Abstract

BASHOR, MICHAEL PAUL. Effects of Carcass Washing Systems on *Campylobacter* Contamination in Large Broiler Processing Plants. (Under the direction of Kevin M. Keener and Patricia A. Curtis.)

Campylobacter, a major food borne pathogen found in poultry products, remains a serious problem facing poultry processors. *Campylobacter* research has primarily focused on detection methods, prevalence and detection on carcasses, with limited research conducted on intervention. The aim of this study was to assess the effectiveness of carcass washing systems in four large broiler-processing plants in removing *Campylobacter* species.

Washing systems evaluated included combinations of inside/outside carcass washers and homemade cabinet washers. Processing aids evaluated were trisodium phosphate (TSP) and acidified sodium chlorite (ASC). The washer systems consisted of 1 to 3 carcass washers and used from 0.57 to 2.57 gallons of water per carcass. The washer systems used chlorinated water with 25 to 35 ppm of total chlorine. These washer systems on average reduced *Campylobacter* populations log 0.5 cfu/ml from log 4.8 cfu/ml to log 4.3 cfu/ml. Washer systems with TSP or ASC reduced *Campylobacter* populations on average by an additional log 1.03 to log 1.26 respectively. Total average reductions in *Campylobacter* populations across the washer system and chill tank were log 0.76 cfu/ml. Washer systems that included antimicrobial systems had total average reductions in *Campylobacter* populations of log 1.53 cfu/ml.

These results suggest that carcass washer systems consisting of multiple washers provide minimal reductions in *Campylobacter* populations found on poultry in processing plants. A more effective treatment for reducing *Campylobacter* populations is ASC or

TSP treatments; however, these reductions, while significant, will not eliminate the organism from raw poultry.

Effects of Carcass Washing Systems on *Campylobacter* Contamination in Large Broiler Processing Plants

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By
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Biography

Michael Paul Bashor was born on October 4, 1978 in Cary North Carolina. He is the son of Paul and Marti Bashor. Michael grew up in Cary with his older sister Kiersten. He attended Cardinal Gibbons High School in Raleigh, NC where he was on the soccer and wrestling team for all four years. After graduation in 1996, Michael attended North Carolina State University to pursue a science degree. It was not until his junior year that he realized that Food Science was the degree for him. During the final summer of his graduate studies, Michael completed an internship with Anheuser-Busch in Cartersville Georgia, as a brewing intern. He will be moving to Minneapolis, MN upon graduation, where he will be working for General Mills in R&D, joining his fiancée Amy.

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Manuscript 1:
Comprehensive review of *Campylobacter* and Poultry Processing

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Abstract

Campylobacter has been recognized as the leading bacterial cause of human gastroenteritis in the United States, affecting about 2.5 million people annually. Epidemiological data suggest that contaminated products of animal origin, especially poultry, contribute significantly to campylobacteriosis. Thus, reduction of contamination of raw poultry would have important impact in reducing incidence of illness. In poultry slaughter plants, routine procedures such as such as carcass washings and the application of processing aids have been shown to reduce populations of *Campylobacter* in the carcasses by 0.5 to 1.5 logs. Populations of *Campylobacter* have been shown to enter a poultry processing plant at levels between log 5 cfu/ml and log 8 cfu/ml. The purpose of this article is to review *Campylobacter*, the infection that it causes, its association with poultry, contamination sources during processing, and intervention methods.

Bacteriology and Ecology

Campylobacter was first described in 1880 by Theodore Escherich (Friedman et al., 2000). The name *Campylobacter* is derrived from the Greek word “kampylos”, which means curved. *Campylobacters* are gram- negative, slender, spiral curved rods having dimensions of 0.2 to 0.8 um wide and 0.5 to 5um long. Extremely rapid, darting, reciprocating motility can be seen with a phase contrast microscope, with a comma-shaped, S, or gull wing-shaped cells.

Campylobacter is a fastidious organism that is capable of surviving in a wide range of environments. It has been isolated from rivers, estuarine and coastal waters, at

populations ranging from 10-230 cfu/100ml (Bolton et al., 1982, 1987). *Campylobacter* is a commensal organism routinely found in cattle, sheep, swine, and avian species. The avian species are the most common host for *Campylobacter* probably because of their higher body temperature (Skirrow, 1977). A study by Hazeleger et al. (1995) investigated the effect of environmental temperatures over different seasons and nutrients on the survival of *C. jejuni*. They found peak isolation during the late fall and winter months. Also, the investigators found that aging *C. jejuni* cells survived the longest at 4°C. Willis and Murray (1997) found *Campylobacter* to be at their highest populations on poultry during the warmer months of the year (May through October). During these months 87-97% of the samples tested were positive for *C. jejuni*. They also reported substantial variability in the intestinal colonization of *C. jejuni* across different broiler flocks at different ages in the production cycle.

Campylobacter jejuni and *C. coli* account for the majority of human infections (Friedman et al., 2000) and are commonly referred to as "thermophilic" campylobacters, being able to grow at 37°C to 42°C with an optimum growing temperature of 42°C, but it is incapable of growth below 30°C. However, a recent study (De Cesare et al., 2002) found that *C. jejuni* will survived in excess of 4 hours at 27°C and 60-62% relative humidity on some common clean or soiled food contact surfaces.

Campylobacter is easily inactivated by heat having a D-value less than 1 minute at 60°C. Freeze thawing also reduces the population of *Campylobacter* (Stern and Kazmi, 1989). *Campylobacter* is inactivated by frozen storage at –15°C in as few as 3 days (Stern and Kotula, 1982); however, freezing does not eliminate the pathogen from contaminated foods (Lee et al. 1998). Hazeleger et al. (1995) discovered that aging *C.*

jejuni cells survived the longest at 4°C. *Campylobacter* will not survive below a pH of 4.9. It is capable of growing in the pH range of 4.9 to 9.0, and grows optimally at pH 6.5 to 7.5.

C. jejuni is unusually sensitive to oxygen and dehydration. Enzymes present in *C. jejuni* such as superoxide dismutases (SODs), catalases, peroxidase, glutathione synthetase, and glutathione reductase are thought to play a vital role in providing protection against oxygen toxicity (Pesci et al., 1994; Purdy and Park, 1994). *Campylobacter* requires a special gas atmosphere for growth in or on laboratory media, which usually consists of 5% oxygen, 10% carbon dioxide, and 85% nitrogen (Stern and Kazmi, 1989).

Doyle and Roman (1982b) examined the sensitivity of *C. jejuni* to drying. They demonstrated that several factors influenced the rate of inactivation of *Campylobacter* when dried on a glass surface, including bacterial strain, temperature, humidity, and the suspension medium used to resuspend the organism. In all instances, greater survival occurred when organisms were dried in Brucella broth rather than in skim milk. The results of Doyle and Roman (1982a) suggest that *C. jejuni* is quite sensitive to drying and storage at room temperature, but at refrigeration temperatures and appropriate humidity, large numbers may survive drying and remain viable for several weeks. Other studies have also found *C. jejuni* to be quite sensitive to drying at room temperature (Luechtefeld et al., 1981).

Campylobacter is oxidase and catalase positive and contains a single polar unsheathed flagellum at one or both ends. *C. jejuni* hydrolyzes hippurate, indoxyl, and acetate, reduces nitrate, but is unable to oxidize or ferment carbohydrates. Most strains

are sensitive to nalidixic acid and resistant to cephalothin. There has been a marked increase in the incidence of fluoroquinolone resistance in *C. jejuni* that may be associated with the increase in the use of fluoroquinolones by the poultry industry (Koenraad et al., 1995).

As *Campylobacter* cells begin to age, they become coccoid in shape (Moran and Upton, 1987). Several investigations have shown an association between the transition from the spiral to coccoid morphology with a nonculturable state (Moran and Upton, 1986; Rollins and Colwell, 1986; Jones et al., 1991; Stern et al., 1994). Recent studies, however, suggest no correlation between culturability and cell morphology (Medema et al., 1992; Hazeleger et al., 1995; Lazaro et al., 1999). There are mixed reports on the existence and characteristics of the viable but not culturable (VBNC) state of *C. jejuni* (Rollins and Colwell, 1986; Jones et al., 1991; Tholozan et al., 1999). The mechanism of survival in the putative VBNC state remains unclear.

Infection

Campylobacter has long been recognized as a cause of diarrhea in cattle and of septic abortion in both cattle and sheep. It is only in the last 25 years that it has been recognized as an important cause of human illness (Friedman et al., 2000). The Centers for Disease Control and Prevention reported that *Campylobacter* is the most common cause of bacterial diarrheal illness in the United States and causes more illness than *Salmonella* and *Shigella* combined (Meer and Misner, 1998), affecting about 2.5 million people annually. Disease control studies have demonstrated that 50 to 70% of human *Campylobacter* illness is attributed to consuming poultry and poultry products, thus the

value of reducing levels associated with raw poultry has drawn considerable attention (Allos, 2001; Tauxe, 1992). Children less than 1 year and young adults aged 15-25, are more susceptible to developing this disease and individuals with immunosuppression can develop prolonged or unusually severe cases of illness (Friedman et al., 2000). Deaths attributed to *Campylobacter* infection in the United States are estimated at 680-730 per year (Saleha et al., 1998).

Does as low as 500 organisms have been reported to cause illness (Black et al., 1988; Friedman et al., 2000). There are several species of *Campylobacter* (*C. jejuni*, *C. coli*, *C. lari*, and *C. uppsaliensis*) capable of causing human illness. However, *C. jejuni* is implicated in about 85% of the cases of human campylobacteriosis, with the remaining cases being primarily caused by *C. coli* (Fricker and Park, 1989; Friedman et al., 2000).

The most common clinical symptoms of campylobacteriosis are fever, abdominal pain, and diarrhea that occurs within 2 to 5 days of ingestion of food or water contaminated with *C. jejuni* (Robinson, 1981; Black et al., 1988). Symptoms are usually self-limiting and are resolved within a period of 3-10 days, and most cases do not require the use of antibiotics. When antibiotics are necessary, erythromycin and fluoroquinolones are usually prescribed.

In about 1 out of 1000 cases, the infection is followed 2-3 weeks later with Guillain-Barre Syndrome (GBS), a debilitating inflammatory polyneuritis characterized by fever, pain and weakness that progresses to paralysis. Other possible autoimmune diseases from *Campylobacter* infections include Miller Fisher syndrome (MFS) and Reiter's syndrome or reactive arthritis (Kuroki et al., 1993; Nachamkin et al., 1998).

Several studies have suggested that the consumption of undercooked poultry, and/or handling of raw poultry are risk factors for human *Campylobacter* infection and illness (Skirrow, 1982; Hopkins et al., 1984; Oosterom et al., 1984; Tauxe et al., 1985; Harris et al., 1986; Kapperud et al., 1992; Blaser 1997; Wallace et al., 1998; Altekruse et al., 1999). A large number of serotypes of *C. jejuni* isolated from chicken carcasses are frequently linked to human cases of campylobacterosis, thus confirming that poultry is an important contributor in the epidemiology of human campylobacterosis (Stern and Kazmi, 1989).

Most *Campylobacter* infections are sporadic, i.e. they involve individual cases. Outbreaks of *Campylobacter* infections have been traced to raw milk and contaminated water, raw milk and contact with pets and farm animals (Kapperud et al., 1992; Altekruse et al., 1999). *C. jejuni* may be present in milk from fecal contamination during milking or an udder infection (Doyle and Roman, 1982; Hutchinson et al., 1985; Orr et al., 1995). Raw milk has been identified as a vehicle in *Campylobacter* human gastroenteritis (Blaser et al., 1979; Robinson et al., 1979; Porter and Reid, 1980; Potter et al., 1983; Korlath et al., 1985). Vogt et al. (1982) and Sacks et al. (1986) reported contaminated water as a source of *Campylobacter* outbreaks. Of eight reported waterborne outbreaks, four involved surface water, two unchlorinated deep-well water, and two chlorinated deep-well systems (Kornblatt et al., 1981; McNeil et al., 1981; Mentzing 1981; Palmer et al., 1983; Taylor et al., 1983; Vogt et al., 1982). Investigation of these waterborne outbreaks either failed to recover *Campylobacter* or recovered strains that were not the same serovar as those isolated from clinical cases in these outbreaks (Sacks et al., 1986). This failure to recover the same serovars from

environmental and clinical samples could be due to the lack of advanced technology for identifying strains at that time.

Fluoroquinolones are used for treatment of infections in poultry. Recently, questions have been raised regarding the involvement of use of fluoroquinolones in antibiotic resistance of *Campylobacter*. Fluoroquinolone-resistant *Campylobacter* was first detected in 1985 (Jacobs-Reitsma et al., 1994b). In the Netherlands, enrofloxacin was first introduced to the poultry industry in 1987. By 1993, 29% of the animal isolates of *Campylobacter* were quinolone-resistant. Resistance among human isolates also increased significantly during the same period (Piddock, 1997).

HACCP

On July 25, 1996, the United States Department of Agriculture (USDA) and the Food and Safety Inspection Service's (FSIS) Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems final rule was published in the *Federal Register* (USDA, 1996b). FSIS stated the goal of its food safety strategy and proposed pathogen reduction/HACCP regulations as follows: "FSIS believes its food safety goal should be to reduce the risk of foodborne illness associated with the consumption of meat and poultry products to the maximum extent possible by ensuring that appropriate and feasible measures are taken at each step in the food production process where hazards can enter and where procedures and technologies exist or can be developed to prevent the hazard or reduce the likelihood it will occur" (FSIS baseline data, 1995).

