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Foodborne pathogens in poultry production and post-harvest control

Rajesh R. Nayak
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Foodborne Pathogens in Poultry Production and Post-harvest Control

Rajesh R. Nayak

**Dissertation submitted to the
College of Agriculture, Forestry, and Consumer Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Doctor of Philosophy
In
Animal and Food Sciences**

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Division of Animal and Veterinary Sciences

**Morgantown, West Virginia
2000**

Keywords: *Salmonella*, zinc chloride, *E. coli*, poultry, foodborne, pathogens
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ABSTRACT

Foodborne pathogens in poultry production and post-harvest control

Rajesh R. Nayak

A comprehensive ecological survey was conducted from April 1997 to June 1999 on four turkey flocks (F5-F8) to identify the preharvest sources of *Salmonella* colonization. Generic *E. coli* and total coliforms were enumerated as indicators of fecal contamination in F7 and F8. Turkey cecal and crop contents, litter, drinkers, air, feed, feeder contents, and environmental swabs were sampled and tested for *Salmonella* and *E. coli*. *Salmonella* was isolated from 13% of litter, 11% of turkey ceca, 10% of drinkers, and 5% of swabs. *Escherichia coli* and total coliforms were detected in 45 and 53% of samples, respectively. *Salmonella heidelberg* was the major serotype isolated from the sampled flocks. About 25% of the *Salmonella* isolates were resistant to antibiotic(s). Identifying preharvest sources of *Salmonella* and *E. coli* colonization would assist integrators and producers in designing hazard analysis and critical control point (HACCP) protocols. On-farm reduction of these pathogens will assist processors in reducing positive carcasses at the plant.

A skin attachment model was used to examine the ability of $ZnCl_2$ to reverse or inhibit *Salmonella* attachment to broiler skin. In reversal experiments, skin samples were first treated with 1 mL of *S. typhimurium* culture (10^8 CFU/mL) for 30 min, followed by the addition of 1 mL of 25 or 50 mM $ZnCl_2$ for 5 or 15 min. For inhibition experiments, this order was reversed. "Firmly" and "loosely" attached salmonellae were enumerated on the skin. Treated skin samples were observed under a scanning electron microscope. In reversal experiments, 25 and 50 mM $ZnCl_2$ reduced ($p < 0.01$) "firmly" attached cells by 77 and 89%, respectively, when compared to the control (water). At 25 and 50 mM concentration, $ZnCl_2$ reduced ($p < 0.0001$) cells in the "discard" by 99.4 and 99.9%, respectively. Micrographs indicated that 25 and 50 mM $ZnCl_2$ reduced ($p < 0.1$) *Salmonella* attachment by 69 and 99.9%, respectively, in the reversal experiments. In the inhibition experiments, 25 and 50 mM $ZnCl_2$ reduced ($p < 0.01$) "firmly" attached cells by 82 and 91%, respectively. Reduction of *Salmonella* may be attributed, in part, to the bactericidal activity of $ZnCl_2$ in addition to detachment of the bacterial cells on skin.

*Dedicated to my parents
and in the loving memory of Amma, Attya and Chacha*

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INTRODUCTION

Fresh and processed foods have been implicated in transmitting more than 200 diseases among humans (Mead et al., 1999). The Center for Disease Control (CDC) and Prevention estimates that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead et al., 1999). Of these, nearly 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths are caused by known pathogens. Bacteria such as *Campylobacter*, *Salmonella* (non-typhoid), *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Vibrio* spp. have been implicated in several foodborne outbreaks. In addition, parasites such as *Cryptosporidium parvum*, *Cyclospora*, *Giardia lamblia*, *Toxoplasma gondii*, and *Trichinella spiralis*, and viruses such as Hepatitis A and Norwalk-like viruses have been implicated in food-related outbreaks (Mead et al., 1999).

Foodborne diseases cause more illnesses but fewer deaths than previously estimated. Outbreaks have been attributed to factors such as demographics and lifestyles, human behavior, changes in industry and technology, changes in travel and commerce, the shift towards global economy, microbial adaptation, breakdown in the public health infrastructure, and the lack of knowledge on food safety and handling practices (Knabel, 1995; Altekruze et al., 1997; Hall, 1997). Foodborne illnesses have incurred medical and productivity costs of \$8 to 23 billion/year to the U.S. economy (Hedberg et al., 1994). Addressing emerging foodborne diseases will require sensitive and rapid surveillance, enhanced laboratory methods of identification and subtyping, and effective prevention and control of pathogens.

The CDC report estimates 1.3 million cases of non-typhoidal *Salmonella*-related illnessness (9.7% of the total foodborne illnesses), 15,600 hospitalizations (25.6% of the total), and 553 deaths (30.6% of the total) in the U.S. each year (Mead et al., 1999). Food vehicles

associated with these outbreaks include chicken, turkey, beef, pork, eggs, milk, and dairy products, (Tietjen and Fung, 1995). Poultry consumption has increased dramatically in the U.S. over last the two decades. In 1993, over 7.5 billion fertile eggs were hatched and about 6.5 billion broilers were produced in the U.S. (NACMCF, 1997). In 1991, broiler consumption per person was 29 kg and turkey and ready-to-cook chicken was 9 kg, a total of 38 kg of poultry consumed per person. Increased poultry consumption has increased poultry-associated foodborne diseases, particularly salmonellosis, caused by *Salmonella*.

Contamination of poultry with *Salmonella* occurs prior to entry into the processing facility and by cross-contamination in the processing plants. It is difficult to improve the microbiological quality of chickens in the processing areas. Several physical and chemical decontamination procedures have been applied to poultry carcasses in an attempt to reduce and/or eliminate *Salmonella* contamination. However, these procedures have not been completely successful in eliminating *Salmonella*. Hence, it is necessary to develop new methods to eliminate the pathogen from poultry carcasses. In order to achieve this objective, a clearer understanding of the mechanism of *Salmonella* attachment to poultry skin/tissue is necessary. To achieve a significant reduction in the level of *Salmonella* contamination among broiler carcasses, *Salmonella*-free chickens must be delivered at the processing plants. A reduction of *Salmonella* on poultry carcasses necessitates comprehensive control at the farm where birds are brooded and raised before shipment to processing plants. Breeder flocks, hatcheries, contaminated feed and water, and environmental sources/vectors such as litter, humans, and insects are potential pre-harvest sources of *Salmonella* contamination in poultry (Jones et al., 1991; Bailey, 1993; Hoover et al., 1997).

Knowledge of key preharvest factors responsible for *Salmonella* colonization would help integrators and poultry producers in defining critical, preharvest control points on the farm. Intervention at these points could reduce or eliminate horizontal transmission and *Salmonella*-positive birds arriving at the processing plants. Treatment of carcasses with novel chemicals can be useful in reducing the level and frequency of *Salmonella* on poultry carcasses at the processing plants. A combination of pre- and post-harvest intervention strategies can ultimately deliver *Salmonella*-free, fresh poultry to consumers, thereby significantly reducing the medical and productivity costs associated with salmonellosis.

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REVIEW OF LITERATURE

***Salmonella* Profile**

Characteristics

Salmonella spp. are facultative anaerobic, gram-negative rods belonging to the family *Enterobacteriaceae* (D'Aoust, 1997). Most members of this genus are motile with peritrichous flagella, except *S. pullorum*, *S. gallinarum*, and *S. singapore* which possess dysfunctional flagella. Salmonellae are chemoorganotrophic and can metabolize nutrients by respiratory and fermentative pathways. These organisms grow optimally at 37°C (range 2 to 54°C), pH 6.7 to 7.5 (range 4.5 to 9.5), and water activity ≥ 0.93 . They can catabolize D-glucose and other carbohydrates producing acid and gas. Salmonellae are oxidase negative and catalase positive, grow on citrate as the sole carbon source, produce hydrogen sulfide, decarboxylates lysine and ornithine, and do not hydrolyze urea. These traits have been useful in the biochemical identification of this organism. *Salmonella* produces acid and gas from glucose in triple sugar iron (TSI) agar medium and does not utilize lactose or sucrose in TSI or in different plating media such as brilliant green, xylose lysine deoxycholate, xylose lysine tergitol, and Hektoen enteric agars. In addition, typical salmonellae produce an alkaline reaction from decarboxylation of lysine to cadaverine in lysine iron agar (LIA) and generate hydrogen sulfide gas in TSI and LIA media.

Over 2,000 serotypes of *Salmonella* have been identified (D'Aoust, 1997). Prevalence of *Salmonella* species as a biochemically homogeneous group of microorganisms has been rapidly diminishing. Biochemical identification of *Salmonella* isolates is coupled with serological confirmation involving agglutination of bacterial surface antigens with *Salmonella*-specific antibodies. These include O lipopolysaccharides (LPS) on the external surface of the bacterial

outer membrane, H antigens associated with the peritrichous flagella, and the capsular (Vi) antigens found only in *S. typhi*, *S. paratyphi C*, and *S. dublin* (Le Minor, 1981).

Reservoirs

Salmonella is ubiquitous in the natural environment, residing mostly in the gastrointestinal tract of warm and cold-blooded animals. Sources of infection include contaminated soil, vegetation, water, and components of animal feeds such as bone, meat and fish meal, particularly those containing milk-, meat-, or egg-derived constituents. Egg and poultry products remain the principal reservoir of *Salmonella*. In addition, beef, pork, and mutton are potential vehicles of *Salmonella* transmission (D'Aoust, 1997). Persistence of *Salmonella* in the poultry, beef, and pork industries originates from exposure of livestock to environmental sources of contamination, contaminated feeds, and by parental transmission.

Disease characteristics and treatment

Salmonella infections among humans can lead to several clinical symptoms such as enteric (typhoid) fever, uncomplicated enterocolitis, and systemic infections by non-typhoid microorganisms. The disease is usually self-limiting but may become systemic in young children and immunocompromised adults. Enteric fever can range from 7 to 28 days and may include diarrhea, prolonged or spiking fever, abdominal pain, and headache (D'Aoust, 1991). Treatments include supportive therapy and/or use of chloramphenicol, ampicillin or trimethoprim-sulfamethoxazole to eliminate the systemic infection. Non-typhoidal *Salmonella* results in enterocolitis that appears 8 to 72 h after contact with the invasive pathogen. Symptoms include abdominal pain and non-bloody diarrhea. Supportive therapy (fluid and electrolyte replacement) has been recommended for clinical cases of enterocolitis. *Salmonella*-induced chronic conditions such as aseptic reactive arthritis, Reiter's syndrome, and spondylitis have

been reported (D'Aoust, 1997). Live attenuated vaccines induce strong and durable humoral and cell-mediated immunity.

Infectious dose (ID)

The ID varies with the immune status of an individual. Newborns, infants, elderly, and immunocompromised individuals are more susceptible to *Salmonella* infections than are healthy adults. Generally, a large number of cells (10^6 - 10^{11}) are required to cause an infection.

However, studies have shown that 1-10 cells in cheddar cheese and chocolate can constitute a human ID (D'Aoust et al., 1985; Kapperud et al., 1990). High fat content has been the common denominator associated with low ID. Entrapment of *Salmonella* within hydrophobic lipid micelles may protect against bactericidal action of gastric acids (D'Aoust, 1997).

Pathogenicity and virulence factors

The ability of *Salmonella* to cause human infection depends on the ability of the organism to attach (colonize) and enter (invade) intestinal columnar epithelial cells (enterocytes) and specialized M cells overlying Peyer's patches. *Salmonella* competes with the indigenous gut microflora for suitable attachment sites on the luminal surface of the intestinal cell wall and evades capture by secretory immunoglobulin A present on the surface of epithelial cells (D'Aoust, 1997). *Salmonella* attaches to the enterocytes by type 1 (mannose-sensitive) or type 3 (mannose-resistant) fimbriae, surface adhesions, nonfimbriate (mannose-resistant) hemagglutinins or enterocyte-induced polypeptides with host (human) glycoprotein receptors located on the microvilli or glycocalyx of the intestinal surface (D'Aoust, 1991; Polotsky et al., 1994).

Following attachment, *Salmonella* can produce one or more virulence factors that cause infection (Table 1).

Colonization of *Salmonella* in Poultry

Avian salmonellosis

Salmonella pullorum and *S. gallinarum* are responsible for pullorum disease and fowl typhoid, respectively. Both diseases are widespread, and unless precautionary measures are implemented to control the spread, mortality is high. Pullorum disease infects the ova of turkey and chickens and can be responsible for the vertical transmission of *Salmonella*. The embryo is infected when the egg is hatched, and the disease can spread by horizontal transmission in the hatchery. An acute septicemia may be responsible for high mortality. Treatment with antimicrobial agents (sulfonamides) reduces mortality in infected birds. Fowl typhoid is a septicemic or chronic disease similar to pullorum, except that mortality from typhoid occurs at any age (North and Bell, 1990). Although both diseases have been successfully eradicated in the U.S., other *Salmonella* serotypes have been responsible for causing salmonellosis in humans. A commensal relationship between *Salmonella*, excluding *S. pullorum* and *S. gallinarum*, and host (bird) makes it difficult to prevent colonization of this organism in the gastrointestinal tract (Bailey, 1988). Because the bird's health is not compromised by the organism, it would be accurate to report that chickens or turkeys are colonized, and not infected, with *Salmonella*.

Sources and transmission

There are several potential sources of *Salmonella* contamination in an integrated poultry operation (Hoover et al., 1997; Holder, 1998). Bird ceca, large intestine, and crop are the primary sites of *Salmonella* colonization (Fanelli et al., 1971; Snoeyenbos et al., 1982; McMeekin et al., 1984; Corrier et al., 1991; Bryan and Doyle, 1995; Hargis et al., 1995). Several factors affect the susceptibility of poultry to *Salmonella* colonization (Bailey, 1987). These factors include: (1) age of birds; (2) *Salmonella* serotype and initial challenge dose level;

(3) stress – environmental, transport, and overt or subclinical disease; (4) feed additives – antimicrobial and anticoccidials; (5) survival through gastric barrier; (6) competition with gut microflora; (7) location of a hospitable colonization site; and (8) host genetic background.

Day-old birds: Day-old chicks and poults are more susceptible to *Salmonella* colonization than are older birds. Milner and Shaffer (1952) demonstrated that 50% of day-old chicks were infected with an oral challenge of 10 cells of *S. typhimurium*. By day 14, however, a challenge dose of a million cells infected only 10% birds, indicating a marked increase in resistance with age. Mortality is normally high among chickens and turkeys during the first two weeks of the grow-out period (Williams, 1984). As birds age (2 weeks post-hatch), they offer greater resistance to natural *Salmonella* challenge due to development of a complex gut microflora (Barnes et al., 1972; 1980). Once the birds are colonized with *Salmonella* they become carriers and are capable of horizontally and vertically transmitting the organism within the flock and to other flocks as well. The severity of transmission will depend on the duration of exposure, sanitary conditions, and presence of other infectious agents in the breeding houses, hatcheries, and grow-out facilities.

Hatcheries and breeder flocks: In an integrated poultry operation, the hatchery takes eggs from broiler-breeder and distributes chicks to grow-out facilities. Thus, if one *Salmonella*-positive breeding flock exists, it could spread easily throughout the entire operation. *Salmonella* transmission may be compounded within the operation if poor hatchery management allows the organism to backtrack and colonize other breeding farms that supply eggs. Studies have shown that *Salmonella* serotypes found on fully processed broiler carcasses originate predominately from hatcheries and breeder flocks (Bhatia and McNabb, 1980; Lahellec and Colin, 1985).

The U.S. poultry industry processes over 8 billion broiler-hatching eggs each year through commercial hatcheries (Cox, 1998). *Salmonella* rapidly penetrates the shell and membranes of a freshly laid egg and enters the interior of the egg via pores (Mayes and Takeballi, 1983). Existing conditions during incubation tend to favor the proliferation of these bacteria. Invading *Salmonella* do not cause extensive decomposition of the fertile egg and the chick hatches (Cason et al., 1994), resulting in the establishment of salmonellae reservoirs in commercial broiler operations and breeder hatcheries (Cox et al., 1990; 1991; Blankenship et al., 1993). Transmission of *Salmonella* has also been reported through direct ovarian shedding of the organism into the egg prior to addition of the cell membrane (Mayes and Takeballi, 1983). Ovarian contamination occurs when *Salmonella* passes from the alimentary canal to the ovaries via blood (Gordon and Tucker, 1965). Once *Salmonella* gets past the egg membranes, there are no effective means to eliminate or prevent further invasion into the egg and developing embryo (Cason et al., 1994). Entrapped *Salmonella* can be ingested by the chick as it emerges from the egg, and during hatching, can horizontally transmit *Salmonella* to other chicks. Bailey and coworkers (1992) demonstrated that a single egg contaminated with *Salmonella* may contaminate all eggs and newly hatched birds within a hatching cabinet.

Feed: Feed is a major source of *Salmonella* contamination in poultry flocks (Snoeyenbos et al., 1970; Dougherty, 1976; Cox et al., 1983; Hoover et al., 1997; McIlroy, 1998). Feed and feed ingredients such as animal proteins/by-products and vegetable proteins can provide an environment of suitable nutrients, moisture, pH, and temperature for survival of *Salmonella*. Feed ingredients of animal (red meat) and vegetable origin, fishmeal, and poultry by-product meal are the major sources of feed contamination (Morris et al., 1969; Simmons and Byrnes, 1972; Cox et al., 1983; Rouse et al., 1988; Smith et al., 1989; Jones et al., 1991). Inadequate

time-temperature control during pelleting, improper storage, and contamination during transportation can contribute to feed contamination with *Salmonella* prior to entering poultry houses (McCapes and Riemann, 1998). Feeder contents are also responsible for horizontal transmission of *Salmonella* within the poultry facilities (Dougherty, 1976; Hacking et al., 1978; Lahellec and Colin, 1985; Hoover et al., 1997).

Salmonella contamination of poultry feed can be controlled by consistently and effectively decontaminating feed and preventing recontamination at the production facilities. Control measures would include purchasing *Salmonella*-free feed ingredients and implementing stringent sanitary measures during feed production and transportation (Hoover, 1996). Organic acids (propionic and formic) and other chemicals (aldehydes, natural terpenes, and surfactants) control *Salmonella* recontamination in poultry feed (McIlroy, 1998). Feed pasteurization (65-70°C) can be effective, but recontamination must be prevented in the post-heat manufacturing environment of the production facility. Irradiation of finished feed would be a promising decontamination technology for future consideration.

Litter: Poultry litter is a vector of *Salmonella* transmission in the grow-out facilities. Frequency and *Salmonella* serotype information on chicken carcasses can be assessed by examination of litter within poultry houses (Simmons and Byrnes, 1972). Litter can be contaminated by droppings of infected birds. Once the bird sheds *Salmonella*, litter can harbor the organism from a few days to several weeks. Litter, contaminated with *Salmonella*, can serve as a reliable indicator of flock contamination (Snoeyenbos et al., 1967; 1969; Olesiuk et al., 1969; Bhatia et al., 1979). *Salmonella* populations in litter are transient and heavy contamination would depend on recontamination from the intestine of the colonized bird. Fanelli and

coworkers (1970) reported that recycling of *Salmonella* between litter and birds appears to maintain intestinal colonization.

Eventually, litter develops a *Salmonella* inhibitory effect (Tucker, 1967; Fanelli et al., 1970; Bhatia et al., 1979). Bactericidal activity of old litter was associated with a rapid increase in pH and ammonia compared to fresh litter (Snoeyenbos et al., 1967; Turnbull and Snoeyenbos, 1973; Opara et al., 1992). Older litter may assist young birds in development of complex gut microflora, excluding *Salmonella* by competition (Fanelli et al., 1970; Hoover, 1996). Several serotypes such as *S. pullorum*, *S. gallinarum*, *S. typhimurium*, and *S. infantis* were susceptible to the bactericidal effects of used litter (Botts et al., 1952; Snoeyenbos et al., 1967; Tucker, 1967; Fanelli et al., 1970; Olesiuk et al., 1971).

Water: Water is an important source/vector of *Salmonella* transmission (Gauger and Greaves, 1946; Bryan et al., 1968; Higgins et al., 1982; Morris et al., 1969; Stersky et al., 1981). Fecal contamination of drinking water by *Salmonella*-infected birds could result in rapid contamination of the poultry house. Water can be initially contaminated with *Salmonella* by bird feces, litter, feed, and dust or by residual contamination associated with drinkers (Poppe et al., 1986; Morgan-Jones, 1980). High levels of *Salmonella* (10^4 CFU/mL) have been found in the drinking water of young (< 1 week of age) poults and chicks (Poppe et al., 1986). Birds could consume 10^5 salmonellae per day re-infecting themselves continuously. Lahellec and Colin (1985) found that 18% of drinker samples were contaminated with *Salmonella* in broiler flocks, and Hoover and coworkers (1997) reported that 64% of drinkers were *Salmonella*-positive in turkey flocks. Addition of chlorine and sanitizer to drinking water reduced *Salmonella* in broilers (Al-Chalaby et al., 1985; Poppe et al., 1986). Type of drinkers used in broiler

production influenced the level of free available chlorine in chlorinated water and thus, the levels of *Salmonella* (Poppe et al., 1986).

Pests: Rodents, insects, birds, and wild animals have been implicated as sources and vectors of *Salmonella* in poultry houses (Jones and Twigg, 1976; Keymer et al., 1991; Nagaraja et al., 1991; Henzler and Opitz, 1992; Hoover, 1996; Amick-Morris, 1998; Wray and Davies, 1998). Rats and mice are the major sources/vectors of *Salmonella*, and they can act as carriers after consuming infected chicken manure. Mice can transmit paratyphoid infections in poultry (Nagaraja et al., 1991). Mice usually stay within close proximity to feed and nesting places and can carry *Salmonella* within the poultry facility. Rats, on the other hand, travel long distances and may transmit *Salmonella* from farm to farm. *Salmonella* transmission may be amplified by mice or rats defecation in feed troughs and floor beds and on egg collection belts.

Contamination can spread throughout the poultry house by automated feeding, egg conveyors, and manure removal equipment (Wray and Davies, 1998). Infected wild birds spread *Salmonella* on farms and pastures where domestic animals may acquire infections. Infected seagulls transmit *Salmonella* to poultry houses (Reilly et al., 1981). Davies and Wray (1994) detected *Salmonella* in several species of wild birds near hatcheries and poultry processing units, and these birds could contaminate equipment left outside buildings. Darkling beetles, maggots, blowfly larvae, cockroaches, flies, and mealworm beetles have been implicated in mechanical transport of *Salmonella* in poultry houses (Greenburg et al., 1970; Jones et al., 1991; Baggesen et al., 1992; Bennett, 1993; Rivault et al., 1993; Davies and Wray, 1995; Amick-Morris, 1998; Wray and Davies, 1998).

Environmental sources: Transmission of *Salmonella* within poultry houses may result from air and dust particles (Baskerville et al., 1992). Movement of personnel and birds, flapping

of wings, and improper ventilation can spread *Salmonella* and other microorganisms in poultry houses (Jones et al., 1984). Wathes and coworkers (1988) reported that transmission occurs by inhalation and ingestion of contaminated airborne particles. Reports indicate that *Salmonella* can be isolated from organs of chicks, hens, mice, and calves after exposing them to aerosols containing the organism (Clemmer et al., 1960; Wathes et al., 1988; Baskerville et al., 1992). Dust in the poultry houses originates from feed, litter, feces, and birds. Wray and Davies (1998) reported that dust particles within selected poultry houses were contaminated (8.5 to 220.4x10² CFU/L air) with microorganisms such as species of *Streptococcus*, *Staphylococcus*, and *Salmonella*.

Other parameters: Management and husbandry practices, transportation, and uncontrollable environmental conditions such as temperature may stress poultry. This stress, in turn, could increase the potential for bird colonization (Bailey, 1988). Thaxton and coworkers (1971) found that day-old chicks reared at 29, 24, and 21°C increased mortality by 0, 28, and 78%, respectively when infected with *S. paratyphi*. Soerjadi and coworkers (1979) found that day-old chicks held at 18 to 22°C were more susceptible to low and moderate challenges of *S. typhimurim* than chicks challenged and held at 32 to 36°C. Broilers withdrawn from feed for several hours before slaughter, crowded into transport coups, deprived of water, and subjected to hot and cold environmental temperatures during transportation could shed *Salmonella* and thus, cross-contaminate birds prior to entry at the slaughter facility (Bailey, 1988).

Control of *Salmonella* During Live Production

The complexity of *Salmonella* colonization of poultry will require a multi-faceted, integrated approach to successfully reduce the incidences of this pathogen. An overview of the

movement of *Salmonella* through the poultry chain and possible means of control during each step of production is illustrated in Fig. 1 (McCapes et al., 1991).

Biosecurity and disinfection

A comprehensive biosecurity program should consist of guidelines for location of farms and facilities (conceptual biosecurity), farm and fence layout (structural biosecurity), and movement of personnel and equipment, manufacture and distribution of feed, water sanitation, rodent and pest control, cleaning and disinfection procedures, monitoring procedures and disease surveillance (operational biosecurity) (Rosales and Jensen, 1998). These guidelines should be simple, cost effective, and reviewed periodically. Protocols should include (1) procedures for poultry house cleaning and disinfection (Appendix, Chapter 1); (2) requirements for decontamination of buildings before and after placement of each flock; (3) requirements for employment and contract growing; (4) restrictions to avoid contact with avian and animal species; (5) requirements for personnel hygiene and health status; (6) disposal procedures for dead birds and manure; (7) source verification of litter suppliers; and (8) steps for decontamination of transport vehicles.

Salmonellosis is a “management” disease since its control depends on controlling sources of *Salmonella* contamination and transmission. Producers must purchase day-old breeding stock from companies with stringent biosecurity practices and participation in programs approved by the National Poultry Improvement Program (NPIP). Poultry feed should be purchased from companies that can deliver *Salmonella*-free feed. Feed mills must implement effective control programs for raw material receipt, storage, grinding and mixing, pelleting, crumbling, cooling, storage and during transportation of finished feed. Feed should be adequately processed (82-

85°C for 10-12 min) in pre-pelleting feed conditioners. Hazard analysis and critical control point (HACCP) protocols may be introduced in feed mills to reduce *Salmonella*.

A biosecurity program to reduce rodent access to feed and water within poultry houses should include appropriate house management and sanitation, professional inspections for rodent baiting and trapping, rodent proofing the facility, and eliminating grass, shrubs, broken equipment, and burrows inside and outside the facility. Feed spills should be removed regularly to avoid attracting wildlife. Insects can be controlled by maintaining stringent sanitary practices at the facility and through careful use of pesticides. Drinking water should be chlorinated (3-5 ppm residual chlorine) to prevent *Salmonella* transmission.

An effective biosecurity program can never be “set in stone,” and must be continuously improved through revisions, updates, and periodic audits by federal agencies and other professionals. Workshops on biosecurity and sanitation protocols must be organized to educate managers, flock supervisors, contract growers, farm employees, vaccination and monitoring crews, veterinarians, and others involved with poultry operations. A biosecurity program should be complemented by the use of antibiotics, competitive exclusion products, feed additives to prevent recontamination, litter and water treatments, and vaccination.

Use of Antibiotics

Antibiotics are added to poultry feed or water (100-200 ppm) for treatment of bacterial diseases (Stavric and D’Aoust, 1993). Williams (1985) reported that neomycin or oxytetracycline, alone and in combination, reduced the level and frequency of *S. typhimurium* in the intestines of broilers. On the other hand, feeding antibiotics such as a combination of lincomycin and ovaparacin, nitrofurazone, and a combination of nitrofurazone and novobiocin

enhanced colonization of *S. typhimurium*, *S. infantis*, and *S. enteritidis*, respectively, in chickens (Glisson, 1998).

Widespread use of antibiotics in the poultry industry have resulted in the emergence of antibiotic resistant *Salmonella* isolates from farm animals and meat-derived products (Novick, 1981; Holmberg et al., 1984; Spika et al., 1987; Pacer et al. 1989; D'Aoust, 1997). Gast and Stevens (1988) found that administering kanamycin to chickens resulted in increased kanamycin-resistant strains of *S. arizonae*. *Salmonella typhimurium* DT 104 has attracted attention from public health authorities in the U.S. due to several reported outbreaks associated with this strain (Anon, 1997; Benson and Munro, 1997). This serotype is resistant to a wide range of antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracyclines, trimethoprim, and ciprofloxacin (Threfall et al., 1997). *Salmonella typhimurium* DT 104 can withstand food-processing conditions such as acids, drying, preservatives, and disinfectants, when compared to other *Salmonella* serotypes. Human infections of DT 104 have been associated with eating contaminated meat products. Controlling multi-drug resistant *Salmonella* would require avoidance of unnecessary antibiotic usage by the poultry industry.

Competitive exclusion (CE)

A promising approach to reduce *Salmonella* in live birds is CE. This approach, the “Nurmi Concept,” was first reported by Nurmi and Rantala (1973). Pivnick and Nurmi (1982) summarized the CE concept as follows: (i) newly hatched chicks may be colonized by a single cell of *Salmonella*; (ii) older birds are more resistant to *Salmonella* colonization because of a mature, complex gut microflora; and (iii) introduction of pathogen-free intestinal/cecal contents or scrapings from a healthy, adult bird into a day-old chick speeds up maturation of gut microflora. This mature microflora increases the resistance of most chicks to *Salmonella*

colonization. Studies have conclusively shown that CE is an effective approach to *Salmonella* control (Snoeyenbos et al., 1978; Pivnick et al., 1981; Bailey, 1988; 1993). Competitive exclusion cultures may exclude *Salmonella* by (1) competing for attachment sites and nutrients in the intestine; (2) excreting metabolic products such as bacteriocins, volatile fatty acids, lactic acid, and hydrogen sulfide that are toxic to *Salmonella*; and/or (3) creating an intestinal environment conducive to the growth of obligate anaerobes. The exact mechanism(s) of action remains unknown.

