IMPROVING THE ENVIRONMENT OF BROILER BREEDER CONFINEMENT FACILITIES BY MEANS OF AN ELECTROSTATIC SPACE CHARGE SYSTEM

by

LARRY JASON RICHARDSON

(Under the Direction of Jeanna Wilson)

ABSTRACT

Dust and microorganisms are associated with poultry confinement facilities. Since, microorganisms in general are carried by dust particles, this provides an excellent vector for horizontal disease transmission between birds. An electrostatic space charge system (ESCS) was investigated in its effectiveness on reducing airborne dust, microorganism and horizontal disease transmission in pullet and lay facilities. The ESCS was designed to reduce airborne dust and bacteria by inducing a strong negative electrostatic charge on airborne particulates and to collect these particulates on the grounded surfaces. Dust concentrations in each room were logged at 10-minute intervals and airborne bacteria levels were measured using media plates exposed to the air. Airborne dust levels were significantly reduced in the pullet and lay facility. Reducing airborne dust particulates, significantly reduced overall bacteria and reduced horizontal transmission of an inoculated *Salmonella enteritidis*. The ESCS was an effective means of reducing dust, bacteria and disease transmission between birds.

INDEX WORDS*:* Electrostatic space charge system, Pullets, Broiler breeders, Broilers, Confinement facilities, Dust, Microorganisms, Salmonella, Disease transmission

IMPROVING THE ENVIRONMENT OF BROILER BREEDER CONFINEMENT FACILITIES BY MEANS OF AN ELECTROSTATIC SPACE CHARGE SYSTEM

by

LARRY JASON RICHARDSON

B. S. A., The University of Georgia, 2000

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2002

IMPROVING THE ENVIRONMENT OF BROILER BREEDER CONFINEMENT FACILITIES BY MEANS OF AN ELECTROSTATIC SPACE CHARGE SYSTEM

by

LARRY JASON RICHARDSON

Major Professor: Jeanna L. Wilson

Committee: Charles L. Hofacre
Bailey W. Mitchell

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2002

DEDICATION

I would like to dedicate this thesis to my beloved family and closest friends. Achieving such goals scholastically could not have been possible if not for my loving parents, Larry and Marilyn Richardson. Throughout my tenor as a student here at The University of Georgia, my mother and father were always there for encouragement and constantly reminding me of the bigger picture in life. I would also like to thank my closest friends who meant so much to me for their support and encouragement when I needed it the most.

ACKNOWLEDGEMENTS

Dr. Jeanna Wilson, I would like to thank you for your guidance and support throughout my tenor here at The University of Georgia in both undergraduate and graduate school. I have learned so much from your teachings and practical thinking. Thank you for being an excellent major professor who always encouraged me to think for myself.

Great appreciation is extended to the members of my committee Dr. Charles Hofacre and Dr. Bailey Mitchell for your ideas, advice, guidance and contribution to the project and thesis.

Tongrui Liu, thank you for always making sure everything in the lab was ordered and your quickness to lend a helping hand with questions or advice.

Mr. Fred Smith and the PDRC, thank you for your technical assistance.

Mr. Johnny Doster, thank you for helping me in data collections throughout the experiments.

Ben Martin, thank you for being an excellent technician and for your assistance in the daily care of the experiments.

Benton, Emily, and Ruth thank you for always offering a helping hand when needed.

Mr. Doug Ard, thank you for the support and help over the years.

Dr. Glen Ware, thank you for your assistance in the statistical analyses of the data from this project.

TABLE OF CONTENTS

INTRODUCTION

A major goal of the poultry industry is to produce a safe, edible and economical product for consumers. The USDA Food Safety and Inspection Service stated in the 1996 Federal Register"…the FSIS food safety goal should be to reduce the risk of foodborne illness associated with the consumption of meat and poultry products to the maximum extent possible by ensuring that appropriate and feasible measures are taken at each step in the food production process where hazards can enter…"(Federal Register, 1996).

The United States total farm income from broiler and turkey production grew from approximately 1 billion dollars in 1960 to over 9 billion dollars in 1992 (Cunningham, 1993). From 1994 to 2001 that number has increased to 10 billion dollars (GDA and USDA, 2002). The number of poultry being commercially processed grew from approximately 7.3 billion to 8.4 billion from 1994-2001, a 15% increase (GDA and USDA, 2002). The per capita consumption of poultry in 1997 was 90 pounds and increased to 93 pounds in 2001 (GDA and USDA, 2002). A problem the poultry industry has encountered with this boost in the production and consumption of poultry is an amplified likelihood of a foodborne illness associated with its product.

Efforts have been taken to reduce the levels of bacteria on edible products in processing plants (Rose *et al*., 1996) but in order to further reduce these levels new strategies at the farm end of the continuum must be implemented (USDA,

1996; Rose *et al*., 1996; Mallinson, 2001). Proper management practices such as: increased bio-security, rodent and insect control, removal of dead, vaccination, house cleaning and litter management have been shown to aid in the reduction of certain enteropathogens in confinement rearing facilities; however development of new technologies will further aid in the reduction of bacteria at the farm.

Certain genus of bacteria, for example *Salmonella* can be found persistently in the environment of poultry confinement rearing facilities even when proper management practices are followed. *Salmonella* contamination of poultry and confinement facilities are inevitable due to environmental persistence and the number of animals, reptile, and insect reservoirs (Hinshaw, 1944b; Goyal and Singh, 1970; Borland, 1975; Henzler and Opitz, 1992; Opara *et al*., 1992). *Salmonella* has been readily found in the feed, dust, litter, and manure of farm environments (Morehouse and Wedman, 1961; Allred *et al*., 1967; Williams, 1975; Williams, 1981; Gay, 1993). Numerous reports have shown that animals readily transmit *Salmonella* by both horizontal and vertical transmission (Schaaf *et al*., 1931; Stokes *et al*., 1956; Clemmer *et al*., 1960; Board, 1966; Smyster *et al*., 1966; Lahellec and Colin, 1985; Timoney *et al*., 1989; Baskerville *et al*., 1992; Nakamura *et al*., 1994; Holt, 1995).

Vertical transmission of *Salmonella* from the breeder hen to its progeny (broiler) via the egg occurs at a low rate; however, if a single hatching egg is infected with *Salmonella* it can be readily dispersed throughout the hatching cabinet infecting adjacent chicks (Muira *et al*., 1964; Shivasprasad *et al*., 1990;

Cason *et al*., 1994). In recent studies, 5-10% of day old chicks leaving the hatchery are infected with *Salmonella* (Bailey, 1987; Cox *et al*., 1990). Byrd and others (1998) stated that if as few as 5% of the chicks leaving the hatchery have been exposed to as few as 100 *Salmonella*, it could infect over half of the uninfected chicks. The chicks (broilers) are then grown on litter floors for 6-8 weeks of age and then transported to processing plants for human consumption. Therefore, it is vital to reduce the number of hens in a breeder flock infected with *Salmonella* in order to reduce the number of *Salmonella* contaminated fertile hatching eggs.

Many pathogens have recently received considerable attention in their role as a human foodborne illness threat. *Salmonella* infection remains among the leading sources of gastroenteritis (Mossel, 1988; Bean and Griffin, 1990; Levine *et al*., 1991; Mishu *et al*., 1994; Olsen *et al*., 2000). The estimated annual incidence of a foodborne illness associated with *Salmonella* ranges from 1 million to 4 million cases in the United States (Tauxe, 1996). Since, *Salmonella* and other bacteria can cause a foodborne illness many methods are being investigated to reduce this potential hazard. High levels of dust concentrations are known to be associated with confinement rearing facilities and the impact of these levels on human and animal health has been of concern in recent years (Pearson and Sharples, 1995; Simpson *et al*., 1999). Airborne dust in poultry confinement rearing facilities can be generated from the litter, feed, birds, incoming air, and secondary suspension due to bird activity. Dust acts as a major carrier of airborne bacteria and a reduction of dust by 50% can decrease

bacteria by up to 100-fold (Carpenter *et al*., 1986; Madelin and Wales, 1989). Developing technology that will reduce airborne dust concentrations in these environments and therefore the number of airborne bacteria could decrease the potential bacterial contamination of chickens by airborne particulates. Control of bacteria at the farm will inevitably result in fewer bacterial infections of eggs, chicks, and broilers with *Salmonella* and other bacteria and result in a reduced number of bacteria on poultry carcasses at the processing plant.

Ionization of air is a method of reducing airborne particulates and therefore bacteria. By negatively charging the air, particulates are attracted to the grounded surfaces such as walls, floor, and equipment and are continually removed from the air. The implementation of this technology in confinement rearing facilities could reduce the airborne horizontal transmission of bacteria among birds. The ionization concept is something that is frequently seen in nature (Kreuger and Reed, 1976). Air is made up of molecules, which contain a core with positively charged protons surrounded by negatively charged electrons. When a molecule of air becomes negatively charged, it tries to find equilibrium by attracting to positively charged molecules. This attraction to positively charged surfaces occurs due to the unstable molecule of air desiring to become a stable or passive molecule.

Mitchell and Stone (2000a) have used this concept to develop an electrostatic space charge system, which has shown significant reductions in environmental airborne particulates and bacteria in caged layer rooms and hatching cabinets. Results from these studies indicate that this device could be a useful tool in confinement rearing facilities where high levels of airborne particulates and microorganisms are known to exist.

OBJECTIVES

The objectives of this thesis were to examine the effects of an electrostatic space charge system in poultry confinement facilities:

a) To evaluate the effect of an electrostatic charge on the reproductive performance of broiler breeders in lay.

b) To determine the optimum airborne bacteria sampling technique and times in the dusty environment of a long term use poultry facility.

c) To study the reductions in airborne dust concentrations and microorganisms during the pullet and lay phase of broiler breeders.

d) To analyze the effect of reducing *Salmonella* contamination in broiler breeder hens and the impact on their subsequent progeny.

LITERATURE REVIEW

Introduction

Environmental and microbial concerns of animals being reared in confinement facilities are the subject of numerous reports in recent years. (Pearson and Sharples, 1995; Simpson *et al*., 1999; Takai *et al*., 1999). Airborne concentrations of dust and other particulates in these confinement-rearing facilities increase over the duration these animals are in the facilities (Hinz *et al*., 1994). It has been shown that bacteria can adhere to dust particles and that there is a direct correlation of reducing the level of airborne dust particulates to the decrease in airborne bacteria (Madelin and Wales, 1989).

Salmonella is the most prominent cause of gastroenteritis associated with the consumption of poultry. An estimated 1 million to 2 million people become infected with *Salmonella* annually from the consumption of poultry or poultry products. The majority of the first part of the 20th century was spent on studying the disease outbreaks in poultry associated with *Salmonella* organisms and eradicating these diseases from commercial poultry; however, with the increase in foodborne illness associated with *Salmonella* over the past 40 years, focus has turned to the reduction of this organism in the environment and in the animal. This comes not from a disease standpoint but from a human health concern.

Current methods are being adapted at the farm end of the continuum to reduce *Salmonella* contamination associated with the birds and their environment. A few methods that are being researched or that have already been implemented to reduce *Salmonella* levels in birds and confinement facilities are competitive exclusion, vaccinations, using built-up litter instead of fresh litter, proper management practices along with bio-security to reduce contamination from outside elements (wild birds, rodents, reptiles, and insects).

The electrostatic space charge system, which negatively charges airborne particulates and causes them to be attracted to grounded surfaces, is a promising new technology that could be implemented to reduce the level of airborne dust, therefore airborne bacteria levels such as *Salmonella*. The negative ionization of airborne particulates would aid in reducing or preventing the spread of bacteria through the air to adjacent animals in these facilities by reducing airborne dust. Decreasing cross contamination of animals along with better air quality could lead to a healthier environment for these animals.

The objectives of this literature review are first to review *Salmonella* and the incidence in poultry and confinement facilities. Secondly, this review will highlight studies pertaining to the isolation, recovery and identification of *Salmonella*. In addition, this review will examine work pertaining to evidences of *Salmonella* infection in all phases of poultry. The final area reviewed is the literature pertaining to the ionization of air as a method to reduce airborne bacteria and particulates in animal and poultry environments.

***Salmonella* incidence in poultry**

A REVIEW

The isolation and impact of *Salmonella* contamination in poultry can be seen as far back as the late 1800's and early 1900's (Klein, 1889; Moore, 1895; Rettger, 1900; Hewitt, 1928; Baumler *et al*., 2000). *Salmonella* can be readily transferred from animal to animal and from animal to human by direct and indirect means (Schaaf, 1936; Edwards, 1956). Around the mid 1900's and until present day, extensive literature has been reported on the effect and occurrence of *Salmonella* in a wide variety of environments, poultry feeds, animals and especially in the incidence of *Salmonella* infections in humans and the foodborne disease salmonellosis associated with the infection (Hinnshaw, 1944a; Edwards *et al*., 1948a, 1948b; Moran, 1959; Faddoul and Fellows, 1966; Cox, 1983; Bean and Griffin, 1990).

In the early 1940's, approximately 60 serological types of *Salmonella* had been discovered and by the 1970's, approximately 1700 had been reported (Bullis, 1977b). Hinshaw and others (1944a) listed 48 *Salmonella* serotypes isolated from poultry in the United States that could be associated with public health. Faddoul and Fellows (1966) found 34 different *Salmonella* serotypes in avian species over a five-year period from 31,247 specimens submitted for diagnostic investigation.

In a five-year epidemiological study on naturally occurring infection of *Salmonella* from 1971-1975 in twelve poultry houses by Zecha and others (1977), 11 serotypes were isolated from a total of 17,858 bacteriological cultures. Bailey and others (2001) looked at the prevalence of *Salmonella* serotypes throughout integrated poultry operation in a multistate epidemiological investigation and identified 36 different serotypes from the hatchery to the end of processing. Present time, over 2300 serotypes of *Salmonella* have been identified but only about 10% of these serotypes have been isolated from poultry (Le Minor and Popoff, 1992; Gast, 1990a).

In a study by Edwards and others (1948a) to determine the rate and division of *Salmonella* types in the United States, they found that approximately 50% of the *Salmonella* positive cultures isolated were from different fowl sources. Edwards regarded fowl as the greatest reservoir of *Salmonella* infection in the United States. In three reports from 1957-1959, the number of *Salmonella* cultures from poultry was 73% in 1957, 87.2% in 1958 and 73.6% in 1959 and three similar reports were published by Moran (1958, 1959, 1960). These independent authors agree that birds are the greatest reservoir for *Salmonella* and that poultry and poultry products are increasingly cited as the source of *Salmonella* infections in humans.

In a 1985 survey by the Centers for Disease Control, in 9,000 isolates from various sources of animals, 52% of the isolates came from poultry (CDC, 1987). Waltman and others (1992) investigated the pooled cecal samples of 81 different poultry laying flocks from nine states and determined that all samples

were positive for *Salmonella* (Waltman *et al*., 1992). Ebel and colleagues (1992) found similar results with 86% of 406 laying houses being positive for *Salmonella*. Bailey and colleagues (2001) reported on a multistate epidemiological investigation where they looked at recovery of *Salmonella* from the broiler hatchery to the end of processing. A total of 10,740 samples were analyzed from 20 on-farm and 6 transport or processing plant locations and all were found to have some level of *Salmonella* present.

SALMONELLA IN FEED

Erwin (1955) published one of the first reports of the isolation of *Salmonella* in poultry feed and since that discovery, wide ranges in the percentage of *Salmonella* cultures in feed and feed ingredients has been reported (MacKenzie and Bains, 1976; Hacking *et al.*, 1978; Cox *et al*., 1983). It has been reported that very low levels of *Salmonella* in feed can cause colonization of the intestinal tract of chicks that are 1 to 7 days of age (Schleifer, 1984). Snoeyenbos and colleagues (1967) found that 51.8% of the animal by-products being shipped to a feed mill were positive for *Salmonella* and that it could be found in the processed feed. In addition, there are reports of feed contamination causing flock infection by ingestion of the feed, but the infection has occurred at a low incidence (Boyer *et al*., 1958; Snoeyenbos *et al*., 1967). Hacking and others (1978) tested a total of 111-pelleted feed samples from a commercial feed mill and found 4 out of the 111 (3%) samples contaminated with *Salmonella*. However, in a report by Cox and others (1983) they found

Salmonella isolates in 15 out of 26 (58%) samples of pelleted and mash poultry feed from 10 feed mills in three different states.

TWO CATEGORIES OF SALMONELLA

Salmonella species can be divided into two major categories (Schaaf, 1936; Edwards, 1956; McCoy, 1975; Le Minor and Popoff, 1992; Clarke and Gyles, 1993):

a) Salmonella species that are host specific.

b) Salmonella species which are non-host specific.

Salmonella are gram-negative facultative anaerobic organisms with morphology being associated with straight, non-spore forming rods and in the family of bacteria *Enterobacteriaceae* (Krieg and Holt, 1984). *Salmonella* species can be distinguished between each other through their biochemical properties and antigenic structure (Krieg and Holt, 1984; Ewing, 1986).

HOST SPECIFIC INFECTIONS

Two major host specific species of *Salmonella*, which has caused major disease outbreaks in poultry and that have been studied extensively over the years, are S. *gallinarum* and S. *pullorum* (Klein, 1889; Rettger, 1900 and 1909; Hinshaw *et al*., 1926; Hewitt, 1928; Schalm, 1937; Moore, 1946). S. *gallinarum* was isolated from poultry in the late 1800's and caused a disease known as fowl typhoid (Klein, 1889; Hewitt, 1928). The disease, fowl typhoid, can be associated with increased mortality, anorexia, decreased egg production, a

watery to mucoid yellow diarrhea, birds are despondent and have rapid breathing (Klein, 1889; Hewitt, 1928; Johnson *et al.*, 1992; Salem *et al.*, 1992; Sato *et al.*, 1997). S. *pullorum* was isolated around the same period and caused a disease first named “fatal septicemia of young chicks” or “white diarrhea” (Rettger, 1900 and 1909). The disease name was later changed to pullorum disease in 1929 at the second Northeast conference on Pullorum Disease (Bullis, 1977a). Pullorum disease causes fatigue, ruffled feathers, a chilled appearance with birds huddling near a source of heat, labored breathing, and bacillary white diarrhea in young poults (Rettger, 1900 and 1909; Sato, 1997).

Fowl typhoid and mainly pullorum disease caused increased mortalities in poultry flocks worldwide and suppressed the industry until proper testing and eradication programs could be implemented (Jones, 1913; Schaffer *et al*., 1931; Chase, 1947; McDermontt, 1947). In 1913, Jones was the first person to develop a specific diagnostic procedure to test for the disease in infected birds. Jones developed a macroscopic tube agglutination test, which tested for the presence of anti-S*. gallinarum* antibodies in the bird (Jones, 1913). Shaafer and others (1931) developed another test for the detection of infected birds with S. *gallinarum* or S. *pullorum*, the whole-blood test for slide agglutination of stained antigen. This test formed the basis for the rapid expansion of testing and elimination of the disease in poultry flocks (Bullis, 1977a). In 1935, the National Poultry Improvement Plan was adopted for the control and eradication of pullorum disease and later revised to include fowl typhoid since the same test was used to detect both diseases, and by the mid 1970’s, no evidence of the two

diseases could be found in commercial poultry (Bullis, 1977a). In 1975, only 16 cases were reported of the disease in the entire United States (AAAP, 1975).

NON-HOST SPECIFIC INFECTIONS

Non-host specific infections of avian species were first noted in 1895 in an infectious enteritis outbreak in pigeons causing mortality (Moore, 1895). Since that time, numerous non-host specific *Salmonella* infections have been documented in poultry and for this reason an infection of this type are commonly referred to as a paratyphoid infection in the bird or salmonellosis in the human. However, severe paratyphoid infection has been shown to cause mortality in poultry (Edwards *et al*., 1948a; Vestal and Stephens, 1966).

Milner and Shaffer (1952) were the first to report that the severity of paratyphoid infections in chicks is dose dependent. Sadler and others (1969) found the level of intestinal infection to be correlated to age and inoculum dose. At 38 days of age, only 8% of the inoculated birds in the study were fecal shedding the inoculum and at 73 days of age the number had dropped to 1% and by 94 days of age only 0.5% were fecal shedding the *Salmonella*. Morris and others (1969) found that naturally occurring paratyphoid infection caused peak mortality in poultry from 3 to 7 days of age. Smith and Tucker (1980) showed similar results of a decrease in mortality in young birds as age increases by artificially challenging birds with S. *typhimurium*. Invasion into the internal organs of chicks has also been shown to occur more frequently in younger chicks than older birds (Turnbull and Snoeyenbos, 1974).

However, in mature chickens paratyphoid infections are rarely associated with high mortality (Brown *et al*., 1976). The most common effect of a paratyphoid infection in adult birds is intestinal colonization, bacteremia, fecal shedding and dissemination into internal organs and persistent colonization of the bird (Hopper and Mawer, 1988; Gast and Beard, 1990b; Shivaprasad *et al*., 1990).

NON-HOST SPECIFIC SALMONELLA AS A FOODBORNE DISEASE

Although over the years, many have reported the effect of paratyphoid infections in animals, in the past few decades the emphasis has shifted to the role that animal infections play in salmonellosis in humans (Surkiewicz *et al*., 1969; Silliker, 1982; Mead *et al*., 1999). Salmonellosis in humans is commonly related to the consumption of meat products and more specifically poultry and poultry products that are infected with non-host specific *Salmonella* infections (Schaaf, 1936; Edwards, 1956; Humphrey *et al*., 1988). *Salmonella* can be introduced into a breeder, egg laying, or broiler flock from numerous different routes. S. *typhimurium* was at one time the most common isolated non-host specific serovar (McCoy, 1975); however, S. *enteritidis* has become an increasing problem in its role as a cause of salmonellosis in humans (Gomez *et al*., 1997). Gomez and others (1997) stated that S. *enteritidis* only comprised 5% of the isolates in poultry in 1985 and by 1995 that number had increased to 25% of the isolates, a 5-fold increase.

Poultry and poultry products have been reported as one of the main sources for infection in humans of non-host specific *Salmonella* (Schaaf, 1936; Edwards, 1956; Bryan, 1980; Humphrey *et al*., 1988). In 1983, the Center for Disease Control stated that poultry could be linked to approximately 9.5% of the 2 million total salmonellosis cases reported in 1981 (CDC, 1983). In more recent literature, *Salmonella* infections account for the majority of gastroenteritis outbreaks in the United States and the consumption of poultry has been implicated in 40% of the cases (Olsen *et al*., 2000). The Center for Disease Control (1996) reported that 582 S. e*nteritidis* outbreaks occurred from 1985-1995 and contributed to 24,058 cases of illness in the United States.

In one report, foodborne transmission accounted for approximately 13.8 million (36%) of the 38.6 million illnesses in the United States annually and of the 13.8 million cases, bacterial pathogens account for 60% of the infections (Mead *et al*., 1999). *Salmonella* infections alone accounted for approximately 1.2 million of the bacterial infections. Outbreaks and surveillance data are widely recognized as underestimates of the true level of *Salmonella*-related enteric disorders and an estimated 400,000 to 4,000,000 causes may actually occur annually (Cohen and Tauxe, 1986).

Environmental and bird sampling

ENVIRONMENTAL SAMPLING

Over the years, numerous reports have documented methods of environmental sampling for optimum isolation and recovery of *Salmonella* in poultry confinement facilities. Multiple methods also exist for the determination of *Salmonella* contamination in poultry hatchery environments and the recovery rates can be found to differ among reports. However, a few of these methods have been recommended in order to increase the probability rate of recovery of *Salmonella* organisms (McCoy, 1962; Tate *et al*., 1990; Bailey and Cox, 1992; Opara *et al*., 1992 and 1994). In a World Health Organization (WHO) consultation in 1994, strategies for the detection and monitoring of *Salmonella* infected poultry flocks was extensively covered (WHO, 1994). Determining reliable techniques for detection of *Salmonella* in the poultry environment is critical in monitoring and controlling *Salmonella* within the poultry industry (Mallinson *et al*., 1989 and 2001).