FSIS established generic *E. coli* performance standards based on national microbiological baseline surveys. The standards provide a benchmark for use by slaughter establishments in evaluating their *E. coli* test results. Test results that do not

meet the performance criterion are considered an indication that the slaughter establishment may not be maintaining adequate process control for fecal contamination and associated bacteria. This performance criterion is directly related to the effectiveness of the sanitary standard operating procedures (SSOP's) and good manufacturing practices (GMP's). Such results are used in conjunction with other information to evaluate and make appropriate adjustments to ensure adequate process control for fecal contamination (FSIS baseline data, 1995).

FSIS also established pathogen reduction performance standards for *Salmonella* that require all slaughter establishments to meet or reduce the incidence of *Salmonella* contamination of finished meat and poultry carcasses below the national baseline prevalence as established by the most recent FSIS national microbiological baseline data for each major species (FSIS baseline data, 1995). FSIS conducts *Salmonella* testing in slaughter establishments to detect whether companies are meeting the pathogen reduction performance standards, and whether they require corrective action. According to the 1996 baseline study, 88% of carcasses sampled were positive for *Campylobacter*, and 20% were positive for *Salmonella* (Conner et al., 2001).

Pathogen specific performance standards for raw products are an essential component of the FSIS food safety strategy because they provide a direct measure of progress in controlling and reducing pathogens. There is indication that FSIS may implement a *Campylobacter* performance standard in the near future (FSIS baseline data, 1995). However, there are much less data available on *Campylobacter* levels on raw poultry carcasses during processing than *Salmonella*.

A critical challenge poultry processing plants are facing in complying with these regulations is the ability of the plant to define and take corrective actions to assure compliance of the zero visible fecal contamination of carcasses (USDA, 1994) and pathogen performance standards (USDA, 1996a). Processors have had a difficult time in developing preventative measures to comply with zero visible fecal contamination regulations (USDA, 1994). Under HACCP, when a deviation occurs the plant must implement a corrective action and preventive measure. The plant's typical response for a corrective action has been to slow down the processing line, add additional line personnel for visual inspection, and as a preventative measure, add additional carcass washers or wash systems. This has resulted in poultry processing plants increasing their daily water usage by 30 to 50% since HACCP plans went into effect (Jackson et al., 1999). A typical poultry processing plant (150,000 to 200,000 birds/day) uses 1 to 2 million gallons of water each day (Sellars and Kiepper, 2001). While the necessity of the added personnel may be justified, the justification for increasing water use and additional washing systems has not been scientifically proven. A study is needed to document the impact of these washer systems on *Campylobacter* populations in the slaughter process.

Poultry and poultry products have often been implicated in the transmission of *Campylobacter*. Case control studies of foodborne infection rates have estimated that 50 to 70% of *Campylobacter* illness is due to poultry and poultry products, thus reducing levels of *Campylobacter* contamination associated with raw poultry may be warranted (Allos, 2001; Tauxe, 1992). There are many factors that contribute to *Campylobacter* contamination of broiler carcasses, and some of these factors may be impossible to control in order to meet performance standards. Many would believe that because of the

strict growth environment requirements for *Campylobacter* that it would be easy to remove the organism from a processing plant; however, the incidence of contamination and their populations are at such high levels (log 5 to log 8 cfu/ml of carcass rinse), the incidence of contamination is high, because the organism is constantly re-introduced with the high number of birds being processed daily (Bashor et al., 2002).

Potential for cross contamination of *Campylobacter* is very high inside the poultry processing plant. Since *Campylobacter* enters the plant at such high levels, it can easily be spread. Poultry enter the processing plant have *Campylobacter* populations ranging from log 5 to log 8 cfu/g, and the bacteria are found in the crop as well as fecal material (Byrd et al, 1998a).

The problem of *Campylobacter* contamination of food must be addressed. To date there are no practical or effective control measures available (Newell and Wagenaar, 2000). At the consumer level, accidental ingestion of one drop of raw chicken juice can easily constitute an infectious dose, which is as little as 500 organisms (Newell and Wagenaar, 2000; Friedman et al., 2000). Infections can occur during the improper handling of raw chicken carcasses, by eating insufficiently cooked chicken, and via cross contamination of other foods by contact with knives or cutting boards used to prepare raw chicken. While some reduction in cross contamination of poultry carcasses can be achieved by improved sanitation during processing, the elimination of *Campylobacter* populations from birds prior to processing is desirable, but may not be practical or feasible.

Various on-farm strategies have been advanced to reduce the incidence rates of poultry contamination: introduction of competing microbial populations into newly

hatched chicks, chlorination of poultry drinking water, vaccination or selective breeding of poultry for resistance to *Campylobacter* colonization. Sound management practices incorporating good husbandry and hygiene practices also play a part in limiting the occurrence of *C. jejuni* in poultry flocks (Saleha et al., 1998). Farms that utilize these practices tend to have lower rates of intestinal colonization with *Campylobacter* spp. (Sjogren and Kaijser, 1989; Humphrey et al., 1993; Kazwala et al., 1993).

Sources of contamination on the farm

Campylobacter contamination of live birds can be traced back to the farm (Byrd et al., 1998b). The available research on bacterial populations in poultry production is mainly from studies on chickens. In a typical broiler processing operation, freshly laid fertile eggs are collected and incubated at a hatchery. After they hatch, the chicks are delivered to farms where they are reared until they are ready for slaughter, and then transported to a processing plant.

Flock colonization

Campylobacter is considered to be a commensal organism in many avian species, including those grown commercially. In most flocks, colonization is not detectable until at least 10 days and continues for many weeks (Newell and Wagenaar, 2000). Spread of C*ampylobacters* among hatch mates is rapid if infected birds are introduced into the population. Under laboratory conditions, three days of contact with artificially inoculated seeder birds is sufficient for the majority of the brood to be colonized (Shanker et al., 1990). Young (1 to 2 week old) chicks are highly susceptible to *Campylobacter* colonization (Stern, 1992). Chickens are coprophagic, which facilitates the fecal-oral spread of C*ampylobacters;* however, the rapidity of the shift from uncolonized to 100%

colonized suggests that *Campylobacters* are also spread from chick to chick via their communal source of drinking water (Montrose et al., 1985).

When a flock of broiler chickens becomes positive for *Campylobacter*, the prevalence of infection among birds is high, often reaching 100% of birds tested (Gregory et al., 1997; Pokamunski et al., 1986). As a commensal organism in poultry, *Campylobacter* colonizes the intestinal mucus layer in the crypts of the intestinal epithelium (Beery et al., 1988). There are two modes of transmission of *Campylobacter* in poultry: horizontal and vertical. Both have been shown to occur.

Horizontal transmission is thought to be mainly through contaminated water, litter, insects, wild birds, rodents, fecal contact and transfer of the bacteria by farm personnel via their boots (Aarts et al., 1995; Evans and Sayers, 2000). Feed has not been implicated in the spread of *Campylobacter* because it is too dry to favor survival.

Chickens can harbor very high levels of *Campylobacter* in the gut, up to 9.0 log CFU/g of cecal content, without symptoms, and the microorganism can be transmitted among birds within a flock (Berndtson et al., 1992; Evans 1997; Altekruse et al., 1999). Once one bird in a flock is colonized, the infection spreads very quickly (Beery et al., 1988). Gregory et al. (1997) found almost complete colonization within a flock by the end of the grow-out period (49 days) and 50 to 100% of birds in a flock were colonized in other studies. Stern et al. (1995a) showed that 9 out of 10 broiler farms tested were positive for *Campylobacter*.

A Dutch study revealed that 67% (29/43) breeder flocks were colonized with *Campylobacter* (Jacobs-Reitsma, 1995). The level of colonization is lower in Norway

and Sweden where 17% (10/176) and 14% (522/3727) of the broiler flocks were shown to be colonized with *Campylobacter* (Kapperud et al., 1993).

Wallace et al. (1998) conducted a comprehensive study on the colonization of turkeys by *Campylobacter*. Colonization of the chicks took place within seven days. They found a 100% carriage rate by day 21 in all samples. The results indicated that peaks in *Campylobacter* populations isolated from fecal samples correlated with peaks in number of organisms found in the water and litter samples, as well as an increase in the number of birds with diarrheal symptoms. The maximum number of *Campylobacters* excreted was 7.78 log. *C. jejuni* was the only species isolated, but was comprised of several different biotypes. From turkeys that were slaughtered and stored at 4^{o}C for seven days, these authors recovered 6.15 log and 6.98 log CFU/ml *Campylobacter* from the cecum and feces, respectively. This is comparable to what was found in chickens; however, in an earlier study Rosef et al. (1984) showed that the carriage rate of *Campylobacter* on turkey carcasses was greater than that of either hens or broiler chickens.

Other studies have shown vertical transmission as a means of contamination of a breeder flock (Van de Giessen et al., 1992). *C. jejuni* isolates from a parent flock were found to be the same clonal origin as those from the offspring in a broiler flock. Egg transmission of *Campylobacter* from the breeder flock has not been recognized as a source of entry because of the inability to culture *Campylobacter* from the hatchery samples or from newly hatched chicks (Acuff et al., 1982; Doyle, 1984; Jones et al., 1991; Neill et al., 1984). Clark and Bueschkens (1985) inoculated fertile chicken eggs with *C. jejuni* and found that 11% of the resulting chicks at hatch had the inoculated

Campylobacter in their intestinal tract. Other studies have demonstrated that chickens raised in a laboratory environment without exposure to any farm environment continued to become colonized by *C. jejuni* (Lindblom et al., 1986). Chuma et al. (1994) determined the carrier rate of *C. jejuni* in the cecal content of newly hatched chicks to be as high as 35%. These data suggest that the chicks were colonized prior to delivery to the farm. Pearson et al. (1996) found no difference between the types of *Campylobacter* isolated in the hatcheries and the types of *Campylobacter* isolated in the subsequent broiler chickens, suggesting that the *Campylobacter* contamination may have occurred by way of vertical transmission.

Feed Withdrawal

Byrd et al. (1998b) studied the effect of feed withdrawal on *Campylobacter* in the crops of market age broiler chickens. The purpose of feed withdrawal is to allow the clearance of the gastrointestinal tract and thus reduce the potential fecal contamination of poultry carcasses during slaughter. Feed withdrawal caused a significant increase in *Campylobacter* positive crop samples taken from seven of nine houses sampled. They found 90 of 360 birds tested before feed withdrawal were positive for *Campylobacter,* whereas 254 of 359 birds tested after feed withdrawal were positive for *Campylobacter*. Feed withdrawal did not significantly alter the *Campylobacter* isolation frequency from ceca. During feed withdrawal, the pH of the crop decreases, affecting the microflora present (Hinton et al., 2000). This has been suggested as an important factor in the increased prevalence of *Campylobacter* contaminated crops. Byrd et al. (1998a) reported the incidence of *Campylobacter* contamination in crop contents may exceed that of cecal

contents by as much as 37 fold in some broiler flocks, and may represent a critical preprocessing control point in reducing *Campylobacter* entry into the processing plant.

Transportation

Commercially grown poultry flocks are collected on the farm, placed into crates, transported to the processing plant and processed on the same day. *Campylobacter* populations have been shown to increase during transport and holding prior to slaughter (Stern et al., 1995a). It is known that stress can cause a disturbance of intestinal functions and may lower the resistance of the live animal and increase spreading of intestinal bacteria. Ceca, blind pouches between the ileum and the colon of the broiler intestinal tract, can harbor large numbers of *Campylobacter* (Duke, 1986). Moran and Bilgili (1990) found that pathogens, including *Campylobacter*, consumed orally by the bird before or during crating and transportation may colonize the ceca where they may be retained throughout processing. Line et al. (1997) studied ways to reduce *Salmonella* and *Campylobacter* populations associated with broiler chickens subjected to transport stress using a yeast culture. They found that feeding *Saccharomyces boulardii*, a non pathogenic yeast, could reduce the frequency of *Salmonella* colonization to lower than pre-stress levels. The frequency of *Campylobacter* isolation from the ceca was not affected by treatment.

A survey by Jacobs-Reitsma et al., (1994a) found *Salmonella* in 27% of 181 broiler flocks surveyed and *Campylobacter* in 82% of 187 flocks. Potential sources of *Campylobacter* contamination on poultry carcasses included fecal contamination of feathers and skin during transport to the slaughter facility, leakage of fecal content from the cloaca, intestinal breakage and contact with contaminated equipment, water, or other

carcasses (Jacobs-Reitsma, 2000). Feathers may carry 8 log total bacteria/g (Barnes, 1975) and skin 6 log bacteria/cm^2 (Wilkerson et al., 1961). It was shown that poultry carcasses can become contaminated with *Campylobacter* from their intestinal contents during the slaughter process (Wempe et al., 1983; Genigeorgis et al., 1986). *Campylobacter* populations on the feathers of cooped and transported birds are ten fold greater than those remaining on the farm (Stern et al., 1995b).