Two types of CE cultures, undefined (bacterial composition unknown) and defined (known bacterial composition) are used (Stavric and D'Aoust, 1993; Mead, 1998). Studies have been carried out during the past decade to determine the laboratory and field efficacy of undefined and defined CE cultures. Undefined CE cultures offered protection to chicks when challenged with a standard dose of *Salmonella* under laboratory conditions (Stavric and D'Aoust, 1993; Nisbet et al., 1996; Palmu and Camelin, 1997; Carrier et al., 1998; Fukata et al., 1999; Hume et al., 1998). However, field study results have been variable. Protective capacity of undefined cultures is influenced by source of microflora, culture conditions, methods of administration, presence of feed additives, in-laboratory or natural environmental *Salmonella* challenge, and hygienic practices on the farm (Bailey, 1987; Savage, 1987). Formulation of undefined cultures has been difficult due to insufficient knowledge of microbial ecology, isolation/detection techniques, underlying protective mechanisms, and host (bird)-microbe and microbe-microbe interactions in the GI tract. Defined cultures are less effective than undefined cultures under laboratory conditions and afford little protection against *Salmonella* colonization (Stavric, 1987; Carrier et al., 1993; Stavric and D'Aoust, 1993). Potency of defined cultures decreases with storage and manipulation of isolates. Commercial CE preparations such as

Aviguard, Avi-Free, Broilact, CF3, Preempt, and Mucosal CE are currently available (Smith, 1997; Nisbet et al., 1998). Yeast (*Saccharomyces boulardii*) has also been used to reduce *Salmonella* associated with broiler chickens (Line et al., 1997). Competitive exclusion treatment should be complemented by biosecurity measures, vaccination programs, HACCP protocols, and stringent hygiene practices on the farm to protect poultry from natural *Salmonella* challenge.

Vaccination

Prophylactic vaccination is another method of preventing vertical transmission of *Salmonella*. Bacterins and live attenuated cultures have been used as vaccines in the prevention of avian salmonellosis (Nagaraja and Back, 1998). Components, such as ribosomes, outer membrane proteins, and polysaccharide-protein conjugates extracted from *Salmonella* have also been investigated as potential vaccines (Nagaraja et al., 1982; 1984; 1985; 1988). An experimental vaccine prepared from an acetone-killed, *S. enteritidis* oil emulsion reduced incidence and level of intestinal colonization by *S. enteritidis* in poultry feces at 1 week when post-challenge with 10^8 CFU (Gast et al., 1993). A vaccine prepared from killed *S. typhimurium*, expressing high amounts of type 1 fimbriae, has been effective in lowering *Salmonella* colonization in the cecal junction, liver and spleen. Killed vaccines do not induce sufficient protection to eliminate non-specific *Salmonella* serotypes colonizing chickens. Vaccines prepared from live but attenuated *Salmonella* strains (*gallinarum*, *typhimurium*, *choleraesuis*, and *enteritidis*) have produced variable protection depending on delivery route and ability of the attenuated strain to survive the host (Pritchard et al., 1978; Barrow et al., 1987; 1998; Barrow, 1990; Barrow et al., 1990a; 1990b; 1998; Hassan and Curtis, 1994).

Control of *Salmonella* During Processing

Processes in a poultry slaughter facility may cause a serious threat if not controlled on a regular basis.

Sources of contamination

Although the frequency of *Salmonella*-positive birds entering a processing plant is generally low (National Advisory Committee on Microbiological Criteria in Foods, 1997), there are several opportunities for carcass cross-contamination during slaughter and subsequent processing (Bryan et al., 1968; Morris et al., 1969; May, 1974; Lillard, 1990). Rate of *Salmonella* contamination, either from processing plants or retail markets, varies from 5 to 100% on broiler carcasses (Todd, 1980; Humphrey et al., 1988; Lammerding et al., 1988; Carraminana et al., 1997; National Advisory Committee on Microbiological Criteria in Foods, 1997). Possible sources of *Salmonella* contamination during poultry processing are summarized in Table 2 (Russell, 1998). Reviews on microbial contamination associated with individual processing steps have been reported (Bryan and Doyle, 1995; National Advisory Committee on Microbiological Criteria in Foods, 1997; Russell, 1998).

Intervention strategies in the processing plant

Although broiler slaughter operations do not include thermal processes that ensure elimination of *Salmonella*, a number of processing steps can be controlled to minimize and reduce microbiological hazards. Implementation of HACCP protocols in the slaughter facility and several chemical and physical treatments of broiler carcasses reduce/eliminate *Salmonella* associated with fresh poultry.

The goals of HACCP for poultry slaughter operations are to prevent, eliminate, or reduce the incidence and level of pathogenic microorganisms such as *Salmonella* and *Campylobacter*

and to control bacterial recontamination and/or outgrowth (National Advisory Committee on Microbiological Criteria in Foods, 1997). In general, HACCP can be defined as a systematic, scientific approach used in food production to ensure food safety (National Advisory Committee on Microbiological Criteria in Foods, 1998). It involves seven principles: (1) to assess hazards and risks associated with the process. A food safety hazard includes any biological, chemical or physical agent that is reasonably likely to cause illness or injury in the absence of its control; (2) to determine critical control points (CCP) for identified hazards. A CCP is any step in the process at which control can be applied in order to prevent/eliminate a food safety hazard or to reduce it to an acceptable level; (3) to establish critical limits at each CCP to control the hazards. Critical limits are based on process parameters such as temperature, time, pH, water activity, salt concentration, and sensory information; (4) to establish reliable procedures to monitor CCP's. Monitoring frequencies must be sufficient to ensure optimal control of CCP's; (5) to establish corrective actions when there is a deviation identified during monitoring a CCP. Actions must eliminate the hazard and ensure safe disposition of the product; (6) to establish record keeping and documentation procedures. The HACCP plan, CCP documentations, and modifications made in HACCP or CCP plans should be included in the file; and (7) to establish verification procedures confirming that the HACCP system is working correctly.

Regulatory agencies have poultry processing companies switch from organoleptic to science-based inspection. This inspection includes microbiological testing and mandatory reporting of any incidence of pathogen recovery, and identification of actual sources of contamination and cross-contamination, and visual examination of carcasses for fecal contamination. Federal regulations mandate all poultry processing plants to implement an in-house HACCP protocol. To verify that HACCP systems are effective, the Food Safety and

Inspection Service (FSIS) has set performance standards for *Salmonella* that slaughter plants producing ground meat and poultry have to meet. According to the “Mega-Reg” policy implemented by the federal agencies, meat slaughtering facilities in the U.S. are required to meet performance standards for *Salmonella* in order to verify the effectiveness of their HACCP plans (Anon, 1996). This policy was mandated on January 27, 1997 for meat processing plants in the U.S. Sampling and testing for *Salmonella* is carried out on an unannounced basis by federal inspectors. Failure to meet performance standards (Table 3) requires a plant to take immediate action to meet the guidelines. If the company fails consistently, the FSIS suspends inspection services.

In the slaughter facility, CCP’s fall into two categories (Fig. 2): (1) preventive measures to avoid colonization of carcasses with *Salmonella*. These measures include adequate live-bird surveillance on the farm, surveillance of personnel handling the birds, pest control, equipment sanitation, safe water supply, and good manufacturing practices in the processing units (McCapes et al., 1991); and (2) risk minimization measures to limit spread of *Salmonella* from contaminated carcasses. These measures include chlorination of carcass rinse water, refrigeration, equipment cleaning and disinfection procedures, and personnel sanitation.

Several intervention strategies have been attempted to rid raw poultry carcasses of *Salmonella* and other poultry pathogens. Presently, commercial processing plants in the U.S. use 20-50 ppm chlorine disinfectant (3-5 ppm residual chlorine) in immersion chillers (Thomson et al., 1979; Tamblyn et al., 1997). Chlorine has been widely used because it is safe (GRAS status), readily available, and inexpensive (Tsai et al., 1991). Chlorine (20 ppm) has also been used by slaughter facilities to disinfect equipment surfaces, carcass rinse cabinets, and the inside-outside bird washer prior to the chiller. However, chlorinated immersion and spray methods of

carcasses have been relatively ineffective against *Salmonella* due to the large amounts of organic matter (blood and feces) (Hargis et al., 1998).

Another factor that affects bactericidal properties of chlorine is pH. In water, chlorine dissociates into hypochlorous acid (powerful disinfectant) and hypochlorite ion (weak disinfectant). Poultry chill water has a pH of 7.6 to 8.0. At this pH, less than 60% of the chlorine is available in the form of hypochlorous acid (Waldroup, 1998). However, if chill water pH is maintained between 6.0-6.5, about 95-98% of the chlorine would be available as hypochlorous acid. Hence, chill water tanks are injected with CO₂ that forms carbonic acid thereby reducing pH of the chill water.

Alternative decontamination methods have been investigated as potential replacements for chlorine as the standard disinfectant. Chlorine dioxide (1.5 to 3.0 ppm residual chlorine) has been permitted in U.S. poultry processing plants (Lillard, 1979; 1980; Waldroup, 1998). However, high cost and skin discoloration of raw carcasses have limited its use. Trisodium phosphate (8 to 12%) has also been approved for use in processing units to reduce *Salmonella* and other foodborne pathogens (Giese, 1993; Tamblyn et al., 1997).

Several physical and chemical approaches such as air scrubbing (Dickson and Cox, 1992), heat (Davidson et al., 1985; Morrison and Fleet, 1985), irradiation (Mulder et al., 1977; Hanis et al., 1989; Thayer et al., 1991; Thayer, 1995; Abu-Tarboush et al., 1997), ozone (Yang and Chen, 1979; Sheldon and Brown, 1986; Waldroup, 1998), and several organic acids and chemicals have been used to reduce/eliminate *Salmonella* on carcasses and to minimize cross-contamination (Conner and Bilgili, 1994; Hwang and Beuchat, 1995; Tamblyn and Conner, 1997; Tamblyn et al., 1997; Xiong et al., 1998; Yang et al., 1998). The Food and Drug Administration (FDA) has approved irradiation of refrigerated or frozen uncooked meat, meat by

products, and certain meat products to control foodborne pathogens and extend product shelf life (FDA, 1997). Organic acids such as lactic, acetic, citric, malic, propionic, and tartaric acid reduce *Salmonella* levels on poultry carcasses (Snijders et al., 1985; van der Marel et al., 1988; Hwang and Beuchat, 1995; Kolsarchi and Candogan, 1995; Tamblyn and Conner, 1997). Among several chemical treatments, phosphates (Kim and Slavik, 1994; Lillard, 1994; Hwang and Beuchat, 1995; Salvat et al., 1997; Wang et al., 1997; Coppen et al., 1998), hydrogen peroxide (Lillard and Thomson, 1983), quaternary ammonium salts (Breen et al., 1995; Wang et al., 1997), glutaraldehyde (Thomson et al., 1977), sorbates (Robach and Sofos, 1982; Kolsarchi and Candogan, 1995), Tween 80 (Hwang and Beuchat, 1995), and a combination of lysozyme and EDTA (Samuelson et al., 1985) have been found to be effective in reducing *Salmonella* contamination on poultry carcasses.

Although these approaches reduce *Salmonella* on fresh poultry, they could adversely affect taste, color, flavor, texture, and/or appearance of the product (Breen et al., 1995; Hargis et al., 1998). Existing technologies reduce, but rarely eliminate, *Salmonella* on carcasses during processing because they are ineffective against bacterial cells that are attached to skin.

***Salmonella* attachment to poultry skin/carcasses**

Salmonella incidence on broiler carcasses increases with successive stages of processing, possibly due to the ability of the pathogen to firmly attach to poultry tissue or skin (Lillard, 1986a; b; 1989a; b). According to Lillard (1989b), “attached” bacteria can be defined as bacteria that remain on the skin after exposure to a bacterial cell suspension for a predetermined time and following rinsing for one min under running water at 50 psi. Lillard (1989a) demonstrated that *Salmonella* are firmly attached to poultry skin before broilers arrive at the slaughter facility, and high levels are recovered after 40 consecutive whole carcass rinses of a single carcass.

Salmonellae were not always recovered in the first whole carcass rinse and this sampling method could result in false negative reports. Attachment of *Salmonella* to poultry skin or the avian intestinal tract is complex and the mechanism(s) of action are not well understood.

The mechanism of bacterial attachment involves two steps (Kim and Doores, 1993). The first step involves retention of bacteria in a liquid film on the surface of skin and meat after immersing the sample in the bacterial suspension (Thomas and McMeekin, 1981; Lillard, 1986a; 1986b; Thomas et al., 1987). Thomas and McMeekin (1984) demonstrated that, in the first 15 s of immersion, over 90% of *S. typhimurium* cells were in the water film and less than 10% were recovered from the skin samples. However, after 30 to 60 min of immersion, 40 and 60% of cells were recovered from the skin, respectively, and fewer were found in the water film. These results indicate that there is a transfer of cells from the water film to the skin as immersion time increases. Contamination of carcasses by *Salmonella* during immersion processes can be reduced by preventing formation of the surface film, and by altering the surface tension. Scanning electron micrographs have shown that *Salmonella* appears to be entrapped in deep crevices or ridges that become more prominent after immersing poultry skin and muscle in water (Lillard, 1988). *Salmonella* cells lodged in these crevices or ridges of the skin are protected and not easily accessible. Certain chemical treatments used in chill water are effective in reducing salmonellae to nondetectable levels, but did not completely eliminate them from the carcasses (Lillard, 1979; 1980; Lillard and Thomson, 1983; Lillard et al., 1987).

Firstenberg-Eden (1981) reported that bacteria are closely associated with each other and entrapped in certain inaccessible sites by physical forces. Attachment at this stage is reversible and balanced between attractive van der Waals and repulsive electrostatic forces (Gristina, 1987). Adhesion depends on characteristics of the bacterium, the surrounding medium, and the

substrate surface to be colonized. Bacteria approach the binding site with flagella in motile organisms or through fluid flow or external propulsion in motile and non-motile organisms (Benedict, 1988). Although both surfaces (bacterial and meat tissue) possess a net negative surface charge favoring repulsion, variations in isoelectric points, through localized changes in the microenvironmental pH, may allow the two surfaces to approach within the minimum van der Waals forces, favoring attraction (Benedict, 1988).

The second step of attachment is characterized by a time-dependent, irreversible exopolymer formation. At this stage, bacteria multiply and form a microcolony, which can lead to the formation of a biofilm. Formation of these extracellular polysaccharides (glycocalyx) or attachment fibrils has been involved in the attachment process (Costerton et al., 1978; Butler et al., 1979; 1980; Schwach and Zottola, 1982).

The strongest form of *Salmonella* attachment involves high-affinity chemical attachment to specific receptor sites. Little is known about the mechanisms involved in the chemical attachment of *Salmonella* to avian intestinal tract or skin surfaces. Duguid and coworkers (1966) found that *S. typhimurium* exhibited adhesive properties due to type 1 pili. Adhesive properties of type 1 pili were inhibited by D-mannose. On the other hand, Jones and workers (1980) found that type 1 pili were not involved in the attachment of *S. typhimurium* to HeLa cells. Cell adhesin, released during homogenization of *S. typhimurium* cells, inhibited attachment of the organisms to the HeLa cell receptor. Authors confirmed the presence of a cell-bound, D-mannose resistant hemagglutinin that was responsible for attachment of *S. typhimurium* to HeLa cells. Inhibition of *in vitro* attachment of *Salmonella* to inverted ceca (McHan et al., 1989) and intestinal segments (Oyofe et al., 1989a) by D-mannose and inhibition of colonization in broilers by D-mannose (Oyofe et al., 1989b) indicated that mannose-sensitive adhesion (associated with

type 1 fimbriae) plays a major role in the colonization of the avian intestinal tract by salmonellae (Craven and Williams, 1998). Lillard (1986b) also found that mannose-sensitive type 1 fimbriae, isolated from *S. typhimurium*, were responsible for attachment of the organism to chicken skin.

Attachment of microorganisms depends on the presence of flagella and their activity (Notermans and Kampelmacher, 1974; Butler et al., 1979; Farber and Idziak, 1984). Flagellated bacteria readily attach and nonflagellated bacteria rarely attach to poultry skin. Bouttier et al. (1997) observed a 90% reduction in *S. choleraesuis* attachment to beef muscle tissues after mechanically removing the flagella or treating the bacteria with specific anti-flagella serum. Authors attributed this reduction to a loss of bacterial mobility leading to a reduction in the number of bacterial cells reaching the tissue during the period of contact. *Salmonella* attachment has also been associated with connective tissue, particularly collagen (Thomas and McMeekin, 1981; Benedict et al., 1991; Kim and Doores, 1993; Walls et al., 1993). Adhesion to hyaluronan has been identified as the likely explanation (Sanderson et al., 1991). Benedict (1988) demonstrated that *S. typhimurium* attached to fresh meat surfaces by selectively binding to collagen fibers, particularly the reticulin type. Lipopolysaccharides and hydrophobic bonding have contributed to *in vitro* attachment of *S. typhimurium* and *S. californica* cells to chicken cecal mucus and enterocytes (Craven et al., 1992; Craven, 1994). The phenomenon of *Salmonella* attachment to poultry skin/tissue or the intestinal tract involves more than one mechanism. Water uptake and type of fimbriae, flagella, and cell wall components may, alone or in combination, influence *Salmonella* attachment to poultry skin.

Reduction of *Salmonella* will depend on the extent of fecal contamination within the production facility. Use of non-pathogenic *E. coli*, as an indicator of fecal contamination, merits

consideration in monitoring fecal contamination and thus, controlling overall sanitation on poultry farms.

Significance of Generic *Escherichia coli* as an Indicator Organism

Fecal coliforms are generally found in the gastrointestinal tract of humans and warm-blooded animals. They include members of at least three genera: *Escherichia*, *Klebsiella*, and *Enterobacter*.

According to FSIS, generic (non-pathogenic) *E. coli* is an ideal indicator of good manufacturing practices within processing units (USDA-FSIS, 1996). This organism has also been chosen as a measure of assessing control in poultry slaughtering facilities (USDA, 1996). The new FSIS Pathogen Reduction/Hazard Analysis and Critical Control Point (HACCP) regulation, also referred to as “Mega-Reg” policy, codifies the principles of pathogen prevention and reduction. This policy, published in July 1996, provides food safety inspectors with systematic science-based tools to carry out inspections of raw carcasses in all federally inspected meat and poultry slaughter and processing operations in the U.S. The FSIS estimated that total cost of implementation by meat and poultry industries will be between \$305 to \$357 million (Anon, 1996).

Sanitation standard operating procedures (SOP's), establishment and maintenance of HACCP plans, and microbiological testing of generic *E. coli* for process control are the focal points of this policy. Sanitation SOP's were required to develop, implement, maintain, and document ongoing sanitary measures in the plant by January 1997. All establishments were required to implement a HACCP protocol for each final product. Implementation was dependent on the plant size. The HACCP plan in large (>500 employees) and smaller (10 to 499 employees) plants was implemented by January 26, 1998 and January 25, 1999, respectively.

Very small plants (<10 employees or annual sales <\$2.5 million) have a HACCP plan in place since January 25, 2000 (Anon, 1996).

In accordance with the “Mega-Reg” policy, slaughter facilities have been testing generic *E. coli* since January 27, 1997. Federal regulations require plants to test for *E. coli* in order to verify that the slaughter process is removing or preventing fecal contamination on carcasses. *E. coli* was selected as the test organism because enumeration is useful to insure that processing parameters are in control, analysis is easy and inexpensive, and *E. coli* can be easily quantified when compared to pathogens such as *Salmonella*, *Campylobacter*, and *Listeria* (Russell, 1996). Smaller plants, annually producing less than 6,000 cattle, 20,000 pigs, 440,000 chickens, or 60,000 turkeys were required to collect one sample per week during June to August period for *E. coli* testing. Sampling criterion for larger plants is shown in Table 4 (Anon, 1996). A survey (1992 to 1998) conducted by FSIS found that 99.6% of broilers, 98.9% of turkey, 99.3% of ground chicken, and 84.4% of ground turkey were contaminated with generic *E. coli*, while 99.9% of broilers, 99.8% of turkey, 99.7% of ground chicken, and 95.5% of ground turkey were contaminated with fecal coliforms (McNamara and Levine, 1998). Establishments sampled in this program were responsible for slaughtering approximately 99.9% of all broiler and turkeys slaughtered in the U.S. and for producing 100% of the ground chicken and turkey under federal inspection.

Generic *E. coli* has been reported as the best indicator of fecal contamination on poultry carcasses (USDA-FSIS, 1996). Fecal contamination is the primary route by which enteric bacteria such as *Salmonella*, *Campylobacter*, and *E. coli* O157:H7 gain access to meat and poultry. *Escherichia coli* can be considered as an “index” organism because its presence could be used to indicate the presence of other pathogenic organisms. However, generic *E. coli* and fecal

coliforms do not have a reliable and defined relationship with poultry pathogens because of differences in physiology, growth requirements, and growth characteristics (Solberg et al., 1977).

While *E. coli* levels may be affected by processing parameters, environmental conditions to which birds are exposed before entering the slaughter facility may also have an impact on the contamination level. Fecal matter present on the outside of birds as they enter the plant may influence *E. coli* levels on the carcasses, regardless of efforts to reduce contamination during processing. Feed withdrawal before transportation could influence the level of *E. coli* contamination. Feed is normally withheld for 6-7 hours before being shipped to the slaughter plants. If they are not withheld from feed for a sufficient period, these birds will have their intestines full of ingesta that are likely to rupture during evisceration and eventually leak onto the carcasses. If feed withdrawal is too long (>12 h), the intestines begin to slough their mucosal lining, which weakens the intestine and makes it more susceptible to tearing during evisceration (Russell, 1996). Although such factors are not plant related, under current guidelines, the processor maybe ultimately responsible for *E. coli* incidences.

Consumer Food Safety

Nearly 85% of outbreaks associated with foodborne diseases occurs as a result of food mishandling in food service establishments and homes (Hall, 1997). Food safety measures implemented by the industry and federal agencies to govern pre- and post-harvest control of foodborne outbreaks/illnesses will be of no avail if the consumer mishandles/abuses raw or processed foods. Therefore, it is important to heighten consumer awareness and encourage food safety training and education among consumers and personnel associated with food service establishments. Knabel (1985) reported the top eleven factors contributing to outbreaks associated with foodborne diseases caused by mishandling of household foods in the U.S. They

were ranked as follows: (1) contaminated raw food/ingredients; (2) inadequate heat processing; (3) procuring food from unsafe source; (4) improper cooling; (5) lapse of ≥ 12 h between preparing and eating; (6) infected person handling the food; (7) improper fermentations; (8) inadequate reheating; (9) toxic containers; (10) improper hot holding; and (11) cross contamination.

General guidelines for handling poultry meat in retail food stores and food service

establishments (National Advisory Committee on Microbiological Criteria for Foods, 1997)

The following precautionary measures should be taken to avoid the risk of foodborne diseases in restaurants and retail food establishments: Fresh raw broilers should be received in good condition at $\leq 41^{\circ}\text{F}$ and visually inspected, and temperatures should be checked for product abuse. Products must be stored below 40°F to minimize growth of foodborne pathogens and they must be rotated on the first-in, first-out basis. All raw materials should be covered, properly dated, and labeled. Raw products must be separated from cooked products to avoid cross contamination. Coolers should be checked regularly for temperature fluctuations. Food service workers must be educated about risks associated with mishandling raw poultry during food preparation and distribution. Fresh products must be separated from processed products during food preparation. Equipment and utensils such as knives, cutting boards and pans used in preparing raw chicken must not be allowed to come in contact with processed foods. Contaminated equipment and utensils should be properly cleaned and sanitized after use. Chicken and turkey (whole and parts) products must be cooked to a minimum internal temperature of 180°F , as measured in the thickest part of the breast muscle. Ground/restructured poultry products must be cooked to a minimum internal temperature of 165°F for 15 s. Products such as rotisserie that are cooked and held for hot display should be kept at a minimum

temperature of 140°F. Leftover processed poultry products should be immediately refrigerated to prevent microbial growth. Leftovers and other precooked broiler parts should be reheated to an internal temperature of 165°C within two hours.

General guidelines for handling poultry meat by consumers (National Advisory Committee on Microbiological Criteria for Foods, 1997)

Consumers should take the following precautions in their kitchens to reduce the risk of foodborne illnesses: Perishable poultry products should be purchased after other grocery items have been selected. Raw products should be bagged separately to avoid contamination of other food products. Raw meat or poultry product should never be allowed to come in contact with a food package that will not be cooked before consumption. Raw poultry products must be immediately refrigerated (< 40°F) or frozen (< 0°F). The refrigerator and freezer should be cleaned and sanitized periodically. Raw products should be kept separately from cooked products. Kitchen countertops, sinks, and cutting surfaces should be cleaned and sanitized after they come in contact with any raw poultry product. Surfaces should be cleaned with soapy water, rinsed thoroughly, and sanitized with chlorine solution (one cap of bleach/gallon of cold water) or any available kitchen sanitizers. Cross-contamination can occur in the kitchen when utensils, plates, and hands are not thoroughly washed and sanitized before handling or preparing cooked foods or foods that will not be further processed (e.g. salads). Never use the same cutting board or plates for raw and cooked poultry products unless they have been washed and sanitized between uses. Frozen products should be thawed overnight in the refrigerator. Guidelines for cooking, refrigerating, and reheating (leftovers) whole and minced poultry products have been mentioned in the previous discussion.

Although the above guidelines are directed toward reducing contamination of pathogens in poultry products, they could be applied to any meat product. Minimum cooking temperatures will vary with the type of meat product. In general, consumers, food safety establishments, and retail outlets should remember four key terms to fight foodborne diseases: (1) Clean: wash hands and surfaces often; (2) Separate: do not cross-contaminate; (3) Cook: cook to proper temperatures; and (4) Chill: refrigerate promptly.

Concluding Remarks

Microorganisms are naturally present in the environment and are found on most raw agricultural products. There are several means by which microbes, particularly foodborne pathogens such as *Salmonella*, *Campylobacter*, *E. coli* O157:H7, and *Listeria*, can enter a food system. Factors such as temperature, stress, and unsanitary conditions in the facility may contribute to pre- and post-harvest contamination. Pathogens can survive minimal preservation treatment(s) and can continue to multiply if food products are abused. Humans may introduce pathogens into food products during production, processing, distribution, storage, and preparation, prior to consumption. Depending on individual susceptibility (old age, infants, or immunocompromised), foodborne pathogens can inflict mild to life-threatening diseases. The public health system in the U.S. needs to develop a more efficient early-warning network than the currently existing Foodborne Disease Active Surveillance Network (FoodNet) and pathogen DNA fingerprinting (PulseNet), to detect, control and/or prevent foodborne outbreaks. Food safety research should involve more sensitive, rapid, and automated methods to accurately detect and characterize pathogens in food products.

Food companies should strictly adhere to the food safety regulations and guidelines implemented by the federal agencies. Food safety educational training programs should be made

mandatory for personnel working in the food companies. Food safety workshops can be held through extension services at the universities to educate the public. More federal dollars must be invested in educating school students and consumers through regular food-safety related programs on television, radio, and the Internet. Controlling the incidence of foodborne pathogens in food products from “The Farm to the Table” must be a concerted effort on the part of all people involved in each segment of the food system – producers, integrators, shippers, retailers, processing companies, retailers, food service establishments, and consumers.

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Table 1. *Salmonella* virulence factors and their mechanisms of action (D'Aoust, 1997).

Virulent factors	Mechanism of action	Invasion genes
Siderophores	Compete with host transferrin, lactoferrin and ferritin ligands for available iron	<i>fur</i>
PhoP/PhoQ regulon	Enables <i>Salmonella</i> to survive phagocytes, high acidity of phagolysosomes and defensins	<i>pag</i> and <i>prg</i>
Toxins A-B enterotoxin, cytotoxin	Fluid exsorption	<i>stx</i>
Vi antigen	Inhibiting opsonization of the C3b host complement factor on surface lipopolysaccharide	<i>viaA</i> and <i>viaB</i>
Enzymes Catalase, superoxide dismutase, peroxidase	Protection against singlet oxygen and H ₂ O ₂	<i>oxyR</i>
Plasmid (30-60 Mda)	Rapidly multiply the bacteria and spread infection	<i>spv</i>
Porins (outer membrane proteins)	Regulates influx of nutrients and antibiotics	<i>ompC</i> , <i>ompD</i> , <i>ompE</i> and <i>ompF</i>
Lipopolysaccharides	Repels lytic attack of the host complement system	-

Table 2. Sources of *Salmonella* (and other microorganisms) contamination during poultry processing (Russell, 1998).

Operation	Workers hands	Equipment and tools	Bird to bird	Other environmental factors ¹
Stunning	-	-	-	*
Scalding	-	**	***	****
Picking	-	****	**	-
Washing	-	*	-	****
Rehanging	***	-	-	-
Evisceration	**	****	**	-
Inspection	****	-	-	-
Reprocessing	****	****	-	-
Washing	-	*	-	****
Chilling	-	-	**	****
Rehanging	****	-	-	-
Sizing	-	***	***	-
Boxing	****	*	*	-

¹processing parameters, water quality, and disinfectant level

* not a significant source of contamination

**** significant source of contamination

Table 3. *Salmonella*-reduction performance standards¹ in the U.S. poultry slaughter plants (Anon, 1996).

Class	Performance standards (% positive)	Number of samples tested	Maximum number of positives to achieve standard
Broiler carcasses	20.0	51	12
Turkey carcasses	18.6 ²	Not available	Not available
Ground chicken	44.6	53	26
Ground turkey	49.9	53	29

¹ Products cannot test positive at rates exceeding the above standards. Frequency and timing of testing will be based on the establishment's previous results and other performance criteria.

