-inspected facility (1.47 MPN/carcass) in our study. The mean log concentration of *Campylobacter* in the USDA-FSIS baseline study (3.56 CFU/carcass) is lower than the mean log CFU/carcass for birds processed in the MPU (5.44 CFU/carcass), yet higher than the *Campylobacter* concentrations for the USDA-inspected facility (0.99 CFU/carcass) and on-farm processors (2.43 CFU/carcass).

In the current study, the mean log concentrations of *Salmonella* were not significantly different in birds processed on-farm and at the USDA-inspected facility; but the concentrations of *Campylobacter* were significantly higher for birds processed on-farm in comparison to those processed in the USDA-Inspected facility. By contrast, the concentrations on MPU-processed birds were significantly lower for *Campylobacter* and higher for *Salmonella*. Freedom Rangers processed on-farm had a significantly higher mean log concentration of *Campylobacter* than the Cornish Cross birds processed on-farm. *Salmonella* concentrations did not differ significantly by breed. Since it has been suggested that Freedom Rangers may adapt to the pasture environment more successfully than the fast-growing Cornish Cross birds (Fanatico, 2010), the increased foraging by this breed may have resulted in higher transmission of *Campylobacter* from the environment. It may be possible that Freedom Rangers are less susceptible to colonization by *Salmonella* present in the farm environment. The pathogen concentration findings in this study suggest that processing broilers in the MPU may pose a relatively higher risk with regard to

contamination of carcasses with *Campylobacter*. However, the findings should be interpreted with caution since different breeds within each processing method were raised by producers and the participating broiler farms were located in disparate areas within the southeastern region of the United States. Additionally, inter-laboratory variability of the methods for pathogen detection and quantification may have contributed to the differences between breeds and processing methods in this study.

The main goals of the study were to establish baseline data on the food safety of pasture-raised broilers processed on-farm, in an MPU pilot plant and at a small USDA-Inspected facility as well as assess the impact of processing waste disposal on the farm environment. As a result, we did not evaluate potential management risk factors which may have contributed to the differences in the prevalence and concentrations of the pathogens in birds processed on-farm and at the small USDA-inspected facility compared to the MPU. Furthermore, information on the breeding flocks and practices of the hatcheries associated with the participating pasture-raised broiler farms was not available.

The absence of soil management practices on the farms coupled with the direct disposal of untreated wastewater to the soil surrounding the processing area may contribute to the high prevalence of *Salmonella* and *Campylobacter* on the broiler carcasses processed on-farm. In a longitudinal study on the *Campylobacter* contamination of 18 commercial broiler flocks, Herman et al. (2003) reported a significant correlation between contamination of broilers during rearing and the carcasses after processing. In general, farm biosecurity was not emphasized on the participating small-scale broiler farms in the current study. In many cases, the farmers did not vary or rotate the location of wastewater disposal on the farm during subsequent processing days. The significance of the high concentrations of both pathogens in the processing wastewater (*Salmonella* in On-farm wastewater and *Campylobacter* in MPU wastewater) shown in Figures 2 and 4 relates to the widely accepted idea among small-scale producers that the direct application of processing wastewater is innocuous and will greatly increase soil fertility (personal communication with farmers). The carcasses processed in the MPU had a 100% prevalence of *Campylobacter*, which is reflected in the high prevalence and the mean log concentration of this pathogen in the MPU wastewater shown in Table 2.

In this study, only the on-farm wastewater was applied directly to soil in the processing area of the farm. Although MPU wastewater was not applied to soil, the possibility for direct disposal in soil exists for MPU units used commercially. Research by Ross & Donnison (2006) reported that 99% of *Campylobacter jejuni* in inoculated dairy farm effluent was retained in the top 5 cm of 4 different soil types for 25 days. Holley et al. (2006) reported that surface application of manure to soil resulted in increased survival time of *Salmonella* compared to incorporation of waste into the soil. By contrast, Hutchison et al. (2004) suggested surface spreading of livestock waste as a potential intervention to limit the survival time of *Salmonella* and *Campylobacter* in soil. Since samples were collected at least 1 week after application of processing wastewater to the soil, the data suggests that between slaughter dates, both pathogens are moderately prevalent and fairly concentrated in the soil surrounding the processing area. Dazzo et al. (1973) reported that after 3 years of continuous exposure to manure slurry irrigation under natural field conditions, the resulting modifications to the soil environment favored the extended survival of *Salmonella* Enteritidis.