Contamination in the processing plant

At the processing plant, birds are unloaded, shackled, killed, scalded, defeathered, eviscerated, washed, cooled, and packaged. (Figure 1.)[1]

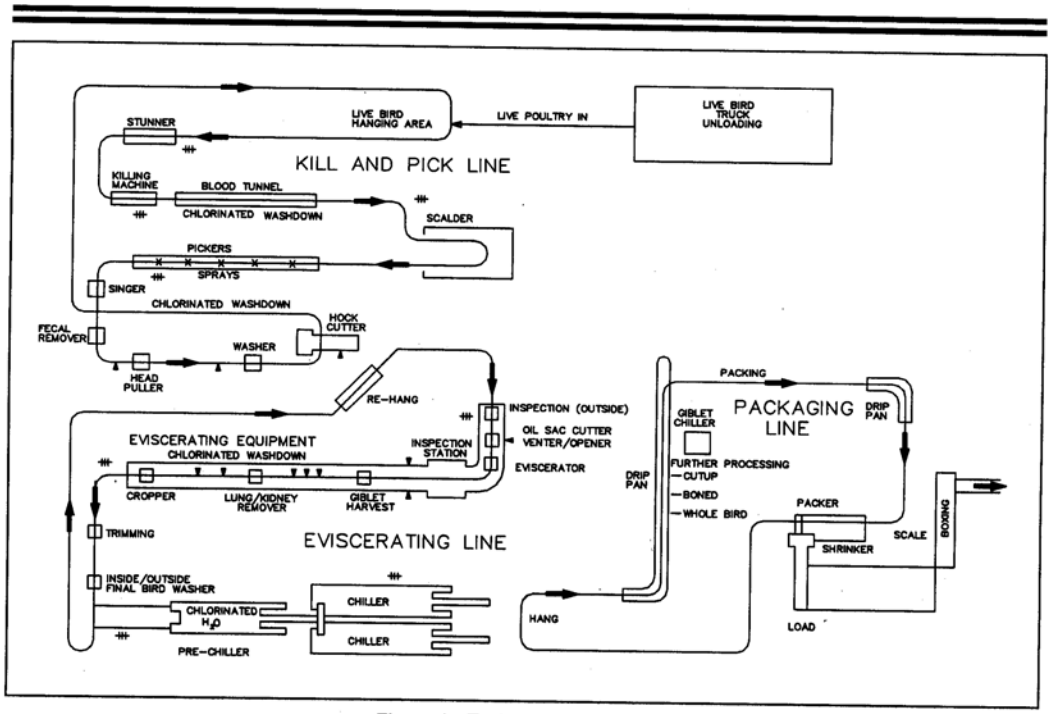

Figure 1 - Typical Poultry Process

[1] Capital Controls. 3000 Advance Lane Colmar, PA. 18915

Scalding

The scalding procedure is used to open the feather follicles in order to facilitate the removal of feathers. The potential for bacterial cross contamination during scalding and picking is well recognized (Mercuri et al., 1974; Bailey et al., 1987). It is hypothesized that the follicles might remain open throughout the processing until the carcass is chilled. When the follicles close during chilling, the microorganisms may be retained. A recent study by Cason et al. (1999) evaluated the microbiological effect of removing feathers from the carcasses while they are out of the scald water and moving between the tanks of a multiple-tank scalder. The data showed no reduction in populations of aerobic bacteria, *E. coli*, or *Campylobacter* on carcasses during scalding and defeathering. Chicken skin has been shown to harbor and support the survival of *C. jejuni* (Lee et al., 1998). Berrang and Dickens (2000) found 3.80 log CFU/g of *Campylobacter* in breast skin before entering the scald tank.

Berrang and Dickens (2000) studied the presence and level of *Campylobacter* on broiler carcasses throughout the processing plant. In this study, samples were collected immediately prior to scald, post-scald, post-pick before transfer to the evisceration line, immediately following the removal of the viscera, after the final washer, and post-chill. *Campylobacter* carcass rinse counts were found to be the highest when carcasses were sampled pre-scald (4.73 log), then the counts dropped significantly after the carcasses were scalded (1.80 log). They also found 3.80 log CFU/g of *Campylobacter* in breast skin before entering the scald tank

Defeathering

Wempe et al. (1983) isolated *C. jejuni* from 94.4% of the feather picker drip water samples, and the population of organisms present was high. They believe that this is an area where cross contamination may occur, since the rubber finger- like projections that beat the feathers from the bird become contaminated and may pass the organism from bird to bird. They observed that the water used in rinsing the birds in the feather picker physically removed the *Campylobacter* organism and thus reduced the number of organisms on the edible parts. They recovered *C. jejuni* from all recycled water samples tested. The use of recycled water to clean the gutters may further contaminate the receiving room with *C. jejuni*. Further distribution of *C. jejuni* may also occur through movement of plant personnel from the receiving area to other areas of the plant.

Berrang and Dickens (2000) found that after defeathering, the counts increased significantly (3.70 log). An increase in *Campylobacter* counts following defeathering has been previously reported (Izat et al.,1988; Acuff et al., 1986). It has been suggested that the rubber fingers in the mechanical picker act to cross-contaminate birds that previously had low or undetectable levels of *Campylobacter* (Acuff et al., 1986; Stern et al., 1995b).

Evisceration

Chicken skin has been shown to harbor and support the survival of *C. jejuni* (Lee et al., 1998). Berrang et al. (2001) studied the presence and level of *Campylobacter*, coliforms, *E. coli*, and total aerobic bacteria recovered from broiler parts with and without skin. Samples were taken from defeathered carcasses before evisceration. No *Campylobacter* were recovered from meat collected from the breasts or thighs, and only 2 of 10 drumstick meat samples had detectable levels of *Campylobacter*. However 9 of 10

breast skin, 10 of 10 thigh skin, and 8 of 10 drumstick skin samples were positive for *Campylobacter*, with levels between 2 and 3 log CFU/g of *Campylobacter*. Kotula and Pandya (1995) found higher counts on breast tissue of broiler meats than on the thigh or drumstick.

The high incidence of contaminated neck flaps and breast tissue suggest that the crop contents may be an important source of *Campylobacter* contamination during processing. The crop has been found to be a significant source of *Campylobacter*, thus potentially contributing to carcass contamination (Byrd et al., 1998a). Berrang et al. (2000) reported that 100% of the crops of eighteen broilers were positive for *Campylobacter*. The study also showed that *Campylobacter* could be found on the skin of carcasses in the early stages of processing even with no contamination from internal organs.

Jeffery et al. (2001) studied the prevalence of *Campylobacter* from skin, crop, and intestine of commercial broiler chicken carcasses at processing. They sampled 6 to 12 carcasses from 22 flocks just prior to evisceration and found skin samples 78% positive, crops 48% positive, and the intestines 94% positive.

Berndtson et al. (1992) isolated *Campylobacter* in 89% of neck skin samples, 93% of peritoneal cavity swab samples and 75% of subcutaneous samples. They also found that muscle samples were only very sparsely contaminated, and believed it was likely that the feather follicles were the orifices where *Campylobacter* is introduced into the subcutaneous layer. Overall, *Campylobacter* counts dropped as the flocks moved through the plant (Berrang and Dickens, 2000). Altmeyer et al. (1985) did not find *Campylobacter* in 50 muscle samples from broilers.

Carcass washing systems

Carcasses are commonly washed with systems of washers using chlorinated water to remove contamination, such as blood, tissue fragments, and fecal contamination as part of the regular processing procedures. Carcass washing has been allowed for poultry since 1978 as an alternative to knife trimming because studies have shown it to be equally effective in removing fecal contamination (Scientific Committee on Veterinary Measures, 1998). The development of new washer systems makes it very difficult for a processing plant to know which type or system would be best for them. Limited studies have been conducted on evaluating the performance and effectiveness of poultry washers and sanitizing treatments within the processing plant (Bautista et al., 1997; Dickens and Cox, 1992; Anand et al., 1989). Sales representatives from carcass washer manufactures have provided design and performance data for their respective wash systems, but no comparison data is available. In addition, many plants have made individual modifications to these systems, and experience indicates that wash systems installed in one plant may not perform equally well in another plant. There are numerous parameters that affect the overall effectiveness/efficiency of the carcass washing system, including number of washers and types. For specific washers, operating parameters include wash water temperature, water pressure, nozzle type, nozzle arrangement, flow rate, line speed, and surfactant/sanitizing agents used.

Carcass washers

There are several types of carcass washers currently used in poultry processing plants. These include brush washers, cabinet washers, and inside/outside bird washers. A brush washer acts similar to that of a car wash. It has many rubber fingers that gently

remove debris with the aid of water from the outside of the carcass. A cabinet washer has a series of nozzles enclosed by a cabinet that spray water on the outside of the carcass. In an inside/outside carcass washer the carcasses enter with the breast facing toward the inside of the machine. As the machine rotates, a probe with a single spray nozzle enters the bird's intestinal cavity and washes the inside thoroughly. Then a series of spray nozzles wash the entire outside of the bird. Many inside/outside bird washers are equipped with pressure nozzles operating between 40 and 180 psi for removing visible fecal contamination on carcasses. These wash systems use 20 to 50 ppm of chlorine as an antimicrobial agent and generally consume 25 to 50 gallons per minute (GPM) of water. The three primary suppliers of poultry carcass washers are Stork Gamco Inc. (Stork Gamco Inc. Airport Parkway, P.O.Box 1258, Gainesville, Georgia 30503), Linco USA (LindHolst & Co A/S, DK 8380 Trige, Denmark), and Baader Johnson (Johnson Food Equipment, Inc., Member of the BAADER Group, 2955 Fairfax Trafficway, Kansas City, KS, 66115).

Stork (Stork Gamco Inc. Airport Parkway, P.O.Box 1258, Gainesville, Georgia 30503) offers a mechanical bird washer MBW-16, which automatically washes the carcasses internally and externally. The carcasses enter with the breast facing toward the inside of the machine. As the machine rotates, a probe with a single spray nozzle enters the bird's intestinal cavity, washes the inside thoroughly and punctures the membrane in the neck cavity for draining. A series of spray nozzles wash the entire outside of the bird. The machine has a posterior port that targets the tail area of the bird. A back flush port lifts the leaf fat for thorough cleaning and flushing in this problematic area. A down

flush port completes the internal cleaning and flushes fecal, ingesta and bacteria down and out of the bird through the neck opening. The machine can wash 3 to 6 lb birds.

Baader Johnson (Johnson Food Equipment, Inc., Member of the BAADER Group, 2955 Fairfax Trafficway, Kansas City, KS, 66115) offers a vertical style inside/outside birdwasher, IO505- 16 or 20, as well as the Sani-Kleen™ Birdwasher IO506- 16 or 20 Head. The vertical style machine keeps the carcasses vertical as they pass through the washer. The IO506 machine uses a tilting cradle and head operation that results in thorough flushing through the abdominal and neck openings as well as leaf fat areas. It uses multiple spray nozzles at critical areas to ensure complete washing of the carcass inside and out. The machine contains a full cabinet enclosure for containment of overspray and easy cleaning. This machine is also adaptable to include any antimicrobial agent. The washer can handle up to 160 BPM weighing 3 to 9lbs. Average water consumption for this machine is 40 PSI-60 GPM, 60 PSI-72 GMP, 80 PSI-82GPM, 100 PSI-90 GPM. Water usage data can vary based on the number of spray nozzles, pump size and location.

Linco USA offers an inside/outside washer (LindHolst & Co A/S, DK 8380 Trige, Denmark). The machine includes special adjustable nylon/stainless nozzles to enter the bird to ensure thorough inside washing, and a positive opening in the neck cavity of the bird to ensure drainage. Water also sprays the outside of the carcass to remove any debris or stains left after eviscerating. The washer can handle high speeds, up to 200 BPM. The machine runs at 80 PSI-60 GPM.

Processing aids

When assessing the right treatment to use, there are many factors that should be considered such as overall efficacy, levels of microbial contamination, potential for introducing other food safety hazards, impact on the environment, effects on sensory properties and quality of the product, feasibility, and consumer perception (Scientific Committee on Veterinary Measures, 1998). Demonstration of efficacy should include not only laboratory tests but also in-plant investigations. Possible treatments for reducing microbial contamination of poultry carcasses during processing or thereafter are listed in.

Table 1: Possible treatments for reducing microbial contamination of poultry carcasses.

Physical	**Chemical**
Cold water, hot water, and high pressure	Organic acids
Steam and Steam vacuum	Inorganic acids/Phosphates
Irradiation	Chlorine and related compounds
Freeze-thaw cycling	Disinfectants/Antimicrobials

The most popular USDA approved treatments of microbial contamination are hot water, steam, lactic acid, acetic acid, citric acid, trisodium phosphate, chlorine dioxide, acidified sodium chlorite, and chlorinated water. Washing with potable water is used extensively in poultry processing and may result in an overall reduction of surface contamination by 90 to 99% (Scientific Committee on Veterinary Measures, 1998). Hot water (74 to 95^{o}C) has been shown to reduce *E. coli* by 99 to 99.9% on beef carcasses. These sprays were most effective when they raised the beef carcass surface temperature to 82^{o}C for 10 seconds (Scientific Committee on Veterinary Measures, 1998). Castillo et

al. (1998) observed that hot water (95°C) reduced *S*. Typhimurium and *E*. *coil* 0157:H7 populations inoculated on beef carcasses by 99.98%, and naturally occurring coliforms by 99.95%. In a study using broiler carcass wings, Rodrigues de Ledesma et al. (1996) compared the effect of water at 95°C for 10 seconds to the effect of dipping in 10% trisodium phosphate or 10% sodium carbonate for 10 seconds. Trisodium phosphate alone caused reductions of 84.3%, 65.3% and 60.2% of *S.* Typhimurium, *Staphylococcus aureus*, and *L*. *monocytogenes, respectively*. Hot water treatment alone caused population reductions in these organisms of 64.5%, 91.3%, and 91.3% respectively. A combination of trisodium phosphate and hot water resulted in reductions of 98.6%, 99.5%, and 99.7% respectively. A pasteurization process using steam at 140°C for 50 milliseconds was found to reduce *L*. *innocua* populations on poultry carcasses by 99.99% (Morgan et al., 1995). It is hypothesized that the increased wetting ability of hot water and trisodium phospate physically remove bacteria, in addition to killing them. In order to conserve water and further improve food safety of poultry, further research into the development of soaps and surfactants specifically designed for removing microbial contamination from poultry carcasses should be initiated.