² McNamara and Levine, 1998

Table 4. *Escherichia coli* control testing criteria in U.S. processing plants (Anon, 1996).

Class	Sampling frequency	Procedure	Acceptable limits ^a	
			Lower limit (m) CFU ^b /cm ²	Upper limit (M) CFU/cm ²
Cattle	1 sample/300 carcasses	Sponge flank, rump, and brisket from carcass in the cooler	Negative ^c	100
Hogs	1 sample/1,000 carcasses	Sponge ham, belly, and jowls from carcass in the cooler	10	10,000
Chickens	1 sample/22,000 carcasses	Rinse whole bird after the drip line	100	10,000
Turkeys	1 sample/3,000 carcasses	Rinse whole bird after the drip line	Not available	Not available

^a No result can exceed the upper limit (M) and three or fewer sample are allowed between the lower limit (m) and the upper limit out of the most recent 13 samples.

^b Colony forming unit

^c Negative is defined by the sensitivity of the method to detect at least 5 CFU/cm²

Figure 1. An overview of the movement of *Salmonella* through the poultry chain (adapted from International Hatchery Practice and International Poultry Production Supplement).

Arrows indicate *Salmonella* movement; D=disinfection; H=heat treatment; M=monitoring; A=acidification; V=vaccination; Ve=vaccination of egg layers only; T=test (if positive reject or reschedule); and CE=competitive exclusion.

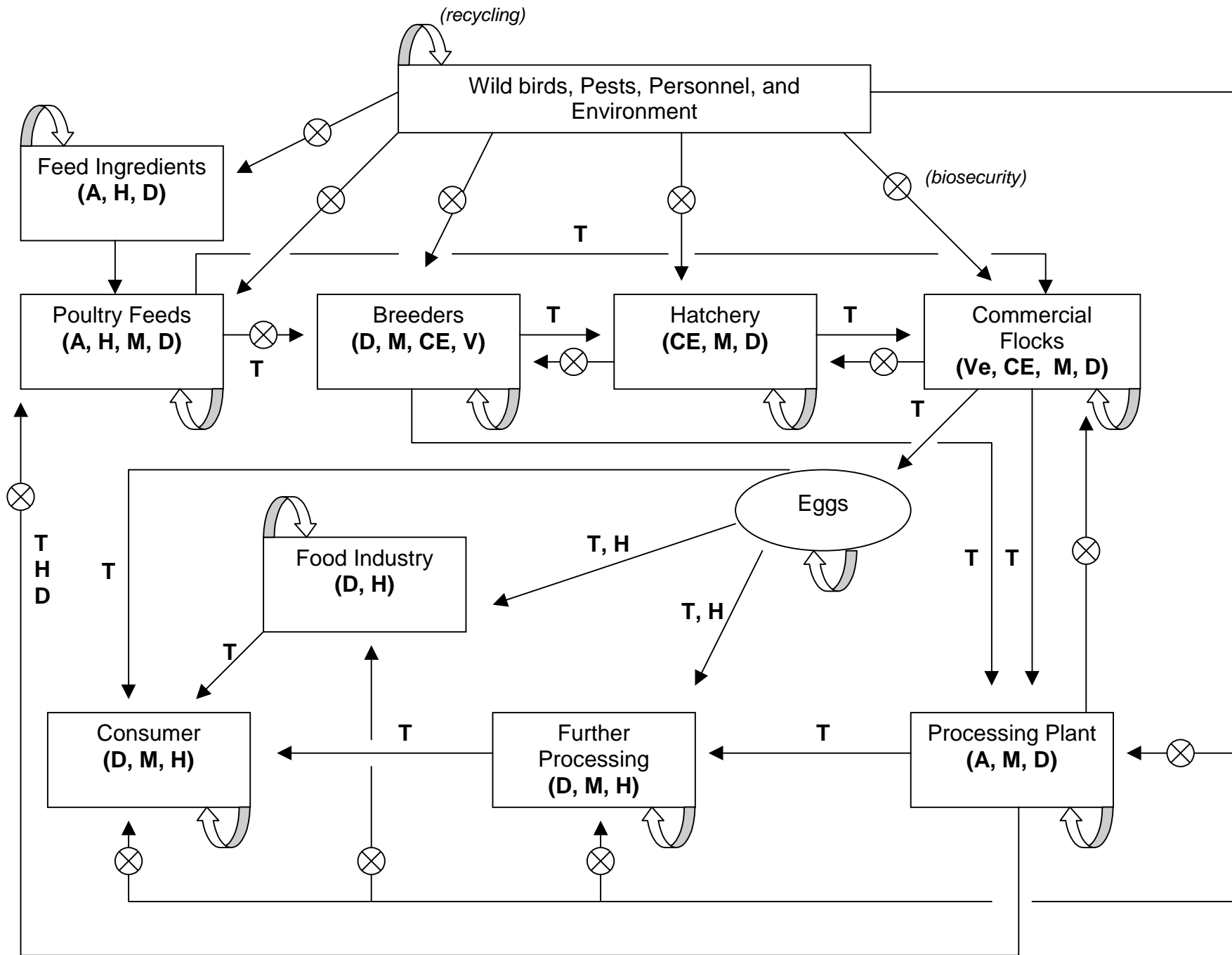
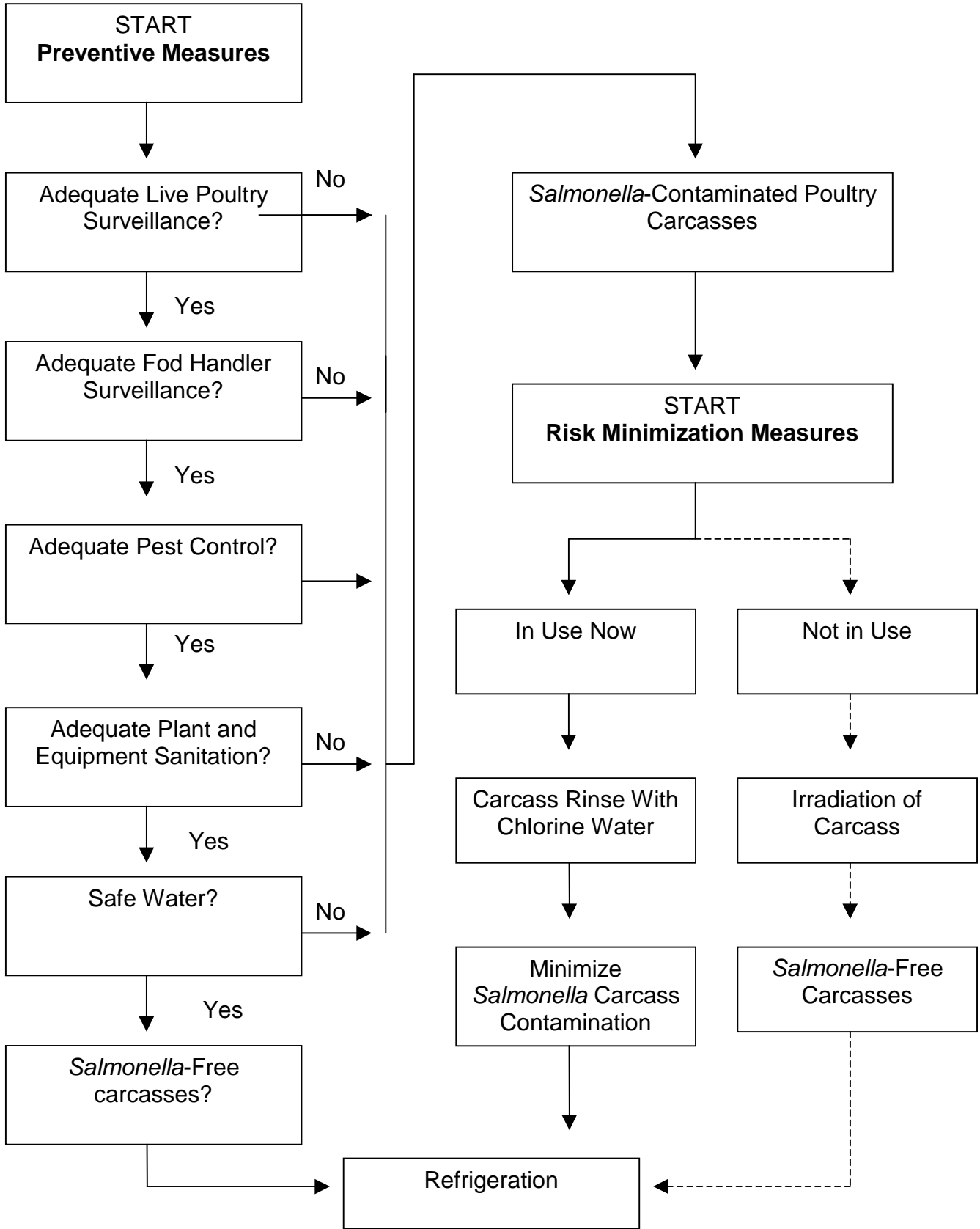


Figure 2. Critical control points for whole carcass processing of poultry salmonellosis (McCapes et al., 1991).



CHAPTER 1
DYNAMICS OF *SALMONELLA* COLONIZATION IN A TURKEY
PRODUCTION FACILITY

ABSTRACT

A comprehensive ecological survey was conducted from April 1997 to June 1999 on four turkey flocks (F5 to F8) to identify the key preharvest sources of *Salmonella* colonization. This study was part of an ongoing survey conducted to evaluate the frequency of *Salmonella* contamination on a turkey production facility that has not been populated with poultry for over 20 years. Turkey cecal and crop contents, litter, drinkers, air, feed, and feeder contents were sampled. Environmental swabs were also collected throughout the facility. For each flock, 5 to 12 pens were sampled during each sampling period. Conventional microbiological and serological procedures were used to isolate, identify, and confirm the presence or absence of *Salmonella*. Isolates were serotyped and screened for antibiotic resistance. *Salmonella* was detected from 21%, 5%, 0%, and 1% of the samples in F5, F6, F7, and F8, respectively. Overall, *Salmonella* was isolated from 13% of litter, 11% of turkey ceca, 10% of drinker, 5% of swab, 3% of feed, and 1% of feeder contents. *Salmonella* was not detected in crop and air samples. *Salmonella heidelberg* (65%), *S. senftenberg* (19%), *S. muenster* (10%), *S. anatum* (3%), and *S. worthington* (3%) were the serotypes isolated from the sampled sources and flocks. Of the 69 serotypes screened, 25% of the *Salmonella* isolates were resistant to the following antibiotics: gentamycin, spectinomycin, tetracycline, tobramycin, and trimethoprim/sulfamethoxazole. Identifying environmental sources associated with *Salmonella* colonization and characterizing serotypes and antibiograms would assist in designing preharvest controls for this poultry-borne pathogen. Integrators and poultry producers may be able to design hazard analysis and critical control point (HACCP) protocols to reduce the level of *Salmonella* arriving at the processing plant.

KEY WORDS: antibiotics, preharvest sources, *Salmonella*, serotypes, turkey

INTRODUCTION

Salmonella contamination has been a persistent problem affecting the poultry industry in the United States. Annual productivity losses and medical costs due to salmonellosis are estimated from \$0.6 billion to \$3.5 billion (Opitz et al., 1993; Buzby et al., 1996). The poultry industry processes over eight billion broiler-hatching eggs through commercial hatcheries each year (Cox, 1998). Much of the traceable human *Salmonella enteritidis* infection has been caused by consumption of egg or poultry products (Bailey, 1993). Poultry meat has been implicated in a large number of salmonellosis cases in humans. *Salmonella* colonizes the intestinal tract of poultry causing contamination of carcasses during processing. The incidences of *Salmonella* contamination among broiler carcasses has been found to vary from 5 to 100% (Carraminana et al., 1997). The Food Safety and Inspection Services conducted a nationwide survey (1992-1998) from federally inspected plants to generate microbiological baseline data (McNamara and Levine, 1998). Establishments sampled in the program accounted for approximately 99.9% of all broiler and turkeys slaughtered in the US, and they produced 100% of ground chicken and turkey product. The study found that *Salmonella* was prevalent in broiler carcass rinses (20%), turkey carcass rinses (18.6%), raw ground chicken (44.6%), and raw ground turkey meat (49.9%).

Reduction of *Salmonella* in poultry requires comprehensive control at the farm where birds are brooded and raised before shipment to processing plants. Chicks are very susceptible to *Salmonella* infection and gut colonization from hatching to 96 h of age (Bailey, 1988). These chicks can be infected by vertical transmission through infected parents or by horizontal transmission through hatcheries, sexing in contaminated hatcheries, cloacal infection, transportation equipment, and feed (Lahellec and Colin, 1985; Opitz et al., 1993).

Environmental factors such as air, litter, and unclean facilities, and vectors such as insects, humans, and rodents are also responsible for *Salmonella* transmission in poultry (Jones et al., 1991; Bailey, 1993; Hoover et al., 1997). Once sources are identified, critical control points can be established at the farms to control horizontal and vertical transmission of *Salmonella*. A reduction in *Salmonella*-positive birds will reduce public health risks associated with poultry products (Simmons and Byrnes, 1972).

Although research has been carried out on *Salmonella* ecology in chickens (Simmons and Byrnes, 1972; Bains and MacKenzie, 1974; Snoeyenbos et al., 1974; Dougherty, 1976; Morgan-Jones, 1980; Bhargava et al., 1983; Lahelec and Colin, 1985; Jones et al., 1991; Bailey, 1993), limited information is available on turkey flocks (Kumar et al., 1971; Hoover et al., 1997; Amick-Morris, 1998). Additionally, rearing turkeys differs from rearing chickens. The production cycle of turkeys is 20 to 22 weeks compared to 6 to 8 weeks in broilers. An ecological survey was conducted on turkey flocks to:

1. Identify key environmental sources/vectors influencing *Salmonella* colonization dynamics in a turkey production facility;
2. Characterize *Salmonella* isolates for serotype and antibiotic resistance patterns;
3. Evaluate the role of temperature and humidity within the production facility, and litter pH and water activity (A_w) on *Salmonella* colonization among sampled flocks and;
4. Monitor sex effect on resistance to *Salmonella* colonization.

MATERIALS AND METHODS

Production parameters

Facility: This survey was conducted at Reymann Memorial Farm, Wardensville, WV, in partnership with British United Turkeys of America (BUTA), Lewisburg, WV. The farm has

been used for beef and sheep production, and it had not been populated with chickens or turkeys for over 20 years prior to initiation of survey work (Hoover et al., 1997). Prior to this study, production trials of three turkey flocks were carried out by BUTA and frequency of *Salmonella* was monitored (Amick-Morris and Kenney, 1997; Guo et al., 1997; Hoover et al., 1997). The facility consists of 24 pens divided equally by the service area (Fig. 1). The dimensions of each pen were 3.6 m (width) x 4.2 m (length) x 2.4 m (height). Four consecutive turkey flocks (F5-F8) were sampled in this facility from April 1997 to June 1999. These were the 5th, 6th, 7th, and 8th flocks produced in this facility. Flock characteristics and responses measured are summarized in Table 1. Biosecurity measures were in accordance with BUTA guidelines (Appendix). Before placement of each new flock, used litter was removed, pens and equipment were disassembled, and the production facility was thoroughly cleaned and disinfected to prevent cross contamination from the previous flock. Fresh litter was added to the pens before placement of new poults in each flock.

Production trials: BUTA trials evaluated effects of strain, gender, and nutrition on growth performance, feed efficiency, and carcass composition of turkeys among sampled flocks. In F5, a strain x gender combination was replicated twice and randomly assigned to the 24 pens. Treatment main effects consisted of six breeds (78x12, 78x25, 38x71, 72x12, 72x71, and 72x25) and two genders (male and female). One hundred and fifty male and 150 female poults for each of 12-treatment combination were placed in a brooding ring (3.6 m diameter). Poults were hatched at the BUTA hatchery in Lewisburg, WV, and transported to the Rocco Farms hatchery in Harrisonburg, VA, to be de-toed. Turkeys from each flock were brooded for a 2-week period, after which the poults were assigned to their respective rearing pens (Table 2). Diet formulation, feeding schedule, and flock densities were not available for this flock.

In F6, a strain x feed combination was replicated four times and randomly assigned to the 24 pens. Treatments main effects consisted of three strains of male turkeys (Big 6, Hybrid, and Nicholas) and two feeding regimens (BUTA-USA feeding program and European High Protein feeding program). Ration formulations and feeding programs are shown in Table 3. Two hundred and fifty poult for each of 6-treatment combination were placed a brooding ring. Approximately 50 poult were placed in assigned pens.

In F7, a strain x feed combination was replicated three times and randomly assigned to the 24 pens. Treatment main effects consisted of four strains of female turkeys (Big 6, Hybrid, Nicholas, and Line 37) and two feeding regimens (Heavy Hen feeding program and Least Cost Hen feeding program). Ration formulation and feeding program are shown in Table 4. Two hundred and fifty poult for each of 8-treatment combination were placed in a brooding ring. Approximately 70 poult were placed in assigned pens.

In F8, a strain x gender combination was replicated three times and randomly assigned to the 24 pens. Treatment main effects consisted of four strains of turkeys (Big 6, Hybrid, Nicholas, and 37-Roaster) and two genders (male and female). The composition of feed is shown in Table 5. Two hundred and twenty five female poult and 175 male poult for each of 8-treatment combinations were placed in a brooding ring. Approximately 70 female and 55 male poult were placed in assigned pens.

Sampling procedures

Litter, drinker, air, feed samples, and environmental swabs were collected for each flock throughout the grow-out period (Table 1). Litter, drinkers, and turkeys were sampled from the same pen. Turkeys were sacrificed by cervical disarticulation in accordance with approved animal care and use procedures (AVMA, 1993).

Turkeys: At day 0, poults arrived from a commercial hatchery within 24 h of hatch. Cardboard liners were sampled by removing three, 16 cm² areas with a sterile scalpel and transferring them to 100 mL Universal Preenrichment (UP) broth (Bailey and Cox, 1992). Liners were sampled only for F7. Samples were collected from boxes that represented three different hatcheries in which the birds were shipped. Four boxes were randomly sampled from each hatchery. In F7, 12 poults from three hatcheries were sacrificed by cervical disarticulation at day 0, and the entire intestine and yolk sacs were transferred to 100 mL UP broth. After two weeks of brooding, 24 poults were sampled from 12 brooding rings in F5, 16 poults from 8 brooding rings in F7, and 16 poults from 8 brooding rings in F8. For F6, sample collection began from week 6 (Table 1). At week 2, intestinal samples were transferred to 100 mL UP broth among the sampled flocks. From week 10 until the end of the grow-out period, the entire ceca were removed, the blind end was snipped with sterile scissors, and cecal contents were emptied into sterile stomacher bags and sealed. Bottles containing UP broth and stomacher bags were held on ice during transport to the laboratory for *Salmonella* testing. Crop contents were sampled in F7 and F8 by removing the crop, making an incision with a sterile scalpel and transferring the contents into a sterile stomacher bag. This bag was sealed and shipped to the laboratory on ice for further analysis.

Litter: Litter samples consisted of a composite sample collected from six random locations within each pen. Samples were collected from the top 5.08 cm of litter preferably mixed with bird feces. Litter samples were placed in sterile bags and held on ice during transport to the laboratory.

Drinkers: At each sampling period, one Plasson drinker in each of the designated pens was sampled. Drinkers were swabbed with 2 or 3 ply sterile cloth gauzes (5 x 5 cm²) held by a

pair of sanitized forceps. Samples were placed in sterile stomacher bags, and bags were sealed and held on ice during transport to the laboratory.

Environmental swabs: Samples were collected from a 16 cm² area at various locations in the facility using a sterile swab moistened with sterile UP broth. Locations included walls, ventilation fans, feathers and open wounds of sick birds, feathers of dead birds, employee shoes, the feed truck, fans inside the pens, feed storage bins, and door handles. Swabs were transferred to 10 mL sterile UP broth and held on ice during transport to the laboratory.

Air: Samples were collected on Rodac plates (65 x 15 mm) containing Brain Heart Infusion (BHI) agar using a SAS portable high flow air-sampler (Model 5203, Spiral Biotech, Inc., Maryland). The air sampler was set to collect 60 liters of air in 20 s, allowing impingement of airborne microorganisms on the Rodac plate (Al-Dagal and Fung, 1993; Hoover et al., 1997). The air sampler cover was sanitized with 70% ethyl alcohol before reuse. Following sampling, the agar was aseptically transferred to sterile stomacher bags, sealed, and held on ice during transport to the laboratory. Air samples were collected from pens, ventilation fans, alleys on each side of the turkey house, the centralized service area, and entrances to the facility during each sampling period (Fig. 1).

Feed and feeder contents: Samples were collected randomly from each feed shipment by placing a sterile bag in the flow of feed from the auger or from the feed cart. Feeder contents were sampled from each of 24 pens every Friday of production for each flock. Samples were stored at – 4°C at the production facility prior to transport to the laboratory for analyses. Approximately 10 to 20% of the feed weigh-back samples were analyzed during each sampling period.

Laboratory procedures

Turkey cecal and crop samples, placed in stomacher bags, were mixed with UP broth in a 1:10 (w/v) ratio and were sealed. Gauze and agar from drinker and air samples, respectively, were mixed with 100 mL UP broth. Approximately 5 g of litter and 10 g of feed or feeder content samples, respectively, were transferred to bottles containing 100 mL UP broth using sterile gloves. Samples were thoroughly mixed with UP broth and incubated (Imperial II Incubator 422, Labline Instrument Inc., Illinois) at 37°C for 24 h. One mL of this preenriched sample was transferred to 9 mL of tetrathionate (TT) broth and incubated at 37°C for 24 h. Selectively enriched samples from TT broth were streaked to isolation on Xylose Lactose Tergitol (XLT4) plates for F5, F6, and F7. Due to the unavailability of tergitol supplement in XLT4 for detecting *Salmonella* in F8, Xylose Lactose Desoxycholate (XLD) plates were used. These plates were incubated at 37°C for 24 h. A single presumptive positive, *Salmonella* colony (black colony on XLT4/XLD plate), per sample, was stabbed and streaked on Triple Sugar Iron (TSI) and Lysine Iron agar (LIA) slants, respectively, using the same inoculating loop. The TSI and LIA slants were incubated at 37°C for 24 h and 48 h, respectively. *Salmonella*-positive, TSI tubes could be identified by a red slant and black butt while LIA tube exhibited a purple slant and black butt. The above transfers were carried out under aseptic conditions in a laminar flow hood (Model NU-425-400, Class II Type A/B3, NuAire™ Inc., Minnesota).

Serology: At least 2 to 3 positive isolates, identified by TSI or LIA, were maintained on BHI agar slants and incubated at 37°C for 24 h. Serology was performed on fresh (18-24 h) BHI cultures using *Salmonella* O (Group A-E; Vi) polyvalent antisera (BBL-Becton Dickenson Microbiological Systems, Maryland) to confirm the presence of *Salmonella*. Using a sterile inoculating loop, sufficient bacterial growth from the BHI slant was gently mixed with 0.5 mL

saline (0.85% NaCl). A drop of this bacterial suspension was placed on a glass slide and mixed with a drop of polyvalent antisera. The mixture was gently rocked for 1 min and agglutination was observed. A positive control of *S. typhimurium* and a negative control of *Escherichia coli* were used to eliminate false-positive readings.

Storage: *Salmonella* cultures, confirmed by serology, were transferred to 5-10 mL UP broth and incubated at 37°C for 18 to 24 h. A loopful of this fresh culture was streaked to isolation on XLT4 plates and incubated at 37°C for 20 to 24 h. A single *Salmonella*-positive black colony from each XLT4 plate was mixed in Micro-Protect tubes (TS/70-B, Technical Service Consultants Ltd., Lancashire, England) containing beads suspended in nutrient broth. These tubes were vertically shaken for 5 s and allowed to stand for at least 30 s allowing enough time for the bacterial suspension to be immobilized on the beads. Subsequently, nutrient broth from each tube was discarded using a sterile dropper and the tubes were stored at -70°C until further use. This procedure was carried out under aseptic conditions in the laminar flow hood.

Serotyping: *Salmonella* isolates were removed from the freezer (-70°C) and 1-2 beads from each Micro-Protect tube were aseptically transferred to 5 mL of sterile UP broth. The tube was immediately placed back into -70°C storage. Beads and broth were incubated at 37°C for 24 h, and subsequently, a loopful of culture was streaked to isolation onto a XLT4 plate. A single black colony from XLT4 was streaked on BHI slant. Isolates were packed, labeled, and shipped to the *Salmonella* Reference Center at the University of Pennsylvania for serotyping and determining their antibiotic resistance profile, in accordance with the Interstate Shipments of Etiological Agents (42 CFR Part 72) regulations.

Environmental parameters: Litter pH and water activity (A_w) were measured, and temperature and relative humidity in the production facility were monitored as potential factors

affecting frequency of *Salmonella* colonization (Hoover et al., 1997). For litter pH, about 10 g of litter were mixed with 100 mL distilled water in a stomacher bag and blended (Model 400, Tekmer, Ohio) at 230 rpm for 30 s. Sample pH was measured with a pH meter (Model 350, Corning Inc., New York). Water activity was measured in duplicate. The Aw meter (Model AquaLab CX2, Decagon Devices, Inc., Washington) was calibrated with 6 M NaCl (Aw=0.76) before measuring samples. Maximum and minimum temperatures inside and relative humidity within the production facility were recorded using temperature and relative humidity sensors (Model ART.NR, Fancom Corp., Netherlands).

RESULTS AND DISCUSSION

Frequency of detection

In any microbiological survey, it is important to select a suitable isolation/detection procedure for the targeted organism. A negative result does not necessarily indicate the absence of *Salmonella* in the sources sampled. Inability to detect *Salmonella* could be due to poor sensitivity of the isolation/detection procedure. In some cases, isolates may be viable but non-culturable. In any case, a sample may still harbor enough cells to transmit *Salmonella* infection among turkeys even though organisms were non-detectable (Amick-Morris, 1998).

Of the 991 samples in the survey, 6% were positive for *Salmonella*. Prevalence of *Salmonella* across all samples was found to vary from 0 to 21% among the sampled flocks. McBride and coworkers (1978) observed considerable flock to flock variation (0 to 72%) among broiler breeds with one-third of the flocks having greater than 10% infection. In the present study, *Salmonella* was isolated from 13% of litter samples, 11% of turkey ceca, 10% of drinkers, 5% of environmental swabs, 3% of feed, and 1% of feeder contents. *Salmonella* was not detected in turkey crop and air samples. Isolation of *Salmonella* from litter samples, drinkers,

feed samples, and feeder contents was evidence of the cross-contamination that occurred within the production facility. A survey conducted by Kumar and coworkers (1971) on three turkey flocks demonstrated that hatcheries, breeder flocks, and environmental sources such as litter, dust, water, and feed were the major sources of *Salmonella* contamination. Jones and coworkers (1991) surveyed two broiler houses for *Salmonella* contamination. *Salmonella* was isolated from 20.8% of feed mills, 13% of breeder houses, 7.1% of hatcheries, 4.5% of broiler houses, and 16.1% of processing plants and 13% of insects and 5.3% of mice collected at the grow-out facility. Among the sources sampled within the broiler houses, authors detected *Salmonella* in 4.3% of dead bird rinses, 2.4% of environmental swabs, and 5.2% of fecal droppings. Hoover and coworkers (1997) reported that 63.8% of drinkers, 53.8% of turkey ceca, 51.1% of litter, 39.1% of feeder contents, 22.8% of air, and 14.8% of feed were positive for the presence of *Salmonella* in two turkey flocks.

In F5, 21% of the 218 samples were positive for the presence of *Salmonella* (Table 6). *Salmonella* was isolated from 31% of turkey ceca, 28% of drinkers, 25% of litter samples, and 12% of environmental swabs. *Salmonella* was not detected in air and feed samples. Shedding of *Salmonella* by turkeys in feces could result in contamination of litter, drinkers, and feeder contents. Simmons and Byrnes (1972) found that 92% of the 56 litter samples isolated from two chicken flocks were contaminated with one or more *Salmonella* serotypes. Drinkers are a primary source of *Salmonella* transmission in poultry houses. Morgan-Jones (1980; 1982) reported that water in drinkers was the major oral route of infection or re-infection among broilers during rearing. Morris and coworkers (1969) found identical serotypes of *Salmonella* from the feces of colonized birds and drinker samples. Feeder contents, within each pen, may be contaminated through turkey feces, feathers, and personnel working within the facility. These

sources, in turn, could be responsible for propagating *Salmonella* transmission among birds. Frequency of *Salmonella* detection increased from 15% at week 2 to 47% at week 18. Although temperature within the facility was not recorded, temperature fluctuations could stress turkeys, inducing them to shed *Salmonella* that might lead to increased rates of horizontal transmission.

In F6, only 5% of the 179 samples were *Salmonella*-positive (Table 7). *Salmonella* was isolated from 22% of litter, 8% of feed, and 4% of feeder content samples, and 3% of turkey ceca. *Salmonella* was not detected from drinkers, environmental swabs, and air samples. Feed and feeder contents have been reported to be the major sources of horizontal transmission of *Salmonella* in poultry houses (MacKenzie and Bains, 1976; Cox et al., 1983; Jones et al. 1991). McIlroy (1998) reported that about 15% of the feed samples tested positive for *Salmonella*. Although *Salmonella* was undetected at week 6, frequency of *Salmonella* decreased slightly from 8% at week 13 to 7% at week 21. The decrease could be attributed to a qualitative and quantitative change in gut microflora that may inhibit *Salmonella* colonization in adult turkeys by competing for nutrients and attachment sites in the gastrointestinal tract. Barnes and coworkers (1972) showed that older birds were more resistant to intestinal colonization by *Salmonella* due to a mature gut microflora.