Another potential source of contamination on small-scale broiler farms is the mortality compost pile. A study by Shepherd et al. (2010) concluded that the conditions on the surface (<5cm) of poultry mortality compost heaps are suitable for pathogen survival during the first phase of composting. Additionally, the authors reported that the temperature and moisture contents of the compost heaps at participating small and medium-sized poultry farms were often below the recommended level for pathogen inactivation. A survey of farmers conducted by Rangarajan et al. (2002) revealed that only 39% (n=31) of compost producers consistently monitored the temperature of their compost heaps for a target peak temperature of 140°F or higher.

The data in Figure 4 suggests that the mortality compost pile may pose a risk for the dissemination of *Campylobacter* in the small-scale poultry farm environment. Since insects, rodents and other small animals are considered a potential vehicle for transmission of enteric pathogens on the farm (Meerburg, 2006), proper construction and management of on-farm mortality composting is necessary to minimize the number of pests associated with the composting process (Carter et al., 1996). All of the

farms in this study lacked structured composting bins; piles were located on the ground near wooded areas or heaps of debris. This arrangement may have provided easy access to the compost pile by animals and other pests. Furthermore, the effects of precipitation and the high moisture content of carcasses may result in runoff from the exposed pile that could contaminate the surrounding soil (Kalbasi et al., 2005). This potential for leaching of mortality compost ingredients supports the recommendation by (Carter et al., 1996) to separate the compost pile from the poultry production and processing area of the farm.

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## CHAPTER 3

### CONCLUSIONS

The high prevalence of pathogens in the soil surrounding the on-farm processing area and mortality compost may be the result of the self-perpetuating dissemination of *Salmonella* and *Campylobacter* on small pasture-raised broiler farms. This may impact the food safety of broilers produced on these farms. Based on the findings of this baseline study, the majority of birds processed on-farm, in the MPU and at the small USDA-inspected facility were positive for *Campylobacter*, but levels of contamination were higher and lower for birds processed in the MPU and at the USDA-Inspected facility, respectively. Birds processed on-farm and at the USDA-Inspected facility were mostly positive for *Salmonella* with levels that correspond with the 2008 USDA-FSIS national *Salmonella* MPN average on broiler chickens. Pasture-raised broilers processed in the MPU had no detectable *Salmonella*. The current work provides insight into small-scale poultry production practices and provides a record of data which may serve as a guide for future improvement of these practices. Further research is needed regarding the small-scale broiler production environment in relation to available processing methods, the breed of bird and potential intervention methods.

Table 1. *Salmonella* prevalence and concentration in small-scale, pasture-raised broiler processing operations. Data within column followed by the same letter were not significantly different (P>0.05). Prevalence data comparison based on chi-squared or Fisher's exact test where appropriate.

	Sample	Prevalence	Mean log MPN	95% CI	
<b>On-Farm</b>	Carcasses	89% <sup>a</sup> n=120	1.779 <sup>a</sup>	1.598 - 1.960	
		Cornish cross	86% n=50	0.919	0.682 - 1.155
		K-22	85% n=20	1.983	1.660 - 2.307
		Freedom Ranger	94% n=50	1.716	1.551 - 1.886
	Environmental	Soil	60% n=42	0.967	0.662 - 1.271
		Compost	72% n=39	0.953	0.665 - 1.245
		Wastewater	48% n=46	1.289	0.870 - 1.707
<b>USDA-IF</b>	Carcasses	78% <sup>a</sup> n=50	1.475 <sup>a</sup>	1.190 - 1.760	
	Red Ranger	78% n=50	1.475	1.192 - 1.759	
<b>MPU</b>	Carcasses	0 <sup>b</sup> n=50	0 <sup>b</sup>	0	
		Cobb 700	0 n=20	0	0
		Ross 708	0 n=10	0	0
		Freedom Ranger	0 n=10	0	0
		Naked Neck	0 n=10	0	0
	Environmental	Wastewater	0 n=9	0	0

Table 2. *Campylobacter* prevalence and concentration in small-scale, pasture-raised broiler processing operations. Data within column followed by the same letter were not significantly different (P>0.05). Prevalence data comparison based on chi-squared or Fisher's exact test where appropriate.