Irradiation

The biological effects of ionizing radiation on cells can be attributed to direct interactions with critical cell components and to indirect actions by molecular entities such as free radicals formed in the water (Scientific Committee on Veterinary Measures, 1998). The DNA of the cell is the most critical target of ionizing radiation and the inactivation of microorganisms is primarily due to damage to the DNA. Gram-positive bacteria seem to show the most resistance. Patterson (1995) investigated the sensitivity

of *C. jejuni*, *C. coli*, and *C. fetus* to irradiation in poultry meat. The D_{10} values ranged form 0.12 to 0.25 kGy. There was a significant difference in the radiation sensitivity between *Campylobacter* species and within strains of the same species. The values indicated that *Campylobacter* was more sensitive to irradiation than *Salmonella* and *L. monocytogenes*. The FDA and USDA have approved irradiation of chicken at a maximum dose of 3kGy to control foodborne pathogens such as *Salmonella* and *Campylobacter* (USDA, 1996C).

Organic Acids

There are several organic acids that have proven to be effective in poultry processing such as acetic acid, lactic acid, citric acid, and succinic acid. They all work very well in killing bacteria, especially *Salmonella*, because of their ability to penetrate and disrupt the cell membrane, and to acidify the cell contents (Scientific Committee on Veterinary Measures, 1998). They are very stable in the presence of organic material often present in poultry processing plants. Because they are acids, they can corrode equipment, and can cause off flavors, odors, and colors. Thomson et al. (1976) compared the effect of acid, heat treatment, and chlorine on inactivating *Salmonella* on broiler carcasses and observed that succinic acid alone (1% at 55^{o}C) reduced *Salmonella* prevalence by 50%. Lillard et al. (1987) reported that 0.2 to 0.5% acetic acid added to the scalding water reduced the total aerobic plate count and Enterobacteriaceae populations. Okrend et al. (1986) added 0.1% acetic acid to scald water and observed a reduction in populations of *S.* Typhimurium and *C. jejuni* from 0.5 to 1.5 log cfu/ml. Bautista et al., (1995) studied the effect of lactic acid, chlorine (50ppm) and trisodium phosphate sprays under various pressures on treating turkey carcasses. They observed

that 1.25% and 4.25% lactic acid caused a 2.4 and 4.4 log reduction in aerobic plate count. The acid spray had an even greater impact on the reduction of coliforms and also reduced *Salmonella*.

Chlorine

Chlorine has been used in poultry processing for more than 40 years to reduce spoilage bacteria, control the spread of pathogens, and prevent build-up of microorganisms on working surfaces and equipment such as chill tanks (Bailey et al., 1986). When sodium hypochlorite is injected into water, it forms hypochlorous acid, the form of chlorine responsible for its antimicrobial properties (Gavin and Weddig, 1995). The addition of chlorine gas to processing water is easily controlled. However, most waters contain organic impurities that will react with the initial amount of added chlorine reducing the amount of available chlorine to form hypochlorous acid. Chlorine added to water will continue to react and be reduced by these impurities until the impurities have been completely oxidized. The amount of chlorine required for this purpose is known as the chlorine demand of the water.

Any chlorine present over the chlorine demand of the water exists as combined residual chlorine or free residual chlorine. The concentration of chlorine where free residual chlorine exists is called the break point. Chlorine combines loosely with nitrogenous (organic) matter to form chloramines and other chloro-nitrogen compounds. These are forms of combined residual chlorine and exhibit relatively weak germicidal properties (Gavin and Weddig, 1995).

The rate at which bacteria are killed is proportional to the concentration of free residual chlorine. The pH of the water after the addition of chlorine determines how fast

the microorganisms will be killed. The lower the pH (below 7.5), the faster the microorganisms are killed, and as the pH increases, the effectiveness of the chlorine decreases (Gavin and Weddig, 1995). Many present day chill tank water treatment programs operate with the cooling water pH in the range of 8.0 to 8.5 or higher. As a result, oxidizing microbiocides such as chlorine are less effective. At a pH of 6.0, chlorine hydrolyzes almost completely to hypochlorous acid (HOCl), which is the most effective form of chlorine for microbiological control; however, at a pH of 8.5, only 8% goes to HOCl, thus requiring a much higher dosage of chlorine to control bacteria. Contamination of processing equipment is progressively reduced by increasing the chlorine concentration to 70 mg/l at pH 6.5 (Bailey et al., 1986). In the chill tank, chlorination with up to 50 mg/l at pH 6.0 was required to control cross-contamination of poultry carcasses due to the increased organic load (Goresline et al., 1951; Ziegler and Stadelman, 1955; McVicker et al., 1958; Mallman et al., 1959; Dawson et al., 1956). .

Chlorine is active against a wide range of microorganisms, with various degrees of susceptibility. At a pH of 6, 0.1 mg/l of free available chlorine killed 99% of *C. jejuni*. (Blaser et al., 1986). The necessary contact time varied between 5 and 15 minutes at 25^{o}C.

Under conditions of commercial processing, not all studies involving chlorine have shown a reduction in carcass contamination. Mead et al. (1975) showed that neither the levels of contamination of bacteria nor the occurrence of cross-contamination were reduced by spray-washing in chlorinated water after evisceration. Sanders and Blackshear (1971) showed little effect of chlorine in the final carcass wash unless at least 40 mg/l were used. Washing carcasses post-chill with water containing 50 mg/l of

chlorine did not reduce the proportion of *Salmonella*-positive samples (Kotula et al., 1967). These studies emphasized the importance of adequate contact time, which is not usually achieved in a washing operation.

A study by Waldroup et al. (1992) examined the modification of broiler processing procedures to include 20 ppm of chlorine through the processing line and include 1 to 5 ppm of free chlorine in the chill tank overflow. These concentrations resulted in a 0.2 to 0.6 log reduction in aerobes, 0.0 to 0.3 log reduction in coliforms, and 0.0 to 0.4 log reduction in *E. coli*.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is an antimicrobial compound recognized for its disinfectant properties since the early 1900's. In 1967, EPA first registered the liquid form of chlorine dioxide for use as a disinfectant and sanitizer. In 1988, EPA registered chlorine dioxide gas as a sterilizing agent. Chlorine dioxide is a synthetic yellowish-green gas with chlorine like odor (Liem, 2002). ClO_2 is unstable as a gas and will undergo decomposition into chlorine gas (Cl_2), oxygen gas (O_2) and heat. However, ClO_2 is stable and soluble in an aqueous solution, and does not form hypochlorous acid or react with ammonia. It functions independent of pH and can provide excellent control at a fraction of the chlorine dosage because it can be used at much lower doses (Lillard, 1979). The smaller dosage also makes chlorine dioxide more cost effective. Chlorine dioxide kills microorganisms by disrupting transport of nutrients across the cell wall. It can be generated in a gas or liquid form and smells like chlorine bleach. The additive may be used to control the microbial population in poultry processing chill water in an amount not to exceed 3 parts per million (ppm) residual chlorine dioxide (Liem, 2002).

Chlorine dioxide reduces microbial contamination of carcasses much the same way as chlorine, but is up to seven times more active, can be used at lower concentrations such at 3 to 5 mg/l in the chill tank, and is less corrosive (Lillard, 1979). Chlorine dioxide does not appear to have an affect on meat flavor, but tends to result in a slightly lighter skin color (Thiessen et al., 1984).

Trisodium phosphate

The trisodium phosphate (TSP) system was created to eliminate the need for off-line reprocessing. It is a white, free-flow crystalline material that complies with the specifications of the Food Chemicals Codex. It has been certified by the National Sanitation Foundation (NSF International) for use in the treatment of drinking water at a maximum dosage of 41.5 mg/l. TSP has a pH of 11.8 at a concentration of 12% (Scientific Committee on Veterinary Measures, 1998). The use of TSP can be costly because of the level needed to clean the carcass. Residual TSP on the carcasses entering the chiller causes the pH to increase dramatically from 7.0 to over 11.0. In plants where TSP is used, chiller water pH can be in the 10 to 11 range. This high pH level greatly negates the antimicrobial properties of chlorine (Scientific Committee on Veterinary Measures, 1998).

The bactericidal effect of TSP is well documented in the scientific literature and confirmed in several industrial studies. TSP is more active on gram negative pathogens such as *Salmonella*, *Campylobacter*, and *E. coli* than against gram positive ones such as *L. monocytogenes*. There are several mechanisms for the TSP mode of action; surfactant properties, destructive effect on bacteria at the high pH (pH 11), removal of bacteria that are not yet firmly attached to the skin surface, removal of some surface fat which

facilitates the removal of bacteria by the washing process, and an effect on the bacterial cell wall.

The overall activity of TSP against *Salmonella* has been well documented (Bender, 1992; Gudmunsdottir et al., 1993; Li et al., 1994; Lillard, 1995; Hwang and Beuchat, 1995). Salvat et al. (1997) and Coppen et al. (1998) investigated TSP treatment of poultry carcasses and found a 2 log reduction of *Salmonella*. Pre-chill spraying of chicken with TSP solution (10%) resulted in 2.1 to 2.2 log reduction in *Salmonella*. The effect of TSP on *C. jejuni* has been demonstrated, with differences noted between in-vitro studies and industrial tests. Using artificial biofilms or cell suspensions, Somers et al., (1994) reported a reduction of 5 logs. Slavik et al. (1994) and Federighi et al. (1995) demonstrated in an industrial trial a reduction of 1.2 to 1.5 log. It has been reported to remove significant numbers of *E. coli*, *Enterobacteriaceae*, *Campylobacter*, *Salmonella*, and total aerobes from poultry carcasses by more than 2 logs when concentrations are between 10 and 12% (pH 11.5 to 13) (Ellerbroek et al., 1992; Federighi et al., 1995; Salvat, 1996; and Somers et al., 1994).

The Rhodia corporation (Rhodia INC. Rhodia Food Ingredients, CN 7500, Prospect Plains Road, Cranbury, NJ, 08512) has conducted several studies using the AvGard® TSP spray and its affect on *E. coli* 0157:H7. Using a 12% solution at 21^{o}C, the results indicate an average reduction of 2 log, and showed an even greater reduction at 38^{o}C. Bashor et al. (2002) found a 1.2 log reduction from broiler carcass rinse samples collected pre and post TSP spray in a commercial poultry processing plant.

Acidified sodium chlorite

Acidified sodium chlorite (ASC) had been used as a disinfectant in hospitals, dental operations, and pharmaceutical clean rooms (Kemp et al., 2001). It has also been approved by the FDA, EPA, and the USDA as an antimicrobial for use on poultry, red meats, fruits and vegetables, and seafood (Kemp et al., 2001). The Alcide Corporation (8561 154th Avenue NE, Redmond, WA 98052) markets the Sanova™ spray system that is a commonly used ASC system in poultry processing plants. The chemistry of acidified sodium chlorite is related to that of chlorine dioxide. When a solution of sodium chlorite is acidified with a weak organic acid, chlorous acid is formed. The formation of chlorous acid is instantaneous on combination of the chlorite and acid. As the pH of the mixed solution decreases from pH 4, the proportion of chlorite that dissociates to chlorous acid increases. ASC typically operates in a pH range of 2.3 to 3.2, and acts as a broad spectrum disinfectant by oxidizing the microbial cell wall, attacking the sulfide and disulfide linkage of proteins. It provides a non-specific attack on the amino acid component of the cell membrane. ASC is applied at ambient temperature, either by a spray or immersion dip, with a concentration averaging 1000 ppm and a dosage rate of 1.3 to 1.5 oz of ASC solution/bird. It is considered a broad-spectrum oxidative antimicrobial, effective on pathogenic bacteria as well as viruses, fungi, yeast, molds, and some protozoa.

Kemp et al. (2001) investigated the effectiveness of using ASC in poultry processing plants under conditions similar to those in commercial poultry facilities. This study showed that the application of ASC was effective in reducing *E. coli* populations on broiler carcasses from an initial level of 3.1 log by 2.2 logs and total coliforms with initial levels of 2.2 by 1.5 logs.

A study conducted by the Alcide Corporation with the Sanova™ system in a broiler processing plant detected populations of *Campylobacter* spp. of 3.7 logs after evisceration, 2.6 logs post wash, and 1.1 logs after the Sanova™ spray (Kemp et al., 2000). It was found that 73.2% of the carcasses were positive for *Campylobacter* after evisceration while 49.1% were positive after the Sanova™ spray. The incidence of *Salmonella spp*. on broiler carcasses decreased from 37% to 10% following the application of the Sanova™ system.

In most poultry processing operations Sanova™ is applied at the end of the evisceration line before chilling or after chilling for whole carcasses or carcass parts. The process does not slow down the processing throughput since the sprays and dips are a very quick process. A typical operation of 70 or 91 birds per minute would require 3 Sanova™ spray cabinets (12 feet) per processing line for a 15 second exposure. Recently, some processors have introduced a second line of defense by adding the Sanova™ system post-chill immediately prior to cut up or packaging.

On-line reprocessing

A study conducted by Fletcher et al. (1997) evaluated on-line reprocessing on visible contamination and microbiological quality of broilers. Before this study, off-line reprocessing procedures for visually contaminated carcasses included the removal of the bird from the processing line, reprocessing in an approved off-line area, then chlorination and finally re-inspection. Approved procedures included washing, vacuuming, and trimming (Fletcher et al., 1997). On-line reprocessing would allow the carcass to proceed through evisceration and a chlorinated inside/outside carcass washer prior to examination for visual contamination at the pre-chill checkpoint. Carcasses that still had visible

contamination would then be removed for manual off-line reprocessing. Results based on visual scores, indicated that the on-line reprocessing reduced the need for off-line reprocessing by 73 to 84%. The incidence of *Salmonella* or *Campylobacter* was not affected by the treatment. This study showed that on-line processing of visually contaminated carcasses could greatly reduce the number of carcasses currently being subjected to off-line reprocessing without negative effects on total bacterial counts or presence of pathogenic organisms.

