INFLUENCE OF METHODOLOGY ON THE RECOVERY OF SALMONELLA FROM RETAIL CHICKEN

by

MUSTAFA SIMMONS

(Under the direction of Daniel L. Fletcher)

ABSTRACT

Two experiments were conducted to determine the influence of methodology on the recovery of Salmonella on retail chicken. The first experiment was designed to determine if there was a difference in the incidence of Salmonella-positive carcasses reported by USDA-FSIS and the incidence of Salmonella-positive carcasses at the retail level using an exhaustive procedure. The second experiment was conducted to determine the effects of sampling methodology on the recovery of Salmonella from whole carcasses.

In the first study 85 of the 251, or 33.9 %, of the retail carcasses were Salmonella-positive. This is higher than both the 20 % from the 1994-1995 baseline and the 10.4 % reported by USDA-FSIS in 1998. The incidence determined in the first study may have been higher due to methodology, different sampling location (retail level versus processing plant), post-process contamination, or the presence of giblets.

In the second study the significantly more (p<0.0001) carcasses were found Salmonella-positive using whole carcass enrichment method than using the 30ml aliquot method recommended by USDA-FSIS. Also the incidence of Salmonella-positive carcasses determined by the whole carcass enrichment method (38 %) is comparable to the incidence determined in the first experiment (33.9 %), and the incidence determined by the Aliquot method (13%) was
comparable to the incidence reported by USDA-FSIS in 1998 (10.4%). Thus showing methodology does have an effect on *Salmonella* recovery when low numbers of *Salmonella* per carcass are expected.

INDEX WORDS: Salmonella, Broilers, Whole Carcass Enrichment, Methodology
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MUSTAFA SIMMONS

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MUSTAFA SIMMONS

Approved:

Major Professor: Daniel L. Fletcher
Committee: Nelson A. Cox
Mark E. Berrang

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
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INTRODUCTION

Salmonellosis is the second most frequently reported cause of bacterial foodborne illness. Each year 8000-39,000 cases are reported. However, estimates are as high as 2 million cases a year (CDC). The Economic Research Service (ERS) estimates that the annual economic costs due to Salmonella infections are $2.4 billion. This estimate includes the medical costs due to acute infection, the value of time lost from work, and the economic value of premature deaths.

Poultry has historically been linked to Salmonella and has consistently received intensive media and political attention. In 1996, the United States Department of Agriculture implemented the Hazard Analysis Critical Control Point Systems ;Final Rule, also known as the Mega Reg. The primary purpose of the Mega Reg is to reduce foodborne illness. One of the requirements of the Mega Reg is that meat and poultry plants implement HACCP plans. HACCP plans identify points where implementation of intervention steps can reduce potential hazards, such as pathogenic microorganisms. To verify the effectiveness of HACCP plans microbiological testing is required. In the case of poultry plants, tests are performed for generic E. coli and Salmonella.

Since the implementation of the Mega Reg the Food Safety and Inspection Service (FSIS) reported a decrease in the percentage of Salmonella-positive broiler carcasses from a baseline of 20% to approximately 10.4%. This reduction is based on aliquot sampling in processing plants. To date no research has been published to determine if this reduction has influenced Salmonella incidence in fresh retail poultry products.
The purpose of this research was to determine the incidence of *Salmonella* positive broiler carcasses at the retail level using an exhaustive methodology. The second part is to determine the effects of sampling methodology on the recovery of *Salmonella* from broiler carcasses.
LITERATURE REVIEW

The genus *Salmonella* has been widely researched and is of historical importance, as is evident by the numerous books and review articles pertaining to the organism such as those by van Oye (168), Cabello et al. (28), Neidhart et al. (115), Saeed et al. (138), Wray and Wray (176), and Bell and Kyriakides (16). This review will cover only the basics of *Salmonella* in general and its importance in poultry with an emphasis of current methodology and occurrences.

**SALMONELLA**

*Salmonella* was first observed by Eberth in 1880 in spleen sections and mesenteric lymph nodes from patients who died from typhoid. At that time the organism was known as typhoid bacillus (64). In 1886 Salmon and Smith isolated *Bacillus cholerae-suis* from swine suffering from hog cholera (46). Other similar organisms were isolated from outbreaks of foodborne disease and infected animals. The genus *Salmonella*, in honor of Salmon, was created in 1900 by Lignieres to accommodate these organisms (36).

The genus *Salmonella* include Gram-negative, facultatively anaerobic, rod-shaped bacteria belonging to the family Enterobacteriaceae. They are distinguished biochemically by their inability to ferment lactose, production of hydrogen sulphide, and decarboxylation of amino acids lysine and ornithine. And serologically they are distinguished by 3 major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen, a superficial antigen overlying the O antigen (71, 81,179).
The principal habitat of Salmonella is the intestinal tract of man and animals. Salmonella serotypes can be host-adapted, or non-host-adapted or ubiquitous (95). Host adapted serotypes usually cause serious systemic diseases. For example S. typhi, S. paratyphi-A, and S. sendai are strictly human serotypes, whereas S. abortus-ovis, S. gallinarum, and S. typhi-suis are ovine, avian, and porcine adapted serotypes, respectively (95). These host-adapted serotypes cannot grow on minimal medium without growth factors, unlike ubiquitous Salmonella serotypes (95). Ubiquitous Salmonella, such as S. typhimurium, can cause a wide range of clinical symptoms, from asymptomatic infection to typhoid-like illnesses in immunocomprsimised individuals or highly susceptible animals such as mice (23).

Most non-typhoidal Salmonella enter the body via food contamination. However, person-to-person spread of Salmonella may also occur (71). Symptoms usually begin 6-48 hours after exposure and include nausea, vomiting, fever, diarrhea and abdominal pain. The duration of symptoms vary but is usually 2 to 7 days (45, 71, 179).

If infection is to occur, Salmonella must survive several of the body’s basic defenses. The first defense being the acidic environment of the stomach. Exposure to such low pH induces the expression of genes involved in pH homeostasis and stabilization of macromolecules (56). Those that survive the stomach must then contend with bactericidal compounds, such as bile salts, of the small intestines (71). However, like other members of the family Enterobacteriaceae, Salmonella copes well with these stress conditions (15). Finally, Salmonella must resist removal by peristalsis. Carter and Collins (31) reported that 80% of the S. enteriditis that survive passage through the stomach of mice are passed with the feces within 6-10 hours post-infection.
Salmonella that survive the defenses of the alimentary tract may then attach and invade the intestinal mucosa (55, 83). Invasion causes the epithelial cells to release various proinflammatory cytokines. They evoke an acute inflammatory response and may be responsible for damage to the intestine (59).

Enterotoxin is also produced by many strains of Salmonella (83, 92, 140, 143). The Salmonella enterotoxin acts by stimulating host adenylate cyclase. This leads to increased levels of cellular cyclic AMP, which causes an increase in the concentration of sodium and chloride ions, and consequently fluid accumulation in the intestinal lumen, which leads to diarrheal symptoms (60).

Cytotoxin production has also been reported in some strains of Salmonella (9,119). Reitmeyer et al. (131) report that Salmonella cytotoxin is an outer membrane component, and thus is in direct contact with host cells, possibly having a role in cell damage or invasion. Koo et al. (88) report Salmonella cytotoxin inhibits protein synthesis.

Salmonella specifically invade lymph follicles that are located in the intestinal wall of the ileum. From there, the pathogens drain to the regional lymph nodes, where macrophages may prevent further spread, causing the infection to remain localized in the intestine. If the macrophages are unable to limit the spread, Salmonella can cause a systemic infection (15).