	Sample	Prevalence	Mean log CFU	95% CI	
<b>On-Farm</b>	Carcasses	70% n=120	2.432 <sup>a</sup>	2.061 - 2.802	
		Cornish cross	40% n=50	1.213 <sup>aa</sup>	0.797 - 1.629
		K-22	90% n=20	3.581	2.756 - 4.403
		Freedom Ranger	92% n=50	4.079 <sup>bb</sup>	3.653 - 4.504
	Environmental	Soil	69% n=39	3.332	2.446 - 4.197
		Compost	61% n=33	3.896	2.775 - 5.018
		Wastewater	46% n=46	2.192	1.362 - 3.023
<b>USDA-IF</b>	Carcasses	74% n=50	0.993 <sup>a</sup>	0.560 - 1.426	
	Red Ranger	74% n=50	0.993	0.560 - 1.426	
<b>MPU</b>	Carcasses	100% n=50	5.438 <sup>b</sup>	5.243 - 5.633	
		Cobb 700	100% n=20	5.119	4.349 - 5.889
		Ross 708	100% n=10	5.296	4.720 - 5.872
		Freedom Ranger	100% n=10	5.563	5.108 - 6.018
		Naked Neck	100% n=10	5.314	4.906 - 5.722
	Environmental	Wastewater	100% n=9	4.344	2.169 - 6.520

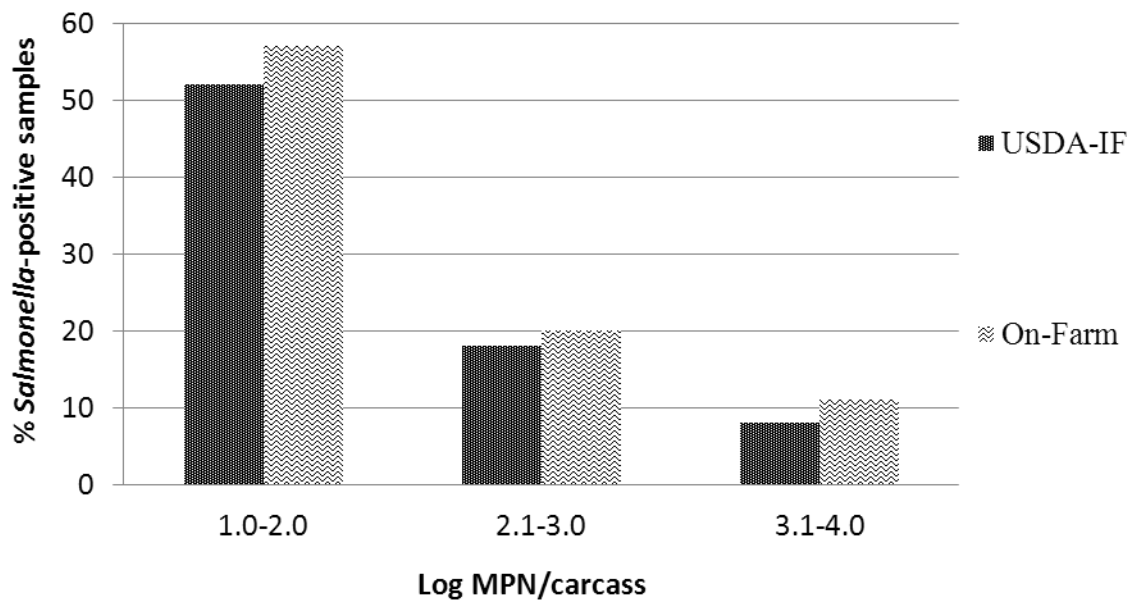


Figure 1. Percentage Bar chart illustrating the Most Probable Number (MPN) of salmonellae on post-chill, pasture-raised broiler carcasses processed on-farm or in a USDA-Inspected Facility (USDA-IF). *Salmonella* was not detected on birds processed at the Mobile Processing Unit (MPU) Pilot plant.