Issues with *Campylobacter* after processing

The Minnesota Department of Health data showed that levels of contamination of retail poultry remain high despite interventions made at the processing plant (Smith et al., 1999). Stern and Line (1992) detected *Campylobacter* spp. in 98% of retail packaged broilers sampled from grocery stores. Another study by Willis and Murray (1997) found 69% (229/330) of raw commercial broilers were positive for *C. jejuni*. A study from New Zealand showed that *Campylobacter* could be isolated from 63% of chicken carcasses at retail outlets (Bongkot, 1997).

Kinde et al. (1983) indicated that the presence of *Campylobacter* in market broilers diminishes over time during refrigerated storage. Blankenship and Craven (1982) detected viable strains of *C. jejuni* stored in sterile ground chicken meat. The growth and extended survival observed at 37^{o}C with ambient atmosphere incubation suggest that the test strains were readily able to locate favorable microaerophilic conditions for growth within the ground meat after surface inoculation.

A recent study in the United Kingdom estimated a population range of *Campylobacter* organisms on the surface of fresh chicken carcasses from 3 to 6 log CFU

per chicken (Friedman et al., 2000). Kanenaka (2000) conducted a survey from two large retail markets in Hawaii to characterize strains of *C. jejuni* isolated from clinical and poultry samples. She found samples collected at Oahu were 83.3% positive for *C. jejuni* on whole chicken samples, 91.7% positive on chicken parts, whereas mainland samples were 93.8% positive for whole chickens, 35.7% for chicken parts, and 56.8% for the total number of *C. jejuni* positive samples. Overall, *Campylobacter* was shown to be present in 70% of samples. It was also observed that certain fryers were found to have substantial contamination with an estimated mean population of log 4.6 CFU/carcass.

Uyttendaele et al. (1999) studied the incidence of S*almonella*, *C. jejuni* and *Listeria monocytogenes* on poultry carcasses and different types of poultry products on sale at a Belgium retail market. *Salmonella* was found in 36.5% of the samples, *C. jejuni* in 28.5% of the samples, and *L. monocytogenes* in 38.2% of the samples. They also found that contamination of poultry meat increases with further processing, but skinless parts were less likely to be contaminated with *Salmonella*, *Campylobacter* and *L. monocytogenes* than those parts with skin. The prevalence of *Campylobacter* spp. in poultry and poultry meat products in Germany was studied by Atanassova and Ring (1999). Of 509 samples taken from poultry flocks, 41.1% were *Campylobacter* positive, whereas broiler carcasses were 45.9% positive.

Most fresh poultry products are marketed in an air atmosphere, but some products are stored in a carbon dioxide atmosphere to extend shelf life. The spoilage flora that develops on poultry during low-temperature air storage is predominantly *Pseudomonas* species, whereas in a carbon dioxide atmosphere *Lactobacillus* species are most prevalent (Balley et al., 1979). Blankenship and Craven (1982) found that *C. jejuni* survived quite

well with spoilage flora that developed during both air and carbon dioxide atmosphere storage.

Thawing of poultry products at ambient room temperature for extensive periods is not recommended. Lee et al. (1998) have reported that *C. jejuni* cells could replicate at room temperatures and under refrigeration at 4^{o}C. Many health authorities recommend thawing poultry rapidly and cooking it thoroughly to an internal end point temperature of 75^{o}C. It is also important to avoid cross contamination of cooked and raw foods.

Dawkins et al. (1984) examined work surfaces, sinks and floors of areas where fresh and frozen chicken had been processed. Cleaning with detergent and hot water (or steam) and drying was sufficient to remove *C. jejuni* from the environment. They reported that drying surfaces after washing was an important factor in controlling persistence in the environment.

A recent study by Kramer et al. (2000) compared the prevalence of *C. jejuni* and *C. coli* in fresh bovine, porcine liver, and chicken portions from retail outlets and compared the strain subtype distribution with those associated with cases of human campylobacteriosis from the same time period and study area. *Campylobacter* were isolated from 73.2% of 489 samples, with chicken exhibiting the highest contamination rate (83.3%). Of the human isolates, 89.3% were *C. jejuni* and 10.7% *C. coli.* A significant proportion of the chicken and lamb isolates shared identical subtypes with the human strains, indicative of their role as potential sources of infection.

Summary

The illness caused by *Campylobacter* contamination is clearly a major issue in our food system. Because such a large majority of contamination is associated with poultry

(50 to 70%), it is important to focus on this vehicle. Control strategies have been improved in recent years including on-farm techniques and methods during poultry processing. However, these strategies still require significant improvements to completely remove or significantly reduce the threat of *Campylobacter* contamination.

Campylobacter populations can be found in chickens entering the plant at levels as high as 5 to 8 logs/gm of fecal material, in the crop, as well as on the feathers of the birds. This contamination is easily spread from carcass to carcass during processing. Although contamination is reduced during processing, they are still present on the carcass after processing at levels of up to 2 to 3 logs/gram. A large portion of the reduction occurs during the carcass washing systems and processing with aids such as TSP and ASC. While these reductions are significant, they still may not reduce the levels of contamination below the threat to public health (as little as 500 organisms can make a person ill).

Table 2. Summary of sources of contamination and levels

Source of Contamination	Levels of Contamination	Reference
Farm	3 log to 9 log	Byrd et al., 1998b
Transport/Initial levels Entering plant	Fecal-6 log Cecal-6.5 log Cecal 7.3 log	Oosterom, 1983 Achen et al., 1998 Berrang et al., 2000
Scalding	Pre-scald 3.8 log	Berrang et al., 2000
Feathers	8 log	Barnes, 1975
Evisceration	Skin-6 log 3.5 log crop Crop-4.7 log	Wilkerson, 1961 Achen et al., 1998 Berrang et al., 2000
Carcass Washers	Pre-Wash 4.78 log Post-Wash 4.3 log	Bashor et al., 2002
Packaging/Retail	4.64 log	Kanenaka 2002 Willis and Murray, 1997

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Manuscript 2:

BROILER CARCASS WASHERS AND CAMPYLOBACTER CONTAMINATION

Effects of Carcass Washers on *Campylobacter* Contamination in Large Broiler Processing Plants

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Abstract

Campylobacter, a major food borne pathogen found in poultry products, remains a serious problem facing poultry processors. *Campylobacter* research has primarily focused on detection methods, prevalence and detection on carcasses, with limited research conducted on intervention. The aim of this study was to assess the effectiveness of carcass washing systems in four large broiler-processing plants in removing *Campylobacter* species.

Washing systems evaluated included combinations of inside/outside carcass washers and homemade cabinet washers. Processing aids evaluated were trisodium phosphate (TSP) and acidified sodium chlorite (ASC). The washer systems consisted of 1 to 3 carcass washers and used from 0.57 to 2.57 gallons of water per carcass. The washer systems used chlorinated water with 25 to 35 ppm of total chlorine. These washer systems on average reduced *Campylobacter* populations log 0.5 cfu/ml from log 4.8 cfu/ml to log 4.3 cfu/ml. Washer systems with TSP or ASC reduced *Campylobacter* populations on average by an additional log 1.03 to log 1.26 respectively. Total average reductions in *Campylobacter* populations across the washer system and chill tank were log 0.76 cfu/ml. Washer systems that included antimicrobial systems had total average reductions in *Campylobacter* populations of log 1.53 cfu/ml.

These results suggest that carcass washer systems consisting of multiple washers provide minimal reductions in *Campylobacter* populations found on poultry in processing plants. A more effective treatment for reducing *Campylobacter* populations is ASC or TSP treatments; however, these reductions, while significant, will not eliminate the organism from raw poultry.

Introduction

Campylobacter, a major food borne pathogen found in poultry products, remains a serious problem for poultry processors. *Campylobacter* organisms have long been recognized as a cause of diarrhea in cattle and of septic abortion in both cattle and sheep. Over the last 25 years it has been recognized as an important cause of human illness (Friedman et al., 2000). The Centers for Disease Control and Prevention reported that *Campylobacter,* which has an infectious does as low as 500 organisms, is the most common bacterial cause of diarrheal illness in the United States causing more illness than *Salmonella* and *Shigella* combined (Meer and Misner, 1998). The disease surveillance data also indicates that campylobacteriosis affects about 2.5 million people every year. Poultry and poultry products have often been implicated in the transmission of *Campylobacter.* Case control studies have estimated that 50 to 70% of *Campylobacter* contamination is due to poultry and poultry products, thus reducing levels associated with raw poultry have drawn considerable attention (Allos, 2001; Tauxe, 1992).

Poultry processing plants are required to have developed and implemented HACCP plans which includes conducting a hazard analysis and identifying critical control points (CCP's) and effective control(s) at these CCP's to ensure compliance with USDA-FSIS regulations (e.g., zero visible fecal, *Salmonella* and *E. coli* performance standards) (USDA, 1996). According to the 1996 baseline study, 88% of the carcasses sampled were positive for *Campylobacter* and 20% were positive for *Salmonella* (Conner et al. 2001). There is every indication that FSIS will implement some type of *Campylobacter* ssp. performance standard in the future. However, there is much less data

available on *Campylobacter* populations on poultry carcasses during the slaughter process than for *Salmonella*.

Processors have had a difficult time in developing preventative measures to comply with zero visible fecal contamination regulations (USDA, 1994). Under HACCP, when a deviation occurs, the plant must implement a corrective action and preventive measure. The plant's typical response for a corrective action has been to slow down the processing line, add additional line personnel for visual inspection, and as a preventative measure, add additional carcass washers or wash systems. This has resulted in poultry processing plants increasing their daily water use by 30 to 50% since HACCP plans went into effect (Jackson et al., 1999). Since the introduction of HACCP, a typical poultry processing plant (150,000 to 200,000 birds/day) uses 1 to 2 millions gallons of water each day (Sellars and Kiepper, 2001). While the necessity of the added personnel may be justified, the justification for increasing water use and additional washing systems has not been scientifically proven to be effective at decreasing the incidence of fecal contamination on the carcasses. A study is needed to document the impact of these washer systems on *Campylobacter* populations in the slaughter process.

Carcasses are commonly washed with chlorinated water to remove contamination, such as blood, tissue fragments, and fecal contamination as part of regular processing procedures. Washing has been allowed in poultry processing since 1978 as an alternative to knife trimming because studies have shown it is equally effective in removing fecal contamination (Scientific Committee on Veterinary Measures, 1998). But, limited studies have been conducted on evaluating the performance and effectiveness of poultry

washers and sanitizing treatments within the processing plant (Bautista et al., 1997; Dickens and Cox, 1992; Anand et al., 1989).

Chlorine has been used in poultry processing for more than 40 years. In poultry processing, the purpose of chlorine addition to water is to prevent cross contamination of carcasses and equipment (Scientific Committee on Veterinary Measures, 1998). Typical, in-plant chlorination of water up is 20 ppm chlorine, with higher levels (50 ppm) required in the chill tank to counter the organic load in the chill water (Goresline et al., 1951; McVicker et al., 1958; Mallman et al., 1959; Dawson et al., 1956).

The rate at which bacteria are killed in with chlorinated water is dependent on the temperature, pH, chlorine concentration, and organic load. As chlorine is added to water it will bind with organic matter, which reduces its antimicrobial efficacy. The goal in chlorine addition is to add sufficient chlorine to produce a free residual, which is unreacted chlorine. When present, this free residual maintains antimicrobial activity for an extended period controlling microbial growth and proliferation in the treated water. For maximum antimicrobial efficacy, a free residual of chlorine at a low temperature and a pH between 4.0 and 6.0 is needed. At a pH between 4.0 and 6.0, chlorine hydrolyzes completely to hypochlorous acid (HOCl), which is a very effective antimicrobial. As pH increases above 6.0, a portion of the HOCL is converted into hypochlorite ions (OCl^-). OCl^- has a lower antimicrobial efficacy than HOCl (Gavin and Weddig, 1995). At a pH greater than 9.0, no HOCL remains in solution. In municipal waters treated with chlorine for extended periods of time, equivalent antimicrobial reductions have been documented for both free residuals of OCL^- and HOCL (Montgomery, 1985); however, in poultry processing plants, contact time and organic load in washer systems are highly variable.

Under commercial processing conditions, studies evaluating chlorine solution as a prechill wash or spray have shown mixed results with regard its antimicrobial efficacy (Barnes, 1965; James et al., 1992; May 1974; Patterson, 1968; Thomson et al., 1976; Wabeck et al., 1968). Mead et al. (1975) showed that neither levels of contamination nor the occurrence of cross-contamination were reduced by spray washing with chlorinated water after evisceration. Sanders and Blackshear (1971) showed little effect of chlorine in the final wash unless at least 40 ppm was used. Carcass shelf life has been shown to be extended by chlorine use in the chill water (Dawson et al., 1956; McVicker et al., 1958; Patterson, 1968; Ranken et al., 1965; Schuler, 1976). Washing carcasses post-chill with water containing 50 ppm of chlorine did not reduce the incidence of *Salmonella*-positive samples (Kotula et al., 1967). A chlorine level of 50 ppm was required to reduce cross-contamination of *Salmonella* incidence from 40% to 8% positive (Thomson et al., 1979). Waldroup et al. (1992) examined the modification of broiler processing procedures to include 20 ppm of chlorine through the processing line and include 1 to 5 ppm of free chlorine in the chill tank overflow. This resulted in a 0.2 to 0.6 log reduction in total aerobes, 0.0 to 0.3 log reduction in coliforms, and 0.0 to 0.4 log reduction in *E. coli*.