**SALMONELLA IN POULTRY**

In the early 1900’s Salmonella pullorum, caused one of the most important diseases in commercial poultry (128). The disease was first described by Rettger in 1900 as a septicemia of young chicks as reported by Snoeyenbos (150). With up to an 80% mortality rate in baby chicks,
pullorum disease is considered the greatest single factor that limited the early expansion of the U.S. poultry industry (150). Another important poultry disease during this period was fowl typhoid, caused by Salmonella gallinarum. It was first described by Klein in 1889 (128). Signs of both diseases are dead embryos in the incubator or early chick mortality, anorexia, dehydration, labored breathing, diarrhea, ruffled feathers, weakness, and adherence of feces to the vent. The diseases also cause decreased egg production, reduced fertility and lower hatchability (147).

In 1935 the National Poultry Improvement Program (NPIP) was established in the United States (150). The goal of NPIP was to prevent the spread of Salmonella pullorum and Salmonella gallinarum by preventing disease transmission to progeny by testing breeder flocks (150). The testing programs and control measures implemented by NPIP resulted in a lower incidence of the diseases in most developing countries during the 1950s and 1960s (126, 150, 154). The NPIP and Auxiliary Provisions is still currently active (3).

Since the 1940s there has been a rapid increase in the isolation of non-host specific Salmonella serovars in humans and animals (57, 65). Reports have shown that poultry and poultry products have been the main sources of non-host specific Salmonella infecting humans (57, 77, 94, 109, 144, 156).

Broiler chickens can harbor Salmonella from hatch through grow-out, however Millner and Shaffer (110) reported that newly hatched chicks are most susceptible to intestinal colonization. They showed colonization was dose dependent and varied with day of challenge. Day old chicks could be colonized with as few as 5 Salmonella cells, and later colonization required higher doses.
Transmission of *Salmonella* from hen to chick may occur as a result of infection of the ovary and oviduct. Timoney et al. (163) inoculated the crop of adult hens with $10^6$ *Salmonella*. The organisms were found in the yolk or albumen of eggs of about 10% of the hens, indicating transovarian transmission. During incubation, contaminated eggs containing gas-forming bacteria may explode thereby contaminating other eggs. The chicks hatching from these eggs will then be contaminated (136). Bailey et al (5) found that only one contaminated egg could lead to substantial *Salmonella* spread throughout the hatching cabinet. Cason et al. (32) showed horizontal spread of *Salmonella* also occurs during hatching of chicks. Cox et al (43) reported that 75% of all hatchery samples were positive for *Salmonella*, and 38 of 40 randomly selected samples contained greater than $10^3$ *Salmonella* cells per sample. However, Lahellec and Colin (93) reported that *Salmonella* serotypes originating in the hatchery were less important in the final product than those present in the grow-out house or those introduced into the house by vectors during rearing.

There are numerous potential sources of *Salmonella* contamination throughout an integrated poultry system, including feed, rodents, insects, litter, wild birds, humans, transport coops, and the processing plant environment (82, 129, 135). Poultry feed is a recognized source of *Salmonella*. *Salmonella* can be isolated regularly from feed ingredients, not only from ingredients of animal origin but from plant origin as well, such as soy, rape, palm kernal, rice bran and cottonseed (174). Davies et al. (48) reported the *Salmonella* serovars which were isolated in the feed mill were also most widely distributed throughout the broiler flock. However, Bailey et al. (8) identified ten different serotypes from feed samples, and only on one occasion was the same serotype found in the feed also found on the fully processed broiler carcass.
Several methods are used to eliminate *Salmonella* from poultry feed, including heating, irradiation and addition of chemical additives. The heat routinely used to manufacture feed often can only reduce coliforms and *Salmonella* by 2 to 3 logs (42). Thus *Salmonella* may survive after pelleting of highly contaminated rations. Leeson and Marcotte (96) showed finished feed can be treated with doses of gamma irradiation up to 10kGy without affecting nutritional quality. However, this method of decontamination is unlikely to be adopted by the industry, due to the high cost of installing irradiation facilities in a feed mill. Nevertheless, neither heat nor irradiation will protect feed from recontamination during storage and distribution. Hinton and Linton (69), however, reported the addition of antimicrobial compounds, such as organic acids, to poultry feed were effective in reducing the prevalence of *Salmonella* infections in poultry.

Insects, rodents and wild animals may also serve as vectors for *Salmonella* in poultry houses (44, 67, 121). Flies that breed in broiler and layer houses are a potential reservoir of *Salmonella* (4, 63). Olsen and Hammack (121) isolated *Salmonella enteritidis* from 2 of 15 pools of houseflies present in caged layer facilities. They also isolated *Salmonella infantis* from house flies and dump flies, and *Salmonella heidelberg* from houseflies.

Rodents also play an important role in the epidemiology of *Salmonella* infection in poultry. Henzler and Opitz (67) reported that on 5 farms where no *Salmonella enteritidis* was isolated 6% of 232 mice were positive for *Salmonella*, while on 5 farms where *Salmonella enteritidis* was present 31.8% of 483 mice were positive for *Salmonella*, 24% for *Salmonella enteritidis*. A bacterial count from the mouse feces yielded more than $10^5$ *Salmonella enteritidis* cells per fecal pellet.
Wild birds may be a source of *Salmonella* in poultry and other domestic animals (47, 72, 78). Craven et al. (44) found that up to 33% of wild bird samples (intestinal and fecal) obtained near poultry houses were positive for *Salmonella*.

Farm workers may also contribute to the spread of *Salmonella*. Bailey et al (8) reported recovery rates of *Salmonella* from boot swabs at 12%, and from the outside dirt near the entrance doors to houses at 6.1%, thus demonstrating how easily human movements and cross-contamination can occur.

Poultry litter has also been shown to be a reservoir for *Salmonella* (19, 53, 165). Poultry may ingest large numbers of *Salmonella* by picking at fecal or cecal droppings of littermates. Snoeyenbos et al. (151) reported *Salmonella* was spread rapidly from infected day old chicks to penmates reared on litter. These infected chickens will also shed *Salmonella* cells in their feces throughout grow out. Tucker (165) reported that *S. pullorum* and *S. gallinarum* persisted for 11 weeks on new litter, but for only 3 weeks in built-up litter. Decreased moisture and high pH due to dissolved ammonia, were shown to be the cause of the higher bactericidal activity of old built-up litter compared with new litter (166). Carr et al (30) demonstrated that water activity ($A_w$) is a critical parameter in the growth of *Salmonella* on the poultry litter surface. Hayes et al (66) suggest that a low $A_w$ environment ($<.84$), achieved via drying of the litter by ventilation or other means, could deter the establishment or continuation of *Salmonella* contamination in a commercial poultry house. Studies by Mallison et al (105) and Himathongkham et al (68) support a reduction in *Salmonella* achieved by lowering the $A_w$ in both laboratory and field studies.
Limawongpranee et al (102) reported *S. blockley* persisted on a farm for more than a year even after all-in-all-out cleansing and disinfection of the poultry house had been applied. This suggest ordinary sanitation and control measures may fail to prevent infection or reinfection with *Salmonella*.

One approach to eliminate *Salmonella* from the live bird environment is competitive exclusion. Nurmi and Rantala (118) showed that *Salmonella* infections could be prevented by feeding chicks anaerobic cultures of normal intestinal flora of adult birds. Competitive exclusion inhibits proliferation or reduces the number of *Salmonella* by the following mechanisms; creation of a restrictive physiological environment, competition for bacterial receptor cites, elaboration of antibiotic type substances, or the depletion of or competition for essential substrates (134).