In F7, *Salmonella* could not be detected in any of the 361 samples (Table 8). Before placement of the poults in F7 (Day 0), litter, drinkers, air, feed, and environmental swabs were collected from various locations in the facility to determine the initial level of contamination. Poults entering the facility and box liners in which the poults were shipped were sampled as an indication of the level of *Salmonella* contamination acquired from the breeder flocks or the hatchery. Mayes and Takeballi (1983) reported that *Salmonella* might either contaminate young chicks directly through ovarian transmission or penetrate the shell membrane after the egg is

laid. Once *Salmonella* transverses the membrane, it is very difficult to remove and destroy them or prevent their further invasion of the egg contents and developing embryo (Cason et al. 1994). Day-old, *Salmonella*-free poults may be contaminated in the rearing house by the environment, feed, water, or litter samples. Simmons and Byrnes (1972) reported that day-old chicks, feed, and litter were the major sources of *Salmonella* contamination for chicken carcasses sampled from two flocks. In order to prevent horizontal transmission of *Salmonella*, it is imperative that poults entering the turkey house are free from *Salmonella*. Hoover and coworkers (1997) reported 18.1% of poult box liners and 25% of yolk sac samples were positive for the presence of *Salmonella* in two sampled flocks. Elsewhere, Jones and coworkers (1991) could detect *Salmonella* in 9.4% of chick yolk sac and 7.1% of hatcheries in a survey of 2 integrated broiler firms.

In F8, only 1% of the 233 samples were *Salmonella*-positive (Table 9). *Salmonella* was isolated from 6% of environmental swabs, 5% of litter, and 3% each of turkey ceca and feeder content samples. *Salmonella* could not be detected in turkey crops, drinkers, air, and feed samples. Reduced detection of *Salmonella* in F6 (5%), F7 (0%), and F8 (1%) could be attributed to Terminate™, a formaldehyde-based feed additive, administered to turkeys.

Distribution of serotypes

A total of 69 isolates were serotyped from 58 *Salmonella*-positive samples in F5, F6, and F8 (Table 10). *Salmonella heidelberg* was the most prevalent serotype, accounting for 65% of the isolates from these flocks. Other serotypes were identified as *S. senftenberg* (19%), *S. muenster* (10%), *S. anatum* (3%), and *S. worthington* (3%). Of the 48 isolates serotyped in F5, 92% were *S. heidelberg*, 4% *S. muenster*, and 4% *S. anatum* (Table 11). *Salmonella heidelberg* was isolated from turkey ceca (46%), drinkers (23%), litter samples (21%), and environmental

swabs (10%). Turkey ceca appeared to be the reservoir for *S. heidelberg*, and it may have been responsible for transmitting this serotype to litter and drinker samples. Differentiating *S. heidelberg* using genotypic methods would be useful in confirming the transmission pattern. In order to prevent *S. heidelberg* from entering the ecosystem of the production facility, control measures should be directed towards reducing this serotype in turkeys and not towards environmental vectors. *Salmonella muenster* was isolated from litter samples, drinkers, and environmental swabs, while *S. anatum* could be detected only in drinkers. Since *S. muenster* and *S. anatum* were detected only in litter and drinker samples, control measures should be implemented on these vectors, and not on the turkeys, because these vectors were responsible for propagation of these serotypes within the production facility. Of the 16 isolates serotyped in F6, 81% were *S. senftenberg*, 13% *S. worthington*, and 6% *S. heidelberg* (Table 12). *Salmonella senftenberg* was isolated primarily from turkey ceca (6%), litter (50%), and feed samples (25%), indicating that these vectors could serve as critical control points. *Salmonella worthington* was isolated from litter (6%) and feeder content samples (6%), while *S. heidelberg* was isolated from 6% of litter samples. *Salmonella muenster* was identified as the serotype for all 5 isolates evaluated in F8. *Salmonella muenster* was detected in 20% of litter samples and 40% each of environmental swabs and feeder content samples.

Serotypes appeared flock and source specific. In F5, *S. heidelberg* (92%) was the most prevalent serotype, while in F6 and F8, *S. senftenberg* (81%) and *S. muenster* (100%), respectively, were predominant. In F5, *S. anatum* was associated with only drinkers. *Salmonella heidelberg*, which was a predominant serotype in F5, could be detected only from F6 litter samples. The presence of *S. heidelberg* in F6 indicates that this serotype could have been transmitted from F5. This observation could have been confirmed by differentiating *S.*

heidelberg using genotypic methods such as plasmid finger printing, pulse field gel electrophoresis (PFGE), PCR-based typing or 16S ribotyping (Olsen et al., 1993; Threlfall et al., 1994). Serotypes were not identified using any such techniques in this study.

Serotype information can be valuable in designing control measures at the production facilities. If certain serotypes are consistently isolated from a particular farm, flock, or source, steps can be taken against those specific *Salmonella* strains in the production facility. Several studies have shown that *Salmonella* serotypes isolated from fully processed broiler carcasses originated from hatcheries, breeder flocks and/or broiler houses (Bhatia and McNabb, 1980; Bhargava et al., 1983; Lahellec and Colin, 1985; Goren et al., 1988; Jones et al., 1991). In a survey conducted by Bhargava and coworkers (1983), *S. kentucky*, *S. saint paul* and *S. infantis* were the major serotypes isolated from three chicken flocks. Lahellec and Colin (1985) found 17.3% of hatcheries and 21.6% of chicken houses infected with *Salmonella*. *Salmonella binza*, *S. infantis*, *S. saint paul*, and *S. schwarzengrund* were detected from chicken viscera, environmental swabs, drinkers, litter, and feeder contents. In addition, *S. coeln*, *S. heidelberg*, *S. montevideo*, and *S. saint paul* were detected consistently in hatchery, grow-out, and processing facilities. Jones and coworkers (1991) reported that *S. typhimurim* (33%) and *S. heidelberg* (13%) were the most prevalent serotypes isolated from breeder houses, broiler houses, feed mills, hatcheries, and processing units. Elsewhere, *S. agona* (47%), *S. saint paul* (26%), and *S. reading* (16%) were the most frequently detected from 25 sampled flocks (McBride et al., 1978). Morgan-Jones (1980) isolated *S. senftenberg*, *S. agona*, *S. montevideo* and *S. infantis* from water troughs, while *S. senftenberg*, *S. agona*, and *S. infantis* were isolated from litter samples from four broiler houses.

Antibiotic resistance profile

Of the 69 *Salmonella* serotypes screened, 25% were resistant to one or more antibiotics such as gentamycin (GM), spectinomycin (SPT), streptomycin (S), tetracycline (TE), tobramycin (TOB), and trimethoprim/sulfamethoxazole (SXT). Among the isolates screened, 100% each of *S. anatum* and *S. worthington*, 29% of *S. muenster*, and 17% of *S. heidelberg* were resistant to the above antibiotics. In F5, F6, and F8, frequency of *Salmonella* serotypes resistant to antibiotic(s) was isolated from drinkers (55%), swabs (43%), feeder contents (33%) turkey ceca (26%), and litter samples (5%) (Table 13).

Of the 48 isolates in F5, 27% of serotypes were resistant to two or more antibiotics (Table 14). Two isolates of *S. anatum* from drinkers at week 2 were resistant to TE and S. Of the 6 isolates of *S. heidelberg* detected in turkey ceca at week 18, 5 isolates were resistant to S, SPT, and GM, while the remaining isolate was resistant to TE, S, SPT, and GM. During this sampling period, two isolates of *S. heidelberg* from drinkers were resistant to S, SPT, and GM and the remaining two were resistant to TE, S, SPT, and GM. One isolate of *S. heidelberg* isolated from an environmental swab at week 18 was resistant to TE and SXT. Antibiotic resistant strains of *S. heidelberg* isolated from drinker samples at week 18 could have been horizontally transmitted by turkeys. However, the origin of TE and S-resistant *S. anatum* in drinkers and TE and SXT-resistant *S. heidelberg* in environmental swab remains unclear. Of the 16 isolates in F6, 13% of serotypes were resistant to TE (Table 15). At week 21 of the grow-out period, *S. worthington*, detected in litter and feeder content samples, was resistant to TE. Of the 5 isolates in F8, 40% of serotypes were resistant to TOB, S, SPT, and GM (Table 16). At week 20 of the grow-out period, two isolates of *S. muenster*, isolated from environmental swabs, were resistant to TOB, S, SPT, and GM.

Antimicrobial agents are fed at subtherapeutic levels to poultry to improve their growth rate and feed conversion, and at therapeutic levels to prevent bacterial infections. Polymixin B and combinations of (1) trimethoprim and sulfadiazine, (2) neomycin and polymixin, and (3) trimethoprim and polymixin B have been administered in feed or drinking water to reduce the levels of *Salmonella* in chickens (Craven, 1995). Williams (1985) reported that feeding oxytetracycline and/or neomycin reduced *S. typhimurium* levels in broiler intestines. On the other hand, feeding antibiotics such as avoparicin and lincomycin favored colonization of *S. typhimurium* while nitrofurazone enhanced colonization of *S. infantis* (Glisson, 1998). Spread of *Salmonella* to contact chickens was subsequently observed. The increase in *Salmonella* colonization could be the result of a decrease in total microbial count of the natural gut microflora. Administering these antibiotics can result in gradual emergence of antibiotic resistant *Salmonella*. One such human pathogen, *S. typhimurium* DT 104, has been found to be resistant to several antibiotics such as ampicillin, chloramphenicol, sulfonamides, streptomycin, and tetracycline. Approval of drugs such as sarafloxacin in poultry may contribute to the emergence and circulation of fluoroquinolone (nalidixic acid and ciprofloxacin) resistant strains of *Salmonella* (Herikstad et al., 1997). Drug resistant strains of *S. enteritidis* PT 4 and *S. typhimurium* DT 104 have been implicated in several outbreaks of salmonellosis. These drug resistant *Salmonella* strains, in turn, could contaminate poultry carcasses eventually leading to salmonellosis outbreaks among humans. It would be difficult to treat illnesses associated with such outbreaks with conventional antibiotics.

Effect of litter pH and water activity

Litter pH and A_w could play an important role in controlling or suppressing the growth of salmonellae in the environment within the production facilities. In F5, the average pH and

Aw measured from the sampled pens at week 10 and 18 were 7.53 and 0.959, and 7.62 and 0.958, respectively (Table 17). Litter pH and Aw measurement data were not available during week 2 of the sampling period. Although frequency of *Salmonella* detection increased from 17% at week 2 to 50% at week 18 in litter samples (Table 6), there was no significant change in litter pH and Aw measurements from week 10 to 18.

In F6, the average pH and Aw at week 6, 12 and 21 were 6.59 and 0.974, 7.48 and 0.981, and 8.10 and 0.870, respectively (Table 18). Although *Salmonella* was not detected in litter samples at week 6, frequency of detection decreased from 50% at week 13 to 17% at week 21 (Table 7). A high litter pH (8.10) and low Aw (0.870) at week 21 ($p < 0.05$) could have reduced presence of *Salmonella* in litter samples, when compared to week 6 and week 13 of the grow-out period. Opara et al. (1992) observed a negative correlation between *Salmonella*-positive, house-representative drag swab (HRDS) samples and litter pH. They concluded that an increase in litter pH was associated with a decrease in *Salmonella*-positive, HRDS samples collected from 13 chicken houses. The authors also reported a positive correlation between HRDS samples and litter Aw indicating that *Salmonella* prevalence increased with increased litter moisture. A high litter pH, as a result of ammonia, and low Aw suppress the growth of *S. typhimurim*, *S. senftenberg*, and *S. thompson* (Opara et al., 1992). These authors attributed the bactericidal activity to a lower Aw that is unfavorable to *Salmonella* viability and to a high litter pH from ammonia dissolved in the available moisture of the litter. A reduction of litter Aw and/or increased litter pH in the turkey grow-out facility may serve as an intervention measure in controlling *Salmonella*.

In F7, the average pH and Aw at week 0, 2, 10, 14, and 21 were 5.26 and 0.410, 6.55 and 0.967, 7.48 and 0.962, 6.96 and 0.979, and 7.70 and 0.953, respectively (Table 19). The low pH

(5.26) and Aw (0.410) of fresh litter may be responsible for no *Salmonella* in litter samples at day 0 (Table 8). Absence of *Salmonella* in fresh litter can also be attributed to heat processing during drying and processing. However, when poult s were assigned to pens, litter pH reached neutrality (6.55-7.70) and a Aw above 0.95 during the grow-out period. These conditions were conducive for harboring salmonellae. *Salmonella* has been reported to proliferate from pH 4.5 to 9.5, with an optimum pH for growth of 6.5 to 7.5 (D'Aoust, 1997). Water activity below 0.94 and above 0.4 are detrimental to *Salmonella* survival (Turnbull and Snoeyenbos, 1973). However, *Salmonella* was not detected in any of the litter samples during the grow-out period (Table 8), indicating that factors other than litter pH and Aw could be responsible for a 0% frequency of detection.

No relationship was observed between litter pH and Aw, and *Salmonella* detection in F8. Average pH and Aw at week 2, 15, and 20 were 6.34 and 0.983, 7.81 and 0.944, and 7.11 and 0.956, respectively (Table 20). *Salmonella* was detected in 13% of litter samples at week 2, but was not detected at week 15 and 20 (Table 9).

Effect of environmental conditions

Maximum and minimum temperatures inside the turkey production facility (averaged across north and south sides; Fig. 1) and percent relative humidity for F6, F7, and F8 are illustrated in Figs. 2, 3 and 4, respectively. Data were not available for F5 due to a malfunctioning of the temperature sensor. In F6, maximum and minimum temperatures inside the facility decreased with the grow-out period. While broad fluctuations were observed in relative humidity, the differences between maximum and minimum temperature measurements were uniform (Fig. 2). No relationship was observed between temperature differences and prevalence of *Salmonella* throughout the grow-out period in F6 because no significant changes

were observed in *Salmonella* frequency (Table 7). In F7, differences between maximum and minimum temperatures were greater from approximately 42 days until the end of the production period (Fig. 3). Relative humidity fluctuations were observed from day 0 to day 40. Large differences between the maximum and minimum temperatures would stress turkeys, inducing them to shed and transmit *Salmonella* within the facility. *Salmonella* remained undetected in sources sampled in F7 during the grow-out period, thus there was no relationship between temperature fluctuation and prevalence of *Salmonella*. In F8, the difference between maximum and minimum temperatures was greater after approximately 70 days of the grow-out period (Fig. 4). Similar to F7, no relationship was observed between temperature differences and prevalence of *Salmonella* in F8 because no significant changes were observed in *Salmonella* frequency (Table 9). No relationship between temperature and prevalence of *Salmonella* in F7 and F8 may have been due to addition of TerminateTM to the feed. The effectiveness of this feed additive in reducing the frequency of *Salmonella* detection was greater than stress-induced shedding and/or proliferation of *Salmonella* caused by temperature fluctuations.

Because the flocks were reared at different times during the year, effect of seasonal changes was evaluated. Flock 6 was sampled from September 1997 to February 1998 (winter), F7 was sampled from March 1998 to August 1998 (summer), and F8 was sampled from January 1999 to June 1999 (spring) (Table 1). Temperature differences were greater during summer months than winter and spring months. Seasonal changes did not affect frequency of *Salmonella* detection in this study. Hoover and coworkers (1997) found that greater temperature fluctuation during summer months contributed to greater *Salmonella* shedding from turkeys. McBride and coworkers (1978) found that *Salmonella* infection among broiler flocks entering the slaughter facility during the summer were not different from flocks arriving in winter. Temperature

differences can have a profound effect on *Salmonella* colonization dynamics (Soerjadi et al., 1979). High environmental temperatures have been reported to depress the immune system in young chickens (Thaxton and Siegel, 1970).

Sex effect

Male and female turkeys were placed only in F5 and F8 of the BUTA production trials. A total of 42 samples from turkey ceca, litter, and drinkers were positive for *Salmonella* in F5. Of these 42 samples, *Salmonella* was detected more frequently in male (57%) than in female (43%) turkeys. In litter and drinker samples, frequency of *Salmonella* detection was higher in males than in females (Fig. 5). A frequency of *Salmonella* detection among male turkeys could be the result of greater sensitivity of male birds to stimuli such as presence of personnel working in the production facility. This stimulus, in turn, could contribute to stress. Male turkeys appeared more active than females. This stress may cause greater shedding of *Salmonella* in their feces, which might cross contaminate litter and drinkers within each pen. Observations regarding the mobility of turkeys were in context with this particular flock, and they do not represent mobility of birds in a typical grow-out facility. In a study conducted by McBride and coworkers (1978) on 25 turkey flocks, the average incidence of salmonellae in male and female turkeys was 7.4 (0 to 36%) and 9.2 % (0 to 44%), respectively. With respect to turkeys in F8, *Salmonella* was detected in 75% males and 25% females. One *Salmonella*-positive sample each of litter, drinker, and feeder content was isolated from male pens, while the remaining sample was isolated from the ceca of a female turkey.

CONCLUSIONS

Contamination of poultry products with *Salmonella* concerns consumers and the poultry industry. Reduction of *Salmonella* is necessary on farms where turkeys are bred, hatched,

brooded, and raised. This study was undertaken to identify and confirm the key sources responsible for *Salmonella* colonization at a turkey production facility. Frequency of *Salmonella* detection varied from 0 to 21% across the four flocks sampled. Turkeys were the major sources and litter and drinkers were the major vectors of *Salmonella* contamination in the turkey production facility. *Salmonella* colonizes the gastrointestinal tract of poultry and, through horizontal transmission, contaminates other birds and eventually carcasses. A knowledge of preharvest factors responsible for *Salmonella* colonization would help integrators and poultry producers design hazard analysis and critical control point (HACCP) protocols to safeguard fresh turkey against *Salmonella* contamination during processing.

Salmonella heidelberg was the most prevalent serotype isolated among the sampled flocks. About 25% of *Salmonella* serotypes were resistant to one or more antibiotics tested. Some serotypes appeared flock specific and were associated with specific preharvest sources. Serotype information and antibiogram patterns can be used, in part, to delineate possible transmission pathways of *Salmonella* in the rearing facilities. A transmission pathway could not be completely established in the present study due to limitation on the number of samples collected and number of isolates serotyped and screened for their antibiotic resistance pattern. Genotypic methods of differentiating *Salmonella* serotypes can provide a complete picture on the transmission pathway. This information could be used to define critical, preharvest control points on the farm. Intervention at these points would reduce and/or eliminate horizontal transmission and *Salmonella*-positive birds arriving at the processing plants. Although antibiotics have improved growth efficiency and reduced bacterial infections in poultry, widespread use could result in emergence of antibiotic resistant strains in farm birds and fresh and processed poultry products.

Environmental conditions, farm management, and husbandry practices within the poultry facility could contribute to stressful conditions increasing the potential for *Salmonella* colonization. No relationship was observed between environmental conditions and *Salmonella* prevalence in this study. *Salmonella* was detected more frequently in male than in female turkeys. A higher frequency of *Salmonella* in male turkeys warrants consideration in designing protocols that reduce *Salmonella* colonization by minimizing stress in rearing male turkeys.

Salmonella is a management disease, and its control depends on controlling the sources of contamination and transmission. To reduce and/or eliminate the incidence of *Salmonella* in turkeys before they enter the processing plants, a concerted effort among hatchery management, breeders, producers, integrators, and feed manufacturers is necessary.

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Table 1. Characteristics of turkey flocks sampled during the survey.

Turkey flocks	BUTA production trials	Sampling period	Sampling frequency (weeks)	Number of pens sampled	Sex of birds	Samples ¹	Environmental characteristics
Flock 5	6 strains x 2 genders	April 1997 to August 1997	2, 10, and 18	12	Males and females	CC, L, D, ES, A, and F	Litter pH and Aw
Flock 6	3 strains x 2 feeds	September 1997 to February 1998	6, 13, and 21	6	Males	CC, L, D, ES, A, F, and FC	Litter pH and Aw, and temperature and relative humidity ²
Flock 7	4 strains x 2 feeds	March 1998 to August 1998	0, 2, 10, 14, and 21	5-10	Females	IY, CC, CrC, L, D, ES, A, F, FC, and BL	Litter pH and Aw, and temperature and relative humidity
Flock 8	4 strains x 2 genders	January 1999 to June 1999	2, 15, and 20	6-8	Males and Females	CC, CrC, L, D, ES, A, F, and FC	Litter pH and Aw, and temperature and relative humidity

¹ IY = intestine and yolk sac samples; CC = turkey cecal contents; CrC = turkey crop contents; L = litter samples; D = drinker samples; ES = environmental swabs; A = air samples; F = feed samples; FC = feeder contents; BL = box liner samples

² Maximum and minimum temperatures and relative humidity were measured inside the production facility.

Table 2. Pen assignments for the sampled flocks.

Pen No.	Flock 5		Flock 6		Flock 7		Flock 8	
	Strain	Sex	Strain	Feed	Strain	Feed	Strain	Sex
1	78x12	Toms	Hybrid	USA ¹	Nicholas	LC ³	Nicholas	Toms
2	72x71	Toms	Big 6	EHP ²	Hybrid	HH ⁴	37-Roaster	Toms
3	78x25	Toms	Nicholas	EHP	Line 37	HH	Big 6	Toms
4	38x71	Toms	Big 6	USA	Big 6	LC	Nicholas	Toms
5	72x12	Toms	Hybrid	EHP	Nicholas	HH	37-Roaster	Toms
6	72x25	Toms	Nicholas	USA	Hybrid	LC	Hybrid	Toms
7	72x17	Toms	Nicholas	EHP	Hybrid	HH	Big 6	Toms
8	38x71	Toms	Hybrid	USA	Big 6	LC	Nicholas	Toms
9	72x71	Toms	Big 6	EHP	Hybrid	LC	37-Roaster	Toms
10	72x25	Toms	Hybrid	EHP	Nicholas	HH	Hybrid	Toms
11	78x12	Toms	Nicholas	USA	Line 37	LC	Big 6	Toms
12	78x25	Toms	Big 6	USA	Big 6	HH	Hybrid	Toms
13	72x71	Hens	Nicholas	USA	Line 37	LC	Nicholas	Hens
14	78x25	Hens	Big 6	USA	Big 6	HH	37-Roaster	Hens
15	72x25	Hens	Hybrid	EHP	Hybrid	HH	Hybrid	Hens
16	72x12	Hens	Nicholas	EHP	Nicholas	LC	Nicholas	Hens
17	38x71	Hens	Big 6	EHP	Line 37	HH	37-Roaster	Hens
18	78x12	Hens	Hybrid	USA	Big 6	LC	Big 6	Hens
19	72x25	Hens	Big 6	EHP	Nicholas	HH	Hybrid	Hens
20	72x71	Hens	Nicholas	USA	Line 37	LC	Nicholas	Hens
21	38x71	Hens	Hybrid	EHP	Nicholas	LC	Big 6	Hens
22	78x12	Hens	Big 6	USA	Hybrid	LC	37-Roaster	Hens
23	78x25	Hens	Hybrid	USA	Big 6	HH	Big 6	Hens
24	72x12	Hens	Nicholas	EHP	Line 37	HH	Hybrid	Hens

Shaded areas indicate brooding pens; ¹BUTA-USA; ²European high protein; ³Least cost; ⁴Heavy hen

Table 3. Dietary specifications of the British United Turkeys of America feeding program used for flock 6.

Diet composition	BUTA (USA) Feeding Program							European High Protein (EHP) Feeding Program					
	Starter I	Starter II	Grower I	Grower II	Grower III	Finish I	Finish II	Starter I	Starter II	Grower I	Grower II	Finish I	Finish II
ME	1290	1310	1350	1425	1475	1525	1550	1275	1340	1425	1475	1540	1550
Protein	28.5	26.0	24.0	22.0	20.0	18.0	17.0	31.50	27.70	24.83	21.03	18.71	17.22
Lysine	1.80	1.65	1.48	1.30	1.12	1.00	0.90	1.88	1.66	1.45	1.21	1.07	0.92
TSAA	1.15	1.10	1.02	0.95	0.90	0.80	0.75	1.22	1.17	1.10	0.97	0.91	0.81
Methionine	0.70	0.65	0.60	0.56	0.51	0.48	0.42	0.68	0.66	0.61	0.54	0.51	0.45
Arginine	1.96	1.78	1.58	1.43	1.21	1.10	1.00	2.03	1.81	1.60	1.39	1.25	1.14
Threonine	1.05	1.00	0.94	0.84	0.70	0.64	0.59	1.20	1.07	0.99	0.79	0.68	0.60
Calcium	1.50	1.40	1.30	1.20	1.10	1.00	0.90	1.50	1.30	1.20	1.10	1.00	0.90
A. Phos.	0.75	0.70	0.65	0.60	0.55	0.50	0.45	0.75	0.65	0.60	0.55	0.50	0.45
Sodium	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.18	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.19	0.16- 0.19
Chloride	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25
Pound/tom	2.2	8.0	12.0	18.0	22.0	24.0	Market	3.5	12.0	22.0	30.0	40.0	Market

Table 4. Dietary specifications of the British United Turkeys of America feeding program used for flock 7.

Diet composition	Heavy Hen (HH) Feeding Program							Least Cost (LC) Hen Feeding Program						
	Starter I (201)	Starter II (202)	Grower I (203)	Grower II (204)	Grower III (205)	Finish I (206)	Finish II (207)	Starter I (401)	Starter II (402)	Grower I (403)	Grower II (404)	Grower III (405)	Finish I (406)	Finish II (407)
ME	1290	1325	1375	1450	1475	1500	1550	1290	1325	1400	1450	1500	1525	1550
Protein	28.5	26.0	24.0	22.0	19.3	18.2	16.2	28.5	26.0	24.0	22.3	19.3	17.5	15.4
Lysine	1.78	1.62	1.45	1.30	1.15	1.02	0.93	1.78	1.62	1.55	1.35	1.15	0.98	0.88
TSAA	1.20	1.15	1.05	0.95	0.88	0.82	0.75	1.20	1.15	1.08	0.95	0.85	0.75	0.68
Arginine	1.96	1.80	1.58	1.43	1.21	1.10	1.00	1.96	1.76	1.58	1.47	1.25	1.10	0.95
Threonine	1.05	1.00	0.94	0.84	0.74	0.67	0.59	1.05	1.00	0.95	0.84	0.72	0.65	0.55
Calcium	1.40	1.30	1.20	1.15	1.10	1.00	0.90	1.40	1.30	1.20	1.15	1.05	1.00	0.90
A. Phos.	0.75	0.70	0.63	0.60	0.55	0.50	0.45	0.75	0.65	0.63	0.60	0.55	0.50	0.45
Sodium	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18	0.16-0.18
Chloride	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25	0.20-0.25
Age (days)	0-21	22-42	43-63	64-77	78-98	99-112	Market	0-14	15-28	29-49	50-63	64-77	78-91	Market
Pound/hen	2.00	5.62	9.75	8.50	15.14	11.50	25.50	1.00	2.50	6.93	6.96	8.50	9.79	42.45
Total lbs	1350	4720	8200	7150	12100	9200	19400	675	2100	5800	5800	7200	7800	32000

Table 5. Dietary specifications of the British United Turkeys of America feeding program used for flock 8.

Diet Composition	Heavy Bird Feeding Program						
	Starter I	Starter II	Grower I	Grower II	Grower III	Finisher I	Finisher II
ME	1290	1325	1375	1450	1475	1500	1550
Protein	28.5	26.0	24.0	22.0	19.3	18.2	16.2
Lysine	1.78	1.62	1.45	1.30	1.15	1.02	0.93
TSAA	1.20	1.15	1.05	0.95	0.88	0.82	0.75
Arginine	1.96	1.80	1.58	1.43	1.21	1.10	1.00
Threonine	1.05	1.00	0.94	0.84	0.74	0.67	0.59
Calcium	1.40	1.30	1.20	1.15	1.10	1.00	0.90
A. Phos.	0.75	0.70	0.63	0.60	0.55	0.50	0.45
Sodium	0.16- 0.18	0.16- 0.18	0.16- 0.18	0.16- 0.18	0.16- 0.18	0.16- 0.18	0.16- 0.18
Chloride	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25	0.20- 0.25
Age (days)	0-21	22-42	43-63	64-77	78-98	99-112	Market
Pounds/hen	2.00	5.62	9.75	8.50	15.14	11.50	25.50
Age (days)	0-21	22-42	43-63	64-84	85-105	106-126	Market
Pounds/tom	2.00	7.85	11.84	17.56	21.00	23.55	15.00

Table 6. Frequency of *Salmonella* detected from various flock 5 samples in the turkey production facility.

Sample	Grow-out Period (number positive/number sampled)			Total
	Week 2	Week 10	Week 18	
Turkey ceca	6/24	1/24	15/24	22/72 (31%) ¹
Litter	2/12	1/12	6/12	9/36 (25%)
Drinkers	2/12	2/12	6/12	10/36 (28%)
Environmental swabs	1/14	0/18	4/10	5/42 (12%)
Air	0/11	0/10	0/5	0/26
Feed	0/0	0/3	0/3	0/6
Total	11/73 (15%)	4/79 (5%)	31/66 (47%)	46/218 (21%)

¹ Figures in parentheses indicate percent positive

Table 7. Frequency of *Salmonella* detected from various flock 6 samples in the turkey production facility.