Processing aids such as trisodium phosphate (TSP) and acidified sodium chlorite (ASC) have also been approved for use in poultry processing (Scientific Committee on Veterinary Measures, 1998). TSP, used at concentrations between 8 and 12% (pH >11.5), has been shown to be an effective decontaminant for poultry carcass (Federighi et al., 1995; Slavik et al., 1994). It has been reported to remove significant populations (>2 logs) of *E. coli*, *Enterobacteriaceae*, *Campylobacter*, *Salmonella*, and total aerobes from

poultry carcasses when TSP concentrations are between 10 and 12% (pH 11.5 to 13) (Ellerbroek et al., 1992; Federighi et al., 1995; Salvat, 1996; and Somers et al., 1994). It is suspected that TSP's surfactant properties contributed to these reductions.

A study by Kemp et al. in 2001 investigated the effectiveness of using acidified sodium chlorite (ASC) in poultry processing as an antimicrobial for carcasses under conditions similar to those in commercial poultry processing facilities. This study showed that the application of ASC was effective in reducing *E. coli* populations from log 3.11 cfu/ml to log 0.91 cfu/ml and total coliforms from log 2.15 cfu/ml to log 0.64 cfu/ml.

The objective of this study was to investigate the efficacy of commercial washer systems and antimicrobial treatments (TSP and ASC) in four large broiler processing plants for removing *Campylobacter* contamination from broiler carcasses and to measure water consumption of washer systems. These results will provide poultry processors scientific data on *Campylobacter* populations and the removal efficiencies of washer systems in commercial poultry processing plants.

Materials and Methods

Sample collection

From January 2001 to December 2002, broiler carcass samples were collected from four large poultry processing plants (150,000 to 250,000 birds/day) labeled as Plant A, B, C, and D, and analyzed for *Campylobacter* contamination. Sample collection for Plant B was repeated in the spring (noted as B1) and fall (noted as B2). Each plant was sampled on three separate days for a total of 15 sampling days. Whole broiler carcass

were aseptically removed from the processing lines at various sample sites. For Plant A, sample sites were before and after each of 3 carcass washers and a post-chill tank, for a total of 5 sample sites. For Plants B, C, and D, the sample sites were post evisceration just prior to the first carcass washer, after the final carcass washer, post antimicrobial spray if present (Plant C-TSP and Plant D-ASC), and post chill tank. In plants B and C individual washers were not accessible for carcass collection. In Plant D, a single carcass washer was isolated and evaluated. A single hot water cabinet spray (63^{o} C, 50 psi, 40 gal/min) was used prior to the single inside/outside washer, but was not evaluated for its reduction in *Campylobacter* populations. At each sample site, 10 carcasses were removed from the line using a new latex glove for each carcass. Carcasses were placed individually into sterile plastic bags that were then sealed and covered with ice. Samples collected after the TSP spray were allowed to drip for 10 seconds, rinsed with 100 ml phosphate buffer, then the pH of the rinse water was immediately neutralized from 11 to 7.2 using a buffered HCl (0.1N) to neutralize any residual TSP (Waldroup, 2001). After collection of all carcasses, the samples were transported to the laboratory (1 hour) and held on ice until analyzed (within two hours of collection). Sample size was determined based on preliminary data collected on *Campylobacter* populations present on the carcasses pre and post wash.

Water consumption data were reported by the poultry processing plants using in line meters. Data was reported on the pressure of the washer spray nozzles, the gallons of water per minute consumed by the washers, as well as the total gallons per bird through the washer systems. The plants also reported the water pH and concentrations of chlorine in the wash water and chill tank.

Microbiology

One hundred ml of sterile phosphate buffered peptone water (pH 7.2) was added to each sample bag for Plants A, B, and C (Cox et al., 1981). For Plant D, because of the residual ASC concerns, Butterfield's phosphate buffer (100 ml) with 0.1% sodium thiosulfate was used (Kemp et al., 2001). Sodium thiosulfate was included in the buffer solution as a neutralizing agent for any residual ASC remaining on the carcass surface. Bags were vigorously shaken by hand for 2 minutes using a back and forth arc motion according to USDA Agricultural Research Service recommended procedures (Kemp et al., 2001). After shaking was complete, carcasses were removed with sterile gloved hands and residual rinse drained back into the sample bag for about 30 seconds, then the carcasses were discarded. Rinsate from each bag was collected in sterile collection tubes. A 0.1 ml aliquot was direct plated in duplicate onto a blood free *Campylobacter* selective agar, *Campylobacter* Charcoal Desoocholate Agar (CCDA)[2], supplemented with CCDA selective supplement (16 mg Cefoperazone and 5 mg Amphotericin B) (Stern and Line 1991). In addition, duplicate 1 ml aliquots were distributed evenly over 4 plates each. Methods recommended by the USDA Food Safety and Inspection Service for enumeration of *Campylobacter* require direct plating of the whole carcass rinse onto ten agar plates (2 each for 0.1 ml and 4 each 0.25 ml aliquots in duplicate). Plates were incubated at 42^{o}C in Glad® plastic freezer bags for 36 to 48 h in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) provided by a certified gas tank mixture.

After incubation, colonies were enumerated. Colonies typical of *Campylobacter* (small gray drop-like or gray slimy colonies) were selected and examined for

[2] Oxoid LTD., Basingstoke, Hampshire, England

conformation of cell morphology and motility (using wet mounts and phase contrast microscopy).

Statistical Analysis

Statistical analysis for the study encompassed several parameters associated with sampling. All non zero microbiological measurements were transformed to log10 prior to conducting an ANOVA using the General Linear Model procedure (PROCGLM) of SAS^{TM} (SAS Institute, 1999) to test the main effects (plant variation, sample site, and replication). ($P < 0.05$) were considered statistically significant. The general linear model used for the analysis was $\Upsilon_{ijk} = \mu + \alpha_i + \beta_{j(i)} + E_{ijk,}$ where μ denotes an overall average, α_i denoted fixed plant effects for $_i = 1, 2, 3, 4$, $\beta_{j(i)}$ denotes site effects nested in plant for $_j = 1, 2, 3, 4, 5$ in plants 1 ,2, 4 or $_j = 1,2$ in Plant 3. Multiple comparisons among site means were carried out using the transformed data. A Bonferroni adjustment for all pairwise comparisons was used to control the experimental error rate at $P < 0.05$.

Results and Discussion

Campylobacter Isolation

Between January 2001 and December 2002 a total of 570 broiler carcass samples were obtained from four large (150,000 to 250,000 birds/day) poultry processing plants. In Plant A individual washers in the washer system were tested. Samples were collected prior to the first carcass washer, after each of the three carcass washers, and after the chiller tank. *Campylobacter* incidence and population results for Plant A are summarized in Table 1. In Plants B, C, and D washer systems were evaluated pre and post washer system, post antimicrobial if present, and post chiller tank. Results are shown in Table 2.

In Plant A, it was observed that a single inside/outside carcass washer was effective in reducing *Campylobacter* populations on average by log 0.31 cfu/ml or 48%, (log 4.69 cfu/ml to log 4.38 cfu/ml) (Table 1). The series of three carcass washers were effective in reducing *Campylobacter* populations by log 0.45 cfu/ml or 64.5%. The number of *Campylobacter* positive carcasses remained statistically equivalent (26/30 vs. 27/30) after the first carcass washer. Samples collected after the second carcass washer had a mean *Campylobacter* population of log 4.36 cfu/ml, only a 0.02 log reduction (2.76% reduction) in comparison to the first washer. The difference in *Campylobacter* populations collected after washer 1 and washer 2 was not statistically different ($P > 0.05$). Samples collected after the third carcass washer had *Campylobacter* populations of log 4.25 cfu/ml, a 0.11 log reduction (24.47%) compared to washer 2. Post-chiller tank samples had mean *Campylobacter* populations of log 4.12 cfu/ml, a reduction of 0.13 log or 15.5% reduction compared to the post-wash samples. The percent positive carcasses did decrease from 86.6% to 73.3% through the washing system and chill tank. Total reduction in mean *Campylobacter* populations through the carcass washer system and chill tank in plant A was log 0.57 cfu/ml. These results suggest that the first carcass washer was the primary removal device for *Campylobacter* populations in the carcass washer system, although it effectiveness was limited.

Initial *Campylobacter* populations were equal across all plants (Table 2). It should be noted that B1 and B2 data were collected in Spring and Fall, respectively. All washer systems reduced *Campylobacter* populations from 0.26 to 0.66 log cfu/ml. In plants C and D, TSP and ASC processing aids were used. These were sprayed on the carcasses, where TSP was applied at a 12% rate, pH 11.0, and a contact time of 15

seconds. ASC was applied at 1000 ppm, pH 2.5, 1.3 oz/lb with a contact time of 15 seconds, resulting in additional log 1.03 cfu/ml and log 1.26 cfu/ml reductions, respectively. This suggests that the addition of a processing aid can further improve the reduction of *Campylobacter* populations.

For all plants, *Campylobacter* populations collected post chill were reduced between log 0.07 cfu/ml and log 0.25 cfu/ml. All plants used a chlorinated water chiller at 2^{o} C with 20 to 50 ppm of total chlorine.

Plant B, using three carcass washers, was sampled in two separate seasons to compare levels of contamination throughout the year (Table 2). When sample site *Campylobacter* population means were compared across seasons, there was no significant difference between the data collected ($P > 0.05$). The initial mean population of *Campylobacter* sampled pre-wash was log 4.75 cfu/ml and decreased to log 4.10 cfu/ml (76.7% reduction) post-wash. Populations on samples collected post-chill were log 3.90 cfu/ml for a 27.8% reduction. The incidence of positive *Campylobacter* carcasses decreased from 85% to 72%. Plant B had a total average reduction in *Campylobacter* populations through the washer system and chill tank of log 0.85 cfu/ml.

Plant C (Table 2) had initial mean *Campylobacter* populations of log 4.88 cfu/ml that decreased to log 4.61 cfu/ml after carcasses were subjected to a series of three carcass washers for a reduction in *Campylobacter* populations of log 0.27 cfu/ml. Samples taken at Plant C were also collected following a TSP spray that produced an additional log 1.03 cfu/ml reduction to a level of log 3.58 cfu/ml. Following chilling, the mean *Campylobacter* population was log 3.42 cfu/ml. Total reduction in mean

Campylobacter populations across the washer system, TSP rinse, and chill tank was log 1.46 cfu/ml.

Plant D had initial mean *Campylobacter* populations of log 4.90 cfu/ml that declined to log 4.64 cfu/ml after going through one single inside/outside carcass washer. Samples collected after an ASC rinse had mean *Campylobacter* populations of log 3.38 cfu/ml. *Campylobacter* populations reduced to log 3.31 cfu/ml following the chiller tank. Total reduction in mean *Campylobacter* populations across the washer system, ASC rinse, and chill tank was log 1.59 cfu/ml.

Overall, 87.2% of the total samples collected post evisceration were positive for *Campylobacter*, 85.3% were positive post wash, 71.7% were positive post-antimicrobial application, and 68.0% were positive post chiller. There was strong evidence that *Campylobacter* populations decreased as the poultry carcasses traveled through the washer systems in all plants ($F=178$, $df = 12$, $P < 0.0001$).

Water Consumption

The data for water consumption provided by the processing plants for each carcass washer at each of the four plants is summarized in Table 4. Plant A, B, and C used three inside/outside washers in series for their washer systems. Plant D used a hot water spray followed by one single inside/outside washer. Water consumption for washer systems ranged from 2.4 gallons per bird in Plant A to 0.57 gallons per bird in Plant D. Nozzle pressures and water consumption of individual washers varied between plants, but for the plants with multiple washers (A, B, and C) the first washer always had the highest pressure. This first washer pressure ranged from 60 psi in Plant C to 180 psi

in Plant B. The pH levels in the wash water ranged from 6.8 in Plant D to 7.2 in Plants B and C. The chiller tank pH levels ranged from 6.8 in Plant D to 11.2 in Plant C. Plants were adding between 25 to 35 ppm of total chlorine to the washer water and between 25 to 35 ppm of total chlorine to their chiller tanks.

The efficiency data for each plants washer system is shown in Table 5. The efficiency was calculated by subtracting the mean *Campylobacter* populations achieved by each washer, then dividing by the volume (gal) of water used per bird at each washer. For example at plant A, the efficiency of washer 1 was 34,231 organisms per gallon of water. Sample calculation for this efficiency is Washer 1 - Washer 2 = 24,988 organism reduction. This number is divided by the gallon of water per bird (Washer 1 55gal H_20/line speed (75bird/min)= 0.73gal/bird). Total efficiency is 24,988 organisms/ 0.73 gallons per bird = 34,231 organisms per gallon of water.