Drag swabbing the environment was reported as one of the best methods for recovering *Salmonella*. Kingston (1981) was the first person to modify the Moore swab for veterinary application. Drag swabs are pre-moistened sterile gauze pads that are attached to a 4 to 5 foot length of string and are used for sampling the environment of poultry (Kingston, 1981; Mallinson *et al*., 1989). Kingston (1981) found that the drag-swab technique was efficient, low cost, and reliable isolation rates could be found when compared to standard litter cultures.

Caldwell and colleagues (1994) found that multiple drag swabbing of poultry environments increased the predictive value of *Salmonella* contamination of vacant or occupied poultry facilities.

In a study comparing pre-moistened to dry swabs, a higher recovery rate was found with the pre-moistened swabs (Byrd *et al*., 1997). *Salmonella* was isolated from 20 out of 30 (66.7%) houses using pre-moistened drag swabs compared to 12 out of 30 (40%) houses using dry swabs. Opara and others (1994) compared the following pre-moistening solutions double strength skim milk (DSSM), canned evaporated skim milk (CESM), physiological saline, buffered peptone water (BPW), and distilled water as to their efficiency in recovering *Salmonella*. The pre-moistening agent found to have the best recovery rate of *Salmonella* was double strength skim milk (DSSM) that yielded a 76% recovery within 24 hours of sampling. Davidson and others (1995) examined pre-moistening agents for drag swab sampling and further confirmed that DSSM drag swabs give the highest recovery rate.

Litter samples can also be taken in poultry houses to determine the presence of *Salmonella* (Snoeyenbos, 1967, 1969; Kingston, 1981). One disadvantage with litter sampling in a large-scale poultry operation is the labor associated with sampling and the possibility of aerial contamination from other samples during laboratory procedure (Kingston, 1981). Litter of commercial poultry farms has been linked to carcass contamination (Kingston, 1981). Litter sampling has been shown to be a reliable and sensitive measure of *Salmonella* infections in poultry flocks (Bhatia *et al*., 1979; Read *et al*., 1994). However,

Hayes and collogues (2000) in a recent report showed that litter sampling method resulted in lower recovery rates (45.8%) when compared to the drag swab method which had a higher recovery rate of 91.6%. They speculated that the level of water activity and moisture content of the litter contributed to the distribution of *Salmonella* in the litter.

Samples can be taken from hatch residue in the incubator environment to determine the level of *Salmonella* contamination in young chicks. Hinshaw and others (1926 and 1928) found that *Salmonella* could be disseminated in incubators by fluff. Miura and others (1964) investigated the survival of *Salmonella* in fluff and determined that fluff was a reasonable way of assessing hatchery contamination. In addition, it has also been shown that collecting chick excretions and eggshell fragments can be a reliable method for detection of *Salmonella* (Cox *et al*., 1990).

BIRD SAMPLING

There is a wealth of literature concerning different methods of recovering *Salmonella* from birds. To sample for the presence of *Salmonella* in individual birds, the cloaca of birds can be swabbed by inserting a sterile, cotton-tipped swab 1-2 inches into the cloaca (Ellis *et al*., 1976). After insertion into the cloaca the swab can be transferred directly to tubes containing approximately 10ml of an enrichment media. Fanelli and collogues (1971) studied the presence of *Salmonella* in the intestinal tract of chickens and found that 40% were positive by cloacal swab when compared to 47% by cecal tonsils, 58% by fecal contents and

85% by cecal contents. The fecal shedding of *Salmonella* has been found to be sporadic (Sadler *et al*., 1969). Cloacal swabbing has been found to be labor intensive, and lack sensitivity when compared to intestinal or environmental culture (Fanelli *et al*., 1971; Mutalib *et al*., 1992). However cloacal swabbing is a nondestructive way to determine *Salmonella* contamination on an individual bird basis.

Salmonella has been shown to invade internal organs, colonize in the intestinal tract and in eggs (Brownell *et al*., 1969; Fanelli *et al*., 1971; Gast, 1990 and 1993; Holt and Porter, 1992). *Salmonella* invasion and colonization of the intestinal tract seems to be the most prominent area for the isolation or recovery of the organism in comparison to other internal sites (Fanelli *et al*., 1971; Turnbull and Snoeyenbos, 1974; Brown *et al*., 1976). Humphrey and others (1989) found that translocation of *Salmonella* to the liver, spleen, ovary, and oviduct occurs but at a low incidence. Gast and Beard (1990) exposed laying hens to *Salmonella* by allowing contact with orally infected birds. In that study, 43% of ceca, 36% of spleens, 21% of oviducts, and 7% of ovaries sampled from the hens exposed to the infected birds were positive for *Salmonella*. These data suggest that *Salmonella* was predominately colonized in the intestines, but translocation of the *Salmonella* occurs in adjacent organs.

Sampling the internal organs, chick yolks and intestines are all methods of determining *Salmonella* infection in individual or a pooled samples of birds (Miura *et al*., 1964; Bailey *et al*., 1994; USDA, 1996). The National Poultry Improvement Plan (NPIP) adapted a protocol for the culturing of baby chicks where organs,

yolk, and intestines are pooled and sampled for *Salmonella* (1994). For investigation of internal organ contamination a portion of the heart, liver, lung and spleen can be aseptically collected (Gast and Beard, 1990a). For examination of eggs, the shell of the egg is disinfected and the contents pooled together (Gast, 1993). To determine *Salmonella* contamination of unabsorbed yolk in chicks, a portion of the unabsorbed yolk sac or yolk stalk is aseptically collected (NPIP, 1994). The method of sampling the intestine, involves removal of the cecal tonsils, duodenum, and ileocecal junction (NPIP, 1994).

Laboratory media and isolation techniques

ENRICHMENT MEDIA

Enrichment of samples for the isolation of *Salmonella* is commonly performed in laboratories for three reasons (North and Bartram, 1953; Dixon, 1961; D'Aoust, 1981; Tate *et al*., 1990; Waltman *et al*., 1993):

1) To increase the typically low levels of *Salmonella* that can be present in poultry samples to a detectable level.

2) To suppress the high numbers of other bacteria that are generally present in poultry samples.

3) To increase the detection of *Salmonella* by plating.

There are numerous enrichment methods that differ in incubation time, temperature and media for the isolation of Salmonella from poultry tissues and

their environments (Waltman and Mallinson, 1995). Three of the more common steps for enrichment of poultry samples that give the best recovery rates are:

i) preenrichment of the sample

ii) selective enrichment of the sample

iii) delayed secondary enrichment of the sample

Preenrichment of samples is mainly performed on isolates from foods, feed, and feed ingredients to recover damaged or stressed organisms (North, 1953; D'Aoust, 1981). Preenrichment samples are usually incubated overnight (16 hours) at 37C and then transported into selective enrichment. Tate and co-workers (1990) compared the isolation of *Salmonella* with and without preenrichment and found a 4% increase in isolation with the use of preenrichment prior to selective enrichment. A number of different broth media have been recommended for recovery of the organism (D'Aoust, 1981). The media of preference in the past was lactose broth (North, 1953), but due to the drop in pH that may occur with the use of lactose broth (Juvan *et al*., 1984), buffer peptone water and universal preenrichment broth have received considerable attention because of their buffering capacity (Fricker, 1987; Cox, 1988; Bailey and Cox, 1992).

Selective enrichment allows *Salmonella* to multiply while inhibiting the growth of other bacteria (Leifson, E., 1936; Galton *et al*., 1968). Researchers (Galton *et al*., 1968) found that recovery of *Salmonella* was difficult if the ratio of coliforms to *Salmonella* was high (10:1). Temperature and incubation times for selective enrichment samples have been studied and reports vary among

laboratories, but the most common range of incubation temperatures is 37C and 42C with an incubation time of 24 hours or 48 hours (Dixon, 1961; Harvey and Price, 1968; Carlson and Snoeyenbos, 1972; Harvey and Price, 1979; D'Aoust *et al*., 1992; Waltman and Mallinson, 1995). In a nation wide survey Waltman and Mallinson (1995), found that 50% of the laboratories in the United States plate their selective broths after 24 hours of incubation, however approximately 25% re-incubate after initial plating for another 24 hours and then plate an additional time.

In addition, Waltman and Mallinson (1995) found that 13 different selective enrichments were used to isolate *Salmonella* from poultry environments and 17 different selective enrichments were used for isolating from poultry tissue samples. In the survey, 69% were using some form of tetrathionate (TT) (Hagna and Damon, 1956) for enrichment of tissue samples and 79% were using tetrathionate (TT) for enrichment of environmental samples. The second most common enrichment media in that survey was selenite enrichment media first described in 1936 by Leifson and later modified by Stokes and Osborne (1955). Only one laboratory in the survey was using rappaport-vassiliadis enrichment media first mentioned in 1956 by Rappaport and others. However, many researches have found that tetrathionate media is better at isolating *Salmonella* from samples than selenite (Smyster *et al*., 1970; Dunn and Martin, 1971; Carlson and Snoeyenbos, 1974; D'Aoust *et al*., 1992).

Delayed secondary enrichments of samples have been shown by numerous researchers to increase the recovery rate of *Salmonella* after selective

enrichment of the samples (Pourciau and Springer, 1978; Rigby and Pettit, 1980; Waltman *et al*. 1991,1992, and 1993). Delayed secondary enrichment of a sample is the process of leaving the original sample of selective enrichment at room temperature if the sample is negative for 5-7 days and then transferring 1ml of the sample into a tube containing approximately 10ml of enrichment media (Pourciau and Springer, 1978). Pourciau and Springer (1978) found that delayed secondary enrichment increased recovery of *Salmonella* by 20% to 25% compared to selective enrichment for 24 hours. In three separate reports, Waltman and others (1991, 1992, and 1993) showed that selective enrichment alone incubated at 24 hours and 48 hours tended to recover the majority of the *Salmonella* present, however the use of delayed secondary enrichment will increase (18%) the recovery rate of *Salmonella* in samples.

PLATING MEDIA

Various plating media have been developed or modified over the years to enhance the selection and identification of enteric organisms (*Salmonella*) in order to inhibit organisms other than the one being selected for growth (MacConkey, 1905; Kristensen *et al*., 1925; Leifson, 1935; Taylor, 1965; Miller *et al*., 1991; Poisson, 1992; Mallinson *et al*., 2000). MacConkey agar is one of the few agars that has not seen much modification over the years and is still in use today as an all-purpose media for the selection of gram-negative enteric bacteria (MacConkey, 1905). However, Tate and others (1990) stated that more specific media for selectivity of *Salmonella* would allow better recovery of the organism.

The less selective the media the wider the variety of pathogens that grow on the media, and therefore the less likely to recover *Salmonella* from the media. Miller and others (1991) stated that most media are not formulated for the isolation of *Salmonella* alone and many are designed as a multipurpose media for public health interest.

Waltman and others (1995) along with Mallinson (1990) suggested that two separate formulated medias be used when plating for the isolation of *Salmonella*. They recommended the use of a modified brilliant green agar, brilliant green novobiocin (BGN) agar (Tate *et al*, 1990) and a modified xylose-lysine-desoxycholate agar (XLD), called xylose-lysine-tergitol (XLT4) agar (Miller *et al*., 1990, 1991, 1995) for recovery of *Salmonella* in poultry and their environments. The use of these two agars in combination resulted in a 95% recovery rate of *Salmonella*. Miller and others (1995) compared different recovery rates from media and found that XLT4 had the highest recovery rate at 98% and the second highest at 85% with BGN. Mallinson (1990) when comparing different selective media found a 98% recovery rate with XLT4 media and the second highest yielded a 91% recovery rate on BGN media.

Moats (1978) observed that the addition of novobiocin in XLD medium when isolating *Salmonella* dramatically reduced false positives from 24% to 3% on the media. Hoben *et al*. (1973), Restaino *et al*. (1977), and Komatsu and Restaino (1981) found similar results that the addition of novobiocin in media improved the recovery rate of *Salmonella* by inhibiting surplus bacteria. Tate and others (1990) added novobiocin to BG agar to increase the selectivity for

Salmonella. In this study, *Proteus* was dramatically reduced with the addition of novobiocin, and the percentage of *Salmonella* isolates was significantly increased. Environmental samples were particularly effected by the addition of novobiocin in the media with isolations of *Salmonella* from drag-swabs increasing 37% and a 44% increase in isolates from collected litter samples.

Miller and others (1991) stated that although numerous modifications to media have been made, very few changes in the selective inhibitors have been made over the years. They report that the most common inhibitors used alone or in combination are bile salts, sulfonamides, and brilliant green. Miller and others (1991) investigated alternative selective inhibitors and added tergitol 4 in combination with xylose-lysine and found this formula of media to strongly or completely inhibit the majority of non-*Salmonella* bacteria and allowed the growth of *Salmonella*. From these results, XLT4 allowed easy differentiation of *Salmonella* from other bacteria and was a highly selective plating media that could reduce false-negatives. Miller and others (1995) slightly modified the agar in order to promote H_2S production in the media from the *Salmonella* for better identification.

SALMONELLA IDENTIFICATION

Identification of *Salmonella* to the species level needs to be performed through a series of steps, which are time consuming but critical in determining the correct *Salmonella* group and type. Suspect colonies from the media plates are usually picked and transferred to triple sugar iron (TSI) or lysine iron agar

(LIA) slants (Cox and Williams, 1976; Waltman and Mallinson, 1995). Phenol red is the pH indicator in TSI slants and the media changes color based on the organism's ability to ferment the different sugars in the formula, and identification is made based on color, gas production, and H_2S presence (Cox and Williams, 1976; Ewing, 1986). LIA involves the decarboxylization of lysine and most *Salmonella* produce lysine decarboxylase causing an alkaline production in the media and identification is made on color and H_2S production (Cox and Williams, 1976; Ewing, 1986). The suspect *Salmonella* can be further tested by serogrouping or by serotyping to determine exact species of the isolate (Ellis *et al*., 1976; Ewing, 1986) or in recent years by polymerase chain reaction (PCR), which allows identification on a molecular level (Liu *et al*., 2002).

Salmonella transmission

Numerous routes of *Salmonella* contamination have been investigated in avian species, and the two most prominent routes of exposure are horizontal and vertical transmission. Transmission of *Salmonella* to birds can occur via horizontal vectors such as rodents (Henzler and Opitz, 1992; Baskerville *et al*., 1992), other birds (Holt, 1995) or by airborne particulates (Nakamura *et al*., 1994; Holt *et al*., 1998). Vertical transmission of *Salmonella* from the parent to the progeny has been shown to occur through ovarian infection (Snoeyenbos *et al*., 1969; Gast and Beard, 1989). The two vectors of interest to be discussed here are:

1) Horizontal transmission through the air.
2) Vertical transmission from the parent to the offspring.

HORIZONTAL TRANSMISSION

Baskerville and others (1992) reported that experimentally administering *Salmonella* at 10^2-10^5 cells to hens by aerosol route, resulted in the dissemination of *Salmonella* in the feces for 28 days and at necropsy was found in the lungs, crops, ovaries, oviducts, spleens, kidneys, and eggshells. Clemmer *et al*., (1960) found similar results that low levels of *Salmonella* could be administered via aerosols. Some researchers found control birds positive for *Salmonella* and suggested that aerosol contamination caused the infection (Snoeyenbos *et al*., 1969). Lever and Williams (1996) reported possible airborne

infection of *Salmonella* occurred in day old chicks that occupied the same environment but did not have direct contact with infected chicks.

In two studies, Holt and Porter (1992) and Holt (1995) looked at the transmission of *Salmonella* in molted versus un-molted hens that were reared in adjacent cages and found increased shedding of the *Salmonella* from the molted birds indicating that it took fewer birds to infect uninfected hens with *Salmonella* during a molt. They reported that the primary method of cross-contamination was from direct bird-to-bird contact. Nakamura and others (1997) followed up on Holts work but examined the effect of the flow of air on the transmission of *Salmonella*. They suggested that infection could be possible from the organisms adhering to airborne particles and the spread of infection in a house could be affected by the airflow rates inside the house.

In a follow up study, Holt and others (1998) looked at the airborne transmission of *Salmonella* in three different experiments and found that the birds going through a molt were contaminated from infected birds 1 meter away, and stated that in his previous work that airborne transmission could not be ruled out as a contributing factor. In addition, these researchers noted that 3 days post-challenge 100% of air samples were positive for *Salmonella* in one experiment and peaked at 70% in another experiment. They concluded as previously reported that the hens infected during the molt, shed progressively higher numbers of the organism and were more susceptible to infection. Holt and colleagues (1998) reported that the density of *Salmonella* in the air was relatively low when examining the number of colony forming units per air sample and

therefore, *Salmonella* could be difficult to detect by air sampling if the shed rate from the birds are in low numbers. Gast and others (1998) examined the airborne transmission of *Salmonella* in environmentally controlled isolation cabinets and found that downstream chicks became positive for *Salmonella* when exposed to infected chicks which were upstream of the flow of air and concluded that reducing the airborne movement of *Salmonella* in poultry houses could help limit the spread of infection within a flock.

VERTICAL TRANSMISSION

Salmonella has been recovered in egg contents laid by commercial broiler breeders and in table egg producing birds (Lister, 1988; Wilding and Baxter-Jones, 1985). The method and prevalence of *Salmonella* being transmitted from the mature bird to the egg contents and then subsequently to the progeny has been studied for many years (Solowey *et al*., 1946; Stokes *et al*., 1956; Olesiuk *et al*., 1969; Lahellac and Colin, 1985). Gordon and Tucker (1965), in hens experimentally infected with *Salmonella*, it was found that the organism was transmitted to the offspring.

Snoeyenbos and others (1969) found many different types of *Salmonella* in the ovaries of naturally infected chickens and suggested that egg transmission could be a factor in salmonellosis. It has been shown that infected hens can deposit *Salmonella* in the yolk or albumen of developing eggs (Humphrey *et al*., 1991). Very little multiplication of the bacteria occurs in egg albumin (Lock and Board, 1992). Gast and Beard (1990b) administered a large oral dose of

Salmonella to hens and recovered the organism in 19% of the albumens and 16% of the yolks from eggs over a 4-week period after inoculation. The majority of the multiplication of the bacteria seems to occur in the egg yolk (Lock and Board, 1992).

O' Brien (1988) also showed that transovarial infection could occur from the parent to the progeny. The incidence of transmission of *Salmonella* from the hen to the progeny is low. Olesiuk and others (1969) experimentally infected hens and studied the dissemination of *Salmonella* and recovered the marker organism from 3 of 5,527 eggs. In a more extensive experiment, *Salmonella* was recovered from 1 of 10,000 hatching eggs over a several year period (Wilding and Baxter-Jones, 1985). Egg contaminations with *Salmonella enteritidis* have also been estimated to be as low as 0.005% (Ebel and Schlosser, 2000). Henzler and others (1998) found in their study that the estimated overall incidence of *Salmonella enteritidis* contaminated eggs is about 2.64 per 10,000 eggs; however, flock-specific egg contamination rates ranged from 0 to 62.5 per 10,000 eggs.

Advancing technology: Negative Ionization of air

AIRBORNE PARTICULATES

The level of airborne particulates in animal confinement rearing facilities has been studied significantly over the last 40 years mainly in swine and poultry facilities (Jacobson and Jordan, 1978; Madelin and Wales, 1989; Hilko *et al*.,

2000). The high levels of airborne particulates in these environments and the time exposed to these conditions have been shown to increase respiratory diseases in humans working in these environments and animals raised in these environments. Iversen and others (2000) evaluated human health effects of dust exposure and showed that there is a dose-response associated with working in animal confinement facilities. Anderson and others (1964) measured particle concentrations in turkey confinement facilities and discovered viable viral and bacterial concentrations as high as 2.4×10^5 organisms per cubic meter of such diseases as Newcastle disease, which could result in airsacculitis in poultry. Hayter and Besch (1974) examined airborne particle deposition in the respiratory tract of chickens and found that larger particles (3.7 to 7 microns) deposited primarily in the anterior portion of the respiratory tract and that smaller particles deposit further down the respiratory tract. They stated that "acceptable environmental conditions in animal rooms cannot be maintained without controlling airborne particles".

In a recent study, Hilko and others (2000) evaluated dust levels inside poultry houses and suggested possible methods of control. Dust concentrations were found to vary inside poultry houses from 0.02 to 81.33 mg/m^3 for inhalable dust concentrations. However, respirable aerosol concentrations of dust inside the facilities were lower, ranging from 0.01 to 6.5 mg/m^3. These researchers indicated that by maintaining about 75% relative humidity inside poultry facilities by fogging with water, inhalable dust concentrations could be reduced by up to 65%; however, these measures had no effect on respirable aerosol

concentrations. Van Wicklen and Czarick (1997) monitored respirable aerosal concentrations inside a poultry house and found that the highest concentrations of respirable aerosols were found where air exited the facility via tunnel ventilation fans. The levels were found to decrease as distance from the ventilation fans increased.

NEGATIVE IONIZATION

The concept of utilizing ionization as a means to reduce or eliminate airborne particulates or microbial levels has been studied for many years (Krueger *et al*., 1957; Phillips *et al*., 1964; Krueger and Reed, 1976; Estola *et al*., 1979; Mitchell and King, 1994). The majority of bacteria (83% to 94%) in poultry houses are found on particles greater than 6 microns (Hugh-Jones *et al*., 1973). Negative ionization of air has the potential to reduce the concentrations of airborne microorganisms by reducing the amount of airborne particulates. Dust acts as a major carrier of airborne bacteria and a reduction of dust by 50% can decrease bacteria up to 100-fold (Carpenter *et al*., 1986; Madelin and Wales, 1989). The ionized aerosol particles have a tendency to move towards the opposite charge and consequently, in a closed space like a confinement rearing facility, may be cleared from the air by adhesion to the walls or other charged surfaces via negatively charging these airborne particulates thus causing them to be attracted to oppositely charged surfaces (Mitchell, 1997). Airflow and the amount of power supplied to an ion unit effects how the ions are distributed

throughout a space and if you increase either the airflow or power, the result will be an increased area of charge by an ion distributor (Mitchell, 1997).

EFFECT ON BACTERIA

It has been suggested the growth of colonies of some microorganisms is altered and the decay of aerosol is faster with the use of ionization (Krueger and Reed, 1976). Phillips and colleagues (1964) noticed in their studies that negative ionization of the air leads to faster decay of the microbial aerosols than positive ionization. Seo and others (2001) with the use of negative ionization, found that *Salmonella enteritidis* was reduced from 72% to 98% in an artificially generated aerosol cabinet and that negative ions had a bactericidal effect on the bacteria; however, the mechanism of action could not be determined.

Robinzon and others (1983) compared the effects of negative and positive air ions on the chicken tracheal surface morphology and found that positive ions increased mucoidal secretions. However, no mucoidal secretions were observed with exposure to negative ions. The exposure to either charge reduced the incidence of bacterial foci in the trachea of the chicks to almost zero and was thought to have occurred due to the reductions in airborne dust and bacteria.
Estola and colleagues (1979) in an ionization experiment with animal respiratory disease suggested that the contamination of the air by droplets that carry bacteria may be prevented by ionization of the air. Gabbay (1990) reported that reductions of microbial pollutants in the air were up to as much as 52% in a dental clinic with the use of ionization.