Sample	Grow-out Period (number positive/number sampled)			Total
	Week 6	Week 13	Week 21	
Turkey ceca	0/12	0/12	1/10	1/34 (3%) ¹
Litter	0/6	3/6	1/6	4/18 (22%)
Drinkers	0/6	0/6	0/6	0/18
Environmental swabs	0/8	0/8	0/8	0/24
Air	0/8	0/7	0/8	0/23
Feed	0/15	1/10	2/13	3/38 (8%)
Feeder contents	0/0	0/0	1/24	1/24 (4%)
Total	0/55	4/49 (8%)	5/75 (7%)	9/179 (5%)

¹ Figures in parentheses indicate percent positive

Table 8. Frequency of *Salmonella* detected from various flock 7 samples in the turkey production facility.

Sample	Grow-out Period (number positive/number sampled)					Total
	Day 0	Week 2	Week 10	Week 14	Week 21	
Turkey ceca	0/12 ¹	0/16	0/12	0/12	0/12	0/64
Turkey crop	0/0	0/0	0/0	0/0	0/12	0/12
Litter	0/6	0/10	0/6	0/6	0/5	0/33
Drinkers	0/6	0/8	0/6	0/6	0/5	0/31
Environmental swabs	0/14	0/8	0/8	0/8	0/8	0/46
Air	0/8	0/8	0/7	0/8	0/8	0/39
Feed	0/2	0/2	0/10	0/4	0/8	0/26
Feeder contents	0/0	0/10	0/28	0/29	0/40	0/107
Liners	0/3	0/0	0/0	0/0	0/0	0/3
Total	0/51	0/62	0/77	0/73	0/98	0/361

¹ turkey intestine and yolk sacs were sampled

Table 9. Frequency of *Salmonella* detected from various flock 8 samples in the turkey production facility.

Sample	Grow-out Period (number positive/number sampled)			Total
	Week 2	Week 15	Week 20	
Turkey ceca	0/15	0/12	0/12	0/39 (3%) ¹
Turkey crop	0/13	0/12	0/12	0/37
Litter	1/8	0/6	0/6	1/20 (5%)
Drinkers	0/8	0/6	0/6	0/20
Environmental swabs	0/6	0/6	1/6	1/18 (6%)
Air	0/6	0/6	0/6	0/18
Feed	0/8	0/24	0/9	0/41
Feeder contents	0/0	0/20	1/20	1/40 (3%)
Total	1/64 (2%)	0/92	2/77 (3%)	3/233 (1%)

¹ Figures in parentheses indicate percent positive

Table 10. Distribution of *Salmonella* serotypes isolated from various samples in the turkey production facility.

Sample	Serotype	Flock 5			Flock 6		Flock 8		Total
		Week 2	Week 10	Week 18	Week 13	Week 21	Week 2	Week 20	
CECAL CONTENTS	<i>S. heidelberg</i>	6	1	15	-	-	-	-	22
	<i>S. senftenberg</i>	-	-	-	-	1	-	-	1
LITTER	<i>S. heidelberg</i>	1	2	6	1	-	-	-	10
	<i>S. muenster</i>	1	-	-	-	-	1	-	2
	<i>S. senftenberg</i>	-	-	-	8	-	-	-	8
	<i>S. worthington</i>	-	-	-	-	1	-	-	1
DRINKERS	<i>S. anatum</i>	2	-	-	-	-	-	-	2
	<i>S. heidelberg</i>	-	1	7	-	-	-	-	8
	<i>S. muenster</i>	-	1	-	-	-	-	-	1
SWABS	<i>S. heidelberg</i>	1	-	4	-	-	-	-	5
	<i>S. muenster</i>	-	-	-	-	-	-	2	2
FEED	<i>S. senftenberg</i>	-	-	-	2	2	-	-	4
FEEDER CONTENTS	<i>S. muenster</i>	-	-	-	-	-	-	2	2
	<i>S. worthington</i>	-	-	-	-	1	-	-	1
Total		11	5	32	11	5	1	4	69

Table 11. Distribution of *Salmonella* serotypes isolated from various flock 5 samples in the turkey production facility.

Serotype	Samples				Total
	Turkey ceca	Litter	Drinkers	Environmental swabs	
<i>S. heidelberg</i>	22	9	8	5	44 (92%) ¹
<i>S. muenster</i>	0	1	1	-	2 (4%)
<i>S. anatum</i>	0	0	2	0	2 (4%)
Total	22	10	11	5	48

¹ Figures in parentheses indicate percent of total isolates

Table 12. Distribution of *Salmonella* serotypes isolated from various flock 6 samples in the turkey production facility.

Serotype	Samples				Total
	Turkey ceca	Litter	Feed	Feeder contents	
<i>S. senftenberg</i>	1	8	4	0	13 (81%) ¹
<i>S. worthington</i>	0	1	0	1	2 (13%)
<i>S. heidelberg</i>	0	1	0	0	1 (6%)
Total	1	10	4	1	16

¹ Figures in parentheses indicate percent of total isolates

Table 13. Frequency of antibiotic resistant *Salmonella* serotypes isolated from various samples in the turkey production facility.

Sample	Flock 5	Flock 6	Flock 8	Total
Turkey ceca	6/22	0/1	0/0	6/23 (26%) ¹
Litter	0/10	1/10	0/1	1/21 (5%)
Drinkers	6/11	0/0	0/0	6/11 (55%)
Environmental swabs	1/5	0/0	2/2	3/7 (43%)
Feed	0/0	0/4	0/0	0/4
Feeder contents	0/0	1/1	0/2	1/3 (33%)
Total	13/48 (27%)	2/16 (13%)	2/5 (40%)	17/69 (25%)

¹ Figures in parentheses indicate percent positive

Table 14. Antibiotic resistance among *Salmonella* serotypes isolated from various flock 5 samples in the turkey production facility.

Sample	Grow-out Period						Total
	Week 2		Week 10		Week 18		
	Serotype	Frequency ¹	Serotypes	Frequency	Serotypes	Frequency	
Turkey ceca	<i>S. heidelberg</i>	0/6	<i>S. heidelberg</i>	0/1	<i>S. heidelberg</i>	6/15 (5-S, Spt, Gm) (1-Te, S, Spt, Gm)	6/22 (27%) ²
Litter	<i>S. heidelberg</i> <i>S. muenster</i>	0/1 0/1	<i>S. heidelberg</i>	0/2	<i>S. heidelberg</i>	0/6	0/10
Drinkers	<i>S. anatum</i>	2/2 (2-Te, S) ³	<i>S. heidelberg</i> <i>S. muenster</i>	0/1 0/1	<i>S. heidelberg</i>	4/7 (2-S, Spt, Gm) (2-Te, S, Spt, Gm)	6/11 (55%)
Environmental Swabs	<i>S. heidelberg</i>	0/1			<i>S. heidelberg</i>	1/4 (1-Te, Sxt)	1/5 (20%)
Total		2/11 (18%)		0/5		11/32 (34%)	13/48 (27%)

¹ Antibiotic resistant isolates/total isolates

² Figures in parentheses indicate percent positive

³ Number of isolates and resistant antibiotics

Te=tetracycline; S=streptomycin; Spt=spectinomycin; Gm=gentamycin; Sxt=trimethoprim/sulfmethoxazole

Table 15. Antibiotic resistance among *Salmonella* serotypes isolated from various flock 6 samples in the turkey production facility.

Sample	Grow-out Period				Total
	Week 13 Serotype	Week 13 Frequency ¹	Week 21 Serotype	Week 21 Frequency	
Turkey ceca	-	-	<i>S. senftenberg</i>	0/1	0/1
Litter	<i>S. senftenberg</i>	0/8	<i>S. worthington</i>	1/1 (1-Te) ²	1/10 (10%) ³
	<i>S. heidelberg</i>	0/1			
Feed	<i>S. senftenberg</i>	0/2	<i>S. senftenberg</i>	0/2	0/4
Feeder contents	-	-	<i>S. worthington</i>	1/1 (1-Te)	1/1 (100%)
Total		0/11		2/5 (40%)	2/16 (13%)

¹ Antibiotic resistant isolates/total isolates

² Number of isolates and resistant antibiotic

Te=tetracycline

³ Figures in parentheses indicate percent positive

Table 16. Antibiotic resistance among *Salmonella* serotypes isolated from various flock 8 samples in the turkey production facility.

Sample	Grow-out Period				Total
	Week 2		Week 20		
	Serotype	Frequency ¹	Serotype	Frequency	
Litter	<i>S. muenster</i>	0/1	-	-	0/1
Environmental swabs	-	-	<i>S. muenster</i>	2/2 (2-Tob, S, Spt, Gm) ²	2/2 (100%) ³
Feeder contents	-	-	<i>S. muenster</i>	0/2	0/2
Total		0/1		2/4 (50%)	2/5 (40%)

¹ Antibiotic resistant isolates/total isolates

² Number of isolates and resistant antibiotics

Tob=tobramycin; S=streptomycin; Spt=spectinomycin; Gm=gentamycin

³ Figures in parentheses indicate percent positive

Table 17. Litter pH and Aw measurements from pens sampled during the grow-out of flock 5

Pen No.	Week 10		Pen No.	Week 18	
	PH	Aw		pH	Aw
1	7.77	0.945	2	6.39	0.961
3	8.37	0.949	4	7.71	0.954
6	8.35	0.949	5	7.33	0.962
7	7.20	0.945	8	7.36	0.976
9	7.44	0.966	10	7.49	0.991
12	8.31	0.950	11	7.73	0.944
13	7.38	0.976	14	8.21	0.928
15	6.07	0.972	16	7.47	0.966
18	5.81	0.976	17	8.26	0.938
19	7.12	0.961	20	8.24	0.969
21	7.94	0.953	22	7.77	0.954
24	8.62	0.970	23	7.42	0.956
Average	7.53	0.959		7.62	0.958

Table 18. Litter pH and Aw measurements from pens sampled during the grow-out of flock 6

Week 6			Week 13			Week 21		
Pen No.	pH	Aw	Pen No.	pH	Aw ¹	Pen No.	pH	Aw ²
1	7.19	0.990	3	7.29	0.982	2	8.34	0.886
9	6.22	0.990	4	6.91	0.979	5	7.97	0.832
11	6.34	0.964	5	7.40	0.982	10	8.16	0.854
15	6.38	0.962	17	7.72	0.976	14	8.28	0.867
22	7.09	0.976	18	7.82	0.988	19	7.59	0.918
24	6.30	0.960	20	7.74	0.979	21	8.25	0.865
Total	6.59	0.974		7.48	0.981		8.10	0.870

¹ Average temperature of the Aw meter 23.5°C

² Average temperature of the Aw meter 25.4°C

Table 19. Litter pH and Aw measurements from pens sampled during the grow-out of flock 7

Day 0			Week 2			Week 10			Week 14			Week 21		
Pen No	pH	Aw ¹	Pen No	pH	Aw ²	Pen No	pH	Aw ³	Pen No	pH	Aw ⁴	Pen No	pH	Aw ⁵
3	5.24	0.326	3	6.02	0.979	1	7.48	0.950	2	7.38	0.977	4	8.33	0.967
4	5.34	0.456	4	6.97	0.985	5	7.32	0.981	3	6.73	0.983	7	7.49	0.971
10	5.25	0.377	9	6.94	0.967	8	7.46	0.966	6	7.11	0.987	16	7.51	0.914
14	5.38	0.385	10	6.21	0.984	11	6.88	0.948	13	7.17	0.976	20	7.33	0.957
16	5.26	0.433	14	6.77	0.967	14	7.67	0.966	17	6.46	0.986	22	7.85	0.954
22	5.09	0.482	15	7.59	0.975	15	8.04	0.960	18	6.91	0.966			
			16	6.07	0.931									
			20	5.93	0.982									
			21	6.86	0.941									
			22	6.16	0.960									
Avg.	5.26	0.410		6.55	0.967		7.48	0.962		6.96	0.979		7.70	0.953

¹ Average temperature of Aw meter 30.3°C

² Average temperature of Aw meter 27.8°C

³ Average temperature of Aw meter 22.6°C

⁴ Average temperature of Aw meter 21.6°C

⁵ Average temperature of Aw meter 21.4°C

Table 20. Litter pH and Aw measurements from pens sampled during the grow-out of flock 8

Week 2			Week 15			Week 20		
Pen No.	pH	Aw ¹	Pen No.	pH	Aw ²	Pen No.	pH	Aw ³
3	6.30	0.979	2	8.35	0.932	1	7.02	0.983
4	6.39	0.979	6	8.37	0.944	5	7.56	0.964
9	6.35	0.983	12	7.68	0.953	11	7.32	0.963
10	6.27	0.978	13	7.31	0.944	14	6.91	0.943
15	6.25	0.989	17	7.66	0.935	18	6.68	0.965
16	6.39	0.988	20	7.51	0.955	19	7.15	0.915
21	6.37	0.990						
22	6.40	0.975						
Total	6.34	0.983		7.81	0.944		7.11	0.956

¹ Average temperature of Aw meter 25.2°C

² Average temperature of Aw meter 22.1°C

³ Average temperature of Aw meter 24.1°C

Figure 1. Turkey production facility.

NORTH SIDE

Feed storage
bins
○

Feed storage
bins
○

SOUTH SIDE

Exit (SA) Exhaust Fan							Rear Entrance	Entrance (SA) Exhaust Fan							
Entrance								Service Area	Exit						
	18	17	16	15	14	13				6	5	4	3	2	1
	24	23	22	21	20	19				12	11	10	9	8	7
Service Alley (SA)							Chlorination unit	Service Alley							
							Iodine spray Entrance								

Figure 2. Maximum and minimum temperatures and percent relative humidity inside the turkey production facility in flock 6.

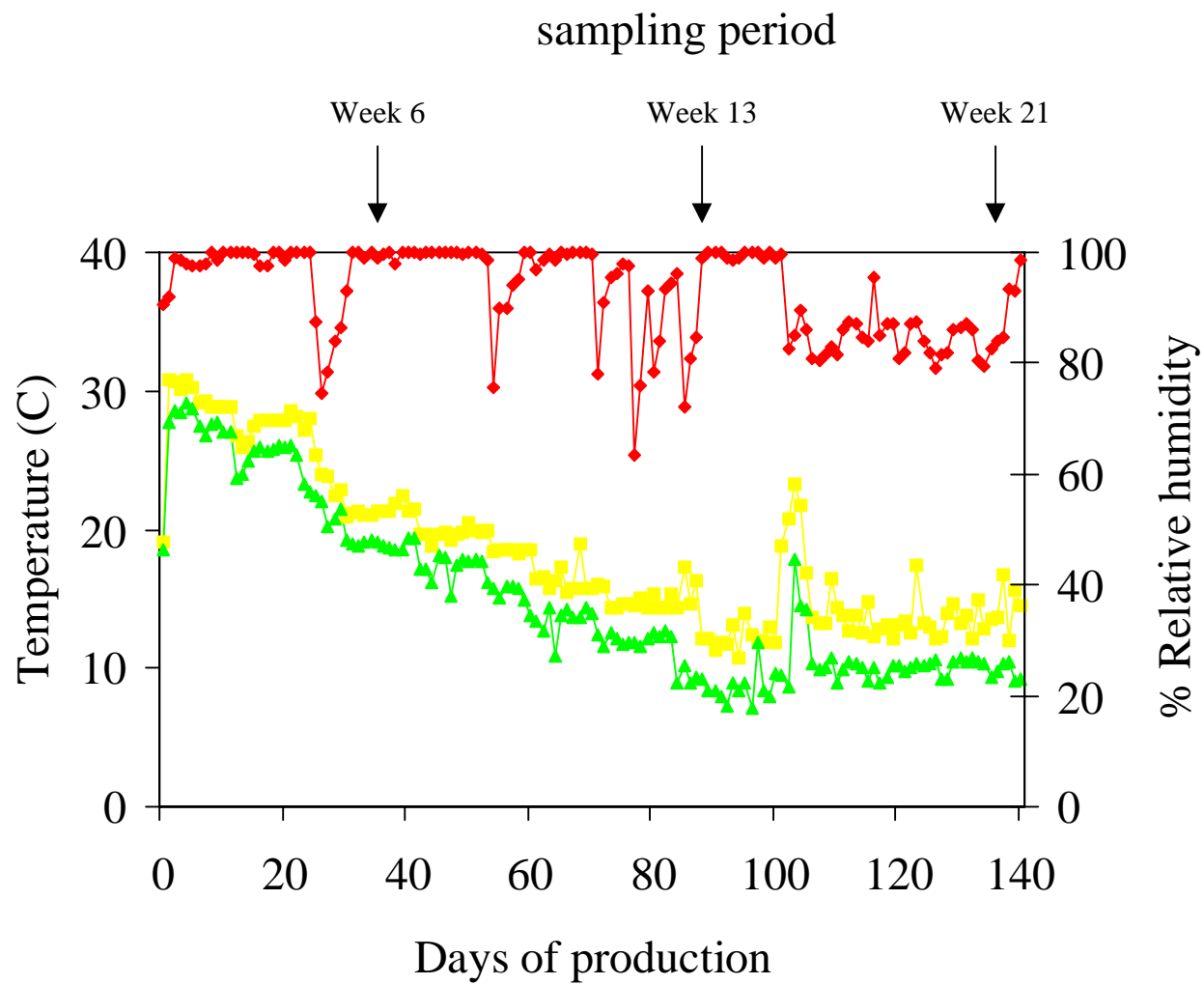


Figure 3. Maximum and minimum temperatures and percent relative humidity inside the turkey production facility in flock 7.

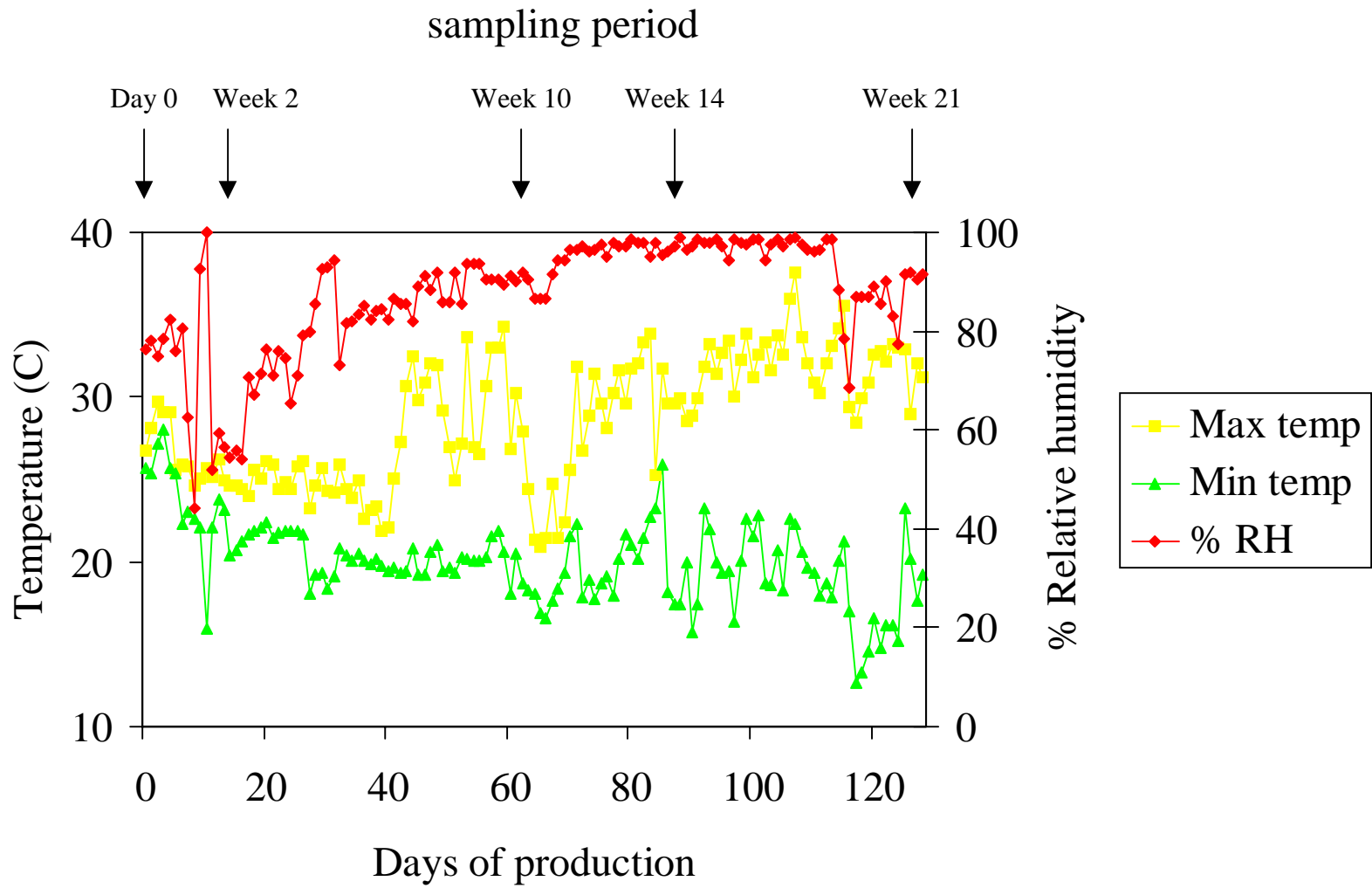


Figure 4. Maximum and minimum temperatures and percent relative humidity inside the turkey production facility in flock 8.

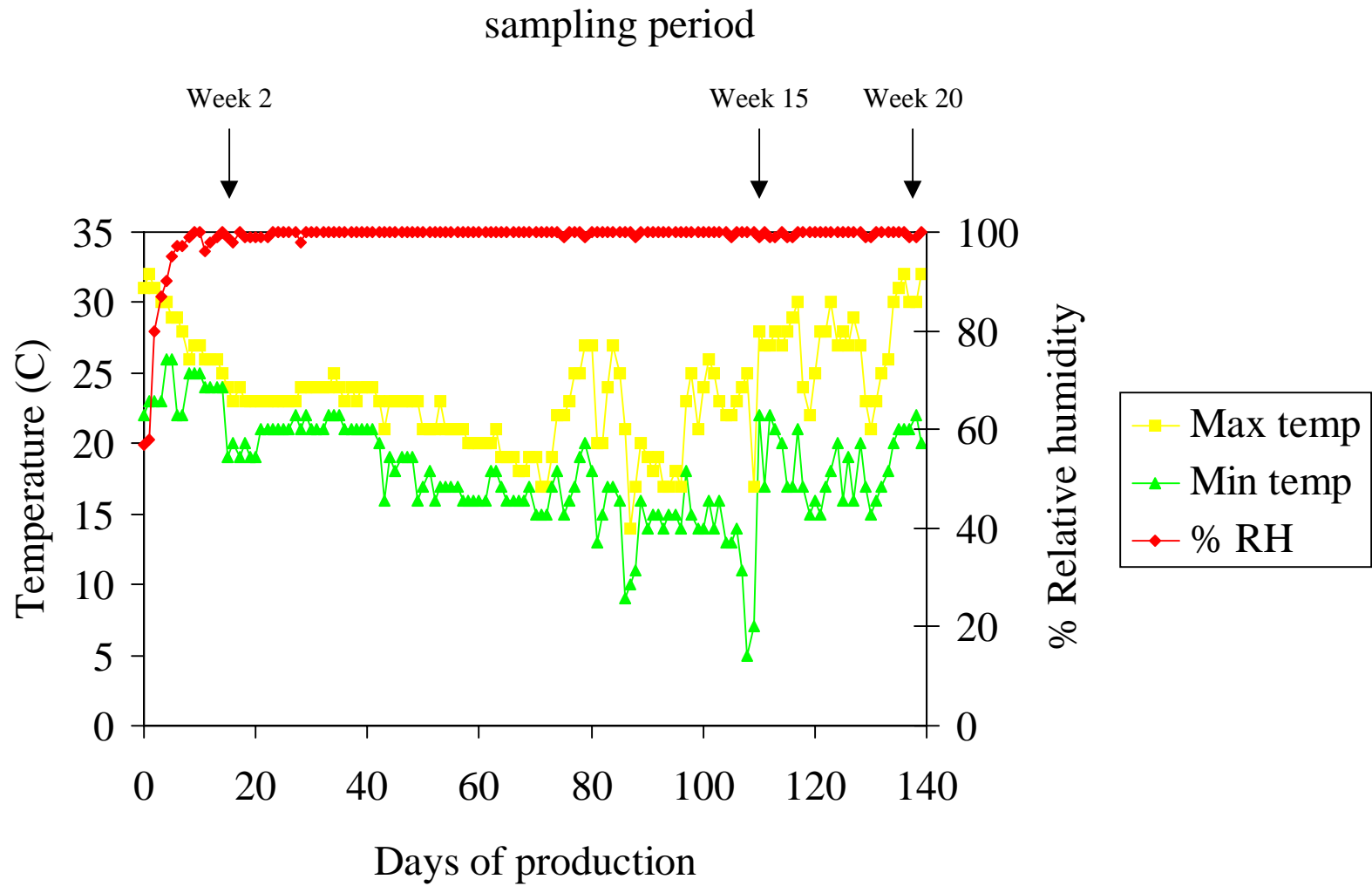
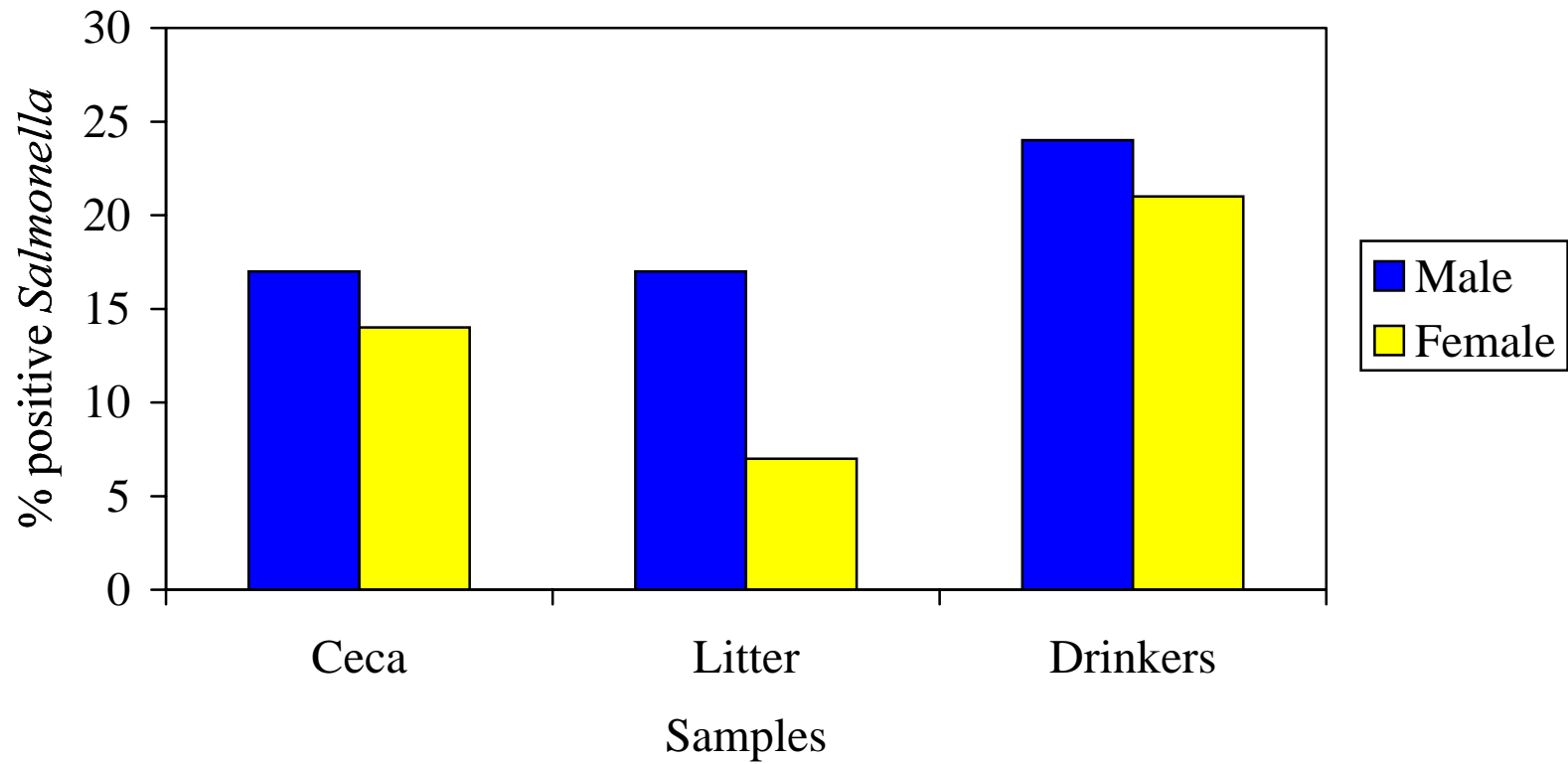


Figure 5. Prevalence of *Salmonella* in male and females turkeys from various flock 5 samples in the turkey production facility.



APPENDICES

FARM BIOSECURITY MEASURES

Following biosecurity measures were implemented on the farm:

1. All barns used to brood, rear, and lay parent stock were operated on a single age, all in-all out basis. Adequate time was allowed for cleaning the facility and equipments before placement of the new flock. Building surroundings were maintained to exclude rodents, wild birds, and other wild animal that could be responsible for transmitting diseases to the birds.
2. Dead birds were removed from the general population and immediately incinerated.
3. Water supply used for drinkers was chlorinated (2-3 ppm residual chlorine) to reduce and control levels of harmful pathogens.
4. Personnel movement inside the facility was controlled. Before entering the facility all personnel were required to shower and change clothes (including footwear) that were provided by BUTA on the farm. Used clothing was washed within the service building. Before entering the turkey house, the rubber boots were rinsed with water containing iodine solution [18.05% (alpha-(p-nonylphenyl)-omega-hydroxypoly(oxyethylene) iodine complex; 1.75% titrable iodine and 16% phosphoric acid].
5. Vehicles transporting poultts and carrying market-size birds were thoroughly cleaned and disinfected to reduce the risk of disease transmission.