In an inside/outside carcass washer the carcasses enter with the breast facing toward the inside of the machine. As the machine rotates, a probe with a single spray nozzle enters the bird's body cavity and washes the inside. Then a series of spray nozzles wash the outside of the bird. The reduction in mean *Campylobacter* populations from washer 1 (log 4.69 cfu/ml to log 4.38 cfu/ml) in Plant A (Stork Gamco inside/outside washer, 55 gal/min, 90 psi) was very similar to reductions from the carcass washer (Stork Gamco inside/outside washer, 60 gal/min, 80 psi) evaluated at Plant D. However, the efficiency is much greater statistically at Plant D (62,773 organisms per gallon of water) than for Plant A effeciency. The hot water rinse in Plant D before the inside/outside washer might aid in reducing adhesion of the *Campylobacter* organisms and improve the efficiency of the inside/outside washer. Another reason might be due to the line speed

(105 birds/min) being faster than Plant A. The total *Campylobacter* removal efficiency of Plant A across all three washers was calculated to be only 12,997 organisms. This is due to the slow line speed (75 bird/min) of the processing line. The efficiency of the second and third washer in Plant A dropped off significantly. The efficiency of Plant B was 40,412 organisms. Plant B was using a high pressure (180 psi, 35 gal/min) inside/outside Stork Gamco washer followed by 2 Johnson inside/outside carcass washers (90 psi, 35 gal/min and 60 psi, 18 gal/min). This increase in efficiency was attributed to the fact that this plant used less water in each washer for a total of only 113 gallons/minute for all three carcass washers. This was considerably less than Plant A (180 gallons/minute) and Plant C (200 gallons/minute). Plant C used a Linco inside/outside carcass washer (60 psi, 80 gal/min), Johnson inside/outside washer (55 psi, 60 gal/min) and a plant build cabinet washer (50 psi, 60 gal/min). The efficiency of Plant C was shown to be only 18,483 organism.

Overall, the application of chlorinated water in carcass washers reduced the *Campylobacter* populations. However, the overall reduction did not appear to be significant when public health was considered. The 0.3 to 0.66 log cfu/ml reduction found in *Campylobacter* populations (average initial levels of log 4.8 cfu/ml) using chlorinated water in washer systems were similar to a previous study by Whyte et al. (2001). The data also suggest that the first or a single carcass washer was the most efficient in removing Campylobacter and contributed log 0.30 cfu/ml reduction (45%).

A reduction in *Campylobacter* populations of log 1.03 cfu/ml using a 12% TSP solution has been previously reported as well (Federighi et al., 1995; Slavik et al., 1994). However there is significant concern within the poultry industry regarding the handling

and treatment of phosphate containing waste streams. The results of the ASC application (1200 ppm, 1.3 oz/bird, 23^{o} C) did show a log 1.26 cfu/ml reduction in *Campylobacter* populations with a 5-s exposure at 1200 ppm. This reduction is greater than that typically seen with 1 h or more of exposure using 20 ppm chlorinated water (Kemp et al., 2001; Waldroup et al., 1992).

Washing systems evaluated included combinations of inside/outside carcass washers and homemade cabinet washers. Processing aids evaluated were trisodium phosphate (TSP) and acidified sodium chlorite (ASC). The washer systems consisted of one to three carcass washers and used from 0.57 to 2.57 gallons of water per carcass. The washer systems used chlorinated water with 25 to 35 ppm of total chlorine. These washer systems on average reduced *Campylobacter* populations log 0.5 cfu/ml from log 4.8 cfu/ml to log 4.3 cfu/ml. Washer systems with TSP or ASC reduced *Campylobacter* populations on average by an additional log 1.03 to log 1.26 respectively. Total average reductions in *Campylobacter* populations across the washer system and chill tank were log 0.76 cfu/ml. Washer systems which included antimicrobial systems had total average reductions in *Campylobacter* populations of log 1.53 cfu/ml.

The current commercial practice of using multiple carcass washers in poultry processing has limited effect on *Campylobacter* contamination. The data suggest a single carcass washer provides the highest efficiency in reducing *Campylobacter* populations, but reductions are not significant when public health is considered. Because the infectious dose is as low as 500 organisms, a reduction of 60,000 organisms per bird to 30,000 organisms per bird may not affect the infection rate. *Campylobacter* infections have not decreased since 1996, while water consumption has increased 30 to 50%.

A manageable strategy for *Campylobacter* reduction in poultry products would be to ensure the lowest possible prevalence of the pathogen in the production process, from "farm to fork". Processing aids such as TSP and ASC could be used to further reduce *Campylobacter* levels, but could not be used to achieve zero visible fecal, a federal regulation. Processing aids are most effective in reducing levels of *Campylobacter* contamination when they are used in conjunction with a single carcass washer using chlorinated water, which removes debris and allows better exposure of the antimicrobial (Kemp et al., 2001). A combination of a chlorinated carcass washer and an antimicrobial treatment serve as a multiple hurdle approach to pathogen control.

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Table 1. Mean log CFU/ml and percent *Campylobacter* positive whole carcass rinse samples taken
at various sites in broiler processing Plant A (n=30)[1]

Sample Site	Pre-wash	After 1st washer	After 2nd washer	After 3rd washer	Post-Chill
CFU/ml	4.69[a]	4.38[b]	4.36[b]	4.24[c]	4.12[c]
Percent Positive	86.6(26/30)	90%(27/30)	90%(27/30)	80%(24/30)	73.3%(22/30)

[1] Population means with the same lower case letter are not significantly different ($P \leq 0.05$).

Table 2. Mean log CFU/ml of rinse for *Campylobacter* from whole carcass rinse
samples at various sites in the processing plant (n=30)

Site	Plant A	Plant B1	Plant B2	Plant C	Plant D
Pre-wash	4.69 (a) (A)	4.73 (a) (A)	4.76 (a) (A)	4.88 (a) (A)	4.90 (a) (A)
Post-wash	4.25 (b) (A)	4.10 (b) (A)	4.10 (b) (A)	4.61 (b) (B)	4.64 (b) (B)
Antimicrobial	N/A	N/A	N/A	3.58 (c) (A)	3.38 (c) (B)
Post-chill	4.12 (b) (C)	3.85 (b) (B)	3.94 (b) (B,C)	3.42 (c) (A)	3.31 (c) (A)

[a] Population means in the same plant with the same lower case letter are not significantly different ($P \leq 0.05$).

[b] Population means within sampling site with the same upper case letter are not significantly different ($P \leq 0.05$).

Table 3. Percent *Campylobacter* positive whole carcass rinse samples taken at various sites in four broiler processing plants (n=30)

Site	Plant A	Plant B1	Plant B2	Plant C	Plant D
Pre-wash	86.6(26/30)(a) (A)	83%(25/30)(a) (A)	86.6%(26/30)(a)(A)	86.6%(26/30)(a)(A)	93.3%(28/30)(a)(A)
Post-wash	80%(24/30)(a)(A)	80%(24/30)(a)(A)	90%(27/30)(a)(B)	90%(27/30)(a)(B)	86.6%(26/30)(a)(AB)
Antimicrobial	N/A	N/A	N/A	80%(24/30)(b)(A)	63.3(19/30)(b)(B)
Post-chill	73.3%(22/30)(b)(A)	70%(21/30)(b)(A)	73.3%(22/30)(b)(A)	60%(18/30)(c)(B)	63.3(19/30)(b)(B)

[a] Population means in the same plant with the same lower case letter are not significantly different ($P \leq 0.05$).

[b] Population means within sampling site with the same upper case letter are not significantly different ($P \leq 0.05$).

Table 4. Data for water consumption for carcass washers at each plant visited

Plant	Washer Type	Nozzle pressure PSI	Gallons per minute	Gallons per bird	pH	Chlorine added PPM
A				2.4		
Washer1	Stork I/O[1]	90	55		7	25
Washer 2	Stork I/O	80	50			
Washer 3	Linco I/O[2]	40	75			
Chill Tank					7.2	25
B				1.08		
Washer 1	Stork I/0	180	35		7.2	35
Washer 2	Johnson I/0[3]	90	60			
Washer 3	Johnson I/0	60	18			
Chill Tank					7	35
C				1.9		
Washer 1	Linco I/0	60	80		7.2	35
Washer 2	Johnson I/0	55	60			
Washer 3	Plant Built Cabinet	50	60			25
TSP Rinse Chill Tank					11.2	
D				0.57		
Washer 1	Hot water cabinet spray (63^{o}C)	50	40			
Washer 2	Stork I/0	80	60		6.8	35
ASC Rinse Chill Tank					6.8	35

[1] Stork Gamco Inc. Airport Parkway, P.O.Box 1258, Gainesville, Georgia 30503

[2] LindHolst & Co A/S, DK 8380 Trige, Denmark

[3] Johnson Food Equipment, Inc., Member of the BAADER Group, 2955 Fairfax Trafficway, Kansas City, KS, 66115

Table 5. Efficiency of carcass washer systems in four large broiler processing facilities

	Pre wash log CFU/ml	Post wash log CFU/ml	GPB[a]	Efficiency[b]
Plant A[1]	4.69	4.25	2.4	12,997
Plant B[2]	4.75	4.10	1.08	40,412
Plant C[3]	4.88	4.61	1.9	18,483
Plant D[4]	4.9	4.64	0.57	62,773

[a] Gallons per bird is calculated using gallons per minute divided by the line speed

[b] Efficiency is read as the mean organisms reduced per gallon of water used on each carcass

[1]Processing line speed of 75 birds/min and 180 gal/min total through washing system

[2]Processing line speed of 105 birds/min and 113 gal/min total through washing system

[3]Processing line speed of 105 birds/min and 200 gal/min total through washing system

[4]Processing line speed of 105 birds/min and 60 gal/min total through washing system

Table 6. Mean *Campylobacter* populations present at Plant A[1] pre wash and post wash for washer 1, washer 2, and washer 3

	Pre washer log CFU/ml	Post Washer log CFU/ml	GPB[d]	Efficiency[e]
Washer 1[a]	4.69	4.38	0.73	34,231
Washer 2[b]	4.38	4.36	0.66	1,636
Washer 3[c]	4.36	4.25	1	5,126

[1] Plant A line speed is 75 birds/min

[a] Washer 1 is a Stork Gamco I/O carcass washer, 55 gal/min, 90 psi

[b] Washer 2 is a Stork Gamco I/O carcass washer, 50 gal/min, 80psi

[c] Washer 3 is a Linco USA I/O carcass washer,75 gal/min, 40 psi

[d] Gallons per bird is calculated uing gallons per minute divided by the line speed

[e] Efficiency is calculated by substracting Post wash mean *Campylobacter* populations from Pre wash mean *Campylobacter* populaions, then dividing by the gallons per minute of water for the carcass washer

Appendix A: SAS Program for Analysis

```
options ls=75;

data one;
   infile "bashornew.dat" firstobs=2 dlm='09'x;
   input Season $ Plant $ rep Site y e8. Bird;
   day=rep;
   ypos=(y>0);
   ty=y/10000;
   ly=log10(ty+1);
   sy=exp(0.1*log(y+1));
run;

proc sort;  by plant; run;

proc means mean std sum n noprint;
   class day site plant;
   var y;
   output out=two sum=sumy mean=meany;
run;

data two;
   set two;
   ly=log(meany);
   sy=sqrt(meany);
   y=meany;
run;

goptions colors=(black) dev=psepsf;

axis1 value=(h=3) offset=(10);

proc gplot;
   title "mean daily bacteria counts";
   plot y*site=plant/haxis=axis1;
run;
proc gplot;
   title "mean daily log-bacteria counts";
   plot ly*site=plant/haxis=axis1;
run;

proc glm;
   class plant site;
   model ly=site(plant) plant;
   lsmeans site(plant);
   lsmeans site(plant)/slice=plant;
   lsmeans site(plant)/pdiff adj=bon;
   output out=three p=meanly r=r;
run;
proc gplot;
   title "mean log-bacteria counts";
   plot meanly*site=plant/haxis=axis1;
run;
```

```
proc gplot;
   title "residuals (log-transform) versus predicted";
   plot r*meanly;
run;
proc univariate normal plot;
   title "residuals (log-transform)";
   histogram r;
   var r;
run;


proc glm data=two;
   class site plant;
   model y=site(plant) plant;
   means site(plant);
   *lsmeans site(plant)/slice=plant;
   *lsmeans site(plant)/pdiff adj=bon;
   output out=two r=r p=p;
run;
proc gplot;
   title "residuals (no transform) versus predicted";
   plot r*p;
run;

proc univariate normal plot;
   title "residuals (no transform)";
   histogram r;
   var r;
run;
```

Analysis of Keener/Bashor data on
Bacterial counts for washer-treated chickens
Jason A. Osborne, Dept. of Statistics, NCSU, December 2002

Bacterial counts were taken on chickens sampled from 4 different plants, one of which was visited in two different seasons. Within each plant, birds were sampled at various sites along a sequences of washers, with labels assigned in the table below:

Label	1	2	3	4
Site	Before 1^{st} washer	After 3^{rd} washer	After microbial rinse	After Chill Tank

Not every site was sampled from every plant. Birds were sampled after the microbial rinse only at plants C and D. So, the total number of treatment combinations for a given day was $17 = 3 \times 3 + 2 \times 4$:

	site label			
Plant	1	2	3	4
A	x	x		x
B(spring)	x	x		x
B2(fall)	x	x		x
C	x	x	x	x
D	x	x	x	x

$n = 10$ birds were sampled for each of 3 days at of these plant $\times$ site combinations for a total of $10 \times 3 \times 17 = 510$ birds.

The response variable was bacterial count (y). Using general linear models to analyze the data was made difficult by the problem of modality at 0. There are a substantial number of birds (106 out of 510)) for which no bacteria were detected ($y = 0$). (See table below.) The distribution of bacterial counts was therefore non-normal and no power transformation will fix this problem.

Bacterial	Frequency				
Count	Site 1	Site 2	Site 3	Site 4	Total
$y = 0$	19	22	17	48	106
$y > 0$	131	128	43	102	404
Total	150	150	60	150	

In addition to demonstrating the modality at 0, the table also provides some evidence of a site effect on bacterial counts, in the sense that the proportion of 0's differs significantly by site, ($\chi^2 = 22.95, p < 0.0001$ on $3df$). The frequency of birds with 0 counts was higher at sites 3 and 4 than at sites 1 and 2.