They also discovered that horizontally positioned plates had more colony forming units than vertically positioned plates and concluded that the ionized particles were attracted to oppositely charged ground. Bacterial aerosols were reduced with the use of ionization in a burn and plastic surgery unit (Makala *et al*., 1979). Variations in bacterial levels were noticed during sampling periods and associated with either nurses or other activity inside the patient rooms. The use of negative air ionization resulted in a reduction from 27.7% to 6.6% in the transmission of Newcastle disease from donor to susceptible chicks in an airborne disease transmission cabinet (Mitchell and King, 1994).

EFFECT IN HATCHING CABINETS

The negative ionization of hatching cabinets have shown a reduction of airborne dust by an average of 79% on day 18 of incubation, increasing to 96% by day 21 of incubation (Mitchell and Stone, 1996). An average reduction of 92.9% for particles up to 10 microns and for particles greater than 10 microns, a 90.8% reduction was seen with the use of a room ionizer system in a hatching cabinet (Mitchell, 1998). Further reductions were noted (98.8% and 99.7%) when the blower of the hatching cabinet was turned off with the room ionizer system lowering the re-entrainment of particulates. The negative ionization of airborne particulates in an experimental hatching cabinet showed similar results with a reduction in airborne dust of 93.6% and a reduction in total and gram-negative bacteria from a range of 85% to 93% (Mitchell *et al*., 2002). In that trial

researchers found that *Salmonella* per gram of cecal contents was significantly reduced by an average, log 10 reduction of 3.4 cfu/g.

EFFECT IN CONFINEMENT REARING FACILITIES

Czarick and colleagues (1985) have shown a 31% reduction in airborne dust with the use of negative ionization in a swine facility. Other researchers reported a 67% reduction in airborne dust in a swine facility, with the use of an electrostatic precipitation dust removal system (Veenhuizen and Bundy, 1990). Holt and others (1999) found that the use of a negative ionization system was responsible for a reduction of *Salmonella enteritidis* in a poultry caged layer facility from 96.5% to 82% over the sampling period. Mitchell and colleagues (2000b) found that the use of negative ionization resulted in a reduction in airborne dust concentrations in a poultry caged layer facility. The ionizer treatment was responsible for a 72% to 91% reduction in artificially generated dust inside the facility and a 52% reduction in natural dust.

REFERENCES

AAAP Disease report summary, 1975, 1976. Avian Dis. 20: 777-779.

Anderson, D. P., F. L. Cherms, and R. P. Hanson, 1964. Studies on measuring environment of turkeys in confinement. Poultry Sci. 42: 305-318.

Allred, J. N., J. W. Walker, V. C. Beal Jr., and F. W. Germaine, 1967. A survey to determine the *Salmonella* contamination rate in livestock and poultry feeds. Am. J. Vet. Med. Assoc. 151: 1857-1860.

Bailey, J. S., N. J. Stern, P. Fedorka-Cray, S. E. Craven, N. A. Cox, D. E. Cosby, S. Ladely, and M. T. Musgrove, 2001. Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. J. Food Protection 64 (11): 1690-1697.

Bailey, J. S., N. A. Cox, and M. E. Berrang, 1994. Hatchery-acquired salmonellae in broiler chicks. Poultry Sci. 3: 1153-1157.

Bailey, J. S. and N. A. Cox, 1992. Universal Preenrichment Broth for the Simultaneous detection of *Salmonella* and Listeria in foods. J. Food Prot. 55: 256-259.

Bailey, J. S., J. Y. Chiu, N. A. Cox, and R. W. Johnston, 1988. Improved selective procedure for detection of salmonellae from poultry and sausage products. J. Food Prot. 51(5): 391-396.

Bailey, J. S., 1987. Factors affecting microbial competitive exclusion in poultry. Food Tech. 41: 88-92.

Baskerville, A., T. J. Humphrey, R. B. Fitzgeorge, R. W. Cook, H. Chart, B. Rowe, and A. Whitehead, 1992. Airborne infection of laying hens with *Salmonella enteritidis* phage type 4. Vet. Rec. 130: 395-398.

Baumler A. J., B. M. Hargis, and R. M. Tsolis, 2000. Tracing the origins of *Salmonella* outbreaks. Science 287: 50-52.

Bean, N. H. and P. M. Griffin, 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles, and trends. J. Food Prot. 53: 804-817.

Bhatia, T. R. S., G. D. McNabb, H. Wyman, and G. P. S. Nayar, 1979. *Salmonella* isolation from litter as an indicator of flock infection and carcass contamination. Avian Dis. 24: 838-847.

Board, R. G., 1966. The course of microbial infection of the hen's eggs. J. Appl. Bacteriol. 29: 319-341.

Borland, E. D., 1975. *Salmonella* infection in poultry. Vet. Rec. 97: 406-408.

Boyer, C. I., D. W. Bruner, and J. A. Brown, 1958. *Salmonella* organisms isolated from poultry feed. Avian Dis. 4: 396-401.

Brown, D. D., J. G. Ross, and A. F. G. Smith, 1976. Experimental infection of poultry with *Salmonella infantis*. Res. Vet. Sci. 20: 237-243.

Brownell, J. R., W. W. Sadler, and M. J. Fanelli, 1969. Factors influencing the intestinal infection of chickens with *Salmonella typhimurium*. Avian Dis. 13: 804-816.

Bryan, F. L., 1980. Foodborne diseases in the United States associated with meat and poultry. J. Food Prot. 43: 140-150.

Bullis, K. L., 1977a. The history of avian medicine in the US. II. Pullorum disease and fowl typhoid. Avian Dis. 21: 422-429.

Bullis, K. L., 1977b. The history of avian medicine in the US. III. Salmonellas. Avian Dis. 21: 430-435.

Byrd, J. E., D. E. Corrier, J. R. DeLoach, D. J. Nisbet, and L. H. Stanker, 1998. Horizontal transmission of *Salmonella typhimurium* in broiler chicks. J. Appl. Poultry Res. 7: 75-80.

Byrd, J. E., D. E. Corrier, J. R. DeLoach, and D. J. Nisbet, 1997. Comparison of Drag-Swab environmental protocols for the isolation of *Salmonella* in poultry houses. Avian Dis. 41: 709-713.

Caldwell, D. J., B. M. Hargis, D. E. Corrier, J. D. Williams, L. Vidal, and J. R. DeLoach, 1994. Predictive value of multiple drag-swab sampling for the detection of *Salmonella* from occupied or vacant poultry houses. Avian Dis. 38: 461-466.

Carlson, V. L., and G. H. Snoeyenbos, 1974. Comparative efficacies of selenite and tetrathionate enrichment broths for the isolation of *Salmonella* serotypes. Am. J. Vet. Res. 35: 711-719.

Carlson, V. L., and G. H. Snoeyenbos, 1972. Relationship of population kinetics of *Salmonella typhimurium* and cultural methodology. Am. J. Vet. Res. 33: 177-184.

Carpenter G. A., W. K. Smith, A. P. C. Macarena, and D. Spackman, 1986. Effect of internal air filtration on the performance of broilers and the aerial concentrations of dust and bacteria. Br. Poultry Sci. 27: 471-480.

Cason, J. A., N. A. Cox, and J. S. Bailey, 1994. Transmission of *Salmonella typhimurium* during hatching of broiler chicks. Avian Dis. 38: 583-588.

Centers for Disease Control, 1996. Outbreaks of *Salmonella* serotype *Enteritidis* infection associated with consumption of raw shell egg: United States, 1994-1995. MMWR 45: 737-742.

Centers for Disease Control, 1987. *Salmonella* Surveillance Report-1985. Annual Summary, Centers for Disease Control, U. S. Department of Health and Human Services, Washington, DC.

Centers for Disease Control, 1983. *Salmonella* surveillance, Annual summary, 1981. U. S. Department of Health, Education, and Welfare, Centers for Disease Control, Atlanta, GA.

Chase, F. E., 1947. *Salmonella* studies in fowl. Can. J. Public Health 38: 82-83.

Clarke, R. C., and C. L. Gyles, 1993. *Salmonella*. In: Gyles, C. L., and C. O., Theons, Eds. Pathogenesis of bacterial infections in animals. Ames: Iowa State University Press, Pages 133-153.

Clemmer, D. J., J. L. S. Hickey, J. F. Bridges, D. J. Schliessmann, and M. F. Shaffer, 1960. Bacteriological studies of experimental airborne salmonellosis in chicks. J. Infect. Dis. 106: 197-210.

Cohen, M. L. and R. V. Tauxe, 1986. Drug-resistant *Salmonella* in the United States: an epidemiological perspective. Science 234: 964-969.

Cox, N. A., J. S. Bailey, L. C. Blankenship, and R. P. Gildersleeve, 1992. Research note: In ovo administration of a competitive exclusion culture treatment to broiler embryos. Poultry Sci. 71: 1781-1784.

Cox, N. A., J. S. Bailey, J. M. Mauldin, and L. C. Blankenship, 1990. Presence and impact of *Salmonella* contamination in commercial broiler hatcheries. Poultry Sci. 69: 1606-1609.

Cox, N. A., J. S. Bailey and J. E. Thompson, 1983. *Salmonella* and other *Enterobacteriaceae* found in commercial poultry feed. Poultry Sci. 62: 2169-2175.

Cox, N. A., and J. E. Williams, 1976. A simplified biochemical system to screen *Salmonella* isolates from poultry for serotyping. Poultry Sci. 55: 1968-1971.

Cunningham, D. L., 1993. The poultry industry in the United States and Georgia: A situation and outlook statement. Department of Poultry Science, The University of Georgia.

Czarick, M. I., G. L. Van Wicklen, and R. A. Clemmer, 1985. Negative ionization for swine during weaning. ASAE Annual Meeting, Paper # 85-4510.

D'Aoust, J. Y., 1981. Update on preenrichment and selective enrichment conditions for detection of *Salmonella* in foods. J. Food Prot. 44: 369-374.

D'Aoust, J. Y., A. M. Sewell, and D. W. Warburton, 1992. A comparison of standard cultural methods for the detection of foodborne *Salmonella*. J. Int. Food Microbiol. 16: 41-50.

Davidson, S., C. E. Benson, and R. J. Eckroade, 1995. Comparison of environmental monitoring protocols for the detection of *Salmonella* in poultry houses. Avian Dis. 39: 475-479.

Dixon, J. M. S., 1961. Rapid isolation of *Salmonella* from feaces. J. Clin. Pathol. 14: 397-399.

Dunn, C., and W. J. Martin, 1971. Comparison of media for isolation of salmonellae and shigellae from fecal specimens. Appl. Microbiol. 22: 17-22.

Ebel, E. and W. Schlosser, 2000. Estimating the annual fraction of eggs contaminated with *Salmonella enteritidis* in the United States. Int. J. Food Microbiol. 61: 51-62.

Ebel, E. D., M. J. David, and J. Mason, 1992. Occurrence of *Salmonella enteritidis* in the U. S. commercial egg industry: Report on a national spent hen survey. Avian Dis. 36: 646-654.

Edwards, P. R., 1956. *Salmonella* and salmonellosis. Ann. N. Y. Acad. Sci. 66: 44-53.

Edwards, P. R., D. W. Bruner, and A. B. Moran, 1948a. Further studies on the occurrence and distribution of *Salmonella* types in the United States. J. Infec. Dis. 83: 220-231.

Edwards, P. R., D. W. Bruner, and A. B. Moran, 1948b. *Salmonella* infections in fowls. Cornell Vet. 38: 247-256.

Ellis, E. M., J. E. Williams, E. T. Mallinson, G. H. Snoeyenbos, and W. J. Martin, 1976. Culture methods for the detection of animal salmonellosis and arizonosis. A manual of the American Association of Veterinary Laboratory Diagnosticians. Iowa State University Press, Ames, IA. p. 87.

Erwin, L. E., 1955. Examination of prepared poultry feeds for the presence of *Salmonella* and other enteric organisms. Poultry Sci. 34: 215-216.

Estola, T., P. Makela, and T. Hovi, 1979. The effect of air ionization on the air-borne transmission of experimental Newcastle disease virus infections in chickens. J. Hyg. 83: 59.

Ewing, W. H., 1986. Edwards and Ewing's Identification of Enterobacteriaceae, 4th ed. Elsevier, New York, NY.

Faddoul, G. P., and G. W. Fellows, 1966. A five-year study of the incidence of salmonellae in avian species. Avian Dis. 10: 296-304.

Fanelli, M. J., W. W. Sadler, C. E. Franti, and J. R. Brownell, 1971. Localization of *Salmonella* within the intestinal tract of chickens. Avian Dis. 15: 366-375.

Federal Register, 1996. Pathogen Reduction; Hazard analyses and Critical Control Point (HAACP) System, Final Rule. USDA-FSIS ACFR part 304, pp. 38806-38989.

Fricker, C. R., 1987. The isolation of salmonellas and campylobacters: A review. J. Appl. Bacteriol. 63: 99-116.

Gabbay, J., 1990. Effect of ionization on microbial air pollution in the dental clinic. Environ. Res. 52(1): 99.

Galton, M. M., G. K. Morris, and W. T. Martin, 1968. Salmonellae in foods and feeds: review of isolation methods and recommended procedures. Centers for disease Control; United States department of Health, Education, and Welfare/Public Health Service.

Gast, R. K., B. W. Mitchell, and P. S. Holt, 1999. Application of negative air ionization for reducing experimental airborne transmission of *Salmonella enteritidis* to chicks. Poultry Sci. 78: 57-61.

Gast, R. K., B. W. Mitchell, and P. S. Holt, 1998. Airborne transmission of *Salmonella enteritidis* infection between groups of chicks in controlled-environmental isolation cabinets. Avian Dis. 42: 315-320.

Gast, R. K., 1993. Detection of *Salmonella enteritidis* in experimentally infected laying hens by culturing pools of egg contents. Poultry Sci. 72: 267-274.

Gast, R. K., 1990. *Salmonella*. In: Gyles, C. L., and C. O., Theons, Eds. Pathogenesis of bacterial infections in animals. Ames: Iowa State University Press, pp. 133-153.

Gast, R. K. and C. W. Beard, 1990a. Isolation of *Salmonella enteritidis* from internal organs of experimentally infected hens. Avian Dis. 34: 991-993.

Gast, R. K. and C. W. Beard, 1990b. Production of *Salmonella enteritidis*-contaminated eggs by experimentally infected hens. Avian Dis. 34: 438-446.

Gast, R. K. and C. W. Beard, 1989. Production of *Salmonella enteritidis* contaminated eggs by experimentally infected hens. Avian Dis. 34: 438-446.

Gay J. M., M. E. Hunsaker, 1993. Isolation of multiple *Salmonella* serovars from dairy two years after a clinical salmonellosis outbreak. Am. J. Vet. Med. Assoc. 203: 1314-1320.

Georgia Department of Agriculture and United States Department of Agriculture, 2002. Georgia Agricultural Statistics Service; Georgia Poultry Facts, (June) pp. 19, 29.

Gomez, T. M., Y. Molerjemi, S. Miyagawa, F. K. Kaferstein, and K. Stohr, 1997. Foodborne salmonellosis. World Health Stat. Q. 50: 81-89.

Gordon, R. F., and J. F. Tucker, 1965. The epizootiology of *Salmonella menston* infection of fowls and the effect of feeding poultry food artificially infected with *Salmonella*. Br. Poultry Sci. 6: 251-264.

Goyal, S. M., and I. P. Singh, 1970. Probable sources of salmonellae on a poultry farm. J. Br. Vet. 126: 180-184.

Hacking, W. C., W. R. Mitchell, and H. C. Carlson, 1978. Salmonella investigation in an Ontario feed mill, Can. J. Comp. Med. 42:400-406.

Hagna, A. A., and S. R. Damon, 1956. New enrichment and plating media for the isolation of *Salmonella* and *Shigella* organisms. J. Appl. Microbiol. 4: 341-345.

Hayes, J. R., L. E. Carr, E. T. Mallinson, L. W. Douglass, and S. W. Joseph, 2000. Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella* spp. in commercial poultry houses. Poultry Sci. 79: 1557-1561.

Harvey, R. W. S., and T. H. Price, 1968. Elevated temperature of incubation of enrichment media for the isolation of salmonellas from heavily contaminated materials. J. Hyg. Camb. 66: 377-381.

Harvey, R. W. S., and T. H. Price, 1979. A review. Principles of *Salmonella* isolation. J. Appl. Bacteriol. 46: 27-56.

Hayter, R. B., and E. L. Besch, 1974. Airborne-particle deposition in the respiratory tract of chickens. Poultry Sci. 53: 1507-1511.

Henzler, D. J., C. K. David, W. M. Sischo, 1998. Management and environmental risk factors for *Salmonella enteritidis* contamination of eggs. Am. J. Vet. Res. 59: (7) 824-829.

Henzler, D. J., and H. M. Opitz, 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. Avian Dis. 36: 625-631.

Hewitt, E. A., 1928. Bacillary white diarrhea in baby turkeys. Cornell Vet. 18: 272-276.

Hilko, H. E., R. W. Boucher, E. von Wachenfelt, and H. Takai, 2000. Dust levels and control methods in poultry houses. J. Agr. Safety and Health 6: 275-282.

Hinshaw, W. R., E. MacNeil, and T. J. Taylor, 1944a. Avian salmonellosis. Types of *Salmonella* isolated and their relationship to public health. J. Am. Hyg. 40: 264-278.

Hinshaw, W. R., and E. McNeil, 1944b. Gopher snakes as carriers of salmonellosis and paracolon infections. Cornell Vet. 34: 248-254.

Hinshaw, W. R., H. M. Scott, and L. F. Payne, 1928. Further studies on dissemination of *Salmonella pullorum* infection in incubators. Am. J. Vet. Med. Assoc. 72: 599-610.

Hinshaw, W. R., C. W. Upp, and J. M. Moore, 1926. Studies in transmission of bacillary white diarrhea in incubators. Am. J. Vet. Med. Assoc. 68: 631-641.

Hinz, T., J. Hartung, and B. Wiegand, 1994. Air quality in a Louisiana-type broiler house. Report No. 94-C-08, AgEng Milano 94, Milano, Italy.

Hoben, D. A., D. H. Ashton, and A. C. Peterson, 1973. Some observations on the incorporation of novobiocin into Hektoen enteric agar for improved *Salmonella* isolation. Appl. Microbiol. 26: 126-127.

Holt, P. S., B. W. Mitchell, K. Seo, and R. K. Gast, 1999. Use of negative air ionization for reducing airborne levels of *Salmonella enterica* serovar *enteritidis* in a room containing infected caged layers. J. Appl. Poultry Res. 8: 440-446.

Holt, P. S., M. W. Bailey, and R. K. Gast, 1998. Airborne horizontal transmission of *Salmonella enteritidis* in molted laying chickens. Avian Dis. 42: 45-52.

Holt, P. S., 1995. Horizontal transmission of *Salmonella enteritidis* in molted and unmolted laying chickens. Avian Dis. 39: 239-249.

Holt, P. S., and R. E. Porter, 1992. Use of pilocarpine-induced alimentary secretions to measure intestinal shedding of *Salmonella enteritidis* in chickens. Avian Dis. 36: 54-58.

Hopper, S. A., and S. Mawer, 1988. *Salmonella enteritidis* in a commercial layer flock. Vet. Rec. 123: 351.

Hugh-Jones, M., W. H. Allan, F. A. Dark, and G. J. Harper, 1973. The evidence for the airborne spread of Newcastle disease. J. Hyg. Camb. 71: 325-339.

Humphrey, T. J., A. Whitehead, A. H. L. Gawler, A. Henley, and B. Rowe, 1991. Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. Epidemiol. Infect. 106: 489-496.

Humphrey, T. J., G. C. Mead, and B. Rowe, 1988. Poultry meat as a source of human salmonellosis in England and Wales. Epidemiol. Infect. 100: 175-184.

Iversen, M S, Kirychuk, H. Drost, and L. Jacobson, 2000. Human health effects of dust exposure in animal confinement buildings. J. Agr. Safety and Health 6: 283-288.

Jacobson, L. D., and K. A. Jordan, 1978. Aerosol concentrations in a turkey barn environment. Transactions of the ASAE 21(2): 325-328.

Johnson, D. C., M. David, and S. Goldsmith, 1992. Epizootiological investigations of an outbreak of pullorum disease in an integrated broiler operation. Avian Dis. 36: 770-775.

Jones F. S., 1913. The value of the macroscopic agglutination test in detecting fowls that are harboring Bact. pullorum. J. Med. Res. 27: 481-495.

Juvan, B. J., N. A. Cox, J. S. Bailey, J. E. Thompson, O. W. Charles, and J. V. Shutze, 1984. Recovery of *Salmonella* from artificially contaminated poultry feeds in non-selective and selective broth. J. Food Prot. 47: 299-302.

Kingston, D. J., 1981. A comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chicken flocks. Avian Dis. 25: 513-516.

Klein, E., 1889. Ueber eine epidemische Krankheit der Huhner, verursacht durch einen Bacillus: Bacillus Gallinarum. Zentralbl, Bakteriol. Parasitenkd. 5: 689-693.

Krieg, N. R., and J. G. Holt, 1984. Bergey's manual of systemic bacteriology, V1, Williams and Wilkins, Baltimore, MD.

Kristensen, M., V. Lester, and A. Jurgens, 1925. Use of typsinized casein, brom-thymol blue, brom-cresol-purple, phenol-red and brilliant green for bacteriological nutrient media. J. Br. Exp. Pathol. 6: 291-299.

Krueger, A. P., and E. J. Reed, 1976. Biological impact of small air ions. Science 193: 1209-1213.

Krueger, A. P., R. F. Smith, and I. G. Go, 1957. Action of air ions on bacteria. I. Protective and lethal effects on suspension of staphylococci in droplets. J. Gen. Physiol. 41: 359-381.

Komatsu, K. K., and L. Restaino, 1981. Determination of the effectiveness of novobiocin added to two agar plating media for the isolation of *Salmonella* from fresh meat products. J. Food Safety 3: 183-192.

Lahellec, C., and P. Colin, 1985. Relationship between serotypes of salmonellae from hatcheries and rearing farms and those from processed poultry carcasses. Br. Poultry Sci. 26: 179-186.

Leifson, E., 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. 40: 581-599.

Leifson, E., 1936. New selenite enrichment media for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. J. Am. Hyg. 24: 423-432.

Le Minor, L., and M. Y. Popoff, 1992. Antigenic formulas of the *Salmonella* serovars, 6th ed. Paris: World Health Organization Collaborating Center for Reference and Research on *Salmonella*. pp. 1-145.

Lever, M. S., and A. Williams, 1996. Cross-infection of chicks by airborne transmission of *Salmonella enteritidis* PT4. Lett. Appl. Microbiol. 23: 347-349.

Levine, W. C., J. F. Smart, D. L. Archer, N. H. Bean, and R. V. Tauxe, 1991. Foodborne disease outbreaks in nursing homes, 1975 through 1987. Am. J. Med. Assoc. 266: 2105-2109.

Liu, T., K. Liljebjelke, E. Bartlett, C. Hofacre, S. Sanchez, and J. J. Maurer, 2002. Application of nested polymerase chain reaction to detection of *Salmonella* in poultry environment. J Food Prot. 65: (In Press)

Lister, S. A., 1988. *Salmonella enteritidis* infection in broilers and broiler breeders. Vet. Rec. 123: 350.

Lock, L. J., and R. G. Board, 1992. Persistance of contamination of hens' egg albumen in vitro with *Salmonella* serotypes. Epidemiol. Infect. 108: 389-396.

MacConkey, A., 1905. Lactose fermenting bacteria in feces. J. Hyg. 5: 333-379.

MacKenzie, M. A., and B. S. Bains, 1976. Dissemination of *Salmonella* serotypes from raw feed ingredients to chicken carcasses. Poultry Sci. 55: 957-960.