Large-scale commercial field trials were conducted in Puerto Rico and Georgia to test the efficacy of mucosal competitive exclusion (MCE) to protect broiler chickens against natural *Salmonella* colonization of the intestinal tract and subsequent contamination of the processed chickens from these treated flocks. In Puerto Rico, incidence rates of intestinal colonization with *Salmonella* were reduced from 11% to 2%, and from 41% to 10% in processed carcasses (18). In Georgia, incidence rates of intestinal colonization were reduced from 2% to undetectable and from 9.5% to 4.5% in processed carcasses (6). However, Bailey et al (7) showed when *Salmonella* contamination is present before the competitive exclusion microflora, significant protection from *Salmonella* colonization is difficult to achieve. Despite the great amount of research in developing competitive exclusion cultures and techniques, this strategy is not totally effective in eliminating *Salmonella* from live birds.
Studies have also been conducted to develop vaccines to eliminate *Salmonella* from live birds (104, 148, 149, 157). Killed *Salmonella* vaccines have not produced convincing levels of protection against ubiquitous *Salmonella* challenge (12, 58, 164). However, poultry can be successfully immunized against host specific species of *Salmonella*, such as *S. gallinarum*, using live attenuated vaccines (149). These vaccines have been used extensively among breeding flocks (14). In contrast, vaccines for *Salmonella* serovars associated with food poisoning, until recently, were used only experimentally in poultry.

An inactivated *S. enteriditis* vaccine, created by iron restriction, has been licensed in European countries, and shown to reduce the level of flock infection (14). Plasmid-cured strains are avirulent, or of greatly reduced virulence, and are immunogenic and protective in the cases of *S. enteriditis* (113) and *S. gallinarum* (10). However, the plasmids of some serotypes, such as *S. typhimurium* (13), are not associated with virulence and plasmid-cured strains of such bacteria would not have the same effect. Thus far, no study has demonstrated good cross-protection between different *Salmonella* serovars for any significant duration after vaccination (14).

A standard management practice in commercial broiler production is the removal of feed immediately prior to transportation to slaughter and processing. Feed-withdrawal programs have been shown to reduce intestinal contents, thus leaving less ingesta and feces available for potential carcass contamination during transport and processing (26, 107, 122, 123, 170).

Rigby and Pettit (132) reported that 8hr feed withdrawal prior to cooping reduced the amount of feces deposited in the coops by one fourth, but did not influence the incidence of *Salmonella*-positive cloacal or cecal contents or the level of *Salmonella* within feces. However, Ramirez et al (130) found that feed withdrawal increased the incidence of *Salmonella* isolation.
from crops of experimentally and naturally infected broilers. Feed withdrawal has been reported to cause an increase in broiler crop pH and a decrease in lactic acid concentration (34, 74). These conditions may provide an improved environment for the proliferation of *Salmonella*. Durant et al. (52) reported the crop contents from hens deprived from feed can cause increased virulence of *Salmonella* by increasing the expression of genes necessary for intestinal invasion. Byrd et al. (27) reported that the use of lactic acid or formic acid in drinking water during feed withdrawal caused a significant reduction in the recovery of *Salmonella typhimurium* from broiler crops.

During transport of birds to the processing plant, birds defecate and consequently step or lie in fecal matter. This leads to an increase in contamination with organisms of fecal origin (125, 145). Unclean coops can also lead to contamination with *Salmonella*. Rigby et al. (133) reported that the incidence of *Salmonella*-positive feathered carcasses increased from 42% pretransport to 93% following transport in contaminated plastic coops. Bailey et al. (8) reported *Salmonella molade* was isolated from broiler carcasses, and on pretransport coops, but not from any on-farm samples, thus its likely that the coops were contaminated from a previous flock.

Wing flapping during unloading and also during hanging and bleeding creates aerosols and dust that are deposited on the feathers and skin of nearby birds. Zottola et al. (180) reported isolating *Salmonella* from the air in poultry plant unloading zones.

Despite efforts to control *Salmonella*, processing plants may actually contribute to the increase in the incidence of *Salmonella* positive carcasses. Lillard (98) reported 5% of broilers entering processing plants harbored *Salmonella*, and the incidence increased to 36% for processed carcasses from the same flock. This is not only due to cross-contamination but also the processing procedure itself changes the bacterial population on the bird’s skin. Thomas and
McMeekin (161) report that during processing, the predominantly Gram-positive microflora of the skin of non-processed poultry carcasses is removed and replaced by heterogeneous population consisting of mostly gram negative bacteria.

Defeathering in commercial poultry operations is achieved by dipping carcasses in hot water immediately before feathers are picked by automatic equipment (124). Scalding affects feather follicles and allows removal of feathers with as much as 80% less force (50). Scalding and plucking of carcasses have been implicated as major sites of cross-contamination by *Salmonella* (112, 116, 175). Humphrey and Lanning (75) reported that feces from the chicken during scalding will dissociate to form uric acid and ammonium urate in the water, and this provides buffering that maintains scald water at pH 6.0, which maximizes the heat tolerance of *Salmonella*. Humphrey (73) reported that at pH 5.9-6.0 *Salmonella typhimurium* had a thermal death value at 52 C (time required to destroy 90% of organisms initially present at a given temperature, D 52 C) of 34.5 minutes. Yang et. al (177) reported destruction of *Salmonella typhimurium* on chicken skin was observed only at a scalding temperature of 55 C or higher. However, studies have shown that scalding at a higher temperature causes increased toughness in cooked chicken meat (85, 127, 146).

Several researchers looked at the effect of scald water pH on the bactericidal activity (73, 100, 106). Humphrey et al. (76) reported the adjustment of chicken scald water to 9.0 ± 0.2 lowered the D 52 C value of *Salmonella typhimurium* from 34.5 to 1.25 min. However, Humphrey and Lanning (75) found scalding at pH 9.0 had no effect on the incidence of *Salmonella* contamination on broiler carcasses. Similarly, Lillard et al. (100) reported that the use
of 0.5% acetic acid scald water would kill bacteria in the scald water but did not significantly reduce the bacteria on the broiler carcass.

Studies report *Salmonella* are isolated more frequently after picking than any other operation (24, 111). Mulder et al. (112) reported picking resulted in cross-contamination when a marker strain of *E. coli* was used to artificially contaminate broilers both internally and externally.

Birds subjected to a sub-scald, above 56 C, have the epidermis removed during picking, and as a result the microorganisms colonizing the stratum corneum are removed. The exposed dermal skin is covered with capillary-sized channels and crevices and provides a new surface for attachment and colonization (161).

The attachment of bacteria to poultry skin is well documented (117, 162, 97). Bacteria attach to carcasses and form biofilms. Biofilms are adherent microcolonies enveloped with an extensive polysaccharide matrix. Costerton et al. (35) state that bacteria within biofilms are more firmly attached to surfaces than individually attached cells. In order to colonize new surfaces, individual cells must be able to disperse from mature biofilms and reattach elsewhere (1).

Chilling is often thought to be key step in reducing bacteria on chicken carcasses (20, 22, 86, 90, 108). However, Surkiewicz et al. (158) found no reduction in the incidence of *Salmonella* contamination after chilling. Morris and Wells (111) found a higher incidence of *Salmonella* on carcasses after chilling than on carcasses prior to chilling. Campbell et al. (29) reported that in federally inspected processing plants 5.5% of the carcasses entered the chiller contaminated with *Salmonella* and 11.6% exited the chiller contaminated with *Salmonella*. 
Immersion of the carcass causes a fluid film to cover the skin. Thomas (160) showed this film contains several serum proteins, amino acids and other soluble or suspended compounds. The presence of organic matter in this fluid film may explain why all skin bacteria are not destroyed by chlorine. Yang et. al (177) showed that there was less than a 1 log reduction of *Salmonella typhimurium* when carcasses spent 50 minutes in the chiller with 20 to 30ppm residual free chlorine present. And how long the chilling water is used significantly reduced the bactericidal activity.