Source: British United Turkeys of America (BUTA) Management Guide

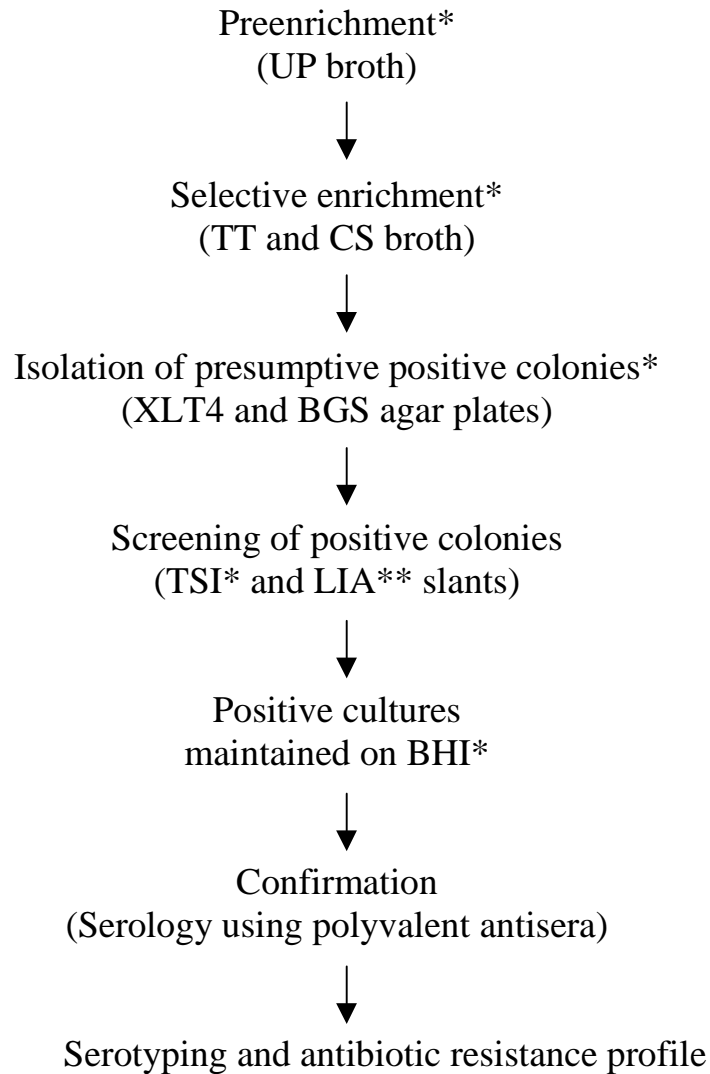
TURKEY HOUSE CLEANING AND DISINFECTION METHODS

Following measures were implemented by BUTA between placement of each flock:

1. Following removal of birds, all remaining feed from bins, augers, hoppers, lines, and feed pans were either disposed off or transferred from the farm.
2. Movable equipments such as drinkers, hoses, feed pans, and feed drops were moved to a centralized location for washing.
3. Personnel clothing from shower/dressing area was washed, dried, and placed into plastic bags for storage until next cycle.
4. A high-pressure water washer was used to rinse dust and litter from the ceiling walls, windowsills, fans, and light traps. Thorough washing was carried out later. Fan motors, electric boxes, and thermostats were dry cleaned.
5. Litter and remaining debris were swept and removed from the facility.
6. Feed tank was washed with a high pressure sprayer containing 20% chlorine bleach and the water was allowed to run out of the boot.
7. Water lines were flushed with 20% chlorinated water for 15-20 min. The chlorinated water was allowed to sit in the water lines over night before flushing with clean water for 15-20 min.
8. Turkey house (ceiling, walls, and floor) was cleaned and disinfected with high-pressure water with mild detergent before placement of new flock.
9. Feed lines and hoppers were cleaned before washing the floor so that all residual feed/dust was removed from the facility.
10. Storage, break room, and shower areas were cleaned.

11. Equipment was removed from the facility, disassembled, and disinfected. After cleaning equipment, each piece was returned to the house for installation.
12. After all equipment was returned to the facility, all doors, and drain holes were closed and secured tightly (rodent proof).
13. Final disinfection of the premise included foaming inside and outside with a disinfectant solution. The facility was closed for at least one day before opening.
14. Upon opening the facility, insecticide and rodent bait was immediately applied and/or distributed before placement of new litter.

SALMONELLA ISOLATION PROCEDURE



(Salmonella Reference Center, University of Pennsylvania)

* Incubated at 37°C for 24 hr

** Incubated at 37°C for 48 hr

UP=universal preenrichment broth; TT=tetrathionate broth; CS=celenite cystine broth;
XLT4=xylose lactose tergitol agar; BGS=brilliant green sulfa agar; TSI=triple sugar iron slant;
and LIA=lysine iron agar slant

SAMPLED PENS

Sampling period	Flock 5	Flock 6	Flock 7	Flock 8
Day 0			3, 4, 10, 14, 16, 22	
Week 2	3, 4, 5, 9, 10, 11, 14, 15, 16, 20, 21, 22		3, 4, 9, 10, 14, 15, 16, 20, 21, 22	3, 4, 9, 10, 15, 16, 21, 22
Week 6		1, 9, 11, 15, 22, 24		
Week 10	1, 3, 6, 7, 9, 12, 13, 15, 18, 19, 21, 24		1, 5, 8, 11, 14, 15	
Week 13		3, 4, 5, 17, 18, 20		
Week 14			2, 3, 6, 13, 17, 18	
Week 15				2, 6, 12, 13, 17, 20
Week 18	2, 4, 5, 8, 10, 11, 14, 16, 17, 20, 22, 23			
Week 20				1, 5, 11, 14, 18, 19
Week 21		2, 5, 10, 14, 19, 21	4, 7, 16, 20, 22	

BIRD DENSITY AND MORTALITY DATA

Pen No.	Flock 5			Flock 6			Flock 7			Flock 8		
	Week 2	Week 10	Week 18	Week 6	Week 13	Week 21	Week 2	Week 10	Week 14	Week 2	Week 15	Week 20
1	0/78 ¹	1/77	0/65	0/51	0/47	1/38	-	0/70	0/65	-	0/50	4/39
2	0/80	0/80	0/68	1/48	0/45	1/34	-	1/69	1/63	-	4/39	4/29
3	0/80	0/79	1/55	0/51	3/44	1/33	0/168	1/61	0/58	3/163	2/50	4/40
4	0/77	0/76	0/50	0/51	5/41	1/31	1/167	0/70	0/66	2/162	0/48	4/36
5	0/79	0/79	0/79	0/52	2/49	1/38	-	0/70	9/55	-	4/40	4/30
6	0/79	0/78	0/72	1/49	2/46	1/37	-	1/69	9/57	-	3/47	4/38
7	0/77	0/77	0/70	0/48	1/44	1/35	-	0/69	3/66	-	0/45	4/36
8	0/77	1/72	0/52	0/48	0/44	1/34	-	0/70	0/63	-	0/49	4/40
9	0/80	0/79	0/65	1/50	0/45	0/37	0/168	2/68	0/64	2/165	4/39	4/26
10	0/81	0/80	0/65	0/50	0/49	0/41	0/167	0/70	9/58	11/148	0/36	0/36
11	0/79	1/78	0/56	0/52	1/48	0/39	-	1/64	0/59	-	0/47	4/38
12	0/79	0/79	0/64	1/49	0/49	0/40	-	0/70	9/57	-	2/49	4/40
13	0/80	0/79	0/60	0/48	1/45	0/39	-	0/62	0/59	-	6/60	0/54
14	0/80	0/78	0/63	1/48	0/43	1/34	1/165	2/66	0/61	-	2/55	0/59
15	0/80	1/77	0/56	0/52	2/47	1/38	1/163	0/69	0/64	2/219	4/62	0/56
16	0/80	0/79	0/58	0/49	0/48	0/37	0/168	0/70	0/66	2/218	4/60	0/54
17	0/80	1/79	0/67	1/50	3/46	1/36	-	0/69	9/54	-	6/56	0/48
18	0/71	0/71	0/43	1/51	2/49	1/40	-	1/68	0/64	-	4/61	0/56
19	0/70	0/70	0/45	1/48	0/46	0/36	-	0/70	9/58	-	5/58	0/52
20	0/80	0/79	1/61	0/52	3/43	0/33	0/166	1/68	0/63	-	6/58	0/52
21	0/80	0/78	0/52	1/49	1/48	0/41	0/168	0/69	0/66	2/217	4/60	0/54
22	0/80	0/79	0/56	1/48	0/44	1/34	1/167	0/69	0/66	2/222	7/57	0/43
23	0/80	0/80	0/64	0/50	0/47	0/31	-	0/69	9/57	-	4/59	0/54
24	0/80	0/80	0/56	1/51	0/48	0/38	-	0/68	18/46	-	3/61	0/54

¹ Dead birds/total birds

CHAPTER 2

**NON-PATHOGENIC *ESCHERICHIA COLI* AND TOTAL COLIFORMS AS
INDICATORS OF FECAL CONTAMINATION IN A TURKEY
PRODUCTION FACILITY**

ABSTRACT

An ecological survey was conducted from March 1998 to June 1999 to determine the prevalence of generic *E. coli* and total coliforms in two turkey flocks (F7 and F8). Flocks were sampled at weeks 10, 14, and 21 for F7 and weeks 2, 15, and 20 for F8. Number of pens sampled during each grow-out period ranged from 5 to 8. Turkey cecal and crop contents, litter, drinkers, air, feed, and feeder contents were sampled. Environmental swabs were collected from various locations in the production facility. Samples from each source were mixed with appropriate amounts of Universal Preenrichment broth, and 1-mL of this enriched sample was immediately transferred to a 3M Petrifilm™ *E. coli*/coliform count plate. Plates were incubated at 37°C for 24 and 48 h to enumerate total coliforms and generic *E. coli*, respectively. *Escherichia coli* and total coliforms were detected from 45 and 53%, respectively, of all sources sampled.

Escherichia coli and total coliforms were isolated from 99 and 100% of cecal contents, 97 and 100% of drinkers, 81 and 95% of litter, 63 and 76% of crop contents, 48 and 52% of swabs, and 18 and 33% of feeder content samples, respectively. Frequency of *E. coli* detection decreased in both flocks as the grow-out period progressed. Identifying preharvest sources of generic *E. coli* and total coliform contamination will help poultry producers and integrators to take necessary control measures in implementing good manufacturing practices within the production facilities. On-farm *E. coli* reduction will assist processors in reducing positive carcasses at the plant.

KEY WORDS: *E. coli*, preharvest sources, total coliforms, turkey

INTRODUCTION

Generic (non-pathogenic) *E. coli* has been used as an indicator organism and its presence indicates a failure in good manufacturing practices at the processing plant. This failure could result in a food product of unacceptable microbial quality (Johnson, 1995). Generic *E. coli* is ubiquitous to the gastrointestinal tract of chickens and turkeys and serves as a microbial indicator of fecal contamination on poultry carcasses. Fecal contamination, in turn, may be responsible for transmitting enteric pathogens such as *E. coli* O157:H7, *Salmonella*, and *Campylobacter* on fresh poultry. Solberg et al. (1977) reported practical implications of using *E. coli* as an indicator of pathogens in raw poultry. The authors demonstrated that if a raw food product contained less than 3 cells of *E. coli*/g, then 7% of the product will contain pathogens. Similarly, if the raw food contained $<10^2$, 10^3 , or 10^4 *E. coli* cells/g, then 42, 52, and 59% of the product, respectively, will contain pathogens. Studies have shown that verotoxigenic (or enterohemorrhagic) *E. coli* O157:H7, a major serotype of generic *E. coli* infections, has been reported predominantly in live beef and dairy cattle resulting from on-farm contamination and/or contamination at the processing units (Hinton et al., 1985; Sisco and Hancock, 1994; Garber et al., 1995; Johnson et al., 1995; Hancock et al., 1997; Wells et al., 1998). Gill et al. (1996) demonstrated that enumerating *E. coli* could be valuable in assessing plant sanitary conditions during beef slaughter. Although *E. coli*-related food borne outbreaks have been associated with raw beef products, raw and processed poultry products should not be excluded.

Federal guidelines stipulate testing of *E. coli* levels on poultry carcasses at the slaughter facility. According to the “Mega-Reg” policy initiated by the Food Safety and Inspection Service (FSIS), meat and poultry slaughtering facilities are required to implement Hazard Analysis and Critical Control Point (HACCP) protocols and conduct testing of generic *E. coli* on

carcasses to ensure process control (Anon, 1996). *Escherichia coli* testing has been carried out using AOAC-approved methods. Regulation requires smaller plants (annually producing 440,000 chickens or 60,000 turkeys) to collect one sample (whole bird rinse after the drip line) per week during the months of June to August (Anon, 1996). Larger plants are required to collect one sample every 22,000 chickens or 3,000 turkeys. Chickens have a lower limit of 100 CFU/cm² and an upper limit of 10,000 CFU/cm². Limits have not been set for turkeys. No result can exceed the upper limit, and only three of fewer samples are allowed in the range from 100 to 10,000 CFU/cm² from the last 13 samples.

However, to facilitate process control at the slaughtering plant, comprehensive control should be implemented at the production facilities. One way of implementation is to identify preharvest sources of generic *E. coli* and total coliforms so that critical control points could be established, preharvest, to reduce their incidence. The objective of this study was to identify key environmental sources/vectors in a turkey production facility that may contribute to generic *E. coli* and total coliform contamination of poultry.

MATERIALS AND METHODS

The survey was conducted at Reymann Memorial Farm, Wardensville, WV, in conjunction with turkey production trials supported by British United Turkeys of America (BUTA), Lewisburg, WV. The facility consists of 24 pens with 12 pens on each side of a central service area. Two turkey flocks (F7 and F8) were sampled from March 1998 to June 1999 on this facility. These are the 7th and 8th flocks produced in this facility. Production trials were conducted by BUTA to evaluate the effect of strain and feed (F7) and strain and gender (F8) on growth performance, feed efficiency and carcass composition of turkeys. Flock characteristics are summarized in Table 1. Standard biosecurity measures were implemented and the houses,

pens, and equipments were thoroughly cleaned and disinfected between each flock in accordance with BUTA guidelines (Appendix, Chapter1).

Sampling Procedures

Turkey cecal and crop contents, litter, drinkers, air, feed, and feeder content were sampled during weeks 10, 14, and 21 for F7 and weeks 2, 15, and 20 for F8. Environmental swabs were collected throughout the facility. Turkeys were euthanized by cervical disarticulation within accepted guidelines. The entire cecum was removed, the blind end was snipped with a pair of scissors, and the cecal contents were transferred to a sterile stomacher bag and sealed. Litter and drinker samples were collected from the same pens used for sampling turkeys. Litter samples were collected from 5-6 random locations within the pen and a composite sample was tested. For water samples, 2 or 3 strips of sterile cloth gauzes were dipped into drinker water and these gauzes were transferred to sterile bags and sealed. Environmental swabs were collected from a 16 cm² area using a sterile swab template; swabs were transferred to 10 mL UP broth. The locations for swab samples included walls, ventilation fans, feathers and open wounds of sick birds, feathers of dead birds, employee shoes, fans inside the pens, feed storage bins, and door handles. Air samples were collected using Rodac plates containing brain heart infusion (BHI) agar with a SAS portable high flow air sampler (Model 5203, Spiral Biotech Inc., Maryland). The air sampler was set to withdraw 60 L of air in 20 s. Air samples were collected from pens, ventilation fans, entrances to the turkey house, centralized service area, and service area on each side of the turkey house during each production period. After collecting the air samples on rodac plates, BHI agar was aseptically transferred to a sterile stomacher bag and sealed. Feed samples were randomly collected from each feed shipment by placing a sterile bag in the feed flow from the auger and/or from the feed cart. Feeder content

samples included feed weighback samples collected from each of 24 pens per week. Samples were stored at -4°C in the facility. About 10 to 20% of the feeder samples were analyzed during each sampling period. Samples were shipped to the laboratory on ice to minimize *E. coli* growth.

Laboratory procedures

Samples of turkey ceca and crop contents were thoroughly mixed with UP broth (1:10 w/v). Drinker and air samples were mixed with 100 mL of UP broth and massaged manually for 30 s. Approximately 5 g of litter, 10 g of feed or 10 g of feeder content samples were transferred to specimen bottles containing 100 mL UP broth and mixed. Bacterial counts from these sources are expressed as log CFU/mL of UP broth. Swab samples, placed in 10 mL UP broth, were directly used for *E. coli* testing. A PetrifilmTM *E. coli*/coliform count plate (Microbiology Products 3M Health Care, Minnesota) for enumerating generic *E. coli* and total coliforms was placed on a leveled surface (Appendix). The top film was lifted and 1-mL of freshly mixed sample was placed at the center of the bottom gel. The top film was rolled down slowly ensuring no air bubbles were entrapped. A plastic spreader was placed on the center of the plate and the sample was distributed evenly by applying gentle pressure on the center of the spreader. The spreader was removed and the gel was allowed to solidify for 1 min. The 3M PetrifilmTM plates were incubated (Imperial II Incubator 422, Labline Instruments Inc., Illinois) at 37°C for 24 ± 2 h to enumerate total coliforms and incubated for an additional 24 ± 2 h at 37°C to enumerate generic *E. coli* colonies. The total coliform count consisted of bright red and blue colonies, and these colonies were closely associated (~ 1 colony diameter) with entrapped gases. *Escherichia coli* colonies appeared blue or red-blue with or without gas regardless of size or intensity of color. All red and blue colonies were counted to obtain a total coliform count. The entire procedure

described above was carried out under aseptic conditions using a laminar flow unit (Model NU425-400, Class II Type A/B3, NuAire™ Inc., Minnesota). Matner and coworkers (1990) reported that the Petrifilm™ plate count method was as good as or better than AOAC-approved Most Probable Number (MPN) method for detecting generic *E. coli* and total coliforms in several food products.

RESULTS AND DISCUSSION

Of the 481 sources sampled from both flocks, 45 and 53% tested positive for generic *E. coli* and total coliforms, respectively. Generic *E. coli* and total coliforms were isolated from 99 and 100% of turkey ceca, 63 and 76% of turkey crop, 81 and 95% of litter, 97 and 100% of drinkers, 48 and 52% of environmental swabs, 0 and 10% of air, 0 and 3% of feed, and 18 and 33% of feeder content samples, respectively. Prevalence of generic *E. coli* has been used in assessing sanitary control of the turkey slaughtering process (USDA, 1996). According to the United States Department of Agriculture (USDA), *E. coli* testing would verify that the slaughtering process was reducing or preventing fecal contamination on carcasses (USDA-FSIS, 1996). Unsanitary conditions on turkey farms could be responsible for harboring *E. coli* and fecal coliforms, contributing to transmission at the slaughter facilities. *Escherichia coli* was selected as the test organism because enumerating *E. coli* could assist in verifying that processing parameters were in control. In addition, detection and quantification of *E. coli* is simple and inexpensive compared to other poultry pathogens such as *Salmonella*, *Campylobacter*, and *Listeria* (Russell, 1996).

In F7, *E. coli* was detected from 100% each of turkey ceca, litter, and drinkers, 75% of crop contents, 50% of environmental swabs, and 26% of feeder content samples (Table 2). *Escherichia coli* remained undetected in air and feed samples. Frequency of detection decreased

from 55% at week 10 to 41% at week 21 of the grow-out period. Since *E. coli* inhabits the gastrointestinal tract, high levels (> 7.0 log CFU/mL) would be expected in turkey ceca and crop contents. *Escherichia coli* found in drinker samples ranged from < 1.0 to 7.0 log CFU/mL, and low counts were observed in environmental swabs (< 1 log CFU/cm²) and feeder contents (< 1 to 2.4 log CFU/mL). In F8, turkey ceca (97%) and drinkers (95%) were the major sources/vectors of *E. coli* contamination (Table 3). Generic *E. coli* were also detected in 65% of litter, 59% of crop contents, and 44% of environmental swabs. Levels of *E. coli* detected from these samples ranged from 1.0 to 7.0 log CFU/mL. Similar to F7, *E. coli* frequency decreased from 73% at week 2 to 31% at week 20. Higher incidences of *E. coli* from litter, drinker, and feeder contents in the sampled flocks indicate that these sources could have been cross-contaminated by turkey droppings. Information on *E. coli* serotypes isolated from the sampled sources would be useful in delineating the possible transmission pathway. Although poultry has not been associated with *E. coli* infections, it is possible that serotype O157:H7 could be among the sources sampled. This strain could contaminate the birds entering the slaughter facility. Nonetheless, frequency and level of O157:H7 were not evaluated in this study. It would be worthwhile enumerating the level and frequency of *E. coli* O157:H7 from the sampled flocks in order to determine a correlation with generic *E. coli* and total coliform data from the present study.

High levels of *E. coli* can be used to predict the presence of other pathogenic bacteria such as *Salmonella* or *Campylobacter*. In other words, *E. coli* can be considered as an “index organism” or a reference for the presence of pathogens (Russell, 1996). In this study, prevalence of *Salmonella* was also determined. In F7, *Salmonella* could not be detected in any of the 361 sources sampled, while in F8, only 1% of the 233 sources sampled tested positive (Table 8 and 9; Chapter 1). *Salmonella* was isolated from 6% of environmental swabs, 5% of litter samples, and

3% each of turkey ceca and feeder content samples. Solberg and coworkers (1977) reported a high correlation coefficient between generic *E. coli* and *Salmonella* (0.708) and coliforms and *Salmonella* (0.704). No correlation could be found in the present study between the presence of *E. coli*/total coliforms and prevalence of *Salmonella* during the grow-out period. Frequency of *E. coli* detection will always be higher than that of pathogens such as *Salmonella* and *Campylobacter*.

Turkey ceca (100%), litter (100%), drinkers (100%), and crop (83%) were the primary sources of total coliform contamination in F7 (Table 4). Total coliforms were also isolated from 50% of environmental swabs, 46% of feeder contents, and 4% of air samples. Turkey ceca, crop, and litter samples had high total coliform counts (> 7.0 log CFU/mL), while swabs, air, and feeder content samples had low counts (< 1.0 to 2.4 log CFU/mL). Although *E. coli*-positive samples decreased in F7 during the grow-out period (Table 1), frequency of total coliforms detected decreased from 60% at week 10 to 54% at week 21. Total coliforms include members of at least three genera: *Escherichia*, *Klebsiella*, and *Enterobacter*. The complex microbial ecology in the turkey intestine may have been balanced by replacement of *E. coli* in the total coliform count with *Klebsiella* and *Enterobacter* during the grow-out period in F7. In F8, total coliforms were detected in 100% each of ceca and drinker samples, 90% of litter, 73% of crop, 56% of environmental swabs, 17% of air, and 5% of feed samples (Table 5). Although 46% of feeder contents were tested positive for total coliforms in F7 (Table 4), no feeder content samples in F8 tested positive. Turkey ceca had high coliform counts (> 7.0 log CFU/mL), while the level of coliforms varied (< 1.0 to 7.0 log CFU/mL) for all other sources sampled. Frequency of total coliforms detection decreased from 80% at week 2 to 43% at week 20.

CONCLUSIONS

Cecal contents, litter and drinker samples were the major preharvest sources/vectors of generic *E. coli* and total coliform contamination in the turkey production facility. Controlling these sources can help the turkey producers and integrators implement good sanitary measures on the farm. Fecal matter present on the outside of turkeys could affect *E. coli* and coliforms levels on the carcasses as these birds enter the slaughter facilities. This is regardless of sanitary conditions maintained at the processing facilities or efforts taken to decrease bacterial load during processing. Feed withdrawal and transportation stress may contribute to *E. coli* contamination before turkeys enter the processing units. These factors are not directly plant related nor are they a measure of personnel sanitation; nonetheless, under the current federal regulations, the integrators would be ultimately responsible for high levels of *E. coli* on carcasses. Since *E. coli* frequently represents the majority of total coliforms, reducing the latter in poultry carcasses could also assist in minimizing risk of *E. coli* infections. Certain serotypes of *E. coli* have been responsible for human hemorrhagic colitis and hemolytic uremic syndrome. Although many of these cases have been linked to consumption of undercooked ground beef, raw and processed poultry products are no exception. It is imperative that turkeys arriving at the slaughter facilities have reduced levels of *E. coli* and/or fecal coliforms. Eliminating generic *E. coli* or total coliforms from turkey houses is impossible because these microorganisms are ubiquitous and they form the natural microflora of the bird's gastrointestinal tract. Hence, efforts should be made in reducing their levels by implementing stringent hygienic practices at the grow-out facilities.

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Table 1. Summary of turkey flock statistics

Characteristics	Flock 7	Flock 8
Sampling period	March to August 1998	January to June 1999
BUTA production trail	4 strains x 2 feeds	4 strains x 2 genders
Sampling frequency (weeks)	10, 14, and 21	2, 15, and 20
Number of pens	5-6	6-8
Sex of birds	Females	Males and females

Table 2. Frequency and enumeration of generic *E. coli* detected from various flock 7 samples in the turkey production facility.

Sample	Grow-out period (no. positive/no. sampled)			Total (% positive)
	Week 10	Week 14	Week 21	
Turkey ceca	12/12 (>7.0) ^a	12/12 (>7.0)	12/12 (>7.0)	36/36 (100)
Turkey crop	0/0	0/0	9/12 (>7.0)	9/12 (75)
Litter	6/6 (>7.0)	6/6 (>7.0)	5/5 (>7.0)	17/17 (100)
Drinkers	6/6 (1.0-7.0)	6/6 (<1.0-7.0)	5/5 (<1.0)	17/17 (100)
Environmental swabs ^b	5/8 (<1.0)	3/8 (<1.0)	4/8 (<1.0)	12/24 (50)
Air	0/7	0/8	0/8	0/23
Feed	0/10	0/4	0/8	0/22
Feeder contents	13/28 (1.0-2.4)	7/29 (<1.0-1.9)	5/40 (<1.0)	25/97 (26)
Total (% positive)	42/77 (55)	34/73 (47)	40/98 (41)	116/248 (47)

^a expressed as range (log CFU/mL) for number of sources sampled

^b counts expressed in log CFU/cm²

Table 3. Frequency and enumeration of generic *E. coli* detected from various flock 8 samples in the turkey production facility.

Sources	Grow-out period (no. positive/no. sampled)			Total (% positive)
	Week 2	Week 15	Week 20	
Turkey ceca	15/15 (>7.0) ^a	12/12 (>7.0)	11/12 (>7.0)	38/39 (97)
Turkey crop	13/13 (1.2-7.0)	6/12 (<1.0-7.0)	3/12 (<1.0-1.6)	22/37 (59)
Litter	8/8 (>7.0)	4/6 (>7.0)	1/6 (<1.0)	13/20 (65)
Drinkers	8/8 (>7.0)	6/6 (<1.0-7.0)	5/6 (<1.0-7.0)	19/20 (95)
Environmental swabs ^b	3/6 (<1.0-2.0)	1/6 (<1.0)	4/6 (<1.0)	8/18 (44)
Air	0/6	0/6	0/6	0/18
Feed	0/8	0/24	0/9	0/41
Feeder contents	0/0	0/20	0/20	0/40
Total (% positive)	47/64 (73)	29/92 (32)	24/77 (31)	100/233 (43)

^a expressed as range (\log_{10} CFU/mL) for number of sources sampled

^b counts expressed in \log CFU/cm²

Table 4. Frequency and enumeration of total coliforms detected from various flock 7 samples in the turkey production facility.

Sample	Grow-out period (no. positive/no. sampled)			Total (% positive)
	Week 10	Week 14	Week 21	
Turkey ceca	12/12 (>7.0)	12/12 (>7.0)	12/12 (>7.0)	36/36 (100)
Turkey crop	0/0	0/0	10/12 (1.4->7.0)	10/12 (83)
Litter	6/6 (>7.0)	6/6 (>7.0)	5/5 (>7.0)	17/17 (100)
Drinkers	6/6 (1.4-7.0)	6/6 (2.0-7.0)	5/5 (1.3-1.6)	17/17 (100)
Environmental swabs ^b	4/8 (<1.0)	3/8 (<1.0)	5/8 (<1.0)	12/24 (50)
Air	1/7 (<1.0)	0/8	0/8	1/23 (4)
Feed	0/10	0/4	0/8	0/22
Feeder contents	17/28 (<1.0-2.4)	12/29 (<1.0-1.9)	16/40 (<1.0-1.5)	45/97 (46)
Total (% positive)	46/77 (60)	39/73 (53)	53/98 (54)	138/248 (56)

^a expressed as range (log CFU/mL) for number of sources sampled

^b counts expressed in log CFU/cm²

Table 5. Frequency and enumeration of total coliforms detected from various flock 8 samples in the turkey production facility.