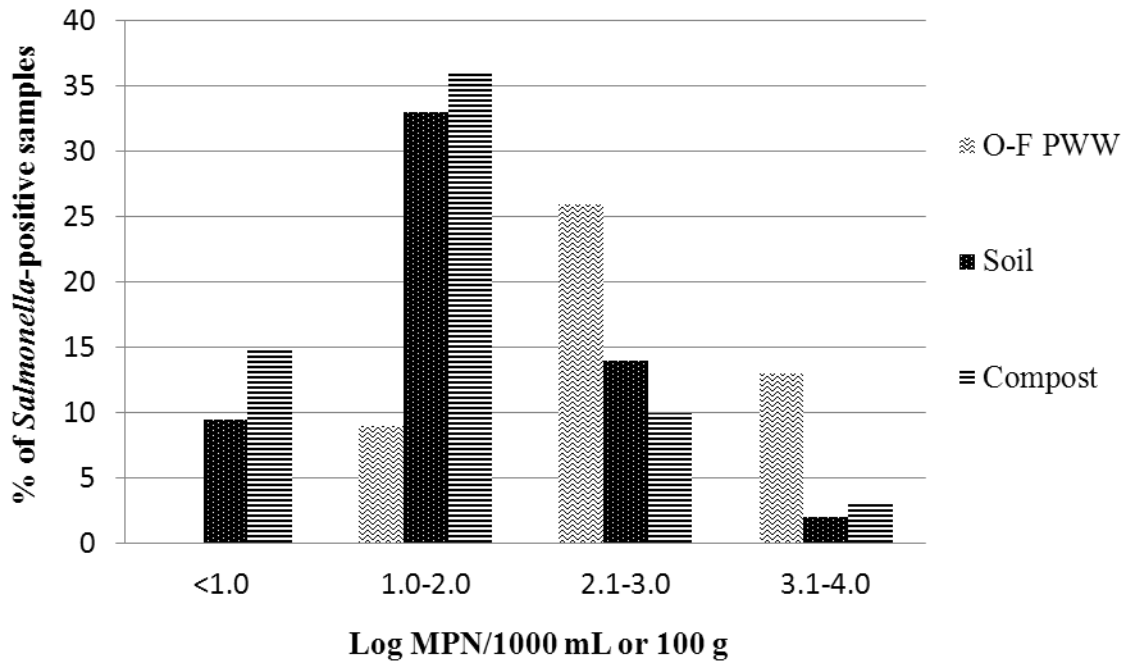


Figure 2. Percentage bar chart illustrating the Most Probable Number (MPN) of salmonellae per 1000 mL of on-farm processing wastewater (OF-PWW) and 100 g of soil and compost in the farm environment. *Salmonella* was not detected in the PWW from the Mobile Processing Unit (MPU) pilot plant.