Lillard and Thomson (101) reported hydrogen peroxide at 6,600ppm or higher in chiller water was effective in reducing populations of aerobic microorganisms by 95-99.5%. However, the reaction of H$_2$O$_2$ with catalase from broiler carcasses causes discoloration and swelling. Fletcher et al. (54) reported that a sodium bicarbonate and H$_2$O$_2$ spray had little or no effect following immediate use, but reduced bacterial loads by 0.3 log after 7 days storage.

Thomas and McMeekin (162) reported immersion chilling also changes the microtopography of the skin causing capillary sized channels to form, which can be colonized by bacteria. Skin swelling, associated with the uptake of water during immersion, opens and exposes channels to contaminants present in the water. Lillard (97) reported an increase in the number of attached bacteria with increased immersion time.

Microbial loads on meats are typically reduced by spraying carcasses with high pressure jets of water or dipping carcasses in solutions containing various antimicrobial agents (e.g., hydrogen peroxide, ozone, chlorine dioxide, lactic acid, or trisodium phosphate [TSP]) (21, 33, 79, 178). TSP is commonly used in the poultry industry (84, 139, 173). Proposed by Bender and Brotsky (17), the process involves immersing whole birds for 15 minutes in a 10% solution of
Av-Gard™ trisodium phosphates and then allowing excess TSP to drip from the bird. Giese (61) suggested that this process works partly by removing a fat coating on poultry skin, allowing the bacteria to be washed from the carcass more effectively.

Coopen et al. (33) reported *Salmonella* incidence was reduced from 74.0% to 9.4% after Av-Gard™ treatment of immersion-chilled broiler carcasses. MPN analysis of the positive samples before and after treatment showed a decrease from an average 115 organisms per carcass to 0.6 organisms per carcass. Salvat et al. (139) reported similar results, obtaining close to a 2 log reduction measured by a miniature MPN procedure. Kim et al. (84) saw a reduction in the number of inoculated *Salmonella* cells on TSP treated broiler skin using a scanning electron microscope. However, it was difficult to see the difference in the number of attached bacteria for non-inoculated skins due to the low number of bacteria present.

Despite these results, the efficacy of TSP in reducing attached *Salmonella* has been questioned. Lillard (99) stated that TSP may not reduce the number *Salmonella* but residual TSP may cause a lower recovery rate due to an increase in pH which causes lethal or sub-lethal injury to *Salmonella* cells. Scantling et al. (142) found that TSP treated turkeys did not have a lower levels of *Salmonella* and *Listeria* at retail level. In fact 56% and 88% of TSP treated turkeys were positive for *Salmonella* and *Listeria*, while 31% and 6% of non-TSP turkeys were positive for the same organisms.

Surface roughness and laminar flow velocity have an effect on efficacy of TSP treatment. The time and temperature dependent changes that occur in the microtopography of poultry skin as a result of water uptake during immersion chilling may hinder the penetration of antimicrobial agents or high-pressure washes, thereby protecting bacteria (89). In a study by Korber et al. (89)
TSP treatment resulted in nearly 100% killing of control biofilms (no crevices on surface), while the viability of remaining cells at the biofilm base was 83 +/- 12% when a crevice width of .15mm was present. Bacteria within biofilms are also generally more resistant to biocides and antibiotics than their planktonic (not in present in biofilm) counterparts. Dhir and Dodd (49) showed that planktonic *Salmonella enteritidis* cells were more susceptible to biocide challenge (phenol, chlorhexidine, and Virkon) than attached cells.

Breen et al. (21) reported quaternary ammonia compounds (QACs), especially cetylpyridinium chloride and hexadecyltrimethylammonium bromide, were effective in both reducing and inhibiting the attachment of *Salmonella typhimurium*. The QACs produced these effects at concentrations lower than the concentration of TSP required to produce the same effects.

Nayak et. al (114) reported zinc chloride at 25 and 50mM concentrations was effective in detaching firmly attached *Salmonella* on chicken skin, and also interfered with the attachment of *Salmonella* to chicken skin. They postulated this is due to electropositive zinc ions selectively binding to negative charges on the cell membrane, and compete for specific receptor sites on the skin surface. Zinc chloride would change the conformation of surface proteins so that receptor sites on the skin surface are no longer exposed.

**RECOVERY OF SALMONELLA FROM POULTRY CARCASSES**

To verify measures taken to control *Salmonella* are effective, it is necessary to have standard methods for the recovery an isolation of *Salmonella* from poultry carcasses. Unlike clinical specimens, direct plating cannot be used to recover *Salmonella* from poultry carcasses.
The physiological state of *Salmonella* can greatly affect its culturability (36). Therefore methods for recovery and isolation from a food product, where *Salmonella* is present in low numbers and may be in a debilitated state due to chilling, heating or chemical treatments, are different from methods used for clinical samples, where *Salmonella* is often in high numbers and in a vegetative state (171).

Methods for the recovery and isolation of *Salmonella* from food products generally include the following steps: sample collection and preparation; nonselective incubation, which allows cells in a debilitated state to recover; selective enrichment, which inhibits other bacteria allowing *Salmonella* to multiply to detectable levels; inoculation of plating media; screening of suspect colonies; and biochemical and serological confirmation (2). The initial step of sample collection and preparation is important in sampling poultry carcasses, because the non-homogeneous nature of the carcass makes getting a representative sample difficult (137). Therefore several sampling techniques have been developed. Some of the most common are tissue swabbing, tissue maceration, and the whole carcass rinse.

Swabbing is a relatively easy and convenient method of sampling and is non-destructive. However, Kotula and Pandya (91) reported *Salmonella* counts were higher on the breast of carcasses than on the thighs or drums. Thus swabbing an arbitrary area of the carcass may produce an inaccurate estimate of bacterial numbers or whether or not they are even present. Cox et al (39) showed this inaccuracy was magnified if carcasses sampled were hard chilled or crust frozen.

Tissue excision and maceration may be more effective than swabbing for recovery of bacteria from whole broiler carcasses. Cox et al (39) reported maceration yielded significantly
higher APC and enterobacteriaceae counts than swabbing on whole birds. However, again the selection area is arbitrary and may not be a true representation of the carcass. Also this method is destructive and carcasses must be downgraded after samples are taken.

Based on the uneven distribution, and relatively low numbers of *Salmonella* per carcass, the whole carcass rinse method is considered the most appropriate for recovering bacteria from fresh poultry carcasses (40, 169). Cox et al. (40) reported the number of *Salmonella*-positive carcasses detected by the whole carcass rinse procedure was significantly higher (p = 0.001) than for rinsing or maceration using neck-skin. Sarlin et al. (141) reported the number of *Salmonella*-positive samples obtained by carcass rinse or skin sampling were significantly higher (p<0.05) than with the swab method. The researchers suggest the sensitivity of the skin sampling was due to using skin directly associated with the thoracic inlet and crop removal as opposed to neck skin.

There are several variations of the rinse procedure, based on the volume of rinse media used, and the aliquot of rinse solution incubated for preenrichment.