Madelin, T. M. and C. M. Wales, 1989. Air hygiene in a broiler house: Comparison of deep litter with raised netting floors. Br. Poultry Sci. 30: 23-37.

Makela, P., J. Ojajarvi, M. Lehtimaki, and G. Graeffe, 1979. studies on the effects of ionization on bacterial aerosols in a burns and plastic surgery unit. J. Hyg. 83: 199-206.

Mallinson, E. T., S. W. Joseph, C. L. E. Derezende, N. L. Tablante, and L. E. Carr, 2001. *Salmonella* control and quality assurance at the farm end of the food safety continuum. Am. J. Vet. Med. 218: 1919-1922.

Mallinson, E. T., R. G. Miller, C. E. de Rezende, K. E. Ferris, J. de Graft-Hanson, and S. W. Joseph, 2000. Improved plating media for the detection of *Salmonella* species with typical and atypical hydrogen sulfide production. J. Vet. Diagn. Invest. 12: 83-87.

Mallinson, E. T., 1990. *Salmonella* monitoring system simplifies evaluation of farms. Poultry Dig. (Sept) pp. 46-47.

Mallinson, E. T., C. R. Tate, R. G. Miller, 1989. Monitoring poultry farms for *Salmonella* by drag swab sampling and antigen-capture immunoassay. Avian Dis. 33: 684-690.

McCoy, J. H., 1975. Trends in *Salmonella* food poisoning in England and Wales 1941-1972. J. Hyg. Camb. 74: 271-282.

McCoy, J. H., 1962. The isolation of *Salmonella*. J. Appl. Bact. 25: 213-224.

McDermontt, L. A., 1947. The K formula stained antigen in the detection of standard and Younie types *of Salmonella pullorum* infection. Can. J. Public Health 38: 83-84.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffen, and R. V. Tauxe, 1999. Food-Related illness and death in the United States. J. Emerg. Inf. Dis. 5(5): 607-625.

Miura, S., G. Sato, and T. Miyamae, 1964. Occurrence and survival of *Salmonella* organisms in hatcher chick fluff from commercial hatcheries. Avian Dis. 8: 546-554.

Miller, R. G., C. R. Tate, and E. T. Mallinson, 1995. Improved XLT4 agar: small addition of peptone to promote stronger production of hydrogen sulfide by salmonellae. J. Food Prot. 58: 115-119.

Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Scherrer, 1991. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Sci. 70: 2429-2432.

Miller, R. G., and C. R. Tate, 1990. XLT4: A highly selective plating medium for the isolation of *Salmonella*. Maryland Poultryman, April, p 2-7.

Milner, K. C., and M. F. Shaffer, 1952. Bacteriological studies of experimental *Salmonella* infections in chicks. J. Infect. Dis. 90: 81-96.

Mishu, B., J. Koehler, L. A. Lee, D. Rodrigue, F. H. Brenner, P. Blake and R. V. Tauxe, 1994. Outbreaks of *Salmonella enteritidis* infections in the United States, 1985-1991. J. Infect. Dis. 169: 547-552.

Mitchell, B. W., R. J. Buhr, M. E. Berrang, J. S. Bailey, and N. A. Cox, 2002. Reducing airborne pathogens, dust and *Salmonella* transmission in experimental hatching cabinets using an electrostatic space charge system. Poultry Sci. 81: 49-55.

Mitchell, B. W., and H. D. Stone, 2000a. Electrostatic reduction system for reducing airborne dust and microorganisms. U. S. Patent No. 6,126,722.

Mitchell, B. W., P. S. Holt, and K. Seo, 2000b. Reducing dust in a caged layer room: an electrostatic space charge system. J. Appl. Poultry Res. 9: 292-296.

Mitchell, B. W., 1998. Effect of negative air ionization on ambient particulates in a hatching cabinet. J. Appl. Engineer. Agr. 14(5): 551-555.

Mitchell, B. W., 1997. Effect of airflow on ion distribution for potential dust reduction applications. J. Agr. Safety and Health 3(2): 81-89.

Mitchell, B. W., and H. S. Stone, 1996. Effect of space charging on dust reduction in a poultry hatching cabinet. Transactions of the ASAE, Paper # 964069.

Mitchell, B. W., and D. J. King, 1994. Effect of negative ionization on airborne transmission of Newcastle disease virus. Avian Dis. 38: 725-732.

Moats, W. A., 1978. Comparison of four agar plating media with and without added novobiocin for isolation of salmonellae from beef and deboned poultry meat. Appl. Environ. Microbiol. 36: 747-751.

Moore, E. N., 1946. The occurrence of fowl typhoid. Circular 19. Newark: University of Delaware, pp 1-20.

Moore, V. A., 1895. On a pathogenic bacillus of the hog-cholera group associated with a fatal disease in pigeons. USDA BAI Bull. 8: 71-76.

Moran, A. B., 1960. *Salmonella* and *Arizona* cultures from agricultural sources: 1959. Avian Dis. 4: 147-149.

Moran, A. B., 1959. *Salmonella* in animals: A report for 1958. Avian Dis. 3: 85-88.

Moran, A. B., 1958. *Salmonella* in animals: A report for 1957. Avian Dis. 2: 85-88.

Morehouse, L. G., and Wedman, E. E., 1961. *Salmonella* and other disease-producing organisms in animal by-products: A survey. Am. J. Vet. Med. 139: 989-995.

Morris, G. K., B. L. McMurray, M. M. Galton, and J. G. Wells, 1969. A study of the dissemination of salmonellosis in a commercial broiler chicken operation. Am. J. Vet. Res. 30: 1413-1421.

Mossel, D. A. A., 1988. Impact of foodborne pathogens on today's world, and prospects for management. Animal and Human Health 1(1): 13-23.

Muira, S., G. Sato, and T. Miyamae, 1964. Occurrence and survival of *Salmonella* organisms in hatchery chick fluff in commercial hatcheries. Avian Dis. 8: 546-554.

Mutalib, A., P. McDonough, S. Shin, V. Patten, and D. Lein, 1992. *Salmonella enteritidis* in commercial layer farms in New York; environmental survey results and significance of available monitoring tests. J. Vet. Diagn. Invest. 4: 416-418.

Nakamura, M., M Takagi, T. Takahashi, S. Suzuki, S. Sato, and K. Takehara, 1997. The effect of the flow of air on horizontal transmission of *Salmonella enteritidis* in chickens. Avian Dis. 41: 354-360.

Nakamura, M., N. Nagamine, T. Takahashi, S. Suzuki, M. Kijima, Y. Tamura and S. Sato, 1994. Horizontal transmission of *Salmonella enteritidis* and effect of stress on shedding in laying hens. Avian Dis. 38: 282-288.

National Poultry Improvement Plan, 1994. A report of a WHO consultation on strategies for detection and monitoring of *Salmonella* infected poultry flocks. Graz, Austria, April 11-15.

A North, W. R., and M. T. Bartram, 1953. The efficiency of selenite broth of different compositions in the isolation of *Salmonella*. Appl. Microbiol. 1: 130-134.

O'Brien, J. D. P., 1988. Salmonella entertidis infection in broiler chickens. Vet. Rec. 123: 214.

Olesiuk, O. M., V. L. Carlson, G. H. Snoeyenbos, and C. F. Smyser, 1969. Experimental *Salmonella typhimurium* infection in two chicken flocks. Avian Dis. 13: 500-508.

Olsen, S. J., L. C. MacKinnon, J. S. Goulding, N. H. Bean, and L. Slutsker, 2000. Surveillance for foodborne disease outbreaks- United States, 1993-1997. Morb. Mortal. Wkly. Rep. 49: 1-62.

Opara, O. O., L. E. Carr, C. R. Tate, R. G. Miller, E. T. Stewart, and S. W. Joseph, 1994. Evaluation of possible alternatives to double-strength skim milk used to saturate drag swabs for *Salmonella* detection. Avian Dis. 38: 293-296.

Opara, O. O., L. E. Carr, E. Russek-Cohen, C. R. Tate, E. T. Mallinson, R. G. Miller, L. E. Stewart, R. W. Johnston, and S. W. Joseph, 1992. Correlation of water activity and other environmental conditions with repeated detection of *Salmonella* contamination on poultry farms. Avian Dis. 36:664-671.

Pearson, C. C. and T. J. Sharples, 1995. Airborne dust concentrations in livestock buildings and the effect of feed. J. Agr. Eng. Res. 60(3): 145-154.

Phillips, G. B., G. J. Harris, and M. W. Jones, 1964. Effect of air ions on bacterial aerosols. Int. J. Biometeorol. 8: 27-37.

Poisson, D. M., 1992. Novobiocin, brilliant green, glycerol, lactose agar: a new medium for the isolation of *Salmonella* strains. Research Microbiol. 143: 211-216.

Pourciau, S. S. and W. T. Springer, 1978. Evaluation of secondary enrichment for detecting *Salmonellae* in bobwhite quail. Avian Dis. 22: 42-45.

Potter, M. E., 1992. The changing face of foodborne disease. Am. J. Vet. Med. Assoc. 201: 250-253.

Rappaport, F., Konforti, N., and B. Navon, 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. 9: 261-266.

Read, S. C., R. J. Irwin, C. Poppe, and J. Harris, 1994. A comparison of two methods for isolation of *Salmonella* from poultry litter samples. Poultry Sci. 73: 1617-1621.

Restaino, L., K. K. Komatsu, and M. J. Syracuse, 1982. A note on novobiocin in XLD and HE agars: the optimum levels required in two commercial sources of media to improve isolation of salmonellas. J. Appl. Bacteriol. 53: 285-288.

Restaino, L., G. S. Grauman, W. A. McCall, and W. M. Hill, 1977. effects of varying concentrations of novobiocin incorporated into two Salmonella plating media on the recovery of four Enterobacteriaceae. Appl. Environ. Microbiol. 33: 585-589.

Rettger, L. F., 1900. Fatal septicemia among young chicks. J. N. Y. Med. 71: 803-805.

Rettger, L. F., 1909. Further studies on fatal septicaemia in young chicken, or “white diarrhea”. J. Med. Res. 21: 115-123.

Rigby, C. E., and J. R. Pettit, 1980. Delayed secondary enrichment for the isolation of salmonellae from broiler chickens and their environment. J. Appl. Environ. Microbiol. 40: 783-786.

Robinzon, B., E. Liffshitz, R. Pyrzak, and N. Snapir, 1983. Effect of negative and positive air ions on the chicken tracheal surface morphology: Study with scanning electron microscopy. Avian Dis. 27: 531-538.

Rose, J. B., C. N. Haas, and C. P. Gerba, 1996. HAACP: An integrated approach to assuring the microbiological safety of meat and poultry. Food and Nutritional Press, Inc., Trumbell, CT. pp. 159-169.

Sadler, W. W., J. R. Brownell, and M. J. Fanelli, 1969. Influence of age and inoculum level on shed pattern of *Salmonella typhimurium* in chickens. Avian Dis. 13: 793-798.

Salem, M., E. M. Odor, and C. Pope, 1992. Pullorum disease in Delaware roasters. Avian Dis. 36: 1076-1080.

Sato, Y., G. Sato, L. Tuchili, G. S. Pandey, A. Nakajima, H. Chimana, and H. Sinsungwe, 1997. Status of S*almonella gallinarum-pullorum* infections in poultry in Zambia. Avian Dis. 41: 490-495.

Schaaf, J. M., 1936. Die Salmonellose (infektiose Enteritis, Paratyphose) des Geflugels, ihre Bedeutung und Bekampfung. Z. infektionskr. Parasitaere Krankheiten Hyg. Haustiere 49: 322-332.

Schaaf, J. M., A. G. MacDonald, W. J. Hall and H. Bunyea, 1931. A stained antigen for the rapid whole blood test for pullorum disease. Am. J. Vet. Med. Assoc. 79:236-240.

Schaffer, J. M., A. D. MacDonald, W. J. Hall, and H. Bunyea, 1931. A stained antigen for the rapid whole blood test for pullorum disease. Am. J. Vet. Med. Assoc. 79: 236-240.

Schalm, O. W., 1937. Study of a paratyphoid infection in chicks. J. Infect. Dis. 61: 208-216.

Schleifer, J. J., B. J. Juven, C. W. Beard, and N. A. Cox, 1984. The susceptibility of chicks to *Salmonella montevideo* in artificially contaminated poultry feed. Avian Dis. 28: 497-503.

Seo, K. H., B. W. Mitchell, P. S. Holt, and R. K. Gast, 2001. Bactericidal effects of negative air ions on airborne and surface *Salmonella enteritidis* from an artificially generated aerosol. J. Food Prot. 64(1): 113-116.

Shivasprasad, H. L., J. F. Timoney, S. Morales, B. Lucio, and R. C. Baker, 1990. Pathogenesis of *Salmonella enteritidis* infection in laying chickens. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses. Avian Dis. 34: 548-557.

Silliker, J. H., 1982. The *Salmonella* problem: Current status and future direction. J. Food Prot. 45: 661-666.

Simpson, J., R. M. Niven, C. Pickering, L. A. Oldham, A. M. Fletcher, and H. C. Francis, 1999. Comparative personnel exposures to organic dusts and endotoxin. Annals of Occupational Hygiene 43(2): 107-115.

Smith, H. W. and J. F. Tucker, 1980. The virulence of *Salmonella* strains for chickens: their excretion by infected chickens. J. Hyg. Camb. 84: 479-488.

Smyster, C. F., G. H. Snoeyenbos, and B. McKie, 1970. Isolation of *Salmonella* from rendered by-products and poultry litter cultured in enrichment media incubated at elevated temperature. Avian Dis. 14: 248-254.

Smyster, C. F., N. Adinarayanan, H. van Roekel, and G. H. Snoeyenbos, 1966. Field and laboratory observations on *Salmonella heidelberg* infections in three chicken breeding flocks. Avian Dis. 10: 314-329.

Snoeyenbos, G. H., C. F. Smyser, and H. van Roeke, 1969. *Salmonella* infections of the ovary and peritoneum of chickens. Avian Dis. 13: 668-670.

Snoeyenbos, G. H., V. L. Carlson, B. A. Mckie, and C. F. Smyser, 1967. An epidemiological study of salmonellosis of chickens. Avian Dis. 11: 653-667.

Solowey, M., E. H. Spaulding, and H. E. Goresline, 1946. An investigation of a source and mode of entry of *Salmonella* organisms in spray-dried whole egg powder. Food Res. 5: 380-390.

Stokes, J. L., W. W. Osborne, and H. G. Bayne, 1956. Penetration and growth of *Salmonella* in shell eggs. Food Res. 21: 510-518.

Stokes, J. L., and W. W. Osborne, 1955. A selenite brilliant green medium for the isolation of *Salmonella*. Appl. Microbiol. 3: 217-220.

United States Department of Agriculture, 1985. Subcommittee on microbiological criteria for foods and food ingredients: Introduction and expansion of the HACCP system in food protection programs. In: An evaluation of the role of microbiological criteria for foods and food ingredients. Washington, DC: National Academy Press, pp. 41-54, 308-328.

Surkiewicz, B. F., R. W. Johnston, A. B. Moran, and G. W. Krumm, 1969. A bacteriological survey of chicken eviscerating plants. Food Tech. 23: 80-85.

Takai, H., J. Seedorf, and S. Pedersen, 1999. Dust and endotoxin concentration in livestock buildings in Northern Europe. Proceedings from

International Symposium on Dust control in Animal Production Facilities, Danish Institute of Agr. Sci., Bygholm, Denmark: pp. 83-89.

Tate, C. R., R. G. Miller, E. T. Mallinson, L. W. Douglass, and R. W. Johnston, 1990. The isolation of *Salmonella* from poultry environmental samples by several enrichment procedures using plating media with and without novobiocin. Poultry Sci. 69: 721-726.

Tauxe, R. V., 1996. An update on *Salmonella*. Health Environ. 10: 1-4.

Taylor, W. F., 1965. Isolation of Shigellae. I. Xylose-lysine agars: new media for the isolation of enteric pathogens. J. Am. Clin. Pathol. 44: 471-475.

Timoney, J. F., H. L. Shivaprasad, R. C. Baker and B. Rowe, 1989. Egg transmission after infection of hens with *Salmonella enteritidis* phage type 4. Vet Rec. 125: 600-601.

Turnbull, P. C. B. and G. H. Snoeyenbos, 1974. Experimental salmonellosis in the chicken. 1. Fate and host response in alimentary canal, liver, and spleen. Avian Dis. 18: 153-177.

U. S. Department of Agriculture (USDA), 1996. National Poultry Improvement Plan and auxiliary provisions. Washington, DC: Animal Plant Health Inspection Service, Veterinary Service, U. S. Department of Agriculture.

Van Wicklen, G. L. and M. Czarick, 1997. Particulate emissions from poultry housing. Transactions of the ASAE, Paper # 974094.

Veenhuizen, M. A., and D. S. Bundy, 1990. Electrostatic precipitation dust removal system for swine housing. ASAE Annual Meeting, Paper # 904066.

Vestal, O. H. and J. F. Stephens, 1966. The relative pathogenicity of selected paratyphoids for chickens. Avian Dis. 10: 502-507.

Waltman, W. D., and E. T. Mallinson, 1995. Isolation of *Salmonella* from poultry tissue and environmental samples: A nationwide survey. Avian Dis. 39: 45-54.

Waltman, W. D., A. M. Horne, and C. Pirkle, 1993. Influence of enrichment incubation time on the isolation of *Salmonella*. Avian Dis. 37: 884-887.

Waltman, W. D., A. M. Horne, C. Pirkle, and D. C. Johnson, 1992. Prevalence of *Salmonella enteritidis* in spent hens. Avian Dis. 36: 251-255.

Waltman, W. D., A. M. Horne, C. Pirkle, and T. G. Dickson, 1991. Use of delayed secondary enrichment for the isolation of *Salmonella* in poultry and poultry environments. Avian Dis. 35: 88-92

Wilding, G. P., and C. Baxter-Jones, 1985. Egg-borne salmonellas: is prevention feasible? In: Proceeding of the International Symposium on *Salmonella*, New Orleans, G. H. Snoeyenbos, ed. Am. Assoc. of Avian Pathologists, Kennett square, PA. pp 126-133.

Williams, J. E., 1981. *Salmonellas* in poultry feed: A worldwide review. Part I. World's Poultry Sci. 37: 6-19.

Williams, B. M., 1975. Environmental considerations in salmonellosis. Vet. Rec. 96: 318-321.

World Health Organization, 1994. World Health Organization consultation on strategies for detection and monitoring of infected poultry flocks. Proceedings from Graz, Austria, WHO-Zoon. 94.173.

Zecha, B. C., R. H. McCapes, W. M. Dungan, R. J. Holte, W. W. Worcester, and J. E. Williams, 1977. The Dillon Beach Project- A five-year epidemiological study of naturally occurring *Salmonella* infection in turkeys and their environment. Avian Dis. 21: 141-159.

MANUSCRIPT 1

Effect of an electrostatic space charge system on airborne dust and subsequent potential transmission of microorganisms to broiler breeder pullets by airborne dust[1]

[1] L.J. Richardson, B.W. Mitchell, J.L. Wilson and C.L. Hofacre, Submitted to Avian Diseases, 10/12/02

Summary

High levels of dust and microorganisms are known to be associated with animal confinement rearing facilities. Many of the microorganisms are carried by dust particles, thus providing an excellent vector for horizontal disease transmission between birds. Two environmentally controlled rooms containing female broiler breeder pullets (n=300) were used to evaluate the effectiveness of an electrostatic space charge system (ESCS) in reducing airborne dust and gram-negative bacteria levels over an eight-week period (starting when the birds were 10 weeks old). The ESCS was used to evaluate the effectiveness of reducing airborne microorganism levels by charging airborne dust particles and causing the particles to be attracted to grounded surfaces (i.e., walls, floor, equipment). The use of the ESCS resulted in a 64% mean reduction in gram-negative bacteria. Airborne dust levels were reduced an average of 37% over a one-week period in the experimental room compared to the control room based on samples taken every 10 minutes. The reductions of airborne dust and bacteria in this study are comparable to earlier results obtained with the ESCS in commercial hatching cabinets and experimental caged layer rooms, suggesting the system could also be applied to other types of enclosed animal housing.

Keywords: electrostatic space charge system, broiler breeder pullets, dust, air quality, bacteria

Abbreviations: ESCS: electrostatic space charge system, FSIS: Food Safety and Inspection Services, TNTC: too numerous to count, C Celsius

Introduction

A major goal of the poultry industry is to produce a safe, edible product for consumers. The USDA Food Safety and Inspection Services stated in the 1996 Federal Register "…the FSIS food safety goal should be to reduce the risk of foodborne illness associated with the consumption of meat and poultry products to the maximum extent possible by ensuring that appropriate and feasible measures are taken at each step in the food production process where hazards can enter…"(3). Levels of S*almonella* have been reduced on edible products in processing plants; however, further reduction of these bacteria may be required at the farm level in order to continue to decrease bacteria in the processed product. *Salmonella* can be introduced into a flock of breeder birds by feed, insects, rodents, drinking water, dust, and air (2, 5, 13). *Salmonella* can then be transmitted from the breeding birds to the broilers through contamination of the egg contents and spread among the newly hatched chicks when chicks consume eggshell fragments and inhale dust and down in the hatching cabinet (7). Dust has been shown to be an important mechanism for airborne transmission of disease-causing agents (4, 6, 9). Studies have shown reducing airborne dust levels by 50% can reduce airborne bacteria by 50 to 100-fold or more (1, 8, 9).

Confinement-rearing facilities are a segment of the poultry industry where high levels of dust and microorganisms are known to exist. Dust is generated from the litter, feed, birds, and incoming air. Bird movement and poultry house equipment can cause secondary suspension (10). An electrostatic space charge system (ESCS) has been designed to reduce airborne dust by charging the air

with negative ions causing airborne particles to be attracted to grounded surfaces (i.e., walls, floor, equipment) (10). The ESCS has shown to significantly reduce airborne particles and bacteria in aerosol generation cabinets, caged layer rooms, disease transmission cabinets and hatching cabinets (4, 9, 11, 12). The highest reduction of bacteria from these studies occurred in the aerosol generation cabinets. The aerosolized levels and transmission of *Salmonella enteritidis* were reduced by 98% or more by the ESCS (12). Dust reduction of 75% in a disease transmission study with chicks, completely eliminated airborne transmission of *Salmonella enteritidis* to the ceca of susceptible downwind chicks (4). These studies indicate that decreasing dust levels in the environment can cause a reduction in bacteria; however, the ESCS has not been tested for extended periods in extremely dusty confinement environments.

For years, dust and microorganisms have been a major concern in confinement rearing facilities. Improving the air quality in poultry houses should reduce bacterial contamination of the final products going to the consumer and aid in improving animal health (1, 3, 4, 6, 8, 9). Broiler breeder pullets are reared on litter floors to 20-22 weeks of age before being moved to laying houses. During this rearing time, airborne dust levels increase and dust accumulates on walls and equipment. The objectives of this experiment were to 1) measure the effectiveness of the ESCS for reducing airborne dust and bacteria counts in an extremely dusty pullet rearing facility over a several week period and 2) determine the optimum bacteria sampling techniques and times in this type of environment.

Materials and methods

EXPERIMENTAL ROOMS

Two essentially identical environmentally controlled rooms (9.1m wide x 7.3m deep x 3.05m high) were equipped with a mechanical trough feeding system, nipple drinkers, and pine litter shavings (Appendix B). Air exchange was supplied by a temperature controlled variable speed negative pressure ventilation system. The rooms were maintained at a neutral static pressure relative to each other and negative pressure relative to the exterior of the room. Incoming air was pulled in one end of the room through inlets equipped with light traps and exited the room via exhaust fans also equipped with light traps. Two circulating fans were used to mix room air and were mounted opposite each other at ceiling height. Ventilation rates for each room were adjusted by fan speed calibrations to ensure each room had identical air exchange to maintain a temperature of approximately 22.2 C.