Sample	Grow-out period (no. positive/no. sampled)			Total (% positive)
	Week 2	Week 15	Week 20	
Turkey ceca	15/15 (>7.0)	12/12 (>7.0)	12/12 (>7.0)	39/39 (100)
Turkey crop	12/13 (1.3-7.0)	8/12 (<1.0-7.0)	7/12 (<1.0-7.0)	27/37 (73)
Litter	8/8 (>7.0)	6/6 (2.1-7.0)	4/6 (<1.0)	18/20 (90)
Drinkers	8/8 (>7.0)	6/6 (<1.0-1.5)	6/6 (1.3-7.0)	20/20 (100)
Environmental swabs ^b	5/6 (<1.0-2.3)	1/6 (1.5)	4/6 (<1.0-1.3)	10/18 (56)
Air	3/6 (<1.0)	0/6	0/6	3/18 (17)
Feed	0/8	2/24 (<1.0)	0/9	2/41 (5)
Feeder contents	0/0	0/20	0/20	0/40
Total (% positive)	51/64 (80)	35/92 (38)	33/77 (43)	119/233 (51)

^a expressed as range (log CFU/mL) for number of sources sampled

^b counts expressed in log CFU/cm²

APPENDIX

INSTRUCTIONS FOR USING 3M PETRIFILM™ E. COLI/COLIFORM PLATES

STORAGE AND DISPOSAL

Store unopened Petrifilm plate pouches at temperatures $\leq 8^{\circ}\text{C}$. Allow pouches to come to room temperature before opening. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than a month.

Exposure to Petrifilm plates to temperature $> 25^{\circ}\text{C}$ and/or humidities $\geq 50\%$ RH can affect the performance of the plates. Do not use plates with brown or orange discoloration.

INSTRUCTIONS FOR USE

Sample preparation (recommended by 3M Microbiology Products, St. Paul, MN)

1. Use appropriate sterile diluents: Butterfield's phosphate buffer (IDF phosphate buffer, 0.0425 g/L KH_2PO_4 , adjust to pH 7.2), 0.1% peptone water, peptone salt diluent, 0.85-0.9% saline solution, thiosulfate-free letheen broth or distilled water. **Do not use buffers containing citrate or sodium thiosulfate with Petrifilm plates.** If citrate buffer is recommended in the procedure, substitute warmed ($40\text{-}45^{\circ}\text{C}$) Butterfield's phosphate buffer.
2. For acidic products, adjust pH of the diluted sample to 6.6-7.2 with 1N NaOH. For alkaline products, adjust pH with 1N HCl.
3. Blend or homogenize sample.

Plating

Inoculate and spread one Petrifilm plate before inoculating the next plate.

1. Place a Petrifilm plate on a level surface.
2. Lift the top film and dispense 1 mL of sample or diluted sample on the center of bottom film.
3. Slowly roll the top film down onto the sample to prevent trapping air bubbles.
4. With the smooth side down, place the plastic spreader on the center of the plate.

5. Distribute sample evenly using a gentle downward pressure on the center of the plastic spreader. Do not slide the spreader across the film.
6. Remove spreader and leave plate undisturbed for at least one min to permit the gel to solidify.
7. Incubate plates in a horizontal position, with the clear side up in stacks of up to 20 plates. Incubator should be humidified. Moisture loss from the plate should not exceed 15% during 48 h of incubation.

Incubation times and temperatures

1. AOAC[®] official methods (991.14 coliform and *E. coli* counts in foods, dry rehydrated film methods): Incubate Petrifilm plates 48 ± 2 h at $35 \pm 1^\circ\text{C}$.
2. AFNOR validated method (certificate number 3M 01/04 - 09/92): Petrifilm incubated at 24 ± 2 h at $42 \pm 1^\circ\text{C}$.
3. Nordic Committee on Food Analysis (NMKL) method (147.1993): For coliforms incubate Petrifilm plates at 24 ± 2 h at 37°C . For *E. coli* incubate at 48 ± 2 h at 37°C .

Interpretation

1. Petrifilm plates can be counted on a standard colony counter or any magnified light source. Do not count colonies on the foam dam since they do not contain certain chemicals present in the gel. Do not count artifact bubbles that may be present. The interpretation of *E. coli* and coliform colonies on Petrifilm EC plates varies by method. Approximately 95% of *E. coli* produce gas.

In general, *E. coli* colonies are blue to red-blue and closely associated (~ 1 colony dia.) with entrapped gases. Coliform colonies are bright red and closely associated (~ 1 colony dia.) with entrapped gases.

Caution: *E. coli* O157:H7 strains are atypical; they do not grow at $\geq 44.5^{\circ}\text{C}$ and are glucuronidase negative. These strains will not produce a blue precipitate. They will appear as non-*E. coli* coliforms (red with gas).

AOAC Method

Enumerate blue to red-blue colonies associated with entrapped gas, regardless of size or intensity of color, as confirmed *E. coli*. Blue colonies without gas are not counted as *E. coli*. Other coliform colonies will be red and associated with gas bubbles. Colonies not associated with gas are not counted as coliforms. The total coliform count consists of both red and blue colonies associated with gas at 24 h. Reincubate plates for an additional 24 ± 2 h at 35°C to detect any additional *E. coli* growth. Regardless of incubation time, whenever a blue colony associated with gas appears, it is a confirmed *E. coli*.

AFNOR Method

Blue to red-blue colonies with or without gas regardless of size or intensity of color are enumerated as *E. coli*.

NMKL Method

Enumerate *E. coli* colonies as noted under AFNOR methodology. Enumeration of total coliforms at 24 ± 2 h as noted under AOAC method.

2. The circular growth area is approximately 20 cm^2 . Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine total count per plate.
3. Petrifilm plates with colonies that are too numerous to count (TNTC) have one or more of the following characteristics: many small colonies, many gas bubbles, and deepening of red color

on the gel. Higher concentrations of *E. coli* will cause the growth area to turn blue while high concentrations of coliforms (non-*E. coli*) will cause the growth area to turn dark red.

When any of these occur, dilute the sample further to obtain a more accurate count.

4. Colonies may be isolated for further identification.
5. After incubation is complete, plates may be stored frozen ($\leq 15^{\circ}\text{C}$) for later enumeration for no longer than one week.

CHAPTER 3

INHIBITION AND REVERSAL OF *SALMONELLA TYPHIMURIUM* ATTACHMENT TO POULTRY SKIN USING ZINC CHLORIDE

ABSTRACT

A skin attachment model was used to examine the ability of ZnCl₂ to reverse or inhibit *Salmonella* attachment to broiler skin. In reversal experiments, skin samples were treated first with 1 mL of *S. typhimurium* suspension (10⁸ CFU/mL) and held at room temperature for 30 min. Skin samples were then treated with 25 or 50 mM ZnCl₂ for 5 or 15 min. Zinc chloride solutions remained in contact with the culture during the time of application. In inhibition experiments, ZnCl₂ solutions were first added; treatment solutions were discarded after 5 or 15 min of application and then the culture was added. Populations of “firmly” and “loosely” attached *Salmonella* were enumerated. Each skin sample was blended with 20 mL of 0.1% peptone water for 30 s. Cells recovered from the skin were denoted as “loosely” attached. The same skin sample was again mixed with fresh 20 mL of 0.1% peptone and blended for 2 min. Recovered cells were denoted as “firmly” attached. *Salmonella* was enumerated on Xylose Lactose Tergitol (XLT4) plates. A section of skin sample, subjected concurrently to the above treatments, was observed under a scanning electron microscope (SEM) to observe changes in skin topography and bacterial attachment. In reversal experiments, 25 and 50 mM ZnCl₂ reduced ($p < 0.01$) “firmly” attached cells by 77 and 89%, respectively, when compared to the control (water). At 25 and 50 mM concentrations, ZnCl₂ reduced ($p < 0.0001$) cells in the “discard” by 99.4 and 99.9%, respectively. Reduction of cells in the “discard” suggests bactericidal activity of zinc on *Salmonella* due to contact between bacterial culture and ZnCl₂ solutions during the time of application in the reversal experiments. Micrographs indicated that 25 and 50 mM ZnCl₂ reduced ($p < 0.1$) *Salmonella* attachment by 69 and 99.9%, respectively, in the reversal experiments. Results from SEM and XLT4 enumeration of “firmly” attached cells in the reversal experiments revealed a consistent reduction in bacterial count on skin with

increasing ZnCl₂ concentration. In the inhibition experiments, 25 and 50 mM ZnCl₂ reduced (p<0.01) “firmly” attached cells by 82 and 91%, respectively. Reduction of *Salmonella* may be attributed, in part, to the bactericidal activity of ZnCl₂ in addition to detachment of the bacterial cells on skin.

KEY WORDS: Zinc chloride, *Salmonella*, poultry skin, attachment, micrographs

INTRODUCTION

Contamination of poultry products with *Salmonella* continues to concern consumers and the poultry industry. Attention has focused on the microbiological quality of broiler carcasses and how processing procedures influence cross-contamination of carcasses. *Salmonella* colonizes the gastrointestinal tract of chickens and, through horizontal transmission, may contaminate other birds and eventually carcasses (Craven and Williams, 1998). Five percent of broilers entering a processing plant harbored *Salmonella*, and the incidence increased to 36% for processed carcasses from the same flock (Lillard, 1990).

Research is needed to find effective, safe, and affordable methods to reduce *Salmonella* contamination of fresh poultry. Physical and chemical methods have been tested with varying degree of success for reducing *Salmonella* contamination on poultry carcasses. Physical methods include air scrubbing, UV and gamma irradiation (Wolfson et al., 1994). Chemical treatments have included chlorine, chlorine dioxide, glutaraldehyde, trisodium phosphate, sodium hypochlorite, sodium metabisulfite, NaCl, NaOH, grapefruit seed extract, Tween 80, cetyl pyridinium chloride, ozone, a combination of hydrogen peroxide and sodium bicarbonate, and organic acids such as lactic, malic, propionic, acetic, and citric acid, (Lillard, 1988; Conner and Bilgili, 1994; Dickson and Siragusa, 1994; Kim et al., 1994; Lillard, 1994; Breen et al., 1995; Hwang and Beuchat, 1995; Tamblyn and Conner, 1997; Salvat et al., 1997; Tamblyn et al., 1997; Wang et al., 1997; Coppen et al., 1998; Xiong et al., 1998a; b; Yang et al., 1998). Currently in U.S. poultry processing plants, 50 ppm chlorine is permitted in carcass washes and immersion chillers as a commercial disinfectant (Tamblyn et al., 1997). Trisodium phosphate (8-12%) is also approved for use in processing plants to reduce the incidence of foodborne pathogens (Tamblyn et al., 1997). Although these treatments reduce *Salmonella* (CFU/cm²) on

skin, they were ineffective against bacterial cells attached to skin. Bacteria are firmly attached or entrapped in the skin before they arrive at the processing plant and are difficult to remove by rinsing (Lillard, 1990). Hence, it is necessary to devise effective methods that interfere with *Salmonella* attachment to poultry skin.

Limited information is available on $ZnCl_2$ as an antimicrobial agent. *Salmonella* is a gram-negative organism with an outer membrane consisting of lipoproteins, lipopolysaccharides, and phospholipids. The strong negative charge on the outer surface of the membrane aids the organism in evading phagocytosis and the action of complement – two components of the host defenses. Cultures of *S. typhimurium* in contact with meat surfaces selectively bind to collagen fibers, particularly the reticulin type (Benedict, 1988). *Salmonella* exhibits adhesion properties due to type 1 pili that are sensitive to D-mannose, while the principal carbohydrate residues of collagen, glucose-galactose and galactose, do not include mannose (Lillard, 1986a; Benedict, 1988). Electropositive zinc ions may interfere with *Salmonella* attachment to poultry skin by selectively binding to the negative charges on the cell membrane. Zinc chloride may also indirectly interfere with *Salmonella* attachment by competing with specific receptor sites on the skin surface. Zinc chloride may change the conformation of surface proteins such that receptor sites on skin surfaces would no longer be exposed for *Salmonella* attachment. The objective of this study was to determine the efficacy of 25 or 50 mM $ZnCl_2$ to reverse or inhibit attachment of *Salmonella* to chicken skin.

MATERIALS AND METHODS

***Salmonella* culture**

Salmonella typhimurium (FY98-DT 12) was obtained from the National Veterinary Services Laboratory (Ames, IA). This organism was selected because it is the most frequently

isolated serotype from raw broiler carcasses (Lillard, 1986a). Cultures were maintained on Micro-Protect beads (TS/70-B, Technical Service Consultants Ltd., Lancashire, England) and stored at -70°C. Two beads containing immobilized *Salmonella* were transferred to 5 mL of Universal Preenrichment (UP) broth and incubated at 37°C for 24 h. Cultures were subsequently streaked on Brain Heart Infusion (BHI) agar slants, incubated at 37°C for 24 h, and stored at 4°C. Freshly inoculated slants were used as starter cultures for experiments conducted in this study.

A loopful of *S. typhimurim* culture, maintained on BHI slants, was added to 5 mL of UP broth and incubated at 37°C for 24 h. This culture was streaked on a Xylose Lactose Tergitol (XLT4) plate and further incubated at 37°C for 24 h. A single isolated colony (*ca.* 1 mm dia.) was transferred from XLT4 to 100 mL of UP broth and incubated at 37°C for 18-24 h. The cell suspension was transferred to three, 50 mL tubes and centrifuged at 3,000 x *g* for 10 min at 4°C (Damon IEC B20A, 870 head, 8x50 mL tube, Fisher Scientific, Pittsburgh). The supernatant in each tube was discarded and the cell pellet was washed with 25 mL of cold, 0.1% peptone water. Resuspended cells were re-centrifuged at 3,000 x *g* for 10 min at 4°C. Supernatant was again discarded, and pellets from the three tubes were suspended in 100 mL of cold, 0.1% peptone water. This suspension yielded a cell count of 10⁸ CFU/mL. The optical density of the culture ranged from 0.55 to 0.58 when measured at 600 nm using a spectrophotometer (Spectronic 20D, Milton Roy Co., New York).

Skin preparation

Skin models were prepared as described by Kim et al. (1996) with a few modifications. Skin samples were obtained from chicken drumsticks purchased at a local supermarket on the day of experiment. Drumsticks were purchased in family packs and these packs were brought to the laboratory for immediate model skin preparation. Skin pieces (*ca.* 36 cm²) were cut from the

proximal, two-thirds of the drumstick and spread evenly on a Styrofoam tray with the external surface of the skin facing up. The tray had been previously sanitized with 70% ethanol. The skin sample was placed on top of a sterile, open capped, 50 mL Nalgene centrifuge tube with the external surface of the skin faced inside the tube. Rubber bands were used to secure the skin section on the tube and the tube was capped (Fig. 1). A hole was inserted at the conical end of the tube for adding culture and test solutions. The above procedure was carried out in aseptic conditions using a laminar flow unit (NU425-400, Class II Type A/B3, NuAire™ Inc., Minnesota).

Skin attachment model experiments

Reversal of *Salmonella* attachment (Appendix): A 1 mL suspension of *S. typhimurium* culture (10^8 CFU/mL) was added to the skin model, and attachment was allowed to take place at room temperature ($\sim 22^\circ\text{C}$) for 30 min. Attachment of *Salmonella* to poultry tissue or skin takes place within 15 s and may continue up to 60 min after immersing the samples in a *Salmonella* suspension (Thomas and McMeekin, 1984; Lillard, 1985; 1986a; b). A treatment solution (sterile) of 5 mL water (control), 25 mM ZnCl_2 or 50 mM ZnCl_2 was added to the tube containing the bacterial suspension. Skin samples were exposed to treatments at room temperature for either 5 or 15 min. The mixture of treatment solution and cell suspension was drained from the skin model for 2-3 min in 10 mL sterile tubes. The mixture was serially diluted and populations of *Salmonella* were designated as “discard” cells. “Discard” pH was measured with a pH meter (Model 350, Corning Inc., New York). The skin sample was cut along the outer circumference of the tube (*ca.* 8.8 cm^2) using a sterile scalpel blade. Each skin sample was placed in a sterile stomacher bag containing 20 mL of 0.1% peptone water and massaged in a stomacher blender (Model 400, Tekmer, Ohio) at 230 rpm for 30 s. Cells present in the wash

solution at this point were denoted as “loosely” attached cells. Aliquots of the skin wash solution were serially diluted and spread plated in duplicate on XLT4 agar to enumerate *S. typhimurium*. The same skin sample was transferred to 20 mL of fresh 0.1% peptone water and massaged in a stomacher at 260 rpm for 2 min. Cells present in the wash solution at this point were denoted as “firmly” attached.

Inhibition of *Salmonella* attachment (Appendix): Attachment of skin pieces to sterile 50 mL centrifuge tubes was carried out as described in the previous experiment. Skin samples were treated with 5 mL water (control), 25 mM ZnCl₂ or 50 mM ZnCl₂ and held at room temperature for 5 or 15 min. Each treatment solution was allowed to drain for 2-3 min and pH of the solution was measured. One mL of *S. typhimurium* suspension (10⁸ CFU/mL) was then applied to each skin sample. Attachment was allowed to take place for 30 min at room temperature. The culture was drained for 2-3 min, and the bacterial count was designated as “discard.” The skin sample was cut from the tube around its circumference. “Loose” and “firmly” attached cells on the skin sample were enumerated as described in the reversal experiment.

One family pack, consisting of 14-16 drumsticks, was used for each replication nested within mechanism of action (reversal versus inhibition). For each treatment combination, skin samples were taken from different drumsticks. Skin samples from 12 different drumsticks (six for enumerating *Salmonella* using the model study and six for electron microscope studies) came from the same drumstick tray. In order to determine a reference baseline, *Salmonella* was enumerated on one skin sample, without treatment, from each family pack. In the reversal and inhibition experiments, each treatment combination was carried out with two separate Nalgene centrifuge tubes. The skin sample from one tube was used for enumerating viable cells on XLT4 while the skin sample from the other tube was used to directly enumerate cells using scanning

electron microscopy (SEM). The skin model experiments were performed first and then a fresh set of tubes was used for SEM studies.

Scanning electron microscopy

Electron microscopy was carried out using the procedure described by Kim and Doores (1993a; b). Skin sample (*ca.* 1 cm²) from each treatment was cut and pinned on a wooden cork such that the external skin surface faced outwards. Bacterial cells were fixed on skin sample by placing each skin in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer (pH 7.1) at 4°C for 8 to 12 h. Samples were washed with distilled water and dehydrated in a graded series of 50, 60, 70, 80, 95, and 100% ethanol (15 min at each ethanol concentration). Specimens were finally dehydrated twice with 100% ethanol for 15 min, washed for three, 5-min intervals with hexamethyldisilazane (HMDS) and air-dried. After complete evaporation of HMDS, skin pieces were mounted on aluminum stubs and sputter coated with 28 nm gold/palladium (Model E5100, Polaron, England). Specimens were examined under a scanning electron microscope (JSM 6400, JEOL, Japan) at 20 kV and 2,500-fold magnification. Ten fields from each treatment were examined and bacterial cells were visually counted from each field. Representative fields are shown.

Experimental design

Six incomplete blocks and treatment within each block were randomized. Each incomplete block consisted of three ZnCl₂ treatments (0, 25, or 50 mM) by two time intervals (5 or 15 min). These six treatment combinations were replicated three times and nested within application of ZnCl₂ (reversal or inhibition). *Salmonella* enumeration using XLT4 plates and SEM data was analyzed by ANOVA using General Linear Model (GLM) procedures (SAS, 1989; Appendix). Means were separated using least square methods. Separate analyses were

conducted for the reversal and inhibition experiments. The following orthogonal comparisons were made: (1) without ZnCl₂ vs with ZnCl₂; (2) 25 mM ZnCl₂ vs 50 mM ZnCl₂; (3) 5 min (25 mM ZnCl₂) vs 15 min (25 mM ZnCl₂); (4) 5 min (50 mM ZnCl₂) vs 15 min (50 mM ZnCl₂); and (5) 5 min (25+50 mM ZnCl₂) vs 15 min (25+50 mM ZnCl₂).

RESULTS AND DISCUSSION

There are several advantages in using a skin attachment model. Conner and Bilgili (1994) indicated that skin model techniques make it easier to evaluate the efficacy of a potential carcass disinfectant and control the optimum conditions of application. Efficacy of a disinfectant could be evaluated under pilot processing conditions in the plant prior to commercial application. An appropriate baseline measurement was made for each treatment combination to account for background *Salmonella* on the skin samples. Activity of ZnCl₂ against the test bacterium (*S. typhimurium*) could be conducted while accounting for background *Salmonella*. In the present study, *Salmonella* could not be detected from the skin samples used for baseline measurements.

Reversal experiments

Limited information is available on effectiveness of ZnCl₂ in reversing and/or inhibiting *S. typhimurium* attachment to poultry skin. This study demonstrated that increasing ZnCl₂ concentration decreased ($p < 0.05$) *Salmonella* attachment to the chicken skin. “Firmly” attached *S. typhimurium* cell count was reduced ($p < 0.01$) from 3.94 log CFU/mL for the control to 3.31 and 2.98 log CFU/mL for 25 and 50 mM ZnCl₂, respectively (Table 1). A similar trend was observed in the ability of ZnCl₂ to detach “loosely” attached *Salmonella* to chicken skin ($p < 0.1$). At 25 and 50 mM concentration, ZnCl₂ reduced ($p < 0.0001$) cells in the “discard” by 99.4 and 99.9%, respectively. Reduction of cells in the “discard” suggests bactericidal activity of zinc on *Salmonella* due to contact between bacterial culture and ZnCl₂ solutions during the time of

application in the reversal experiments. Orthogonal comparisons for the enumerated cells indicated a significant difference ($p < 0.05$) between treatments with or without ZnCl_2 (Table 1). The efficacy of ZnCl_2 to detach “firmly” attached cells improved with an increase in the time of application. Zinc chloride reduced ($p < 0.05$) “firmly” attached cells from 3.64 log CFU/mL at 5 min to 3.18 log CFU/mL at 15 min.

Inhibition experiments

Zinc chloride was effective in interfering with attachment of *S. typhimurium* to chicken skin. Results from inhibition experiment indicated that “firmly” attached *S. typhimurium* count was reduced ($p < 0.01$) from 4.39 log CFU/mL for the control to 3.64 and 3.35 log CFU/mL for 25 and 50 mM ZnCl_2 , respectively. Zinc treatment reduced ($p < 0.05$) “firmly” attached cells. Levels of ZnCl_2 had no effect ($p > 0.05$) on “loosely” attached *Salmonella* or cells in the “discard.” Time of ZnCl_2 application also did not seem to influence the detachment of *Salmonella* on chicken skin for the inhibition experiments.

Studies have been carried out to determine effectiveness of divalent chloride salts in reducing *in vitro* attachment of *S. typhimurium* to chicken cecal mucus (Craven and Williams, 1998) and *Listeria monocytogenes* growth (Zaika et al., 1997). These studies were carried out using divalent cations such as calcium, magnesium, barium, iron, and manganese. Attachment of *S. typhimurium* to immobilized cecal mucus was inhibited by 10 mM zinc, lanthanum, and a combination of EDTA and citrate (Craven and Williams, 1998). However, *Salmonella* attachment was enhanced in the presence of 10 mM calcium, barium, and manganese ions.

Effect of ZnCl_2 in cell suspension

When averaged across ZnCl_2 concentration and the time of application, a combination of “firm” and “loosely” attached cells and cells in the “discard” for the reversal and inhibition

experiments equaled 5.37 and 7.79 log CFU/mL, respectively. Although most of the cells were accounted for in the inhibition experiments, when compared to the initial inoculum volume (8 log CFU/mL), a large reduction in the bacterial count was observed in the reversal experiments. In reversal experiments ZnCl₂-treatment solutions were in contact with *S. typhimurium* culture for 5 or 15 min, depending on the treatment combination. Unaccounted cells in reversal experiments indicated that ZnCl₂ might possess bactericidal activity against *S. typhimurium* in cell suspensions. Increasing levels of ZnCl₂ significantly reduced ($p < 0.0001$) pH of the “discarded” solutions containing the bacterial culture and ZnCl₂ (Table 1). “Discard” pH was reduced from 6.85 for the control to 5.63 for 25 mM ZnCl₂ and 5.45 for 50 mM ZnCl₂. This reduction in pH could also be attributed to the reduction of *Salmonella* in cell suspension. Preliminary experiments conducted prior to this study indicated that bacterial cells tended to clump together when 25 or 50 mM ZnCl₂ was added to pure *Salmonella* suspension (Fig. 2). Cell clumping could reduce the overall number of *Salmonella* colonies isolated on XLT4 agar plates. The bactericidal activity and/or the clumping effect may account for an overall reduction in *Salmonella* cell count by ZnCl₂ treatment in the reversal experiments. The exact mechanism of action needs further investigation.

Bacterial attachment is a complex process and involves more than one mechanism. Adherence of bacteria to poultry skin depends on the bacterial strain, substratum, and environmental conditions such as medium temperature, and pH. Butler and coworkers (1980) demonstrated that bacterial attachment takes place during the first minute of immersion; although in some instances, attachment continues over a 60-min period. Bacterial attachment involves a two-step process (Kim and Doores, 1993a). The first step involves retention of bacteria in a liquid film on the surface of skin or meat. Bacteria are closely associated and entrapped in

inaccessible sites by physical forces. Reversible adhesion at this stage is associated with an optimal energy balance between attractive van der Waal forces and electrostatic repulsion. The second stage involves a time-dependent, irreversible exopolymer (polysaccharides) formation by bacteria. At this stage, bacteria multiply and form a microcolony that can lead to the formation of a biofilm. Although the exact mechanism was not defined in the present study, a strong affinity of zinc ions for *S. typhimurim* cells could override the attachment of these bacterial cells to specific receptor sites on skin samples in the reversal experiments.

Zinc ions play an important role in offering protection against *Salmonella* infections in live birds. Hill and coworkers (1977) found that poultry dietary supplementation with 200 mg of $ZnSO_4$ /kg of feed and 1g of Na-EDTA /kg of feed reduced mortality associated with *S. gallinarum* infections. Kidd and coworkers (1992) demonstrated that supplementing zinc-methionine (72 mg/kg of feed) in hen diets increased cellular immune responses in their progeny by enhancing primary antibody titers to *S. pullorum* antigen. Popova (1997) reported a significant decrease in blood zinc concentrations when pigs were challenged with 5×10^9 CFU of *S. choleraesuis*. Zinc ions were found to be effective against other strains of *Salmonella*. Zinc-methionine (30 mg/kg of feed) reduced *S. arizonae* incidence in turkey spleens (Kidd et al., 1994). As a future experiment, it would be interesting to challenge chicks/poults with a known inoculum volume of *Salmonella* and supplement drinking water with $ZnCl_2$ to evaluate whether zinc ions offers resistance to *Salmonella* colonization in the gastrointestinal tract.

Scanning electron microscopic studies

The antibacterial efficacy of $ZnCl_2$ treatments in reversing or inhibiting attachment of *S. typhimurium* to chicken skin was also evaluated by enumerating the bacterial cell count using scanning electron microscopy. Figures 3 and 4 represent micrographs of chicken skin subjected

to different ZnCl₂ treatments for the reversal and inhibition experiment, respectively. Level of ZnCl₂ was effective in reducing *Salmonella* attached to chicken skin. Enumeration of bacterial cells on the skin samples indicated that ZnCl₂ reduced ($p < 0.1$) *Salmonella* count from 5.27 log CFU/cm² for the control to 4.76 log CFU/cm² for 25 mM ZnCl₂ and 2.26 log CFU/cm² for 50 mM ZnCl₂ (Table 1). Direct enumeration of *Salmonella* by SEM supports data derived from enumeration of cells using XLT4. Results from SEM and from XLT4 enumeration of “firmly” attached cells revealed a consistent reduction in *Salmonella* on poultry skin with increasing ZnCl₂ concentration. No significant changes in the skin topography were observed between the control and ZnCl₂ treatment for reversal and inhibition experiments.

Salmonella cells clustered in certain sections of the skin samples (Fig. 3d, 4a), indicating these regions were more conducive to *Salmonella* attachment than others. *In vitro* attachment of *Salmonella* to intestinal segments (Oyofe et al., 1989), inverted ceca (McHan et al., 1989), and immobilized cecal mucus and enterocytes (Craven et al., 1992) has been associated with type 1 fimbriae (mannose-sensitive adhesion). In addition, lipopolysaccharides play a major role in *S. typhimurium* attachment of to chicken cecal mucus (Craven, 1994). Certain skin sections may have a predominance of type 1 fimbriae and lipopolysaccharide receptor sites facilitating attachment. Immersion of poultry skin in an aqueous bacterial medium results in expansion of collagen and formation of deep channels on the skin surface or deep ridges in the muscle (Thomas and McMeekin, 1984). The authors indicated that bacteria lodged in these crevices were not visible on electron micrographs. The skin samples in the present study should have been shredded before cell enumeration to account for bacteria attached to the skin within the deep crevices. Such deep channels and crevices were observed in the present study (Fig. 3c and 4a). It is possible that these deep ridges could protect *Salmonella* from the treatment solution.

Unfortunately, the skin samples were not shredded in this study, suggesting that the actual cell count could be higher than reported.

CONCLUSIONS

Zinc chloride at 25 and 50 mM concentrations was effective in detaching “firmly” attached *Salmonella* on chicken skin in the reversal and inhibition experiments. Bacterial detachment was more pronounced at 15 min of ZnCl₂ application in the reversal experiments. Reduction of *Salmonella* in cell suspension in the reversal experiments may be attributed, in part, to the bactericidal activity of ZnCl₂ in addition to the pH and clumping effect. Data from inhibition experiments suggested that zinc ions may interfere with binding of *Salmonella* to receptor sites on skin surface or zinc ions may compete with *Salmonella* for the same receptor sites involved in the attachment process. Further studies are necessary to determine the exact mechanism of zinc ions against *Salmonella* attachment to poultry skin. Initial levels of attachment and a combination of time and level of ZnCl₂ could influence efficacy of zinc ions as an antimicrobial agent. Since most *Salmonella* serotypes attach to poultry skin by the same mechanism(s), effectiveness of ZnCl₂ treatments could be extended to other serotypes involved in contamination of poultry carcasses. However, the physiological characteristics of individual serotypes may influence ZnCl₂ activity. In poultry processing plants antimicrobials are routinely added to the chill tank to reduce the level and frequency of pathogens on carcasses. Future investigations should determine whether ZnCl₂ could effectively reduce *Salmonella* contamination on carcasses when added to chill water in combination with 50 ppm chlorine. The authors did not evaluate the efficacy of ZnCl₂ at chill temperatures. Lower temperature may alter the skin topography and/or the dynamics by which *Salmonella* gets attached to skin. These alterations may affect the mechanism(s) by which ZnCl₂ interferes with *Salmonella* attachment

to chicken skin. The practicality of using ZnCl_2 as a chemical disinfectant on poultry carcasses in a slaughter facility merits additional consideration.