These variations in volumes and aliquots can affect the ability to recover *Salmonella*. Surkiwiez et al. (158) who reported that incubation of 270ml out of 300ml lactose broth used to rinse poultry, as opposed to a 10 ml aliquot was four times more effective in recovering *Salmonella*. Cox et.al (41) rinsed carcasses in 100ml of sterile water, incubated all of the recoverable rinse, and reported as few as 20 inoculated cells could be recovered from carcasses, even if the carcasses had been stored at –23 C for 3 or 6 months. To eliminate this variation Cox and Blankenship (38) rinsed carcasses in 500ml of lactose broth and then incubated the entire carcass in the rinse solution for 24h at 37 C. They reported as few as 8 inoculated *Salmonella* cells per carcass could be recovered.
IDENTIFICATION OF SALMONELLA

There are several methods for confirming the identity of *Salmonella* including biochemical, serological, and nucleic acid-based assays (36, 167, 171). The primary biochemical methods employ triple sugar iron (TSI) and lysine iron agar (LIA) slants, which are based on carbohydrate fermentation (TSI), lysine decarboxylation (LIA), and $H_2S$ production (51). However, these tests alone should not be used for ultimate confirmation; as there are atypical strains of *Salmonella* which can for example ferment lactose or not produce $H_2S$, thus giving false negative results (25). Another disadvantage of biochemical test is they require isolated colonies, and the isolation procedure is time consuming (167).

There are several serological assays available for the detection of salmonella, including agglutination-based serology and enzyme-linked immunosorbant assay (ELISA) (11). Advantages of using serological techniques is they are less time consuming, and have a high specificity (167). In terms of reliability, the false positive rates for environmental samples ranged from 2.3 to 5.8% and the false negative rates from 0.4 to 22% (129, 159). Some non-*Salmonella* such as citrobacters may cross-react with some somatic antisera, causing false positives (171). Also, Hoorfar et al. (70) reported some proportion of presumptive *Salmonella* isolates identified by biochemical testing lack either O-antigen or H-antigen or both, thus cannot be identified by conventional serological procedures.

Nucleic-acid based assays would detect *Salmonella* that lack antigens (70). One commercial assay available, based on DNA probes, is the colorimetric Gene Trak *Salmonella* assay (37). The assay employs *Salmonella*-specific DNA probes that hybridize with the
ribosomal RNA of the bacteria and uses a colormetric system for detection (167). Giese (62) reports the probe assay requires the presence $10^3$-$10^5$ cells thus pre-enrichment would most likely be necessary. False-negative rates for this assay of 1.25 to 13.4% and false-positive of 0.57 to 4.9% have been reported (129, 155, 159) for raw chicken, poultry feed, and environmental samples. One disadvantage is this assay is more labor-intensive than commercially available ELISA test, and cost for the instruments are required (167).

Polymerase chain reaction (PCR) can be used with the rfb gene cluster, which contains the genes responsible for the biosynthesis and assembly of the O-antigen repeat unit. This amplified DNA can then be tested for hybridization with a labeled DNA probe. (87, 70). The entire process, in which an amount of DNA equivalent to an overnight culture is obtained, can be completed in 2 hrs, and requires at least $10^2$-$10^3$ cells per ml (167).

**SALMONELLA IN RETAIL POULTRY**

A search of the Web of Science Database (80) in May, 2002 for references containing both *Salmonella* and poultry resulted in 2,969 hits. However, less than 50 of these references report the incidence of *Salmonella* on products at the retail level. Several of these retail references report a high incidence of *Salmonella* (>50%). While some more recent papers report relatively low incidences (<10%).
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CHAPTER 1

RECOVERY OF SALMONELLA FROM RETAIL BROILERS USING A WHOLE CARCASS ENRICHMENT PROCEDURE

1Simmons, M. S., D. L. Fletcher, J. A. Cason, and M. E. Berrang. Submitted to Journal of Food Protection.
ABSTRACT

Whole, fresh broiler carcasses were purchased from grocery stores over a 20-week period. Carcasses were selected based on having intact packages, and a unique USDA plant number and sell-by-date, such that each bird represented a single processing plant and processing day combination. Carcasses were tested for *Salmonella* using a rinse aliquot obtained after whole bird incubation in the rinse media for 24 h. Based on the number of unique processing plants (based on USDA plant number) and expiration dates, the number of birds available each week ranged from 6 to 17. Over the 20 week period, 251 independent carcasses from 14 processing plants were tested. *Salmonella*-positive carcasses ranged from 0 (for one week) to over 60% (for three weeks). Only four of the 20 weeks had an incidence of less than 20% positive carcasses. For the entire 20-week study, 85 of the 251 total carcasses tested, or 33.9 %, were found to be *Salmonella*-positive. For those processing plants from which more than 10 carcasses were obtained, *Salmonella*-positive carcasses ranged from less than 20 % (two plants) to over 40 % (four plants). These results indicate that a whole carcass enrichment may be more sensitive for detecting *Salmonella*-positive carcasses when low numbers of *Salmonella* per carcass are expected.

Keywords: salmonella, broilers, whole carcass enrichment
The incidence of *Salmonella* on poultry carcasses has been well documented and is of international importance. Although there are numerous references reporting *Salmonella* isolations from live poultry, production environments, and processing plants, fewer references are available which report *Salmonella* incidence on carcasses, parts, meat, or products at the retail level. Thirty-nine reports of *Salmonella* incidence in retail poultry products from 17 countries during the period from 1961 through 2001 are listed in Table 1. Recent interest in *Salmonella* in retail poultry is shown by more than half of the reports during this 40 year period being conducted since 1990. Sampling and microbiological methods are not consistent and reported *Salmonella* incidence is extremely variable (1.2 to 76.6 %). However, it is interesting that there has been no obvious change in the retail incidence of *Salmonella* in the period from 1961 through 1990, and since 1990 when approximately half of the studies were conducted.

Because of the association between food borne illness and meat and poultry, the United States government mandated that all meat and poultry plants in the United States comply with the Pathogen Reduction and Hazard Analysis Critical Control Point Rule, commonly referred to as the Mega-Reg (3). Part of this rule requires *Salmonella* testing conducted by the Food Safety and Inspection Service (FSIS) and compliance with a baseline performance standard. Since the implementation of the Mega-Reg, FSIS reports that the percentage of *Salmonella*-positive poultry carcasses has decreased from the baseline incidence of 20.0 % to 10.4 % when sampled immediately after chilling (6).

A rinse sampling method was used in about a third of the studies shown in Table 1, but rinse volumes and the volumes of liquid that were cultured have been quite variable. The official FSIS procedure requires that a whole carcass be taken immediately after chilling and placed in a
bag to which 400 ml of sterile buffered peptone water is added. The bag is shaken for approximately 1 minute, and a 30 ml aliquot is removed for subsequent analysis (3).

Although widely used, rinse sampling does have some limitations. Lillard (28) reported that only a small percentage of bacteria are recovered by rinsing, while the majority of organisms remain firmly attached to the carcass. Treatment with chlorine, trisodium phosphate (TSP), or other antimicrobial agents used during processing may also hinder recovery of *Salmonella*. Lillard (29) stated that TSP may not reduce the number of viable *Salmonella*, but residual TSP may have a negative effect on *Salmonella* detection due to sub-lethal injury to *Salmonella* cells. Given the opportunity to resusitate, however, these cells can be detected in samples taken at a later time, such as at the retail level.

Differences in rinse sampling procedures can also cause variation in results. However, this variation can be minimized by enrichment and incubation of the whole carcass at 37 C for 24 h as described by Cox and Blankenship (16). In that study, as few as 8 inoculated *Salmonella* cells per carcass could be recovered when a carcass was rinsed in 500ml of lactose broth and the whole carcass was incubated with the rinse. The purpose of the present experiment was to determine the incidence of *Salmonella*-positive poultry carcasses at the retail level using the whole carcass enrichment procedure.

**MATERIALS AND METHODS**

**Sample Collection**

Whole, fresh broiler carcasses were purchased, at weekly intervals from grocery stores in the Northeast Georgia area over a 20-week period. All carcasses were purchased on a Monday or
Tuesday to ensure that birds were at least two to three days post-processing. Carcass selection was based solely on intact packaging and a unique processing plant (USDA establishment number) and sell-by-date combination. Thus each carcass represented a unique plant and processing day.