ELECTROSTATIC SPACE CHARGE SYSTEM (ESCS)

Each ESCS unit measured 33.02cm x 83.82cm and consisted of three 71.12cm long ionizer bars with 42 sharp point electrodes and a wire grid ground plane 7.62cm behind the electrodes (Appendix B). The combination of high voltage (-30kVdc) power to the electrodes and the ground plane generated high negative air ion output causing air passing through the unit to be charged. The high-voltage power supply for the ESCS was current limited internally to 0.5mA

to assure safety. Accidental touching of the unit would result in a mild static shock similar to touching a grounded surface after walking across carpet in the winter.

In the experimental room, two configurations of the ESCS were used. In the first configuration, an air ionizer unit was installed at the air inlet on each side of the room (Figure 1.1). The second configuration added an air ionizer unit in front of each of the two recirculating fans. The high velocity air streams at the inlets and in front of the recirculating fans helped to distribute charged air throughout the room (Figure 1.1).

EXPERIMENTAL DESIGN

Each room housed 300 Cobb female broiler breeder pullets. The experiment was started when birds 10 weeks of age when bird size, and surface dust accumulations were high. The room equipped with the ESCS unit served as the treatment and the other room without the ESCS served as the control. Bacterial levels were measured in each room with 1-3 samples taken per week including measurement periods when the ESCS unit was cycled on or off for portions of a day to compare the two rooms with no treatment. Dust concentrations in each room were measured at 10-minute intervals in the center of the rooms at 1.5m above the litter with a Dust Trak laser-based instrument (Model 8520, TSI Incorporated, St. Paul, MN). Dust accumulation on the positive ground plane of the ESCS required it to be cleaned twice a week to assure proper operation. For cleaning, which took about 5-10 minutes, the high voltage

power was turned off and a brush with flexible plastic bristles was used to remove dust.

Bacteria levels were evaluated using open-air MacConkey agar plates (Remel, Lenexa, KS), XLT4 agar plates (Remel), and blood agar plates (Remel). The MacConkey agar plates were used to enumerate total gram-negative bacteria, while XLT4 plates were used to obtain levels of S*almonella* in each experimental room. The blood agar plates were used to enumerate total bacteria counts inside the rooms. To determine the presence of background *Salmonella* was present at the beginning of the experiment, samples of the litter were taken from each room and placed in sterile plastic bags. *Salmonella* cultures were enriched as described by Waltman, *et al*. utilizing tetrathionate brilliant green broth (TTB, Difco Laboratories, Detroit, Mich.) and incubated at 41.5˚C for 18 h (14, 15). For *Salmonella* isolation and identification a loop of TTB enrichment broth was streaked onto an XLT4-BGN biplate (Difco) and incubated at 37˚C overnight. Individual *Salmonella* colonies were then confirmed using triple sugar iron slants (Difco) followed by serogrouping using poly O *Salmonella*-specific antiserum (Difco).

BACTERIAL SAMPLING TIMES AND TECHNIQUES

To determine the most effective method to recover room bacteria, several sampling times and techniques were evaluated. Blood agar plates were exposed to the air for 25 sec, 30 sec, 45 sec, 1 min, 5 min, 10 min, and 1 h and 24 h in the center of the rooms approximately 1.5 m from the floor litter surface. MacConkey

and XLT4 agar plates were exposed to the air for 1 min, 5 min, 10 min, 1 h, and 24 h inside the room 1.5 m above the floor near the room exhaust. In addition, MacConkey, XLT4, and blood agar plates (Remel) were exposed for 1 min (30 sec after exhaust fans started running) by placing the plates in the exhaust air stream.

A specially designed cover tray (16.5cm x 26cm x 6.4cm), which held up to two agar plates, was also tested to allow for a sampling period of 24 h without overloading the plates with bacteria (Appendix B). The plastic cover tray (Home Essentials, Troy, MI) which had a solid top and slotted side-walls was lined with a small mesh perforated rubber shelf liner (Rubbermaid, Wooster, OH) across the slotted side-walls (10mm in diameter), allowing diffusion of small dust particles in the room through the side vents, eliminating direct settling of dust or bacteria onto the plates. The cover tray allowed for longer sampling times, thus sampling a larger volume of air over a longer period. This allowed for sampling the air during various levels of bird activity (i.e. during feeding, resting etc.) in this extremely dusty environment and a reduction in sample variability due to the brief periods of unusual bird activity near the sample area.

DUST CONCENTRATION

Dust concentrations were continuously measured throughout the study in intervals of 10 min in each room using a Dust Trak laser-based instrument (TSI) positioned 1.5m above of 10 min in each room using a Dust Trak laser-based

instrument (TSI) positioned 1.5m the floor in the center of the room. Data was down loaded onto spreadsheets and analyzed.

STATISTICS

The analysis of data was calculated with the statistical program SAS 8.1 (SAS Institute Inc., Cary, N.C.). The general linear model procedure in SAS was used to evaluate the total plate counts and dust concentrations taken during the experiment between the control and experimental rooms. The significance level was set at P=0.05 and evaluated using analysis of variance.

Results

Blood, MacConkey and XLT4 agar plate samples taken at each exhaust, 30 sec after the fans started resulted in too numerous to count (TNTC) colonies. Air samples taken at 25 sec, 30 sec, 45 sec, 1 min, 5 min, 10 min, 1 h, and 24 h inside each room using blood agar plates also resulted in colonies TNTC. Air samples at 1 min, 5 min, 10 min, 1 hr, and 24 hr inside each room using MacConkey without the cover tray resulted in a range of 9 colony forming units (CFU) per plate at 1 min to TNTC at 1 h and 24 h for the treatment and a similar range for the control at 11 CFU/plate at 1 min to TNTC at 1 h and 24 h. The ESCS did result in some reduction in bacterial counts on the MacConkey agar plates at the lower sampling times, but due to the large particles occasionally falling onto the plates, contaminating the samples and the short sampling period, the results were not consistent.

Results from MacConkey agar plates using the cover tray over the eight-week period for 24h sampling times (n=14) with the ESCS treatment are shown in Figure 1.2. The treatment room mean was 156 CFU/plate over the eight-week period on MacConkey agar plates compared to 439 CFU/plate mean count in the control room (Appendix B). When the ESCS was turned off in the treatment room (thus no treatment effect present), the control room mean count on the MacConkey agar plates was 406 CFU/plate compared to the treatment room mean of 401 CFU/plate on the 24 hr sampling time (n=10). These plate counts were not significantly different ($P>0.05$). The cover tray method of air sampling, which prevented the large particles from affecting plate counts, demonstrated the ESCS significantly ($P<0.0001$) reduced the total gram-negative bacteria counts by an average of 64%. Even with the cover tray, blood agar plates were TNTC at these sampling times. Both rooms were S*almonella* serogroup C-2 positive by litter culture, however, all air samples using XLT4 were negative for *Salmonella* throughout the experiment.

Dust concentrations in the rooms ranged from less than 1mg/m^3 to over 25 mg/m^3 during the 8-week sampling period. When dust concentrations were measured in the two rooms over a 24 h period with the ionizer off, dust concentrations ranged from 7.0-8.5mg/m^3 and not significantly different. Dust levels were significantly reduced ($P<0.05$) by an average of 37%, with the ESCS treatment averaging 5mg/m^3 over a 7-day period compared to 7.8 mg/m^3 in the control room (Figure 1.3). Higher outside temperatures usually were associated

with higher ventilation rates and generally lower airborne dust concentrations as can be seen for days 5-7 (Figure 1.3).

Discussion

Dust concentrations and bacterial counts varied from day to day especially as ventilation rates changed, however the variation was similar across both rooms. The dust concentrations and bacteria counts variations were often attributable to caretaker activity in the rooms such as weighing or vaccinating and bird activity on certain days.

Bacterial air sampling at the exhaust fans was found not to be a meaningful location for sampling, because large particles exiting through the exhaust fans encountered the plate. In addition, the TNTC may be due to high velocity of the airborne dust and bacteria exiting through the small exhaust area onto the plates.

The technique of sampling bacteria with the open agar plates was also found to be ineffective for measuring the bacteria levels inside the two rooms. Short-term air sampling for bacteria was variable and did not adequately represent the overall average bacteria counts in the rooms.

It was found that the more effective technique for measuring bacteria levels in the air was by use of the cover tray. This prevented direct settling of extremely large dust particles on the agar plate and allowed longer sampling times. The longer sampling periods were less affected by short-term variations associated with ventilation, bird activity or animal care activity and thus provided

more consistent results. Total bacteria counts using blood agar plates were not possible even with the cover tray; however, total bacteria counts may be possible with a shorter sampling time.

A reduction of 64% in mean total gram-negative bacteria counts was recorded over the 24 h sampling times using the cover tray in the room equipped with the ESCS. The ESCS reduced the amount of airborne dust within the treatment room therefore reducing the number of airborne gram-negative bacteria when compared to the control room. A reduction in airborne gram-negative bacteria could benefit the overall health or *Salmonella* levels of a flock, by reducing bacteria transmission via airborne dust particles.

Litter samples were positive for S*almonella* group C-2 but *Salmonella* was not found on the XLT4 agar plates for the 24 h sampling time with or without the cover tray. This may have been due to the low number of *Salmonella* present in the room.

The ESCS also reduced dust concentrations by an average of 37%. The reduction in bacteria counts appeared to relate to the reduction in airborne dust. The reduction of airborne dust and bacteria in this experiment indicates that the ESCS may be an effective tool for improving air quality and potentially reducing transmission of bacteria from bird to bird in confinement rearing facilities.

References

1. Carpenter G. A., W. K. Smith, A. P. C. MacLaren, and D. Spackman. Effect of Internal air filtration on the performance of broilers and the aerial concentrations of dust and bacteria. British Poultry Sci. 27:471-480. 1986.
2. Davies, R. H., and M. H. Hinton. Salmonella in animal feed. In: Salmonella in Domestic Animals. CABI Publishing, London, U. K. Pages 285-300. 2000.
3. Federal Register. Pathogen Reduction; Hazard Analyses and Critical Control Point (HAACP) System; Final Rule. USDA-FSIS ACFR part 304, et al. Pages 38806-38989. 1996.
4. Gast R. K., B. W. Mitchell, and P. S. Holt. Application of negative air ionization for reducing experimental airborne transmission of Salmonella enteritidis to chicks. Poultry Sci. 78(1): 57-61. 1999.
5. Henzler, D. J., D. C., Kradel, and W. M. Sischo. Management and environmental risk factors for Salmonella enteritidis contamination of eggs. Avian Journal Veterinarian Research. (59): 824-829. 1998.
6. Holt, P. S., B. W. Mitchell, K. H. Seo, and R. K. Gast. Use of negative air ionization for reducing airborne levels of Salmonella enteritidis serovar Enteritidis in a room containing infected caged layers. J. Appl. Poultry Res. 8: 440-446. 1999.
7. Humphrey, T. J. Important and relevant attributes of the Salmonella organism. Proceedings of International Symposium on Foodborne

Salmonella in poultry. American Association of Avian Pathologists, New Bolton Center, Kennett Square, PA. Pages 43-53. 1998.

8. Madelin T. M., C. M. Wathes. Air hygiene in a broiler house: Comparison of Deep Litter with Raised Netting Floors. British Poultry Sci. 30: 23-37. 1988.
9. Mitchell, B. W., P. S. Holt, and K. H. Seo. Effectiveness of electrostatic space charge system for reducing dust in a caged layer room. J. Appl. Poultry Res. 9: 292-296. 2000.
10. Mitchell, B. W., and H. S. Stone. Electrostatic reduction system for reducing airborne dust and microorganisms. U.S. Patent No. 6,126,722. 2000.
11. Mitchell, B. W., R. J. Buhr, M. E. Berrang, J. S. Bailey and N. A. Cox. Reduction of airborne bacteria in the hatching cabinet with an electrostatic space charger. Poultry Sci. 77 (S1): 151. 1998.
12. Seo, K. H., B. W. Mitchell, P. S. Holt, and R. K. Gast. Bacterial effects of negative ions on airborne and surface *Salmonella enteritidis* from an artificially generated aerosol. J. Food Protection. 64(1): 113-116. 2000.
13. Shapcott, R. C. Practical aspects of *Salmonella* control: Progress report on a program in a large broiler integration. Proceedings of International Symposium on Foodborne *Salmonella*. American Association of Avian Pathologists, New Bolton Center, PA. 109-114. 1984.

14. Waltman, W. D., A. M. Horne, and C. Pirkle. Influence of enrichment incubation time on the isolation of *Salmonella.* Avian Dis. 37: 884-887. 1993.

15. Waltman, W. D., A. M. Horne, C. Pirkle, and T. Dickson. Use of delayed secondary enrichment for the isolation of *Salmonella* in poultry and poultry environments. Avian Dis. 35: 88-92. 1991.

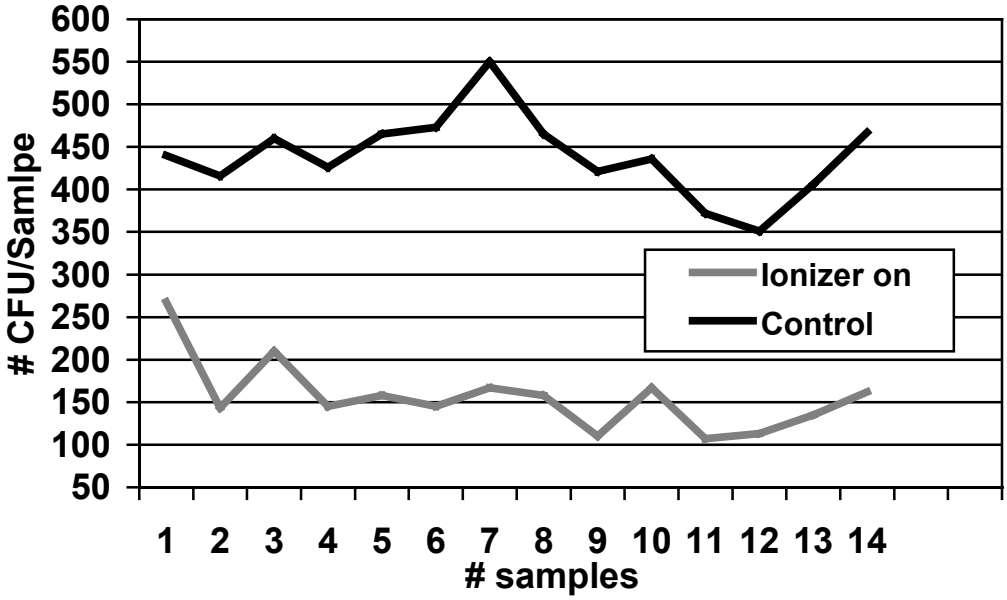
Figures

Figure 1.1. ESCS configuration for the ionization-treated pullet room. (a) One ESCS was placed in front of the lower room air inlet on each side of the room. Dust accumulation after one week can be seen on the wall beside the area that had just been scraped cleaned. (b) One ESCS was placed in front of the recirculating fan on each side of the room.

a.) ESCS CONFIGURATION AT THE LOWER INLET OF THE TREATMENT ROOM

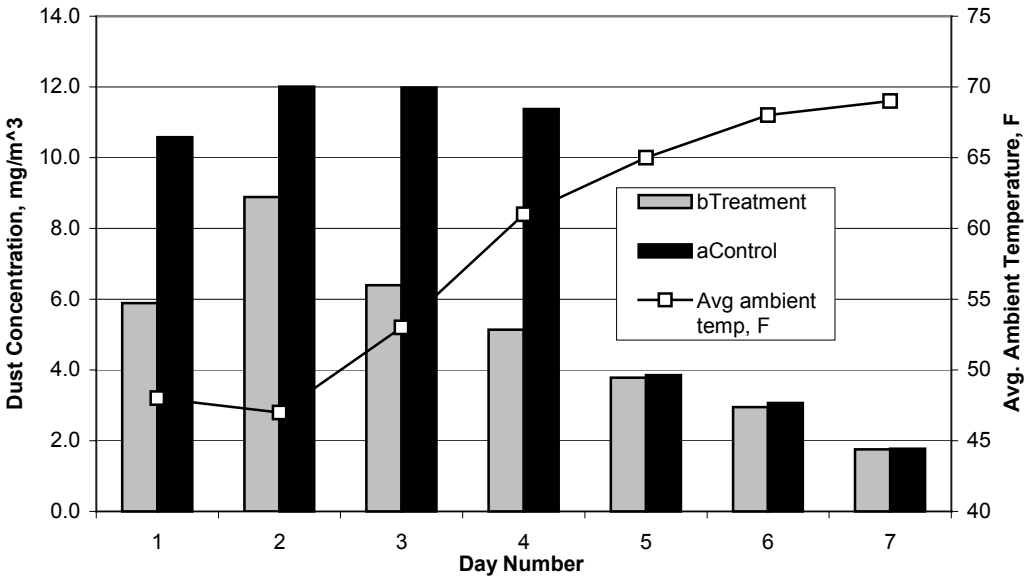
b.) ESCS CONFIGURATION AT THE RECIRCULATING FANS

Figure 1.2. Results from 14 air samples taken utilizing MacConkey agar plates with the cover tray air sampling method at 24 hr sampling times[*].



[a]Gram-negative bacteria was reduced by 64% in the treatment and found to be significantly (P<0.0001) less than the control room.

Figure 1.3. Ionizer effect on average daily dust concentration for a one-week period, and average daily ambient temperature*.



[b]Airborne particulate matter was found to be significantly (P<0.0001) reduced in the treatment room when compared to the control room. The treatment room averaged 4.3mg/m^3 while the control room averaged 6.5 mg/m^3, a 37% reduction in airborne particulate matter.

MANUSCRIPT 2

Effect of electrostatic space charge on reduction of airborne transmission of *Salmonella* and other bacteria during the lay cycle and to the progeny of broiler breeders[2]

[2] L. J. Richardson, C. L. Hofacre, B. W. Mitchell and J. L. Wilson, To be submitted to *Avian diseases*.

Summary

The average broiler breeder laying cycle is 40 weeks and during this period a large amount of dust becomes airborne, and accumulates on walls, ceiling and equipment. Many microorganisms adhere to dust particles; and therefore, dust is an excellent vector for horizontal disease transmission between birds. Ten percent of the broiler breeder hens in the control and treatment room were inoculated with a nalidixic acid resistant S. *enteritidis*. Two environmentally controlled rooms containing commercial broiler breeders were used to evaluate the effectiveness of an electrostatic space charge system (ESCS), which induced a strong negative electrostatic charge, in reducing airborne microorganism levels. The ESCS caused the dust to become negatively charged therefore moving to the grounded floor in the treatment room. *Salmonella* in birds can be transmitted via the air and the infection merits concern due to the human food-borne illness associated with the consumption of poultry. Salmonella was one of the bacteria used to monitor the effectiveness of the ESCS.

In the treated room, the ESCS significantly reduced airborne dust and microorganisms. The use of the ESCS resulted in a 61% reduction in airborne dust levels, which resulted in a 76% reduction in total airborne bacteria and a 48% reduction in airborne gram-negative bacteria in the treatment room. A 63% reduction of gram-negative bacteria on the egg collection belts was also recorded in the treatment room, which yielded in a 28% reduction of gram-negative bacteria on the eggshell surface. The ESCS resulted in fewer S. *enteritidis*

positive hens and their progeny from the treatment room due to reductions of dust and airborne bacteria.

Keywords: Electrostatic Space Charge System, Broiler Breeder Hens, *Salmonella*, Bacteria, and Broilers

Abbreviations: ESCS: electrostatic space charge system, XLT4: xylose-lysine-tergitol 4, CFU: colony forming units, C: Celsius, RAPD: Rapid Amplification of Polymorphic DNA, PCR: Polymerase Chain Reaction

Introduction

Salmonella colonization of broiler breeder hens has been reported to effect bird health, egg production and is a potential cause of human food-borne illness (5, 10). Even though many pathogens have recently received considerable attention, *Salmonella* remains among the leading sources of human gastroenteritis (4, 24, 28, 37). The estimated annual incidence of a foodborne illness associated with *Salmonella* ranged from 1 million to 4 million cases in the United States (47). *Salmonella* are gram-negative bacteria, which can be found in poultry and their environment (19, 48, 7, 18, 45). There have been many methods developed to control *Salmonella* contamination of poultry in processing plants such as identifying critical control points, inspection, chemical treatments and proper sanitation techniques (42, 46, 40). However, to make further reductions in *Salmonella* will require implementation of methods at the farm (2, 26, 36, 25).

Salmonella has the ability to persist in hostile environments and can be transmitted both vertically and horizontally (45, 39,38). *Salmonella* can be transmitted vertically by transovarian transmission from the broiler breeder hen to its progeny via the egg (39). *Salmonella* has also been shown to be readily transmitted horizontally by airborne particulates (3, 9, 17, 35). Current methods to aid in reducing these vectors include competitive exclusion, vaccinations, proper sanitation and biosecurity practices (15, 13, 32).

One approach to further aid in reducing *Salmonella*, is decreasing the level of airborne dust and bacteria in their environment, therefore reducing horizontal and then theoretically vertical transmission. The use of negative ionization could be one method of reducing high levels of dust and bacteria in these confinement facilities. Negative ion generators have been shown to significantly reduce airborne levels of dust in caged layer rooms, hatching cabinets, and broiler breeder confinement rearing facilities (30, 31, 41, 29). Significant reductions in airborne bacteria have also been associated with the use of negative ion systems (33, 16, 12, 41).

Kreuger and others have reported as the viable counts of bacteria in the air are reduced, a lethal effect occurs from the high levels of negative ions (20, 21). Negative air ions reduced horizontal transmission of Newcastle disease by as much as 27.7% from donor chickens to susceptible chickens (33). An electrostatic space charge system (ESCS) developed by Mitchell and others has been tested in aerosol generation cabinets where S*. enteritidis* was reduced by 98% (32, 43). The ESCS has shown complete elimination of airborne S*. enteritidis* horizontal transmission downwind to chicks in isolation cabinets and an 85% reduction in total airborne bacteria in hatching cabinets (30, 12). The ESCS has also been associated with a 64% reduction in gram-negative bacteria during the rearing of broiler breeder pullets (29).

Reducing the amount of airborne bacteria, and thereby the number of *Salmonella* positive broiler breeder hens in a flock could reduce the incidence of *Salmonella* positive progeny at hatch. One *Salmonella* positive fertile egg in the

hatchers increases the possibility that during pip and hatch other chicks will be contaminated before leaving the cabinet (8, 34, 44, 1, 11). The objective of this experiment was to investigate the effectiveness of the ESCS in reducing bacteria, including *Salmonella*, during the lay cycle of broiler breeders and subsequent progeny.

Materials and methods

EXPERIMENTAL DESIGN

To ensure a uniform level of *Salmonella* colonization between the treated and control room, 72-week-old Ross hens (Aviagen, Huntsville, AL) and 24-week-old Cobb roosters (Cobb-Vantress, Siloam Springs, AR) had all *Salmonella* removed by treatment with enrofloxacin at 10mg/kg body weight in their drinking water for eight days. These hens had been conventionally molted and brought back to lay condition over the previous eight weeks prior to treatment. The birds were randomly divided (n=308 hens and n=33 roosters per room) and placed in identical rooms.