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Table 1. Effect of ZnCl₂ on “discard” pH and *Salmonella typhimurium* attachment to chicken skin in the reversal experiments.

ZnCl ₂ level (mM)	pH	“Firmly” attached cells		“Loosely” attached cells		“Discarded” cells		Scanning electron microscope data	
		Log CFU/mL	% reduction	Log CFU/mL	% reduction	Log CFU/mL	% reduction	Log Cells/cm ²	% reduction
0	6.85 ^c	3.94 ^b	-	4.86 ^c	-	6.80 ^b	-	5.27 ^c	-
25	5.63 ^b	3.31 ^a	77	4.42 ^a	64	4.61 ^a	99.4	4.76 ^{bc}	69
50	5.45 ^a	2.98 ^a	89	4.44 ^a	62	4.40 ^a	99.6	2.26 ^a	99.9
Significance	p<0.0001	p<0.01		p<0.1		p<0.0001		p<0.1	
Orthogonal comparisons	A vs B C vs D (p<0.05)	A vs B (p<0.05)		A vs B (p<0.05)		A vs B (p<0.05)		A vs B C vs D (p<0.1)	

A vs B = without ZnCl₂ versus with ZnCl₂
 C vs D = 25 mM ZnCl₂ versus 50 mM ZnCl₂

Figure 1. Schematic diagram of a skin attachment model.

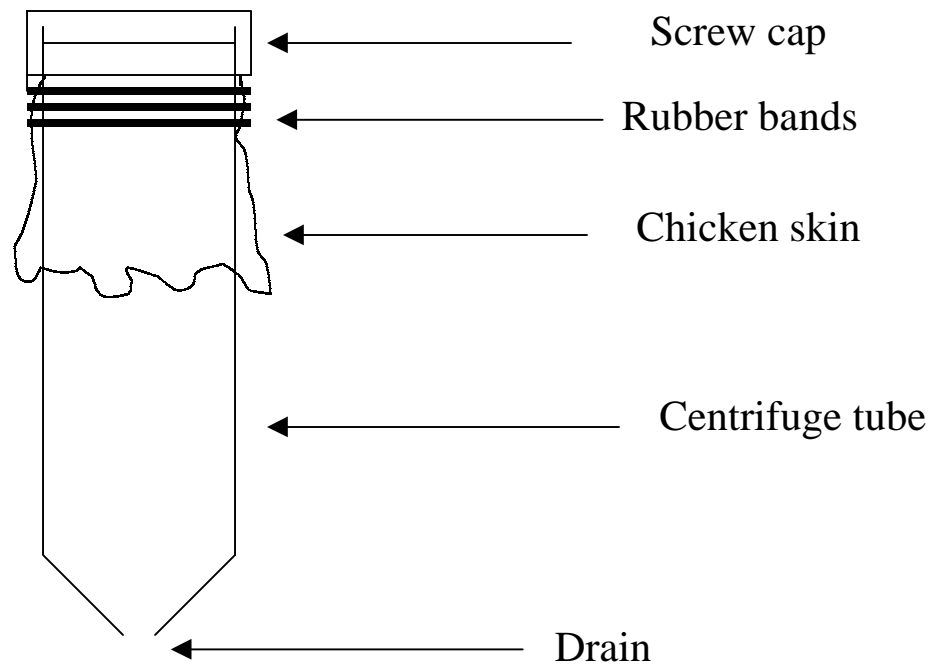
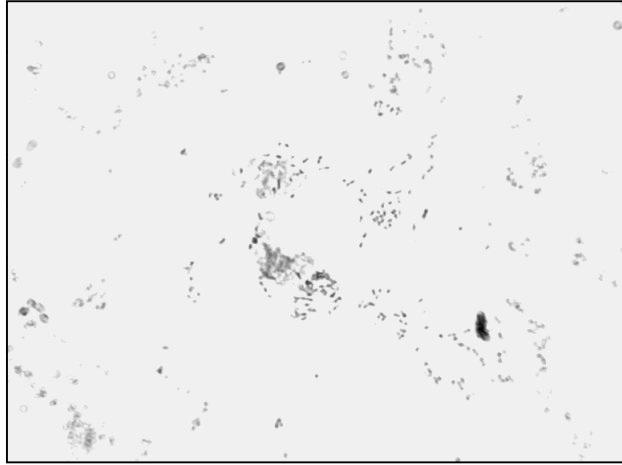
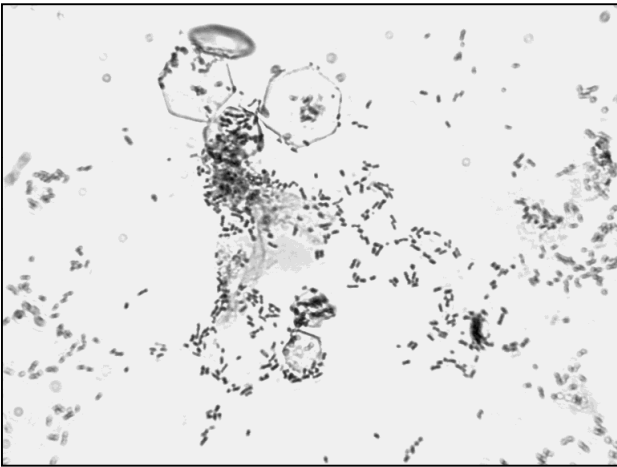


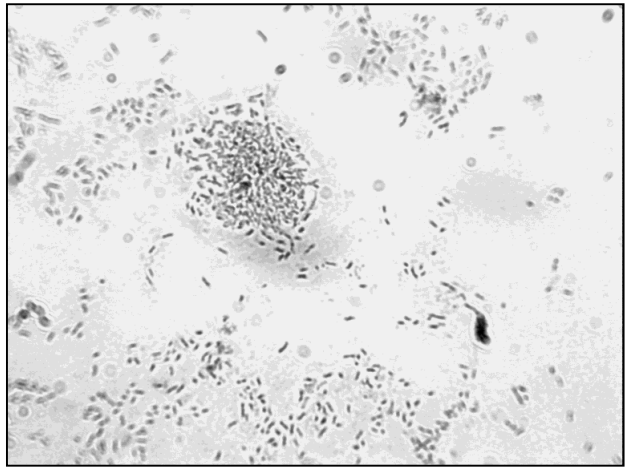
Figure 2. *Salmonella typhimurium* cell suspension (10^7 CFU/mL) mixed with (a) control; (b) 25 mM ZnCl₂; and (c) 50 mM ZnCl₂ and held for 15 min at room temperature ($\sim 22^\circ\text{C}$) (1000 X).



(a)

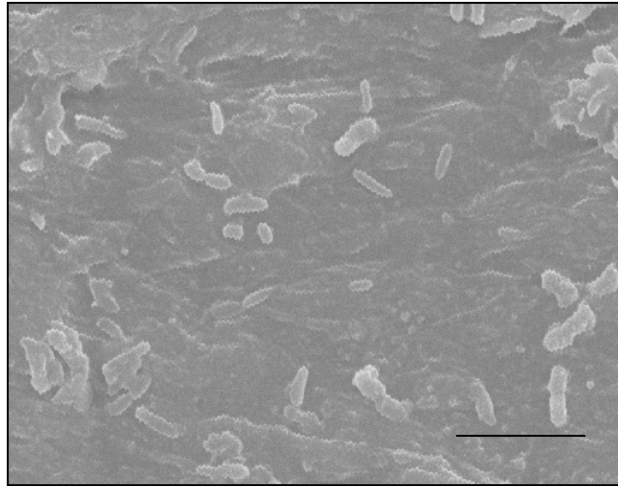


(b)

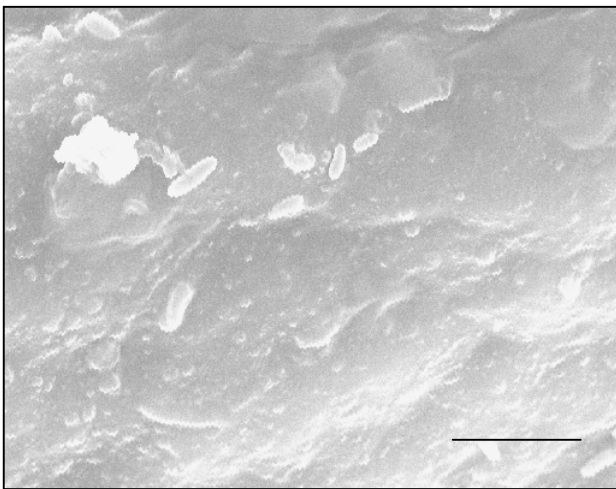


(c)

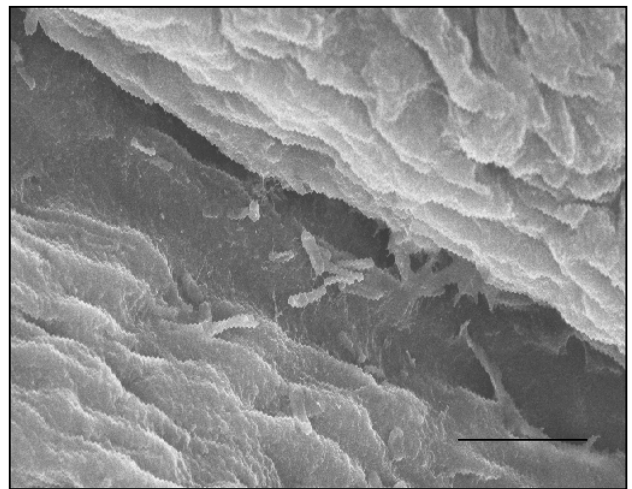
Figure 3. Scanning electron micrographs of chicken skin samples from reversal experiments: (a) control, 5 min; (b) 25 mM ZnCl₂, 5 min; (c) 50 mM ZnCl₂, 5 min; (d) control, 15 min; (e) 25 mM ZnCl₂, 15 min; and (f) 50 mM ZnCl₂, 15 min (bar 10 μ)



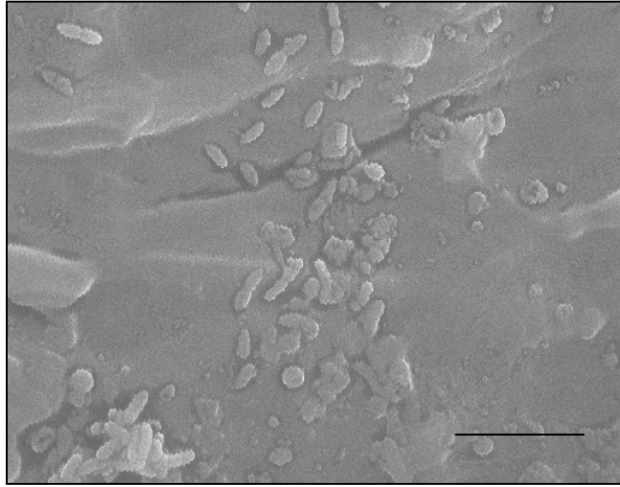
(a)



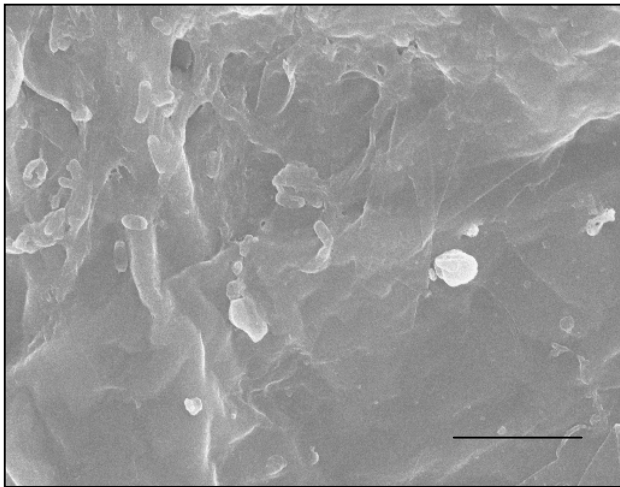
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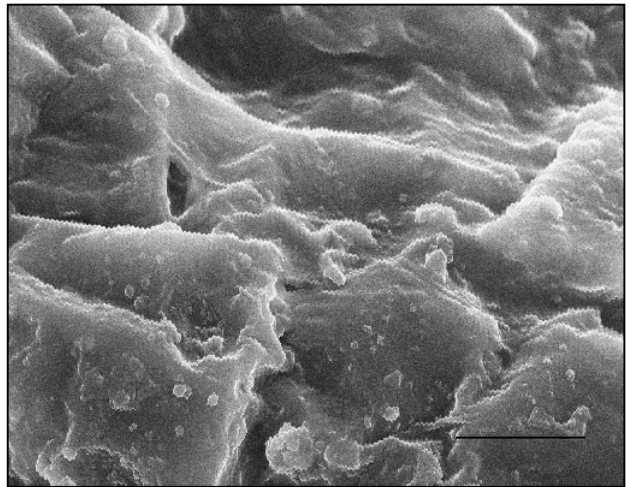
(c)



(d)

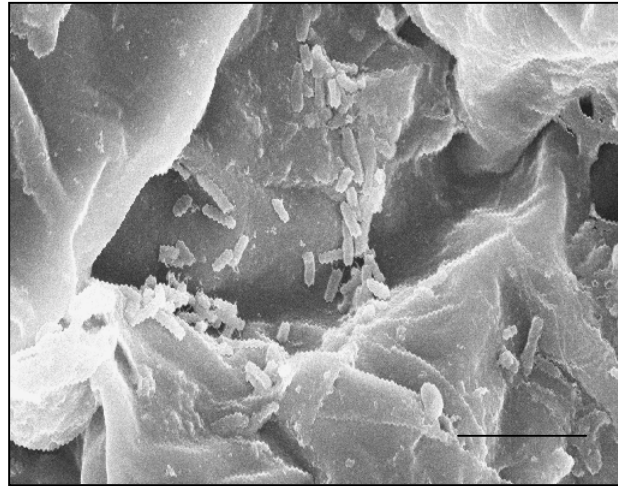


(e)

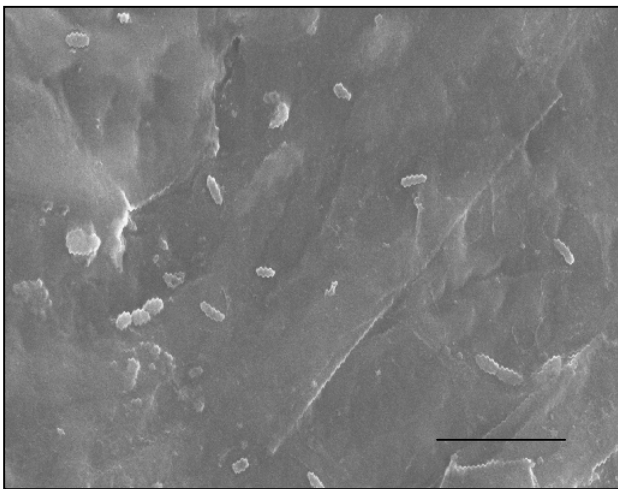


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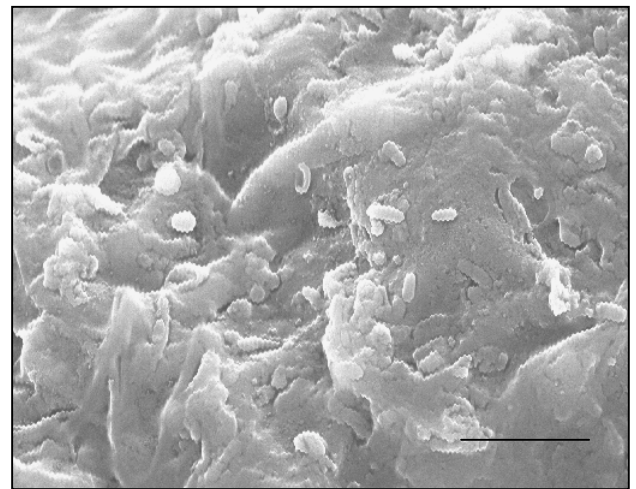
Figure 4. Scanning electron micrographs of chicken skin samples from inhibition experiments: (a) control, 5 min; (b) 25 mM ZnCl₂, 5 min; (c) 50 mM ZnCl₂, 5 min; (d) control, 15 min; (e) 25 mM ZnCl₂, 15 min; and (f) 50 mM ZnCl₂, 15 min (bar 10 μ).



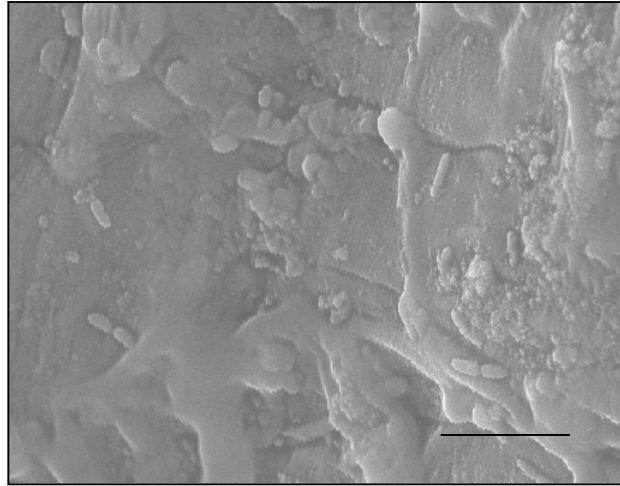
(a)



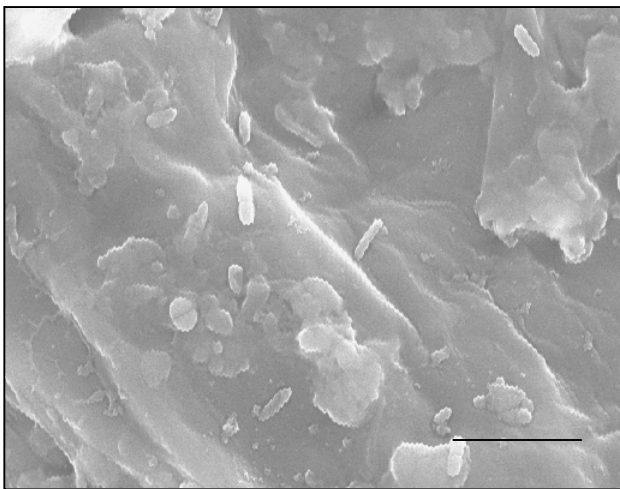
(b)



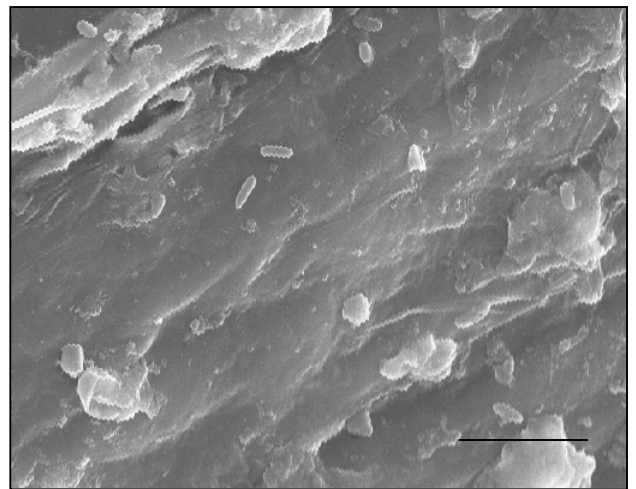
(c)



(d)



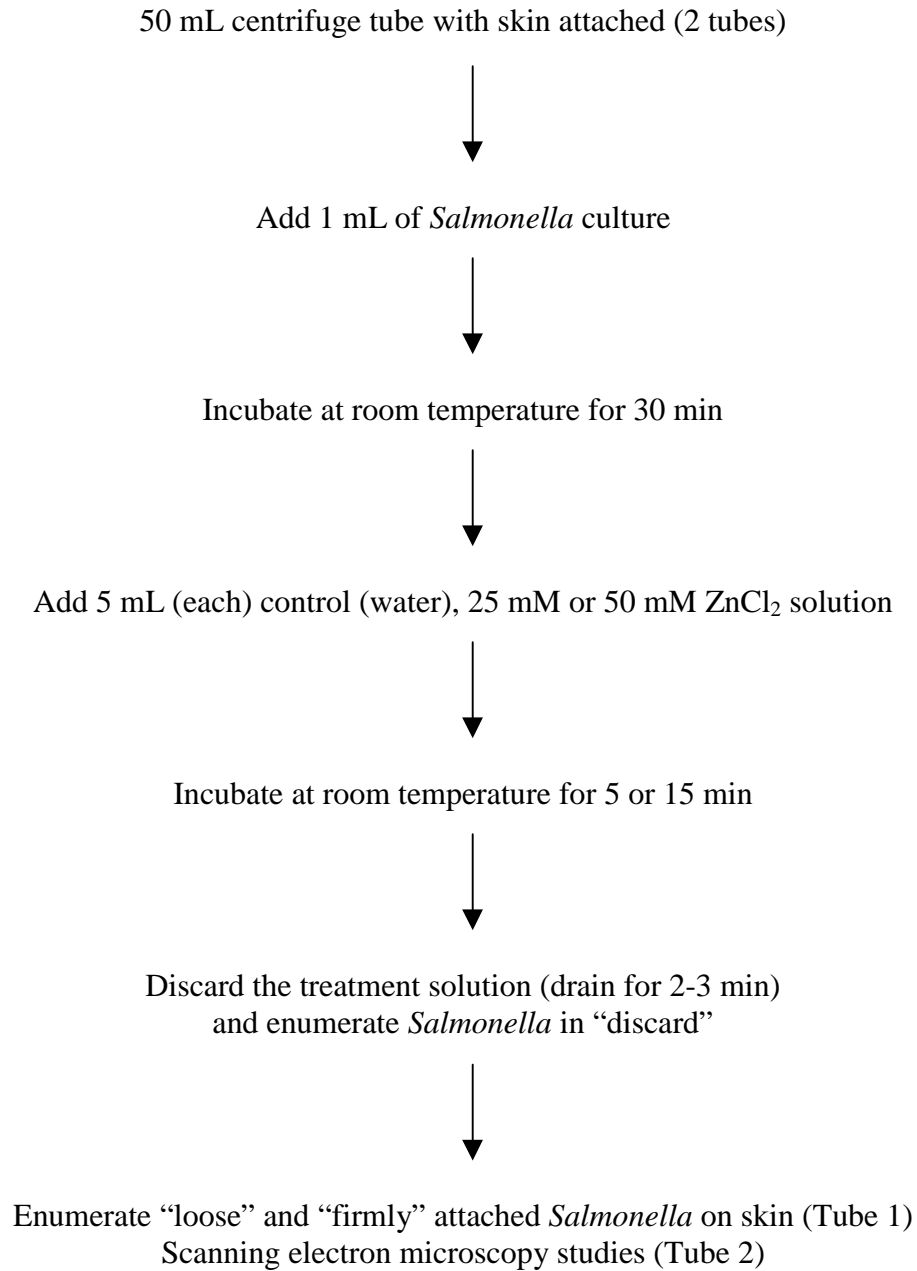
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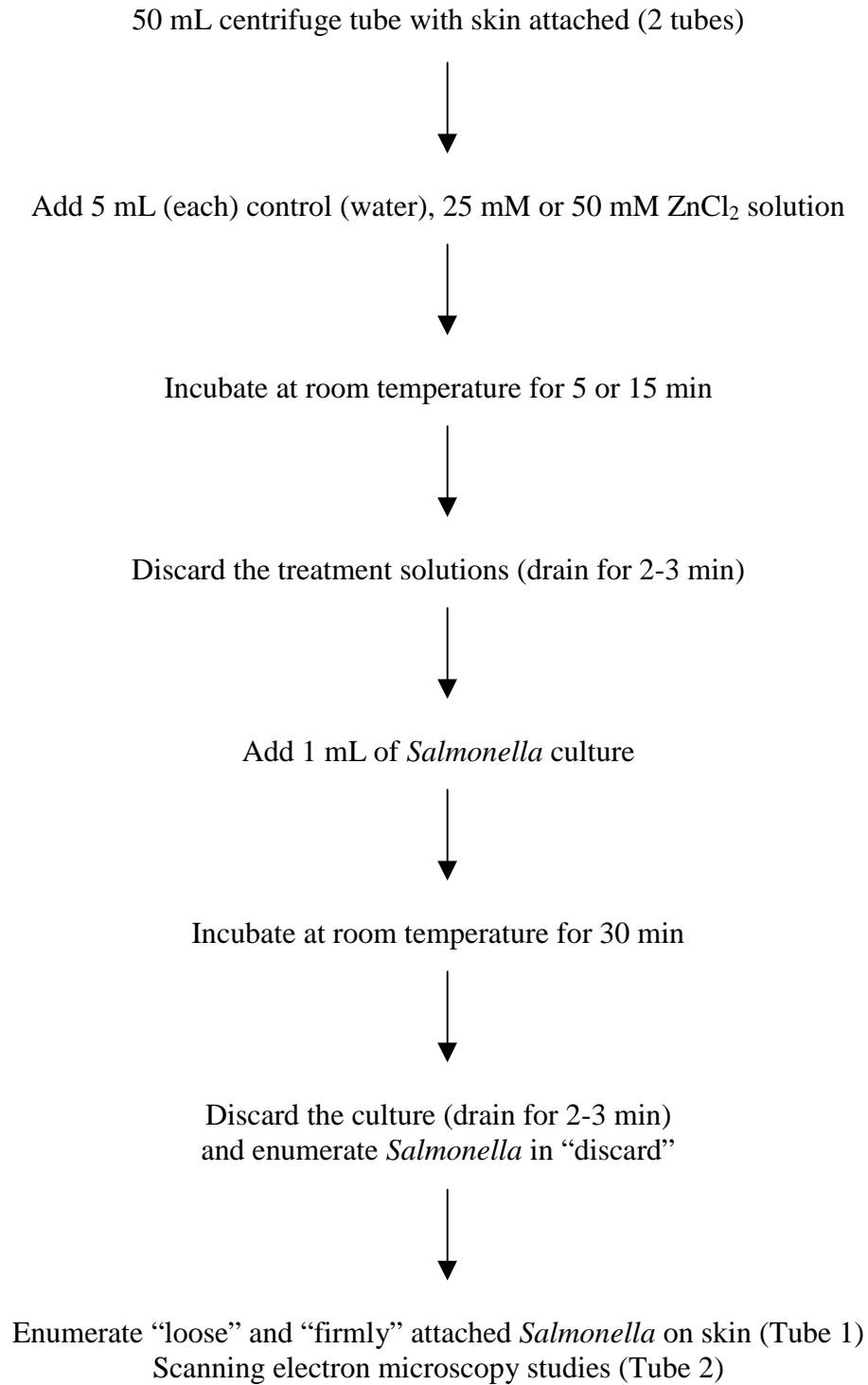
(f)

APPENDICES

REVERSAL EXPERIMENT FLOW DIAGRAM



INHIBITION EXPERIMENT FLOW DIAGRAM



STATISTICAL PROGRAM

INHIBITION OR REVERSAL EXPERIMENTS

```
OPTION LS=80 PAGESIZE=60 NO NUMBER;
TITLE '2X3 FACTORIAL RCB – REVERSAL/INHIBITION EXPERIMENT PLATE
COUNT';
DATA MAZE; INPUT REP TIME LEVEL PH LOOSE FIRM DISCARD;
CARDS;
{PASTE DATA}
PROC PRINT;
PROC GLM; CLASSES REP TIME LEVEL;
MODEL PH LOOSE FIRM DISCARD=REP TIME LEVEL TIME*LEVEL;
LSMEANS TIME LEVEL TIME*LEVEL/PDIFF;
CONTRAST 'A VS B' LEVEL 2 -1 -1;
CONTRAST 'C VS D' LEVEL 0 1 -1;
CONTRAST 'E VS F' TIME 1 -1
          TIME*LEVEL 0 1 0 0 -1 0;
CONTRAST 'G VS H' TIME 1 -1
          TIME*LEVEL 0 0 1 0 0 -1;
CONTRAST 'I VS J' TIME*LEVEL 0 1 -1 0 -1 1;
RUN;
```

SCANNING ELECTRON MICROSCOPE STUDIES

```
OPTION LS=80 PAGESIZE=60 NO NUMBER;
TITLE '2X3 FACTORIAL RCB – REVERSAL/INHIBITION SEM EXPERIMENT';
DATA MAZE; INPUT REP TIME LEVEL CFU;
CARDS;
{PASTE DATA}
PROC PRINT;
PROC GLM; CLASSES REP TIME LEVEL;
MODEL CFU=REP TIME LEVEL TIME*LEVEL;
LSMEANS TIME LEVEL TIME*LEVEL/PDIFF;
CONTRAST 'A VS B' LEVEL 2 -1 -1;
CONTRAST 'C VS D' LEVEL 0 1 -1;
CONTRAST 'E VS F' TIME 1 -1
          TIME*LEVEL 0 1 0 0 -1 0;
CONTRAST 'G VS H' TIME 1 -1
          TIME*LEVEL 0 0 1 0 0 -1;
CONTRAST 'I VS J' TIME*LEVEL 0 1 -1 0 -1 1;
RUN;
```