Since the purpose of the experiment was to sample carcasses obtainable at the retail level, no effort was made to balance retail outlets or processing establishments. Therefore, once the samples were identified as having a unique plant and day identification, the samples were randomly reassigned to arbitrary plant identifications and original source information relative to retail or processing plant identification was purged from the data set.

**Salmonella Analysis**

On the day of purchase, the exterior of each package was swabbed with 100% ethanol and opened with a sterile scalpel. Using a fresh pair of sterile gloves for each carcass, the giblets were removed and discarded. The entire carcass and package exudate were transferred to a sterile 16x16 polyethylene bag containing 500ml of buffered peptone water\(^2\). The carcasses within the bags were then vigorously shaken for 1 minute. The bags containing the whole bird and rinse solution were incubated for 24h at 37 C similar to the method described by Cox and Blankenship (16), but using the 1% buffered peptone water instead of lactose broth.

After incubation, 0.5ml of incubated rinse solution was transferred to 10ml Rappaport-Vassiliadis broth\(^2\) and also to 10ml TT\(^2\) (Hajna) and incubated at 42 C for 24h. Each broth was

\(^{2}\)Becton Dickinson, Sparks, Maryland 21152
then streaked onto BG sulfa agar\textsuperscript{3} and modified lysine iron agar\textsuperscript{3} plates, and incubated for 24hr at 35 C. Suspect \textit{Salmonella} colonies were picked and used to inoculate triple sugar iron\textsuperscript{2} and lysine iron agar\textsuperscript{2} slants and incubated for 24h at 35 C. Presumptive positives were confirmed using Poly O\textsuperscript{2} and Poly H\textsuperscript{4} serological agglutination tests.

**Statistical Analysis**

To test sampling integrity against the possibility of cross contamination, the order of samples was analyzed using linear regression in the General Linear Models (GLM) procedure of SAS (36) and by calculating Chi-square and Fisher’s Exact Test values using PROC FREQ of SAS to test the independence of adjacent pairs of carcasses sampled each day. The last carcass sampled on each of the 20 sampling days was not included in this statistical test because it had no following carcass, so 231 pairs of carcasses were tested.

**RESULTS AND DISCUSSION**

The results for \textit{Salmonella} incidence for the 251 carcasses sampled over the 20-week period are presented by week and total in Table 1.2. \textit{Salmonella} incidence was variable over the 20-week period, ranging from 0 % (week 8) to over 60 % (weeks 11, 14, and 15). For the entire 20-week period, there were 85 \textit{Salmonella}-positive carcasses out of the total of 251 carcasses tested (33.9%).

\textsuperscript{3}Oxoid, Basinstoke, Hampshire RG24 8PW, UK

\textsuperscript{4}Microgen, Camberley, Surrey GU15 3DT, UK
For those plants from which more than 10 carcasses were sampled, the incidence of *Salmonella* positive carcasses varied from less than 20% (Plants B and H) to over 40% (Plants C, D, E, and G) (Table 3). The plant data is presented only to illustrate that the higher than expected incidence of *Salmonella* noted in Table 1.3 was not entirely due to a few plants. Although all of the carcasses were from USDA-inspected facilities, only two of the 8 plants were below the 20% *Salmonella* standard when sampled at the retail establishment and tested using a more stringent recovery methodology.

To test whether cross contamination of samples occurred during the experiment, the data were subjected to both linear regression and frequency response analyses. If cross contamination occurred during sampling the incidence of *Salmonella* would increase as birds were individually sampled each week (thus would result in a positive slope) or that the occurrence of a *Salmonella* positive carcass would result in a higher frequency of subsequent positive as opposed to negative carcasses. The incidence of *Salmonella*-positive carcasses by sampling order (1 = first carcass sampled each week, 2 = second carcass sampled each week, and so on through those weeks that had 17 carcasses sampled) are presented in Table 1.4. Linear regression of the incidence of positive carcasses by sample position showed that the slope was not significantly different from zero ($P = .915$). Frequency analysis of samples indicated that the incidence of *Salmonella*-positive carcasses was 36.2% following a *Salmonella*-negative carcass and 34.2% following a *Salmonella*-positive carcass. Chi-square ($P = .762$) and Fisher’s Exact Test ($P = .439$) both indicated that the incidence of *Salmonella* on a carcass was independent of the incidence on the previous carcass. Therefore, there was no statistical indication of serial cross-contamination between samples.
A *Salmonella* incidence of 33.9 % in whole chicken carcasses at retail would be substantially higher than the FSIS reports of 20.0 % from 1994-1995 baseline (4) and the 10.4 % reported in 1998 (6) for carcasses sampled immediately after chilling. The higher incidence of *Salmonella* in the current study may be due to several differences from FSIS surveys: different sampling methodology (incubating a 30 ml aliquot from a 400 ml rinse versus whole carcass incubation in a 500 ml rinse), different sampling location (processing plant versus retail meat case), possibility of post-processing contamination, presence of giblets in retail carcasses (as opposed to in-plant sampling of carcasses without giblets), sampling retail whole carcasses which represent only about 10% of broiler production as opposed to USDA sampling of all carcasses being processed, regional versus national sampling, and seasonal versus year-round sampling. The overall incidence of *Salmonella*-positive carcasses reported in this study is consistent, however, with relatively recent work that also used rinse sampling of retail chicken in the United States. A survey in Ohio in 1990 found 43.0 % of chicken carcasses and parts were *Salmonella* positive (12) and one in Arkansas in 1991 found that 33.3 % of whole carcasses were *Salmonella* positive (25).

An exhaustive recovery method should be expected to find more positives than a partial aliquot method if relatively few bacteria are expected on the carcasses. In a study of the effectiveness of rinse sampling, Cox et al. (17) wrote, “...When numbers of cells are low, incubation of a small aliquot...after rinsing is not adequate and could result in a gross underestimation of the incidence of *Salmonella*-contaminated carcasses among processed broilers.” The *Salmonella* testing procedure used by FSIS as described in the Mega-Reg is similar to the method used in the baseline study, in which aliquots of the rinse liquid were taken
for culturing of several different types of bacteria, rather than using the entire rinse volume to test for *Salmonella*. The value of the FSIS baseline is that it established a consistent methodology for later comparisons, but that methodology may underestimate the incidence of *Salmonella*.

Waiting a few days before sampling may also allow injured bacteria to recover, as in the case of bacteria on carcasses that are bought in supermarkets several days after processing. Jerngklinchan et al. (26) reported that carcasses from processing plants had a lower incidence of *Salmonella* than carcasses sampled at the retail level.

It is possible that HACCP-related changes in poultry processing reduced the number of *Salmonella* cells on positive carcasses. In the Arkansas study of retail chicken (25), the number of cells in rinses of positive carcasses ranged from 5 to 34 MPN per 100 ml of rinse liquid or approximately 1.5 to 10.2 MPN in a 30 ml aliquot. A recent study in the Netherlands (19) reported *Salmonella* MPNs of 10 or fewer on 89 % of fresh chicken parts and whole carcasses that were sampled at the retail level. The FSIS baseline study (4) of broiler chickens found that 87.3 % of carcasses that were *Salmonella* positive (20.0 % of all carcasses) had MPNs of less than 0.3 per ml of rinse, and the Canadian baseline study (7) found that 60.7 % of carcasses that were *Salmonella* positive by the qualitative method (21.1 % of all carcasses) were below the level of detection by the MPN method. If *Salmonella* bacteria are usually found on positive carcasses at relatively low levels, then any reduction in numbers caused by HACCP-related activities such as more vigorous washing, increased chlorine, antimicrobial rinses, TSP, or fewer opportunities for cross-contamination might reduce the number of *Salmonella* to the point where the size of the aliquot removed from a rinse can influence the probability of recovering the organism. Further research is needed to determine the incidence of *Salmonella* in retail poultry
and the relative contributions of various differences in sampling and methodology to the incidence level reported in the present study.