The environmentally controlled rooms (9.1m wide x 7.3m deep x 3m high) were equipped to U.S. poultry industry standards for broiler breeder flocks with $2/3^{rd}$ of the floor covered by wooden slats and $1/3^{rd}$ of the floor in the middle as pine litter scratch area (Appendix C). There were double-sided mechanical nests (Shenandoah Manufacturing, Harrisonburg, VA), nipple drinkers, a chain feeding system on the slats for the females, and pan feeders in the scratch area for the

males to simulate a commercial broiler breeder facility. A 1.2m x 6.1m x 3m work area was constructed at the entrance of the rooms which consisted of two, egg collection tables, footbath, and feed hopper. The only difference between the rooms was the addition of an electrostatic space charge system (ESCS) that provided the ionizer treatment while the room without the ESCS served as the control (29) (Appendix C).

To move the ionized air into the air stream of the room, each room had two sealed 4-paddle ceiling fans. Suspended below the fans in the treatment room was a 6 bar ionizer unit (29). The ionizer was operated at –30kV(at 0.2mA) and designed in conjunction with the ceiling fans to direct negatively charged particles to the grounded litter. The rooms were cleaned, disinfected and fumigated with formaldehyde and potassium permanganate to ensure that the rooms were uncontaminated. The two experimental rooms were isolated from each other and access was limited. Separate coveralls, boots, hairnets, gloves and footwear bathes were used for each room for added biosecurity. The experimental period simulated the normal lay cycle of a broiler breeder flock (35 weeks).

Ten percent of the hens (n=30) in each room were identified with plastic tags at week 2 (after placement in the experimental facility) and inoculated orally with a 0.5 ml concentration of rifampicin resistant strain of S. *typhimurium* (Holt, USDA, ARS, Athens, GA) at a titer of 1.94×10^7 CFU/ml. The tagged hens were inoculated a second time at week 10 by both oral (0.5 ml) and subcutaneous (0.5 ml) at a titer of 7.8×10^6 CFU/ml. Since the birds did not colonize and shed the

S. *typhimiurum*, at week 11, the same 10% of hens were inoculated orally (0.5 ml) and by subcutaneous injection (0.5 ml) with a nalidixic acid resistant strain of S. *enteritidis* (Holt, USDA, ARS, Athens, GA) at a titer of 4 x 10^8 CFU/ml. To continue the *Salmonella* shedding at 26 weeks an additional 10 % of the hens (n=30) were tagged and inoculated orally (0.5 ml) and by subcutaneous injection (0.5 ml) with the same nalidixic acid resistant strain of S. *enteritidis* at a titer of 6 x 10^9CFU/ml.

AIR SAMPLING FOR DUST CONCENTRATIONS

Dust concentrations were continuously measured throughout the study from week 8-35 at intervals of 10 minutes inside the control and treatment rooms using a Dust-Trak laser-based instrument (Model 8520, TSI Incorporated, St. Paul, MN) (29).

AIR SAMPLING FOR BACTERIA

A specially designed cover tray (15.24 cm x 11.43 cm x 29.21 cm), which held three agar plates at a time, was used to sample airborne *Salmonella* and gram-negative bacteria (41). The plastic cover tray (Home Essentials, Troy, MI) had a solid top and slotted side walls lined with a small mesh perforated rubber shelf liner (Rubbermaid, Wooster, OH), which allowed diffusion of small dust particles but reduced direct settlement of dust or bacteria onto the plates. The cover tray allowed longer sampling periods for a better long-term average and reduced the potential of overloading of the plates.

Bacteria sampling of the control and treatment rooms did not begin until week 8 of the experiment to allow dust and bacteria to be generated by the birds activity inside the rooms. Blood agar plates (Remel Laboratories, Lenexa, KS) were used to evaluate the level of total bacteria inside the control and treatment room from week 8 to week 12. Samples were taken 2-4 times per week simultaneously from both rooms with the plate left exposed openly for a period of 10 seconds. The samples were then incubated at 37 C overnight and colony-forming units (CFU) counted.

MacConkey agar plates (Becton Dickinson Laboratories, Sparta, MD) were used to enumerate total airborne gram-negative bacteria while Xylose-Lysine-Tergitol 4 (XLT4) agar plates (Difco Laboratories, Detroit, MI) were used to enumerate total airborne *Salmonella* levels in order to determine if the hens were shedding the S. *enteritidis* into the air (27). The air samples were taken in the center of each room, approximately 1.5m from the litter scratch area and also 61cm above the nests on each side of the rooms. The MacConkey agar plates were exposed 2-3 days per week in each room from week 9-35, while the XLT4 agar plates were exposed from week 9-28. The MacConkey and XLT4 agar plates were put into the cover tray sampler and left exposed in each room for 5.5 h period and incubated overnight at 37 C and the CFU counted. Black colored colonies from the XLT4 plates were transferred by a 1ul disposable inoculating loop to XLT4 and BGN and incubated overnight at 37 C and then confirmed by serogrouping using poly O *Salmonella*-specific antiserum (Difco Laboratories, Detroit, MI).

EGGSHELL AND EGG BELT CULTURE

Egg impressions were taken twice weekly from 13 to 35 weeks from 32 eggs from each room. Eggs were taken from the collection tables for sampling. A gloved hand held the eggs and the large end of the egg was lightly touched onto a MacConkey agar plate (Bector Dickinson Laboratories, Sparta, MD). This cultured approximately 1/3 of the shell surface of the large end of each egg. The samples were incubated overnight at 37 C and bacteria colonies counted.

MacConkey agar poured into Rodak touch plates (Becton Dickinson Laboratories, Sparta, MD) was used to sample for gram-negative bacteria on the egg collection belts inside the rooms. Two to four Rodak touch plates per sample day were used to enumerate the bacteria on the egg belts. The samples were incubated at 37 C overnight and visually counted. The egg collection belts were sampled during weeks 15-18, 21, and 25 inside both rooms at the same time on each sample day.

SALMONELLA SAMPLING

Prior to and after placement of the flock, the ionizer treated and control room were sampled by dragging a swab soaked in double strength skim milk across the birds bedding material (slats and litter), walls and equipment on both the left and right sides of the room (6). In addition, the four egg belts inside each room were also sampled with a drag swab. To reduce the possibility of cross contaminating a sample, gloves were changed before and after completing each

drag swab. All drag swabs were placed in individual sterile plastic sample bags (Fisher Scientific, Pittsburgh, PA) and labeled. The samples were then enriched as described below. A total of 7 drag swabs per room were taken weekly from week 1-35.

Prior to inoculation of the hens with *Salmonella*, the tagged birds were swabbed cloacally with a sterile cotton tip swabs (40). At week 4, cloacal swabs were performed on 100% of the hens from the control and treatment rooms. The cloacal swabbing was repeated on 30% percent of the inoculated hens and 20% of the uninoculated hens at weeks 15, 17, 28, and 34 in each room. The culture procedure for the enrichment, in order to isolate *Salmonella* was performed as described below.

CHICK AND FLUFF CULTURE

Eggs from weeks 13 (n=408/room), 15 (n=528/room), 17 (n=393/room), 19 (n=377/room), 21 (n=440/room), 26 (n=418/room), 27 (n=430/room), and 28 (n=440/room) were incubated and hatched in separate machines. At each hatch, 30 chicks were randomly selected from each treatment. The chicks were euthanized by cervical dislocation and aseptically opened. A portion of the liver and yolk sac was aseptically removed along with the junction of the ceca to the large intestines from each chick (n=5), pooled together and placed in a 50 ml sterile polypropylene conical tube (Becton Dickinson Laboratories, Franklin Lakes, NJ) containing 30ml of tetrathionate brilliant green broth (TTB) (40).

A sample of the fluff and broken eggshell material from each hatch was aseptically collected and placed in sterile plastic sampling bags and then

weighed (Fisher Scientific, Pittsburgh, PA) from the control and treatment hatching cabinets (40). The samples (n=1/hatching cabinet) then had 100ml of TTB enrichment broth added.

BROILER PLACEMENT AND GROW-OUT

Sixty chicks from the control and 60 treatment chicks from hatches 2, 6, 7, and 8 were randomly selected and placed at 1 d of age in an open sided poultry floor pen house in separate pens with empty pens between to prevent any cross contamination. These broiler pens (1.22m x 3.05m) were equipped with a bell drinker, tube feeder, and pine litter. Twenty broilers per treatment from hatch 2 were euthanized by cervical dislocation at 42 and 56 days of age and the ceca were aseptically removed and placed into sterile plastic sampling bags (Fisher) (40). To these samples, 100ml of TTB with iodine was added and the samples were then put in a stomacher (Technar Company, Cincinnati, OH) for 1 minute. These samples were then inoculated at 41.5 C and cultured for *Salmonella* as described below. Thirty broilers per treatment were sampled at 42 days of age from hatches 6, 7, and 8 using the same methods as described above.

ENRICHMENT AND DELAYED SECONDARY ENRICHMENT

All enrichments were performed on primary samples (drag and cloacal swabs, hatch fluff, chick cultures, broiler cecum and litter cultures), which were utilized for the detection of the nalidixic acid resistant challenge strain of S. *enteritidis*. Tetrathionate broth (Hagna) was added to all samples accordingly

and incubated at 41.5 C for 18 h (49, 50) and isolation and identification performed.

Following the initial plating of all samples the original sample containing TTB with iodine was left out on the bench and allowed to incubate at room temperature for 5 days. After 5 days of incubation, all samples, which were culture negative from initial plating, underwent delayed secondary enrichment. A 1ml solution of the original sample containing TTB was added to 10ml of fresh TTB and incubated at 41.5 C overnight and subsequently plated (49, 50).

ISOLATION AND IDENTIFICATION OF SALMONELLA

A 1ul loop of the TTB was streaked onto XLT4 and BGN agar plates and incubated at 37 C overnight for all enrichment and delayed enrichment samples. The H_2S-positive isolated colonies were then placed onto triple sugar iron slants (Difco Laboratories, Detroit, MI.) and incubated at 37 C overnight (14). Suspect *Salmonella* colonies were then confirmed with poly O *Salmonella*-specific antiserum. All *Salmonella* were then stored for PCR comparison to the challenge strain.

DNA FINGERPRINTING OF SALMONELLA ISOLATES

Salmonella isolates were saved in order to demonstrate that the S. *enteritidis* isolated from the birds and their environment was the same isolate inoculated. Sixteen *Salmonella* isolates were randomly selected (4 drag swab

positives, 6 cloacal swab positives, and 6 ceca positives from broiler grow-out) and were DNA fingerprinted using the method described by Liu (23).

STATISTICAL ANALYSIS

The analysis of data was performed using the general linear model procedure of SAS (SAS V8.2, SAS Institute Inc., Cary, NC) to calculate means and standard deviations. Dust concentrations were evaluated by non-paired t-test. All bacterial sampling data were subjected to square root transformation. For *Salmonella* detection from drag swabs and broiler grow-outs the arcsign transformation was completed. All data subjected to transformations was converted back for reporting purposes in a percent reduction format. The significance level was set at P=0.05 and evaluated using analysis of variance.

Results

Dust concentrations in the control room averaged 3.75mg/m^3 and were found to be significantly different (P<0.0001) from the 1.45mg/m^3 average dust concentration in the ionizer treatment room. During weeks 8-12 air sampling from the treated room showed significantly reduced total airborne bacteria (Figure 2.1) (Appendix C). When airborne gram-negative bacteria were examined from weeks 9 through 35, the treated room had significantly lower (P<0.0001) numbers of bacteria enumerated (Figure 2.2). Air samples from weeks 11 through 28 were negative for the marker strain of *Salmonella enteritidis*. The use of XLT4 plates with the cover tray sampling method to

monitor the level of airborne S. *enteritidis* was not found to be a definitive technique to measure airborne S. *enteritidis* levels. Samples of the egg belts and eggshell surface showed significant reductions ($P<0.0001$) in the level of gram-negative bacteria present (Figure 2.3 and 2.4).

Drag swabs taken at week 1 and 2 prior to the inoculations were negative for *Salmonella* suggesting the enrofloxacin eliminated any *Salmonella* present in the birds. Drag swabs taken weekly from week 2 to 10 and cloacal swabs from all hens at week 4 were negative for the rifampicin resistant strain of S. *typhimurium*. The nalidixic acid resistant marker strain of S. *enteritidis* did colonize in the birds as determined by the positive drag and cloacal swabs and therefore reports on *Salmonella* transmission started at week 11 with S. *enteritidis* inoculants.

The drag swabs of the rooms (litter, walls, equipment, etc.) differed ($P<0.05$) in percentage positive samples. The control room averaged 36% (2.5 per 7 samples) positive, while the treatment room averaged 49% (3.4 per 7 samples) positive swabs. Fluff and 1 d old chick samples from all eight hatches were negative for the inoculated strain of S. *enteritidis*. The number of S. *enteritidis* positive cloacal swabs from the control and treatment rooms are shown in Figure 2.5 with the treatment room having 0/60 positive in comparison to the control room 2/60 positive at 4 weeks post inoculation of the S. *enteritidis* (week 11). At 2 weeks post inoculation of the second challenge inoculation (week 26), the control room had 28/60 positive and the treatment room had 12/60 positive ($P<0.05$). All other cloacal sampling times were negative in the control

and treatment rooms. The number of positive broilers from the four grow-outs is shown in Figure 2.6. Hatch 2 broilers resulted in 2/20 positive from the control birds and 0/20 being positive from the treatment birds at 42 days of age; however, at 56 days of age 9/20 broilers were positive from the control birds and 1/20 from the treatment birds ($P<0.05$). Hatches 6, 7, and 8 after a 42-day grow-out resulted in all 30 broilers from both rooms being negative for the challenge S. *enteritidis*. The S. *enteritidis* isolated from the environment, breeders and broilers during the experiment was determined by PCR to be the identical to the original S. *enteritidis* administered at week 11 and 26 (Appendix C). No other *Salmonella* isolates other than the challenge S. *enteritidis* were detected during this experiment.

Discussion

Salmonella is one of the prominent causes of food-borne illness related to the consumption of poultry meat. A reduction of *Salmonella* at the broiler breeder farm could reduce *Salmonella* transmission to the broiler progeny, and ultimately reduce *Salmonella* contamination of poultry products sold to the consumer. S. *enteritidis* (SE) was inoculated orally and subcutaneous to 10% of the hens in the control and treatment rooms and allowed to shed for several weeks. The shedding of S. *enteritidis* by the inoculated hens was monitored with drag swabs and a second inoculation of S. *enteritidis* was administered to an additional 10% of the hens when the level of shed reached low numbers on drag swabs. The ESCS treated room had a higher number of drag swab positive

samples on a weekly basis than the control. This higher number was expected since the ESCS causes airborne particles to be negatively charged and attracted to grounded surfaces (i.e., equipment, litter surface, and walls). Drag swab sampling of these surfaces should yield a higher number of positive samples in the treatment room, because the ESCS removed the bacteria along with the dust from the circulating air. This is very advantageous in a broiler breeder house that has two/thirds of the floor space slated allowing the contaminated dust to be attracted to areas out of reach of the birds.

The ESCS treated room had a 61% reduction in the amount of airborne dust within the room when compared to the control room without the ESCS. This resulted in the reduction of total bacteria over a five-week period of 76% in the treatment room versus the control room. The rooms were also sampled for a 26-week period measuring airborne gram-negative bacteria, where the ESCS treatment resulted in a 48% reduction in gram-negative bacteria when compared to the control. Gast and others have shown that S. *enteritidis* can be transmitted via the air; however, this was not observed in this study and perhaps was due to S. *enteritidis* shed occurring at too low a level to be detected by the air sampling technique used in this study (45, 33, 34, 35, 36).

When sampling the egg belts and eggshell surface gram-negative bacteria were significantly reduced. Sixty-three percent fewer gram-negative bacteria were recorded in the ESCS treated room on the egg-belt surface, which contributed to a 23% decline in gram-negative bacteria on the eggshell surface. This reduction of bacteria on the eggshell surface could potentially lower the

number of bacteria invading the egg especially if eggs are not stored under proper conditions and condensation develops on the shell surface. The reason for reduction of bacteria on the eggshells of the ESCS room was most likely due, to lower amounts of airborne bacteria laden dust present to fall on the freshly laid eggs.

Large variations in the level of total and gram-negative bacteria were seen throughout the study, and as previously reported with the use of the ESCS in pullet rearing facilities, this variation appears to be primarily associated with the daily activity of the workers feeding, weighing, and collecting eggs and ventilation rate changes with changes in ambient temperature (46).

To monitor the level of horizontal spread of S. *enteritidis* from the inoculated hens to the un-inoculated hens, cloacal swabs were used. The level of horizontal transmission was higher in the control room during two sample times as detected by cloacal swabs but these differences were not consistently detectable. This could be due to the low rate of shed at the latter sample times and the low incidence of recovery associated with *Salmonella* even when the shed rate is high. It has been known that cloacal swabs are not an extremely sensitive means of detecting *Salmonella* due to the sporadic shed of *Salmonella* from the intestinal tract. However, cloacal swabs were done in an attempt to demonstrate the horizontal bird to bird spread of the challenged *Salmonella* in each room.

When the shed of S. *enteritidis* was at its highest, a higher number of S. *enteritidis* positive broilers were recovered in the hatched broilers, but these

findings were not repeated in broilers from subsequent hatches. This finding may be attributed to the traditionally low occurrence of *Salmonella* transmission via the egg. The incidence of S. *enteritidis* egg transmission has been shown to be approximately 1 in 10,000 (51). This may also explain the low incidence of S. *enteritidis* positive samples from the fluff from the hatchers, and the internal organ cultures of the day old chicks.

The electrostatic space charge system reduced airborne dust levels, which resulted in a reduction in both total bacteria and gram-negative bacteria. The ESCS was also associated with reductions in egg belt surface gram-negative bacteria and hence a reduction in the potential eggshell contaminants. The ESCS treated room had fewer cloacal swab S. *enteritidis* positive hens resulting in fewer S. *enteritidis* positive broilers. The ESCS caused dust and bacteria to adhere to the walls, equipment, and litter instead of constantly being circulated in the air and inhaled by the bird. Therefore, this research has demonstrated that, the ESCS can be used to reduce airborne bacteria in a broiler breeder flock and reduce horizontal *Salmonella* transmission between birds and in turn transmission to eggs and progeny.

References

1. Bailey, J. S., R. J. Buhr, N. A. Cox, and M. E. Berrang, 1996. Effect of hatching cabinet sanitation treatments of *Salmonella* cross-contamination and hatchability of broiler eggs. Poultry Sci. 75: 191-196.
2. Bailey, J. S., N. A. Cox, and L. C. Blankenship, 1990. Persistence and spread of external *Salmonella* contamination during broiler production. Poultry Sci. 69S: 154.
3. Baskerville, A., T. J. Humphrey, R. B. Fitzgeorgel, R. W. Cook, H. Chart, B. Rowe, and A. Whitehead, 1992. Airborne infection of laying hens with *Salmonella enteritidis* phage type 4. Vet. Rec. 130: 395-398.
4. Bean, N. H. and P. M. Griffin, 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles, and trends. J. Food Prot. 53: 804-817.
5. Bender, F. E., and E. T. Mallinson, 1991. Healthy birds are lower cost birds. Broiler Industry. January, pp. 62-64.
6. Byrd, J. A., D. E. Corrier, J. R. Deloach, and D. J. Nisbet, 1997. Comparison of drag-swab environment protocols for the isolation of *Salmonella* in poultry houses. Avian Dis. 41: 709-713.
7. Carr L. E., E. T. Mallinson, C. R. Tate, 1995. Prevalence of *Salmonella* in broiler Flocks: effect of litter water activity, house construction, and watering devices. Avian Dis. 39: 39-44.

8. Cason, J. A., N. A. Cox, and J. S. Bailey, 1994. Transmission of *Salmonella typhimurium* during hatching of broiler chicks. Avian Dis. 38: 583-588.
9. Clemmer, D. J., J. L. S. Hickey, J. F. Bridges, D. J. Schliessmann, and M. F. Shaffer, 1960. Bacteriological studies of experimental airborne salmonellosis in chicks. J. Infect. Dis. 106: 197-210.
10. Committee on Public Health Risk Assessment of Poultry Inspection Programs, 1987. Application of the model to the current FSIS inspection system. Poultry inspection: the basis for a risk-assessment approach. National Academic Press, Washington, D. C. pp. 33-36, 84.
11. Cox, N. A., J. S. Bailey, J. M. Mauldin, and L. C. Blankenship, 1990. Presence and impact of salmonellae contamination in commercial integrated broiler hatchery. Poultry Sci. 69: 1606-1609.
12. Gast, R. K., B. W. Mitchell, and P. S. Holt, 1999. Application of negative air ionization for reducing experimental airborne transmission of *Salmonella enteritidis* to chicks. Poultry Sci. 78: 57-61.
13. Gordon, R. F., and J. F. Tucker, 1965. The epizootiology of *Salmonella menstn* infection of fowls and the effect of feeding poultry food artificially infected with *Salmonella*. Br. Poultry Sci. 6: 251-264.
14. Hagna, A. A. and S. R. Damon, 1956. New enrichment and plating media for the isolation of *Salmonella* and *Shigella* organisms. Appl. Microbiol. 4: 341-345.

15. Henzler, D. J., and H. M. Opitz, 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. Avian Dis. 36 625-631.
16. Holt, P. S., B. W. Mitchell, K. H. Seo, and R. K. Gast, 1999. Use of negative air ionization for reducing airborne levels of *Salmonella Enterica* serovar *enteritidis* in a room containing infected caged layers. J. Appl. Poultry Res. 8: 440-446.
17. Holt, P. S., 1995. Horizontal transmission of *Salmonella enteritidis* in molted and unmolted laying chickens. Avian Dis. 39: 239-249.
18. Jones, F. T., 1992. Breeder flock study shows *Salmonella*-causing factors. Feed Stuffs 64(March): 1, 22-23.
19. Jones, F. T., 1990. *Salmonella* control in grow out, in Proceedings. 25th Natl Meet Poultry Health Proc. pg. 73-76.
20. Krueger, A. P., and E. J. Reed, 1976. Biological impact of small air ions. Science 193: 1209-1213.
21. Krueger, A. P., R. F. Smith, and I. G. Go, 1957. The action of air ions on bacteria. J. Gen. Physiol. 41: 359-381.
22. Lahellec, C., and P. Colin, 1985. Relationship between serotypes of salmonellae from hatcheries and rearing farms and those from processed poultry carcasses. Br. Poultry Sci. 26: 179-186.
23. Liu, T., K. Liljebjelke, E. Bartlett, C. L. Hofacre, S. Sanchez and J. J. Maurer, 2002. Application of nested polymerase chain reaction to detection of *Salmonella* in poultry environment. Avian Dis. (In Press)

24. Levine, W. C., J. F. Smart, D. L. Archer, N. H. Bean, and R. V. Tauxe, 1991. Foodborne disease outbreaks in nursing homes, 1975 through 1987. J. Am. Med. Assoc. 266: 2105-2109.

25. Mallinson, E. T., S. W. Joseph, C. L. E. Derezende, N. L. Tablante, and L. E. Carr, 2001. *Salmonella* control and quality assurance at the farm end of the food safety continuum. Am. Vet. Med. 218(June): 1919-1922.

26. Miles, R. D., and G. D. Butcher, 1993. *Salmonella*- controlling it in the broiler, egg industry. Feedstuffs 65: 1, 24-45.

27. Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Scherrer, 1991. Xylose-Lysine-Tergitol 4 an improved selective agar medium for the isolation of *Salmonella*. Poultry Sci. 70: 2429-2432.

28. Mishu, B., J. Koehler, L. A. Lee, D. Rodrigue, F. H. Brenner, P. Blake and R. V. Tauxe, 1994. Outbreaks of *Salmonella enteritidis* infections in the United States, 1985-1991. J. Infect. Dis. 169: 547-552.