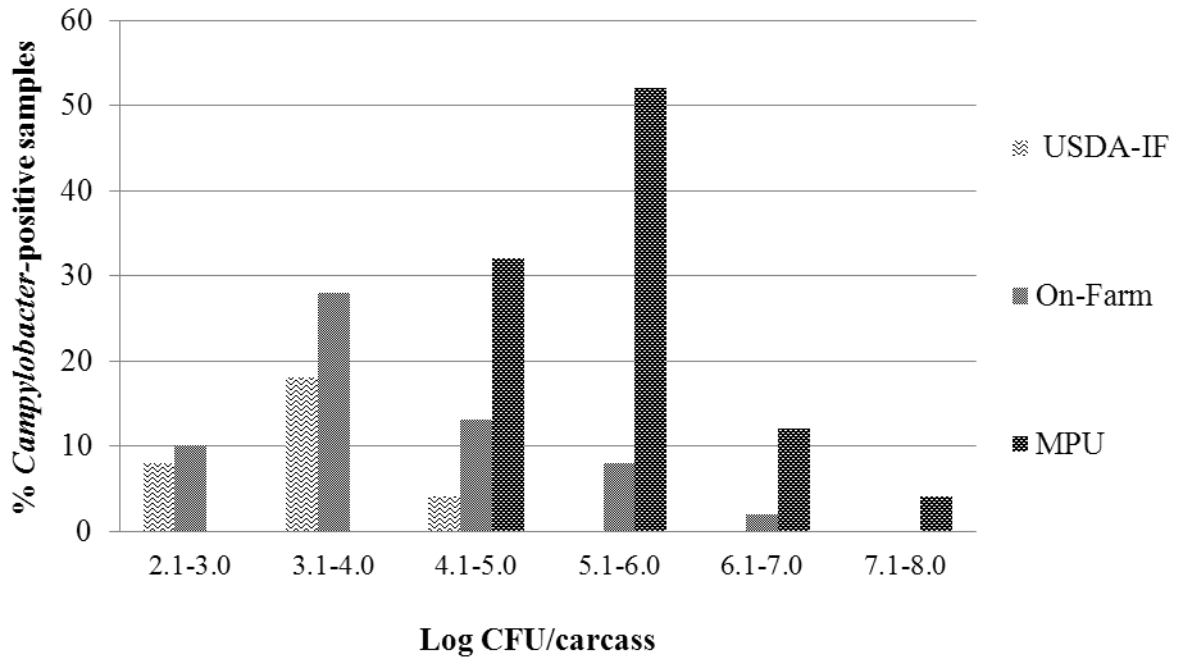


Figure 3. Percentage bar chart illustrating *Campylobacter* counts (CFU) on post-chill, pasture-raised broiler carcasses processed on-farm, in an MPU or in a small USDA-inspected facility (USDA-IF).

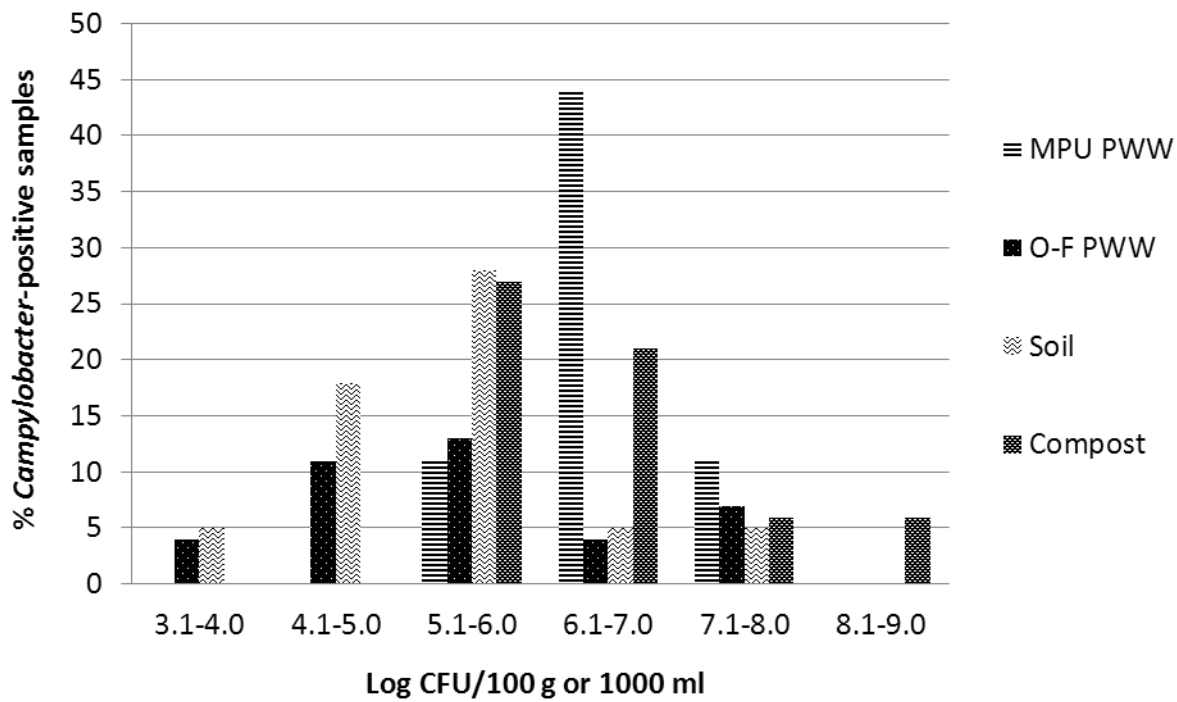


Figure 4. Percentage bar chart illustrating *Campylobacter* counts (CFU) per 1000 mL of processing wastewater (PPW), CFU per 100 g of soil or compost samples collected from the farm environment or the mobile processing unit (MPU) pilot plant.