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   (March):12-18.


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</tr>
<tr>
<td>Harrison et al., 2001 (23)</td>
<td>UK</td>
<td>whole carcass</td>
<td>rinse</td>
<td>95</td>
<td>53.0</td>
</tr>
<tr>
<td>Zhao et al., 2001 (47)</td>
<td>USA</td>
<td>whole carcass</td>
<td>rinse</td>
<td>212</td>
<td>4.2</td>
</tr>
<tr>
<td>Murakami et al., 2001 (31)</td>
<td>Japan</td>
<td>parts</td>
<td>rinse</td>
<td>90</td>
<td>37.8</td>
</tr>
</tbody>
</table>

*Percentage of samples from which *Salmonella* was isolated. Results from MPN tables were reported in the published paper (J. DuFrenne, personal communication, 2001).
TABLE 1.2: Total number of carcasses sampled, number of processing plants represented, number and percentage of *Salmonella* by week and total for the 20 week test period.

<table>
<thead>
<tr>
<th>Week</th>
<th>Total number of carcasses sampled</th>
<th>Number of plants sampled</th>
<th>Total number of <em>Salmonella</em> positive</th>
<th>% <em>Salmonella</em> positive carcasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>40.0</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>8</td>
<td>6</td>
<td>35.3</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>27.3</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>11</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>8</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>7</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>8</td>
<td>9</td>
<td>64.3</td>
</tr>
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<td>12</td>
<td>13</td>
<td>8</td>
<td>6</td>
<td>46.2</td>
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<tr>
<td>13</td>
<td>14</td>
<td>7</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>63.6</td>
</tr>
<tr>
<td>15</td>
<td>11</td>
<td>7</td>
<td>7</td>
<td>63.6</td>
</tr>
<tr>
<td>16</td>
<td>13</td>
<td>7</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>26.7</td>
</tr>
<tr>
<td>18</td>
<td>13</td>
<td>8</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>20</td>
<td>17</td>
<td>9</td>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>Totals</td>
<td>251</td>
<td>85</td>
<td></td>
<td>33.9</td>
</tr>
</tbody>
</table>
TABLE 1.3: Incidence of *Salmonella* contamination on retail broilers by processing plant\(^1\) over a 20-week period.

<table>
<thead>
<tr>
<th>Processing Plant</th>
<th>Total number of carcasses sampled</th>
<th>% <em>Salmonella</em> positive carcasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>49</td>
<td>26.5</td>
</tr>
<tr>
<td>B</td>
<td>37</td>
<td>18.9</td>
</tr>
<tr>
<td>C</td>
<td>35</td>
<td>40.0</td>
</tr>
<tr>
<td>D</td>
<td>32</td>
<td>43.8</td>
</tr>
<tr>
<td>E</td>
<td>31</td>
<td>45.2</td>
</tr>
<tr>
<td>F</td>
<td>22</td>
<td>31.8</td>
</tr>
<tr>
<td>G</td>
<td>19</td>
<td>42.1</td>
</tr>
<tr>
<td>H</td>
<td>16</td>
<td>18.8</td>
</tr>
</tbody>
</table>

\(^1\)Only plants from which greater than 10 carcasses were sampled are included in this table.
TABLE 1.4: Incidence of *Salmonella* on retail broilers by sampling position (e.g. 1 = first carcass sampled, 2 = second carcass sampled, and so on) over the 20-week period.

<table>
<thead>
<tr>
<th>Sample Position</th>
<th>Total number of carcasses at sample position</th>
<th>% <em>Salmonella</em> positive carcasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>15.0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>35.0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>35.0</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>30.0</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>52.6</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>31.6</td>
</tr>
<tr>
<td>9</td>
<td>19</td>
<td>36.8</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>36.8</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>31.3</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>30.8</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>25.0</td>
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<td>14</td>
<td>7</td>
<td>28.6</td>
</tr>
<tr>
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<td>3</td>
<td>0.0</td>
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<tr>
<td>16</td>
<td>2</td>
<td>50.0</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>50.0</td>
</tr>
</tbody>
</table>
CHAPTER 2
COMPARISON OF SAMPLING METHODOLOGY FOR THE DETECTION OF
SALMONELLA ON WHOLE BROILER CARCASSES

Simmons, M. S., D. L. Fletcher, M. E. Berrang, and J. A. Cason. To be submitted to Journal of Food Protection
ABSTRACT

An experiment was conducted to compare two Salmonella recovery methods from the same carcass. One hundred fresh, whole broiler chickens were purchased from retail outlets over a 5-week period (20 carcasses per week). After carcasses were aseptically removed from the packages and giblets removed, the carcasses were placed in sterile bags containing 400 ml of buffered peptone, shaken for 60 s, and then a 30 ml aliquot was removed and incubated for 24h at 37 C (aliquot sample). Immediately, an additional 130ml of buffered peptone was added to the bag with the same carcass, bringing the volume to 500 ml, after which the carcass was reshaken and the whole carcass and rinse were incubated for 24h at 37C (whole carcass enrichment sample). Following incubation, a 0.5 ml sample from each method was placed into 10 ml each of Rappaport-Vassiliadis and tetrathionate (Hajna) broth and incubated at 42 C for 24 h. Each broth was then streaked onto BG Sulfa agar and modified lysine iron agar, and incubated for 24 h at 35 C. Suspect Salmonella colonies were inoculated on triple sugar iron and lysine iron agar slants and incubated at 35 C for 24 h. Presumptive positives were confirmed using Poly O and Poly H agglutination tests. Over the five week period, the Aliquot sample had 13 % Salmonella-positive carcasses compared to 38 % for the whole carcass enrichment (WCE) sample, from the same carcasses. Recovery ranged from 0/20 to 4/20 for Aliquot method, and 4/20 to 10/20 for WCE method over the 5 week period. These results indicate that when low numbers of Salmonella are expected, sampling methodology has a major influence on the identification of Salmonella-positive carcasses.

Keywords: Salmonella, recovery methodology whole broilers, Salmonella incidence
Simmons et al. (7) reported that 34% of retail broilers sampled using a whole carcass enrichment method were Salmonella-positive. This incidence was higher than the Salmonella incidence of 10.4% from broiler carcasses sampled in processing plants reported by United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) (3). However, the results were consistent with several other references where Salmonella incidence was determined in retail poultry products (7). Simmons et al (7) suggested that the difference in results with USDA-FSIS may have been due to sampling methodology, or postmortem sampling time and location (in-plant sampling versus retail sampling).

In the baseline study reported by USDA-FSIS (1) the Salmonella positive carcasses, approximately 20% of those sampled, were subjected to an MPN estimate of Salmonella numbers. FSIS reported approximately 42% of the Salmonella-positive carcasses had less than 12 cells per carcass and that 45% had less than 120 cells per carcass. Thus approximately 87% of the Salmonella-positive carcasses had less than 120 cells per carcass.

It has been previously reported that salmonellae occurring in low numbers are difficult to detect (5, 8). The official USDA-FSIS Salmonella sampling procedure is based on rinsing the whole carcass with 400ml of water, from which a 30ml aliquot is used for Salmonella recovery (2). Based on the aliquot dilution (30/400 or 7.5%) and the expected low number of Salmonella cells per carcass, the current USDA-FSIS sampling may well be underestimating the true incidence of Salmonella-positive carcasses.