29. Mitchell, B. W., L. J. Richardson, C. L. Hofacre, and J. L. Wilson, 2002. Application of an electrostatic space charge system for dust, ammonia and pathogen reduction in a broiler breeder house. ASAE, Annual International Meeting/CIGR XVth World Congress.

30. Mitchell, B. W., R. J. Buhr, M. E. Berrang, J. S. Bailey, and N. A. Cox, 2002. Reducing airborne pathogens, dust and *Salmonella* transmission in experimental hatching cabinets using an electrostatic space charge system. Poultry Sci. 81: 49-55.

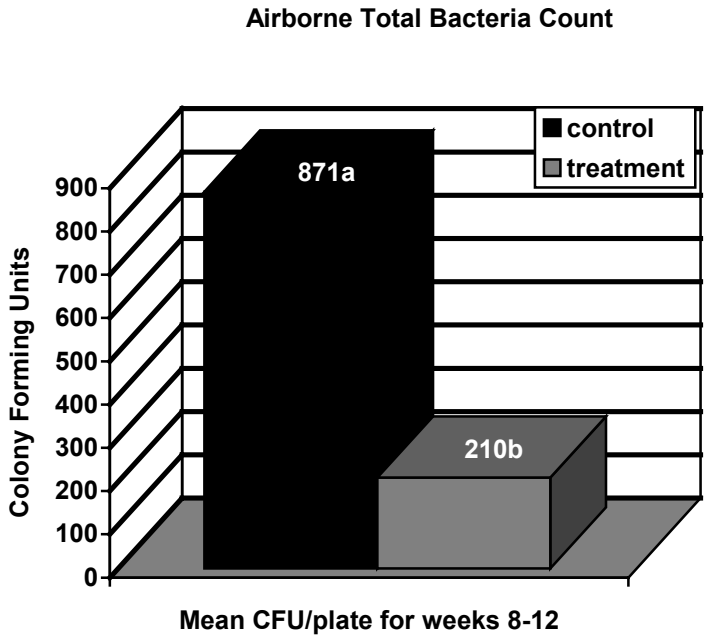
31. Mitchell, B. W., P. S. Holt, and K. S. Seo, 2000. Reducing dust in a caged layer room: An electrostatic space charge system. J. Appl. Poultry Res. 9: 292-296.
32. Mitchell, B. W., and H. S. Stone, 2000. Electrostatic reduction system for reducing airborne dust and microorganisms. U. S. Patent No. 6,126,722.
33. Mitchell, B. W., and D. J. King, 1994. Effect of negative air ionization on airborne transmission of Newcastle disease virus. Avian Dis. 38: 725-732.
34. Muira, S., G. Sato, and T. Miyamae, 1964. Occurrence and survival of *Salmonella* organisms in hatchery chick fluff in commercial hatcheries. Avian Dis. 8: 546-554.
35. Nakamura, M., M. Takagi, T. Takahashi, S. Suzuki, S. Sato, and K. Takehara, 1997. The effect of the flow of air on horizontal transmission of *Salmonella enteritidis* in chickens. Avian Dis. 41: 354-360.
36. O'brien, J. D. P., 1990. Aspects of *Salmonella enteritidis* control in poultry. World's Poultry Sci. 46: 119-124.
37. Olsen, S. J., L. C. MacKinnon, J. S. Goulding, N. H. Bean, and L. Slutsker, 2000. Surveillance for foodborne disease outbreaks- United States, 1993-1997. Morb. Mortal Wkly. Rep. 49: 1-62.
38. Opara, O. O., L. E. Carr, E. Russek-Cohen, C. R. Tate, E. T. Mallinson, R. G. Miller, L. E. Stewart, R. W. Johnston, and S. W. Joseph, 1992. Correlation of water activity and other environmental conditions with repeated detection of *Salmonella* contamination on poultry farms. Avian Dis. 36:664-671.

39. Poppe, C., R. P. Johnson, C. M. Forsberg, and R. J. Irwin, 1992. *Salmonella enteritidis* and other *Salmonella* in laying hens and eggs from flocks with *Salmonella* in their environment. Can. J. Vet. Res. 56: 226-232.
40. U. S. Department of Agriculture (USDA), 1996. National Poultry Improvement Plan and auxiliary provisions. Washington, DC: Animal Plant Health Inspection Service, Veterinary Service, U. S. Department of Agriculture.
41. Richardson, L. J., B. W. Mitchell, C. L. Hofacre, and J. L. Wilson, 2002. Effect of an electrostatic space charge system on airborne dust and microorganism reduction during the rearing of broiler breeder pullets. Avian Dis. (In Press).
42. Rose, J. B., C. N. Haas, and C. P. Gerba, 1996. HAACP: An integrated approach to assuring the microbiological safety of meat and poultry. Food and Nutritional Press, Inc., Trumbell, CT. Pages 159-169.
43. Seo, K. H., B. W. Mitchell, P. S. Holt, and R. K. Gast, 2000. Bacterial effects of negative ions on airborne and surface *Salmonella enteritidis* from an artificially generated aerosol. J. Food Prot. 64(1): 113-116.
44. Shivasprasad, H. L., J. F. Timoney, S. Morales, B. Lucio, and R. C. Baker, 1990. Pathogenesis of *Salmonella enteritidis* infection in laying chickens. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses. Avian Dis. 34: 548-557.

45. Smyster, C. F., N. adinarayanan, H. van Roekel, and G. H. Snoeyenbos, 1966. Field and laboratory observations on *Salmonella heidelberg* infections in three chicken breeding flocks. Avian Dis. 10: 314-329.
46. Subcommittee on microbiological criteria for foods and food ingredients, 1985. Introduction and expansion of the HACCP system in food protection programs. In: An evaluation of the role of microbiological criteria for foods and food ingredients. Washington, DC: National Academy Press pp 41-54, 308-328.
47. Tauxe, R. V., 1996. An update on *Salmonella*. Health Environ. 10: 1-4.
48. Waltman, W. D., 2000. Research examines *Salmonella* detection. Poultry Times 47 (12): 1-3.
49. Waltman, W. D., A. M. Horne, and C. Pirkle, 1993. Influence of enrichment incubation time on the isolation of *Salmonella.* Avian Dis. 37: 884-887.
50. Waltman, W. D., A. M. Horne, C. Pirkle, and T. Dickson, 1991. Use of delayed secondary enrichment for the isolation of *Salmonella* in poultry and poultry environments. Avian Dis. 35: 88-92.
51. Wilding, G. P., and C. Baxter-Jones, 1985. Egg-borne salmonellas: is prevention feasible? In: Proceeding of the International Symposium on *Salmonella*, New Orleans, G. H. Snoeyenbos, ed. Am. Assoc. of Avian Pathologists, Kennett square, PA. pp 126-133.

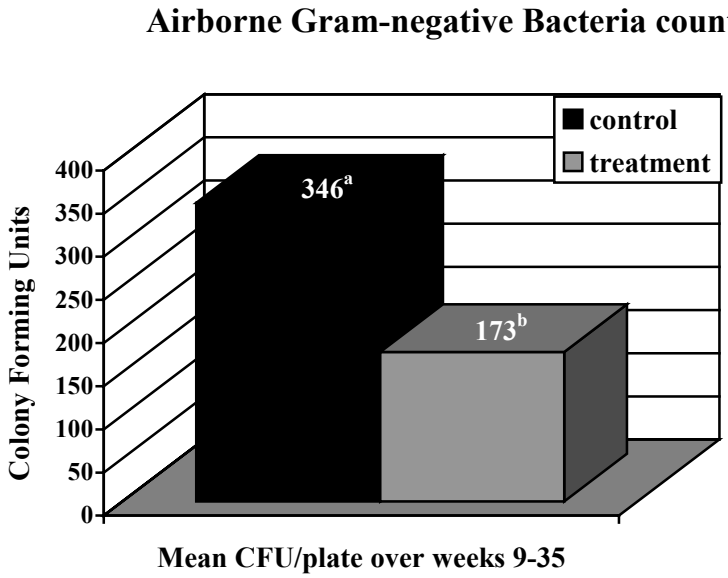
Figures

Figure 2.1. Mean number of colony forming units (CFU) from open-air blood agar plates[*].



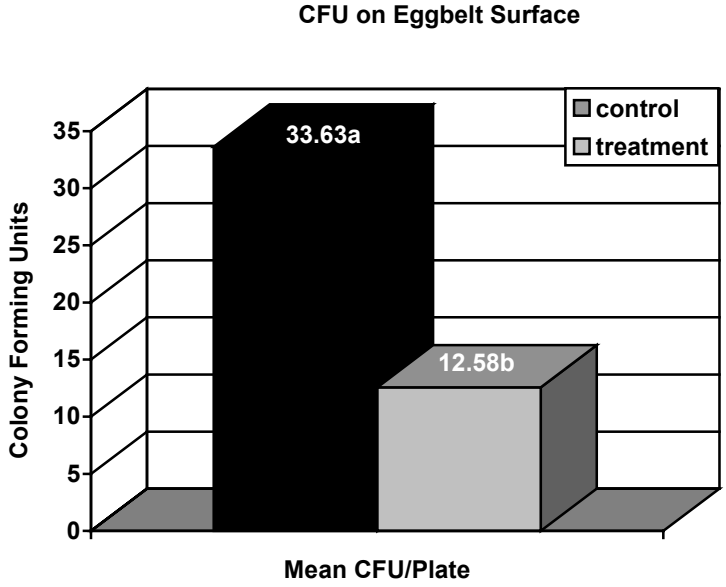
[b]The mean number (210cfu/plate +/- SD 118cfu/plate, n = 36) of total bacteria inside the treatment room was significantly (P<0.0001) less during weeks 8 to 12 than that (871cfu/plate +/- SD 217cfu/plate, n = 36) of the control room.

Figure 2.2. Mean colony-forming units of gram-negative bacteria*.



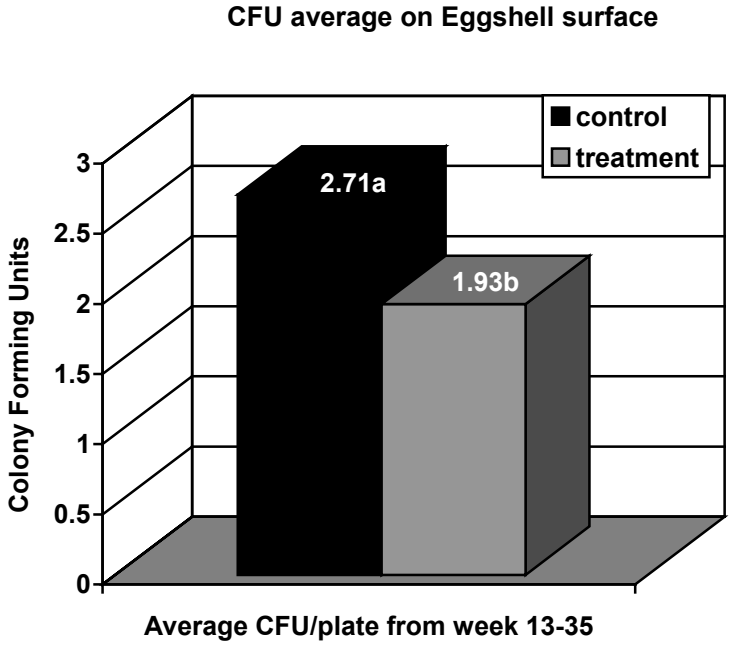
[b]The mean number (173cfu/plate +/- SD 58cfu/plate, n = 190) of gram-negative bacteria in the treatment room was significantly (P<0.0001) less than that (346cfu/plate +/- SD 104cfu/plate, n = 190) of the control room.

Figure 2.3. The number of gram-negative bacteria on the egg belt surface[*].



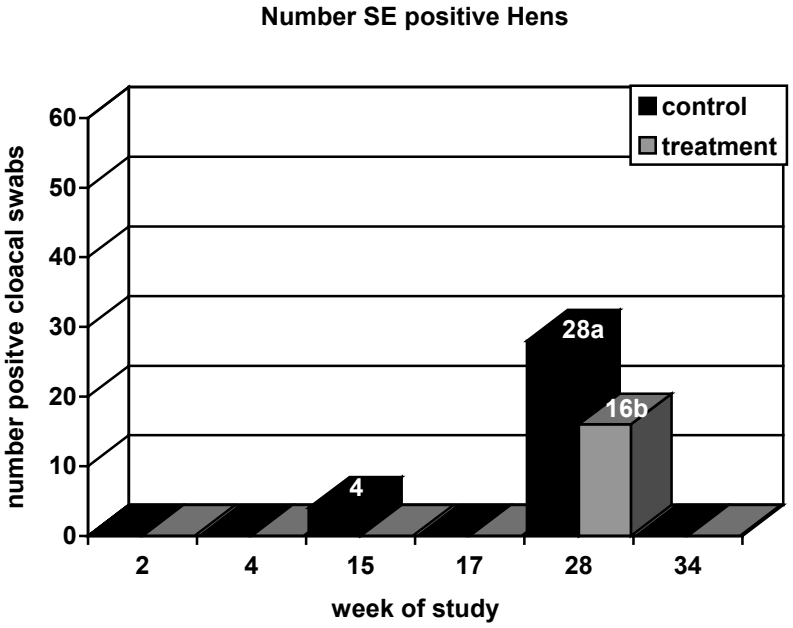
[b]The mean number (33.63cfu/plate +/- SD 22.58cfu/plate, n = 19) of gram-negative bacteria on the egg-belt surface in the treatment room was significantly ($P<0.05$) less than (12.58cfu/plate +/- SD 9.41cfu/plate, n = 19) of the control room.

Figure 2.4. The number of gram-negative bacteria on the eggshell surface[*].



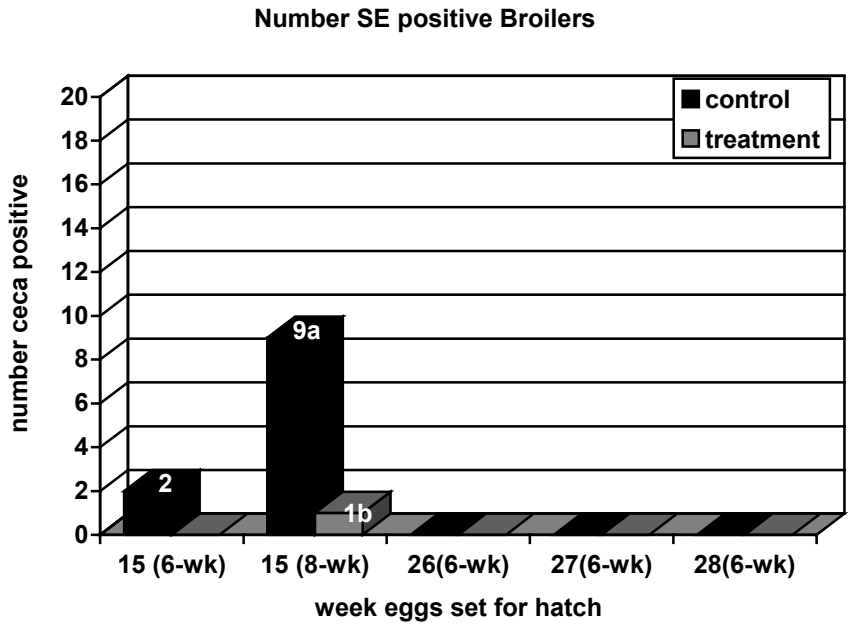
[b]The mean number (1.93cfu/plate +/- SD 4.95cfu/plate, n = 1021) of gram-negative bacteria on the eggshell surface in the treatment room was significantly (P<0.0001) less than that (2.71cfu/plate +/- SD 6.95cfu/plate, n = 1021) of the control.

Figure 2.5. The number of SE positive cloacal swabs from the broiler breeder hens*.



[b]The number (16, n = 60) of SE positive hens in the treatment room was significantly (P<0.05) less at week 25 than that (28, n = 60) of the control room.

Figure 2.6. A graph showing the number of SE positive broilers from ceca examination*.



[b]The number (9, n = 20) of SE positive broilers hatched from the treatment room eggs saved at week 15 was significantly (P<0.05) less than that (1, n=20) of the control room saved eggs.

CONCLUSION

These experiments showed that a reduction in airborne dust concentrations correlated with reductions in airborne bacteria and also that a reduction in *Salmonella* transmission was capable with the use of an electrostatic space charge system in broiler breeder confinement rearing facilities. By reducing the horizontal airborne mode of transmission of *Salmonella*, a reduction in the vertical mode of transmission via the egg was accomplished.

High levels of dust and microorganisms were associated with the experimental broiler breeder pullet and lay confinement-rearing facilities. The amount of airborne dust and bacteria present in the air varied from day to day in both experiments. The variation in both experiments was due to ambient temperature changes and daily differences in activity inside the rooms. Open-air plate method of sampling was not found to be effective in these types of environments for long sampling periods. However a cover tray method of air sampling was found to give a longer sampling period by allowing diffusion of small particulates and exclusion of large debris such as feathers.

Many of the microorganisms were carried by dust particles, thus provided an excellent vector for horizontal disease transmission between birds. The ESCS negatively charged airborne particulates in both experiments and caused these particulates to be attracted to the grounded surfaces. This reduced the amount of airborne dust and bacteria in the birds environment.

Airborne dust levels were reduced by an average of 37% over a one-week period in the experimental pullet treatment room compared to the control room. The use of the ESCS resulted in a 64% mean reduction in gram-negative bacteria in the pullet facility over the eight-week period. However, the naturally occurring *Salmonella* group C-2 could not be detected in the air.

In the broiler breeder study, during the experimental lay cycle of 35 weeks, high levels of circulating dust was noted in the control room. As this dust became airborne, accumulations of the dust could be seen on the walls, ceiling and equipment. The use of the ESCS resulted in a 61% reduction in airborne dust levels, which resulted in a 76% reduction in total airborne bacteria and a 48% reduction in airborne gram-negative bacteria in the treatment room. A 63% reduction of gram-negative bacteria on the egg collection belts was also recorded in the treatment room, which resulted in a 28% reduction of gram-negative bacteria on the eggshell surface. The ESCS resulted in fewer S. *enteritidis* positive hens and their progeny (broilers) in the treatment room due to reductions of airborne dust and bacteria. The ESCS also had no negative effect on bird performance such as body weights, egg production, mortality, or fertility.

The reductions of airborne dust and bacteria in these experiments are comparable to similar studies where ionization has been used to reduce airborne particulates and bacteria and could aid in improving the air quality of poultry confinement rearing facilities and reduce horizontal air transmission of microorganisms and therefore vertical disease transmission inside these facilities.

APPENDICES

The appendix is added to give the reader a clearer understand on the effects of the electrostatic space charge system in these confinement-rearing facilities and briefly discuss material that was not extensively conveyed in the manuscripts without an extensive review of the literature:

1) Production parameters (manuscript 2)

and also to have a visual illustration of the:

2) ESCS system
3) Experimental room design
4) Reductions in airborne bacteria on media plates
5) PCR illustration

in order for the reader to have a better appreciation of the ESCS system in these types of environments.

Appendix A

PRODUCTION PARAMETERS (MANUSCRIPT 2)

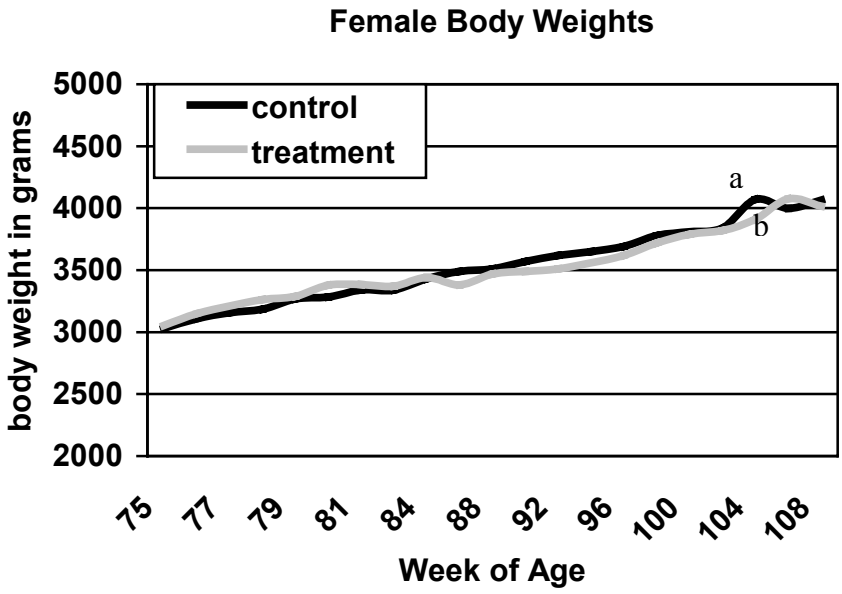
Broiler breeder female body weights were not found to be significantly different in the treatment room compared to the control (Figure A.1). The broiler breeder rooster's body weights for the control and treatment rooms were also found not to be significantly different (Figure A.2). Egg production peaked out for

the control room females at 75% where in the treatment room egg production peaked out at 76% and was not found to be significantly different (Figure A.3). The females in the treatment room had 8% (25/308) mortality compared to the treatment rooms 9% (27/308) mortality, but no significant difference was found ($P>0.05$). The males in the control room had 18% (6/33) mortality compared to the treatment rooms mortality of 12% (4/33) but was not found to be significantly different ($P>0.05$). Reproductive performance was not found to be significantly different ($P>0.05$) in the control and treatment room (Table A.1).

The dissimilarity in body weights between the control and treatment room were minor and the variation was not associated with the use of the electrostatic space charge system. If you factor in the age of the broiler breeder hens and that these birds were molted and carried until 108 weeks of age, this would account for the variations in uniformity between the birds in the control and treatment room. The broiler breeder roosters also had variations in body weights but as commonly known this is typically seen in these heavy breed lines. Since no significant differences was reported in body weights, this suggest that the electrostatic space charge system did not cause a static charge that would deter the broiler breeder males or females from eating out of the pan and trough feeding systems as previously thought. Egg production was also not effected from the use of the ESCS, indicating no effect to the laying efficiency of the broiler breeder hens. There was also not a major difference in hen mortality but a non-significant difference in rooster mortality was observed in the control room. The number of males placed in each experimental room was low and a statistical

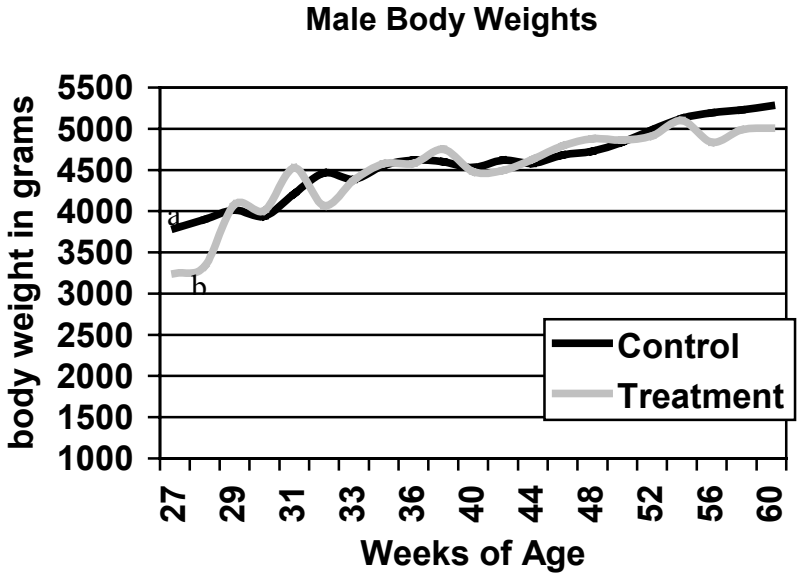
difference could not be stated however a 6% divergence in mortality was observed. The fertility in the treatment room was slightly higher than the fertility in the control room but the difference was not large enough to be significant and many other factors could have led to the increase of fertility in the treatment room. The higher fertility in the treatment room could have been due to the lower number of mortality associated with roosters in the treatment room allowing a lower female to male ratio. The electrostatic space charge system proved to be an effective tool in reducing environmental and microbial concerns without affecting any of the production parameters measured in this study and therefore could be used in a poultry confinement rearing facility housing broiler breeders.