Rather than pursuing more complicated *zero-inflated* models for the analysis, DAY can be taken as the experimental unit, as opposed to bird. So, for each day, the average of the bacterial counts for the 10 birds will be taken as the response in a simplified analysis. Taking averages in this way takes care of the discreteness problem, so that there was no longer modality at zero, but subsequent residual plots reveal an inhomogeneity of variance problem. As mean

daily *Campylobacter* counts decrease across the sites, so does their variability. Subsequent residual plots indicate that $\log_{10}$-transformed data do not obviously violate the homogeneity of variance assumption underlying the analysis of variance.

A nested, fixed effects model for the log-daily mean bacterial count y_{ijk} on day k at site j in plant i is then given by

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + E_{ijk}.$$

where μ denotes an overall average log-bacterial count, α_i denote fixed plant effects, $\beta_j(i)$ denote site effects, nested in plant.

Inspection of the ANOVA table below reveals that almost all (98.4%) of the variability in mean daily log-bacterial counts was explained by this model with site effects nested in plants, with most of the variability coming from the site effect. The F-test for equality of mean counts across sites within the plants was $F = 178.1$ on $12, 34df$ with a p-value less than 0.0001.

As subsequent multiple comparisons

```
                          The GLM Procedure
                    Number of observations    51

                                      Sum of
Source                     DF       Squares   Mean Square  F Value  Pr > F
Model                      16   14.57845006    0.91115313   139.00  <.0001
Error                      34    0.22287432    0.00655513
Corrected Total            50   14.80132438

           R-Square     Coeff Var      Root MSE       ly Mean
           0.984942      1.930271      0.080964      4.194423

Source                     DF   Type III SS   Mean Square  F Value  Pr > F
Site(Plant)                12   14.01276405    1.16773034   178.14  <.0001
Plant                       4    0.56568601    0.14142150    21.57  <.0001
```

Tables of mean *Campolybacter* counts on the transformed scale appear below. Multiple comparisons among site means were carried out using the transformed data. A Bonferroni adjustment for all pairwise comparisons was used to control the experimentwise error rate at $\alpha = 0.05$.

In the subscripts we use the following notation to indicate whether or not two mean bacterial counts are significantly different:

- two site means in the same plant with the same lower case letter are not significantly different.
- two plant means from the same site with the same upper case letter are not significantly different.

Site	Plant	ly LSMEAN	LSMEAN Number	Within Plant Grouping
1	A	4.69339665	1	a
2	A	4.24753582	2	b
4	A	4.12494397	3	b
1	B	4.73104910	4	a
2	B	4.10354122	5	b
4	B	3.85145600	6	b
1	B2	4.76321932	7	a
2	B2	4.10495989	8	b
4	B2	3.94361876	9	b
1	C	4.88164377	10	a
2	C	4.61145577	11	b
3	C	3.57794633	12	c
4	C	3.41957768	13	c
1	D	4.90258070	14	a
2	D	4.66066078	15	a
4	D	3.37513705	17	b
3	D	3.31245969	16	b

Site	Plant	ly LSMEAN	LSMEAN Number	Within Site Grouping
1	A	4.69339665	1	A
1	B	4.73104910	4	A
1	B2	4.76321932	7	A
1	C	4.88164377	10	A
1	D	4.90258070	14	A
2	B	4.10354122	5	A
2	B2	4.10495989	8	A
2	A	4.24753582	2	A
2	C	4.61145577	11	B
2	D	4.66066078	15	B
3	D	3.31245969	16	A
3	C	3.57794633	12	B
4	D	3.37513705	17	A
4	C	3.41957768	13	A
4	B	3.85145600	6	B
4	B2	3.94361876	9	BC
4	A	4.12494397	3	C

For insight into these pairwise comparisons, a matrix of Bonferroni adjusted p-values follows on the next two pages. For example, consider the difference plants C and D at site 3. In the table, these are means #12 and #16, and the corresponding adjusted p-values is 0.0421.

Least Squares Means for effect Site(Plant)
Pr > |t| for H0: LSMean(i)=LSMean(j)
Adjustment for Multiple Comparisons: Bonferroni

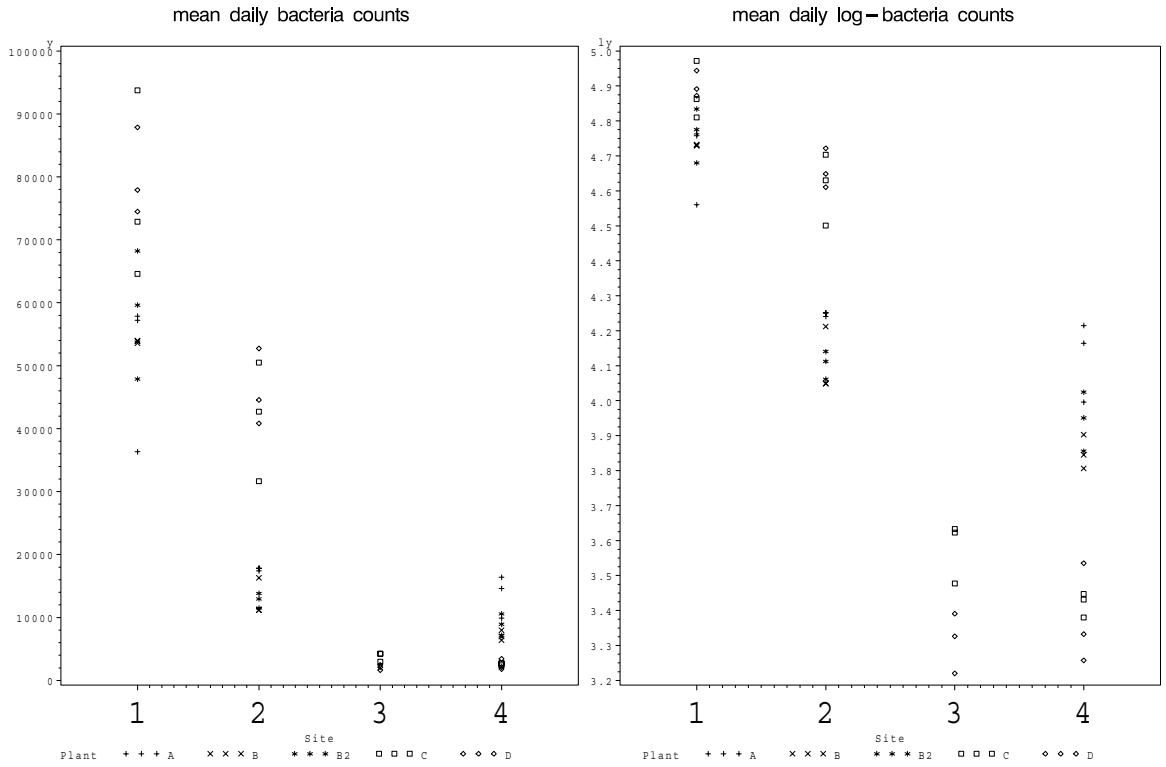
Dependent Variable: ly

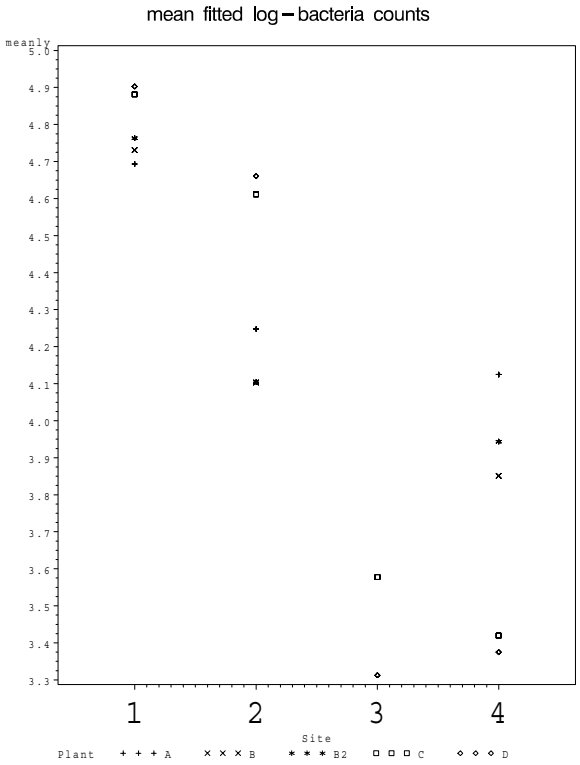
i/j	1	2	3	4	5	6
1		<.0001	<.0001	1.0000	<.0001	<.0001
2	<.0001		1.0000	<.0001	1.0000	0.0001
3	<.0001	1.0000		<.0001	1.0000	0.0297
4	1.0000	<.0001	<.0001		<.0001	<.0001
5	<.0001	1.0000	1.0000	<.0001		0.0749
6	<.0001	0.0001	0.0297	<.0001	0.0749	
7	1.0000	<.0001	<.0001	1.0000	<.0001	<.0001
8	<.0001	1.0000	1.0000	<.0001	1.0000	0.0705
9	<.0001	0.0077	1.0000	<.0001	1.0000	1.0000
10	1.0000	<.0001	<.0001	1.0000	<.0001	<.0001
11	1.0000	0.0005	<.0001	1.0000	<.0001	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	0.0297
13	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	0.4446	<.0001	<.0001	1.0000	<.0001	<.0001
15	1.0000	<.0001	<.0001	1.0000	<.0001	<.0001
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

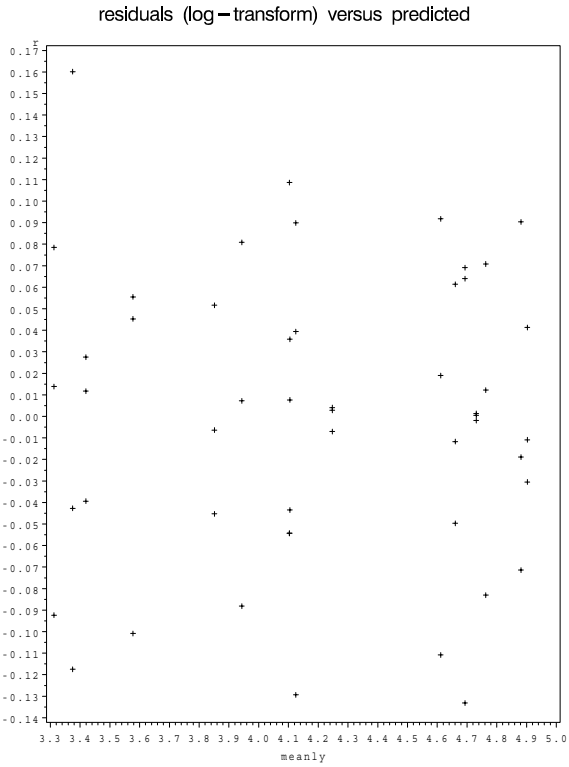
i/j	7	8	9	10	11	12
1	1.0000	<.0001	<.0001	1.0000	1.0000	<.0001
2	<.0001	1.0000	0.0077	<.0001	0.0005	<.0001
3	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001
4	1.0000	<.0001	<.0001	1.0000	1.0000	<.0001
5	<.0001	1.0000	1.0000	<.0001	<.0001	<.0001
6	<.0001	0.0705	1.0000	<.0001	<.0001	0.0297
7		<.0001	<.0001	1.0000	1.0000	<.0001
8	<.0001		1.0000	<.0001	<.0001	<.0001
9	<.0001	1.0000		<.0001	<.0001	0.0005
10	1.0000	<.0001	<.0001		0.0343	<.0001
11	1.0000	<.0001	<.0001	0.0343		<.0001
12	<.0001	<.0001	0.0005	<.0001	<.0001	
13	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000
14	1.0000	<.0001	<.0001	1.0000	0.0136	<.0001
15	1.0000	<.0001	<.0001	0.2758	1.0000	<.0001
16	<.0001	<.0001	<.0001	<.0001	<.0001	0.0421
17	<.0001	<.0001	<.0001	<.0001	<.0001	0.5728

i/j	13	14	15	16	17
1	<.0001	0.4446	1.0000	<.0001	<.0001
2	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	1.0000	1.0000	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001
7	<.0001	1.0000	1.0000	<.0001	<.0001
8	<.0001	<.0001	<.0001	<.0001	<.0001
9	<.0001	<.0001	<.0001	<.0001	<.0001
10	<.0001	1.0000	0.2758	<.0001	<.0001
11	<.0001	0.0136	1.0000	<.0001	<.0001
12	1.0000	<.0001	<.0001	0.0421	0.5728
13		<.0001	<.0001	1.0000	1.0000
14	<.0001		0.1155	<.0001	<.0001
15	<.0001	0.1155		<.0001	<.0001
16	1.0000	<.0001	<.0001		1.0000
17	1.0000	<.0001	<.0001	1.0000	

Conclusions: certainly, there is strong evidence $F = 178.1, df = 12, 34 df, p < 0.0001$) that bacterial counts decrease as birds go through the washer systems. There is also strong evidence ($F = 21.6, df = 4, 34, p < 0.0001$) of plant-to-plant variability. However, pairwise comparisons among sites within each plant indicate that there were no significant differences between sites 3 and 4 in either of the two plants where these measurements were taken. Plant B was visited in two different seasons. When site means are compared across plants, no significant differences between plants B and B2 were found.




mean fitted log – bacteria counts
meanly
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
1
2
3
4
Site
Plant + + + A × × × B * * * B2 □ □ □ C ◇ ◇ ◇ D


residuals (log – transform) versus predicted
r
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
-0.10
-0.11
-0.12
-0.13
-0.14
3.3 3.4 3.5 3.6 3.7 3.8 3.9 4.0 4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8 4.9 5.0
meanly

residuals (no transform) versus predicted