The purpose of this experiment was to determine the effect of sampling method on Salmonella recovery from the same carcass. This experiment is based on the paired recovery of
Salmonella from individual carcasses using both the official FSIS aliquot method and the whole carcass enrichment procedure reported by Simmons et al. (7).

**MATERIALS AND METHODS**

**Sample Collection**

Twenty whole, fresh broiler carcasses were purchased each week from grocery stores in Northeast Georgia over a 5-week period. Only carcasses with intact packages were selected. All carcasses were purchased on a Monday to ensure that birds were at least 3 days post slaughter. Carcasses were selected at random from four grocery stores without regard to plant, brand or sell-by-date.

**Isolation Procedures**

On the day of purchase, the exterior of each package was swabbed with 100% ethanol and opened with a sterile scalpel. Using a fresh pair of sterile gloves for each carcass, the giblets were removed and discarded. The entire carcasses and package exudate were transferred to a sterile 16x16 polyethylene bags containing 400ml of buffered peptone water\(^6\). The carcasses within the bags were then vigorously shaken for 1 minute, and then a 30 ml aliquot was removed (Aliquot). Immediately, an additional 130ml of buffered peptone was added to the bag with the same carcass, bringing the volume to 500 ml, after which the carcass was again shaken and the carcass and rinse solution kept together in the rinse bag as described by Simmons et al. 2002. Both the Aliquot and whole carcass enrichment (WCE) samples were incubated for 24 h at 37 C.

\(^6\)Becton Dickinson, Sparks, Maryland 21152
After incubation, from both th Aliquot and WCE sample, 0.5ml of incubated rinse solution was transferred to 10ml Rappaprt-Vassiliadis broth and also to 10ml TT (Hajna) and incubated at 42 C for 24h. Each broth was then streaked onto BG sulfa agar (BGS) and modified lysine iron agar (MLIA) plates, and incubated for 24hr at 35 C. Suspect *Salmonella* colonies were picked and used to inoculate triple sugar iron (TSI) and lysine iron agar (LIA) slants and incubated for 24h at 35 C. Presumptive positives were confirmed using Poly O and Poly H serological agglutination tests.

**Statistical Analyses**

To test for differences in frequency of *Salmonella*-positive carcasses between the two methods, a chi-square test was performed using the GENMOD procedure of SAS, with a binomial distribution and logit link function (6).

**RESULTS AND DISCUSSION**

Weekly recovery ranged from 0/20 to 4/20 for the Aliquot method, and 4/20 to 10/20 for WCE method (TABLE 2.1). Over the five week period, the Aliquot samples had 13 % *Salmonella*-positive carcasses compared to 38 % for WCE samples, from the same carcasses. This difference was significant (p-value <.0001).

All of the carcasses found to be positive using the Aliquot sampling method were found to be positive using the whole carcass enrichment method, except for 1 carcass in week 4. It is

7Oxoid, Basinstoke, Hampshire RG24 8PW , UK

8Microgen, Camberley, Surrey GU15 3DT, UK
conceivable that the 30ml aliquot removed the small number of *Salmonella* that may have been on the carcass.

These results are consistent with those obtained by Surkiwiez et al. (9) who reported that incubation of 270ml out of 300ml lactose broth used to rinse poultry, as opposed to a 10 ml aliquot was four times more effective in recovering *Salmonella*. The sensitivity of the whole carcass enrichment procedure was reported in a study by Cox and Blankenship (4) in which as few as 8 inoculated cells could be recovered from a carcass.

The results from the whole carcass enrichment method are consistent with those obtained by Simmons et al. 2001(34%). Also the results from the aliquot method are closer to those reported by FSIS (10.4%). The sampling location (retail level vs plant) may cause these results to be slightly higher than those reported by FSIS.

This study showed that methodology has an effect on *Salmonella* recovery when expected numbers of *Salmonella* per carcass may be low. It also showed that the difference in the results of Simmons et al. (7) and FSIS (3) is in part due to sampling methodology. The current carcass sampling methodology recommended by FSIS may be underestimating the incidence of *Salmonella* on poultry, especially if current industry practices are reducing the number of *Salmonella* per carcass. Cox and Blankenship (4) reported that there was no difference in *Salmonella* incidence when comparing the incubation of a 500ml rinse to a whole carcass incubation in 500ml of lactose broth. Therefore, one solution, not as extreme as whole carcass enrichment, would be to incubate all of the rinse solution instead of just the 30ml aliquot.
Acknowledgements

This study was supported in part by state and Hatch funds allocated to the Georgia Agricultural Experiment Station. The authors express their appreciation to Nicole Bartenfeld, Mark Freeman, David McNeal and Lynda Jones for technical assistance.
REFERENCES


TABLE 2.1: Recovery of *Salmonella* from whole broiler carcasses using a 30ml aliquot following a 400 ml buffered peptone rinse (Aliquot) or a 500ml buffered peptone rinse and incubation of carcass and rinse together (WCE) over a 5-week period, analyzed using Chi-square ($\chi^2$) and p by week and for the entire experiment.

<table>
<thead>
<tr>
<th>Week</th>
<th>Aliquot</th>
<th>WCE</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/20</td>
<td>9/20</td>
<td>2.9</td>
<td>0.0914</td>
</tr>
<tr>
<td>2</td>
<td>3/20</td>
<td>10/20</td>
<td>5.6</td>
<td>0.0181</td>
</tr>
<tr>
<td>3</td>
<td>0/20</td>
<td>8/20</td>
<td>10.0</td>
<td>0.0016</td>
</tr>
<tr>
<td>4</td>
<td>4/20</td>
<td>7/20</td>
<td>1.1</td>
<td>0.2881</td>
</tr>
<tr>
<td>5</td>
<td>2/20</td>
<td>4/20</td>
<td>0.8</td>
<td>0.3758</td>
</tr>
<tr>
<td>Total</td>
<td>13/100</td>
<td>38/100</td>
<td>21.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
SUMMARY

Two experiments were conducted to determine the influence of methodology on the recovery of *Salmonella* on retail chicken. The first experiment was designed to determine if there was a difference in the incidence of *Salmonella*-positive carcasses reported by USDA-FSIS and the incidence of *Salmonella*-positive carcasses at the retail level using an exhaustive procedure. The second experiment was conducted to determine the effects of sampling methodology on the recovery of *Salmonella* from whole carcasses.

In the first study 85 of the 251, or 33.9 %, of the retail carcasses were *Salmonella*-positive. This is higher than both the 20 % from the 1994-1995 baseline and the 10.4 % reported by USDA-FSIS in 1998. The incidence determined in the first study may have been higher due to methodology, different sampling location (retail level versus processing plant), post-process contamination, or the presence of giblets. The results do indicate that a whole carcass enrichment may be more sensitive for detecting *Salmonella*-positive when low numbers of *Salmonella* per carcass are expected.

In the second study the significantly more (p<0.0001) carcasses were found *Salmonella*-positive using whole carcass enrichment method than using the 30ml aliquot method recommended by USDA-FSIS. Also the incidence of *Salmonella*-positive carcasses determined by the whole carcass enrichment method (38 %) is comparable to the incidence determined in the first experiment (33.9 %), and the incidence determined by the Aliquot method (13%) was comparable to the incidence reported by USDA-FSIS in 1998 (10.4 %). Thus showing
methodology does have an effect on *Salmonella* recovery when low numbers of *Salmonella* per carcass are expected.

The results from these two experiments indicate further research is necessary to determine the effects of sampling methodology on *Salmonella* recovery. Also future research should be conducted to determine the actual number of *Salmonella* cells present per carcass.