Figure A.1. Graph of the female body weights in the control and treatment rooms throughout the 35-week laying cycle[*].



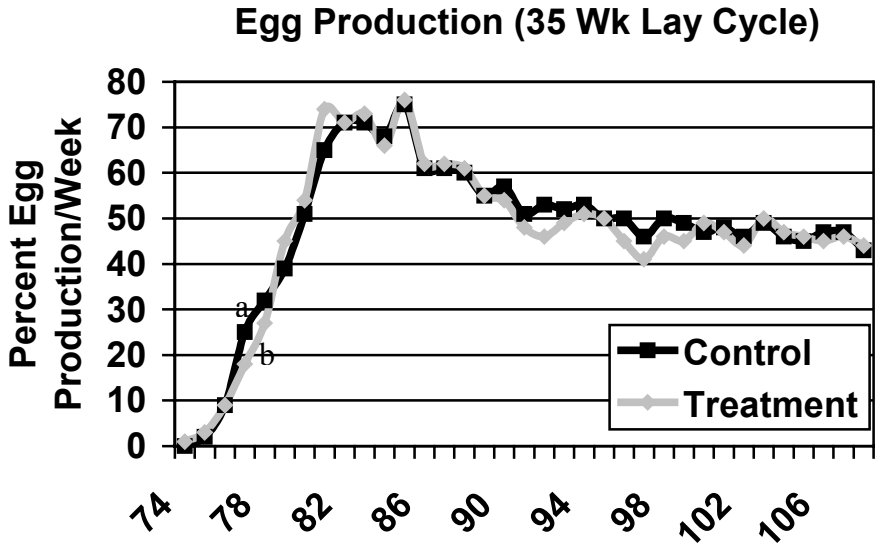
[a]The mean body weights in the control room (3535g +/- SD 315.1g, n = 35) were not found to be significantly (P>0.05) different to the treatment (3519g +/- SD 281.3g, n = 35) room.

Figure A.2. Graph of the male body weights in the control and treatment rooms throughout the 35-week lay cycle*.



[a]The mean body weight (4584g +/- SD 439.6g, n = 35) of the control room was not found to be significantly (P>0.05) different from that (4502g +/- SD 508g, n= 35) of the treatment room.

Figure A.3. Weekly egg production from the control and treatment rooms throughout the 35-week laying cycle*.



[a]The mean percent of eggs laid (47.8%) in the control room was not significantly (P>0.05) different than (47.1%) the mean percent laid in the treatment room.

Table A.1. Reproductive performance in the control room and the treatment room from the eight hatches from the experiment*.

REPRODUCTIVE PERFORMANCE		
Break Out Analysis	**[a]Control**	**[b]Treatment**
Fertility	**92.37%**	**93.04%**
Early Dead	**3.62%**	**4.19%**
Mid Dead	**0.01%**	**0.03%**
Late Dead	**1.97%**	**1.66%**
Pips	**0.05%**	**0.07%**
Hatchability	**86%**	**87%**
Contaminated	**0.03%**	**0.02%**

[a]There were no significant (P>0.05) differences in the reproductive performances in the control room when compared to the treatment room.

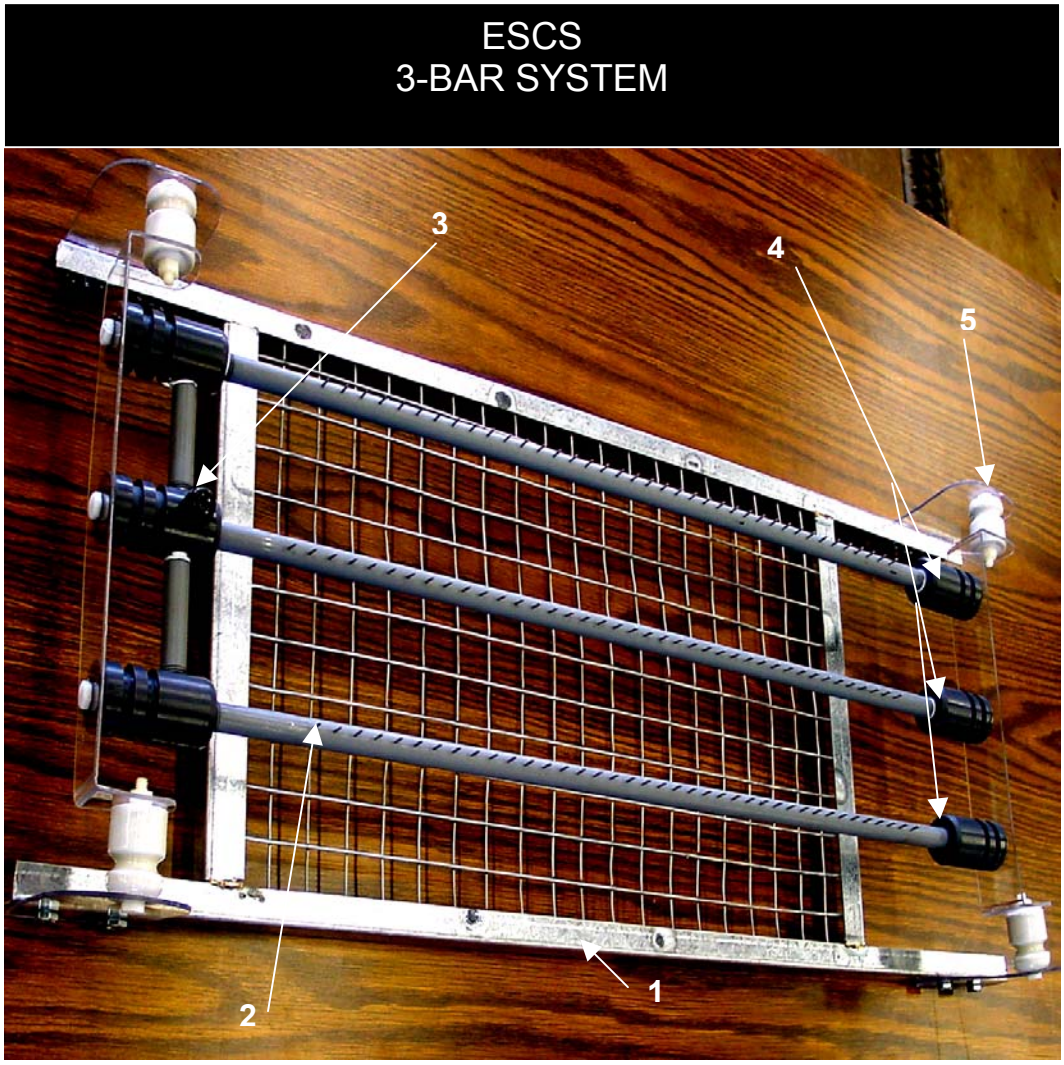
APPENDIX B

PULLET EXPERIMENT

Figure B.1. Visual illustration of the experimental room design for the pullet confinement rearing trial.

EXPERIMENTAL ROOM DESIGN IN THE PULLET CONFINEMENT REARING TRIAL

Figure B.2. Representative picture of the electrostatic space charge system*.



1. Ground plane
2. Pine point electrode
3. High voltage input connector
4. High voltage conductors
5. High voltage insulators

Figure B.3. Representative picture of the reduction in gram-negative bacteria in the pullet confinement facility experiment. Illustrates the reduction in the treatment room compared to the control room on MacConkey agar plates.

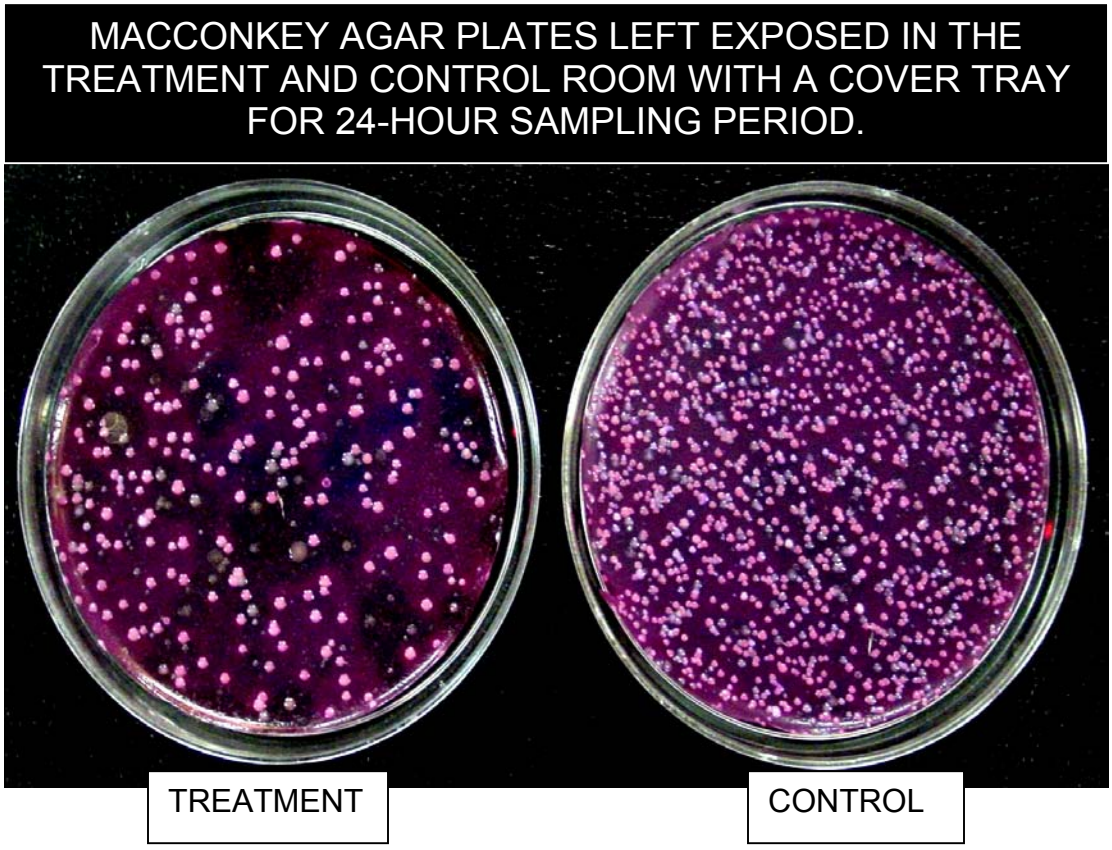
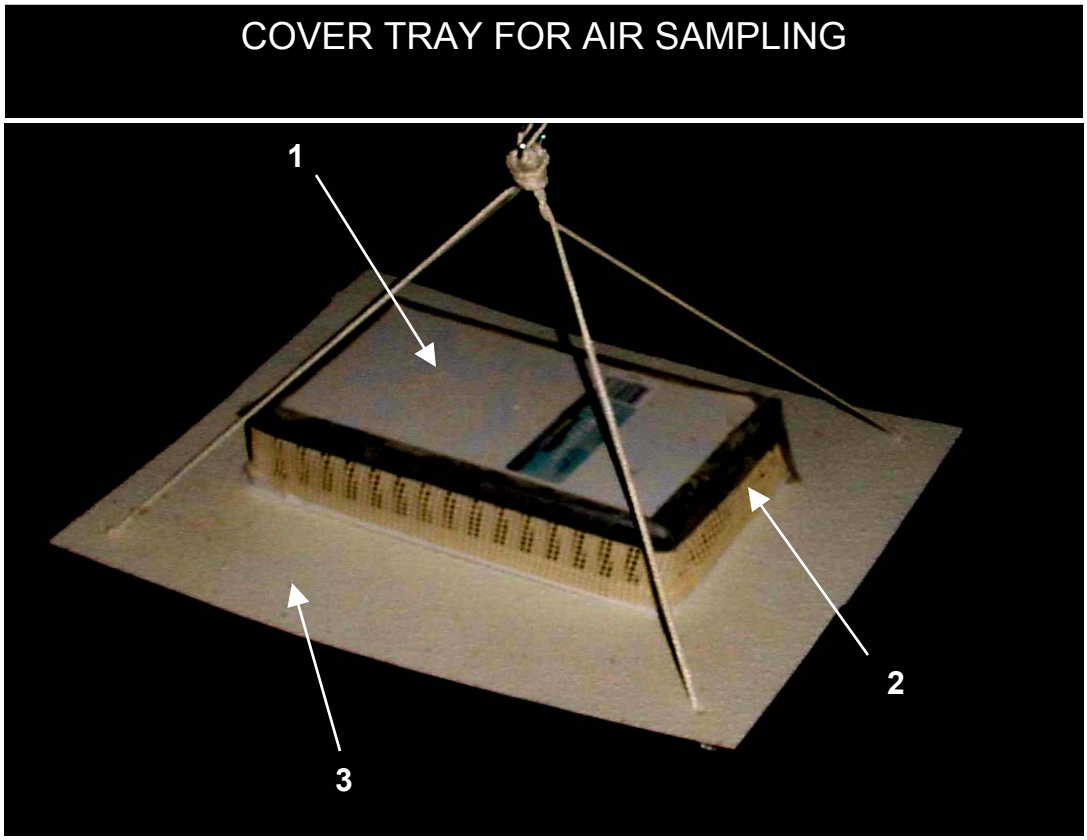


Figure B.4. Cover tray developed for use in air sampling airborne bacteria within the confinement rearing facilities*.



1. Utility bin
2. Perforated rubber shelf liner
3. Sampling platform

APPENDIX C

LAY EXPERIMENT

Figure C.1. Visual illustration of the experimental room design for the broiler breeder laying cycle trial in confinement rearing trial.

EXPERIMENTAL ROOM DESIGN

Figure C.2. ESCS system installed in the center of the room directly below two circulating fans.

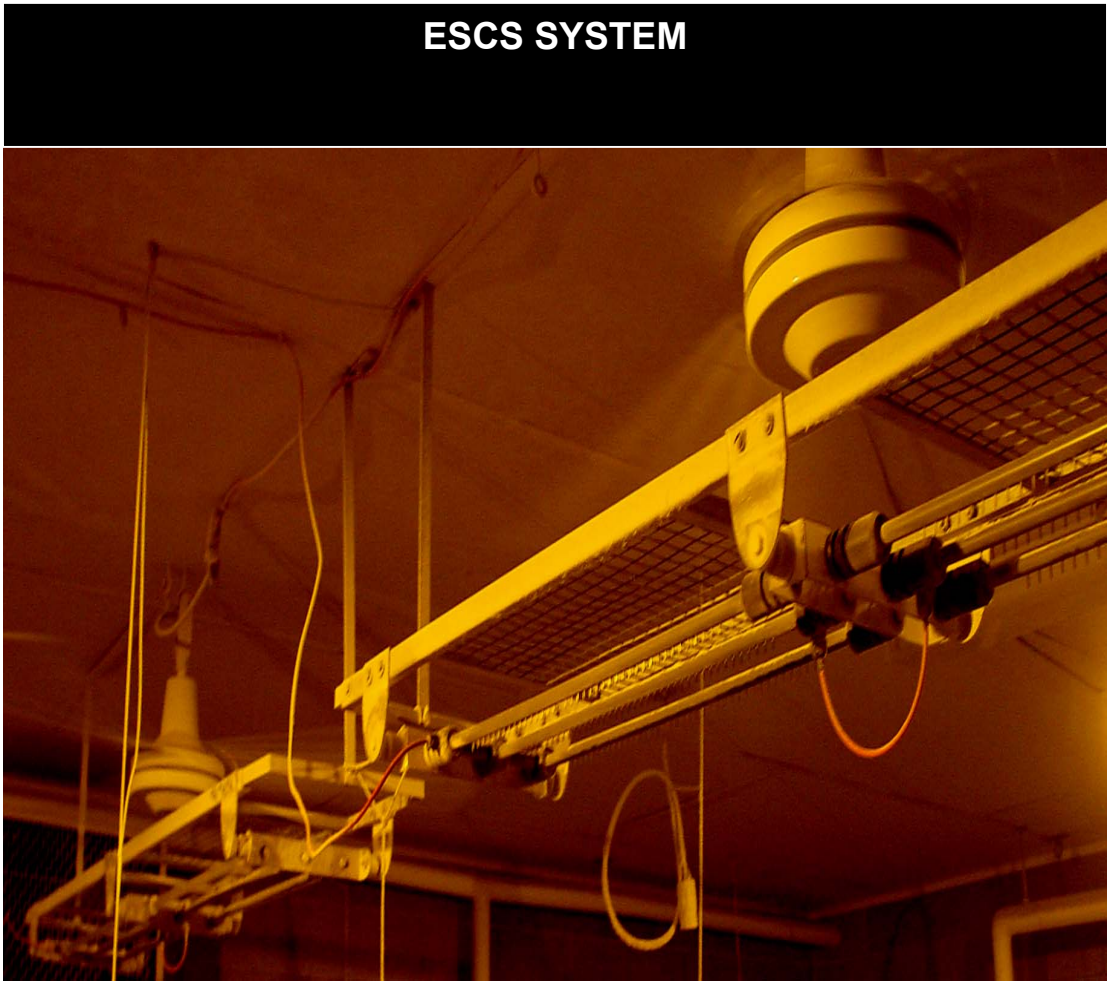


Figure C.3. Representative picture of the reduction in total bacteria in the broiler breeder lay experiment. Illustrates the reduction in the treatment room compared to the control room on Blood agar plates.

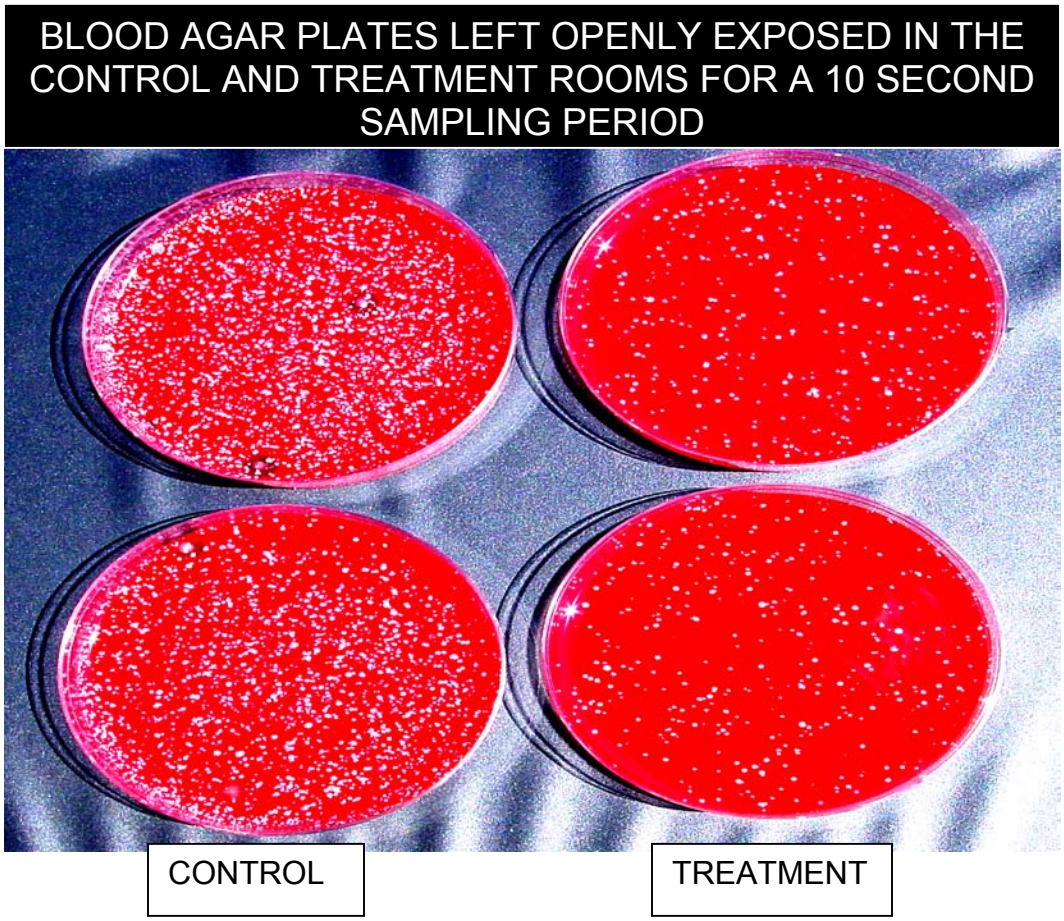
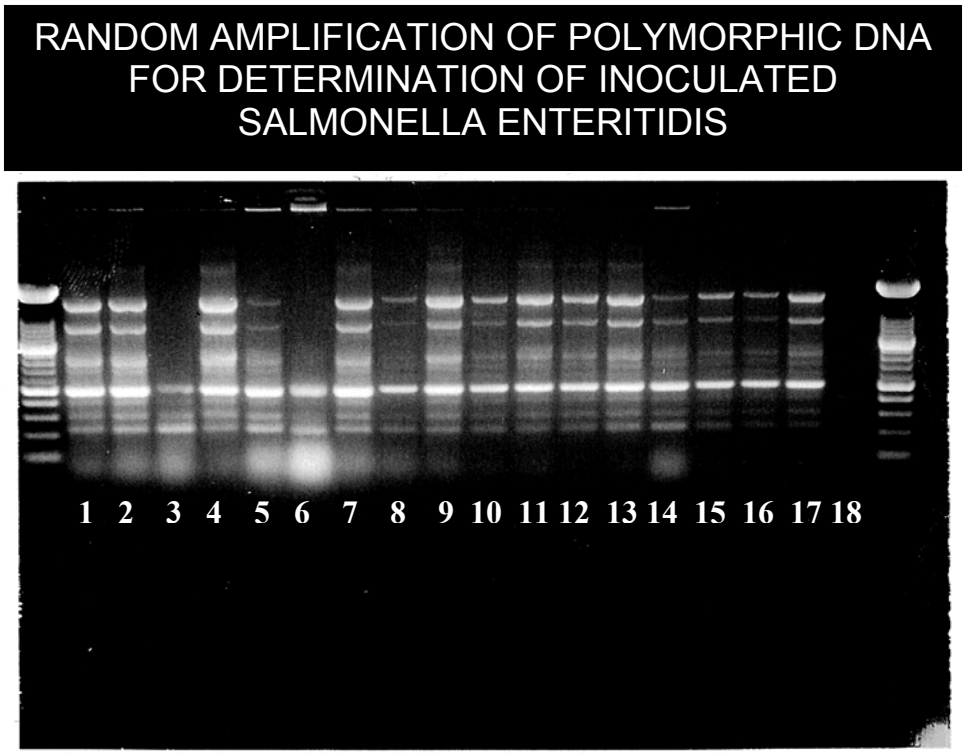


Figure C.4. Rapid Amplification of Polymorphic DNA utilizing PCR in order to confirm that the inoculated S. *enteritidis* is the same S. *enteritidis* recovered from environmental samples*.



1. Original S. enteritidis inoculated into the broiler breeder hens at week 11 and 25.
2-6. Randomly picked S. enteritidis recovered from drag swab samples.
7-11. Randomly picked S. enteritidis recovered from cloacal swab samples.
12-17. Randomly picked S. enteritidis recovered from ceca of broilers.
18. Control.