EFFECTS OF PROCESSING ON THE MICROBIOLOGY OF COMMERCIAL SHELL EGGS

by

MICHAEL THOMAS MUSGROVE

(Under the Direction of Mark A. Harrison)

ABSTRACT

Since passage of the Egg Products Inspection Act in 1970, there have been many changes in the way shell eggs are processed. Modern operations have been designed to improve external and internal physical quality while maintaining or improving the bacteriological quality of eggs. Currently, egg regulations are being restructured to emphasize safety. Scientific data from commercial operations are required for the design of effective regulations. This information is also of use to the egg industry as they formulate Hazard Analysis and Critical Control Point (HACCP) plans. Experiments were performed to provide data on how modern processing conditions affect survival of aerobic mesophilic microorganisms, yeasts/molds, *Enterobacteriaceae*, *Escherichia coli*, and *Salmonella*. While none of these populations was eliminated from the shells of processed eggs, microbial prevalence and population numbers were reduced early in the process. Isolates obtained from *Enterobacteriaceae* analyses were identified to genus or species to determine prevalence and disappearance of bacteria as eggs were processed. All species decreased as a result of processing. Additionally, information was obtained concerning appropriate methods for recovery of aerobes, *Enterobacteriaceae*, and *Salmonella* from pre-process, in-process, and post-process egg shells.

INDEX WORDS: commercial shell eggs, microbiology, *Salmonella*, yeasts, molds, *Escherichia coli*, *Enterobacteriaceae*

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To Paul Blais and Patsy Mason.

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CHAPTER 1

INTRODUCTION

Purpose of the Study

Eggs, a nutritious and inexpensive food, are an important part of human diets world-wide (McNamara, 2003). Modern operations allow for the washing and packaging of thousands of eggs an hour (Klippen, 1990). Since large-scale operations became prevalent in the 1970s, there have been many modifications to the process (Moats, 1978; Hutchison et al., 2003). Understanding how shell egg microbial populations are affected by processing is important to ensuring product quality and safety.

Hazard Analysis and Critical Control Point (HACCP) management systems are used by the Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA) to ensure the safety of meat, poultry, seafood, and other foods (USDA, 1996). The effectiveness of HACCP relies heavily upon published scientific data. FSIS is currently drafting HACCP documentation for the shell egg processing industry that will mimic regulations already in place for meat and poultry plants. A great deal of work has been published on the effect of processing on broiler carcass (Bailey et al., 1987). As a result, step-by-step fluctuations in various microbial populations on broiler carcasses have been determined. This has assisted researchers, industry, and regulators in pin-pointing specific steps that were ultimately determined to be critical control points in a HACCP plan. However, comparable information for shell egg processing facilities is not currently available. Data obtained in this study would provide the needed information.

Recently, we conducted sanitation surveys of egg processing facilities in the Southeast (Jones et al., 2003; Musgrove et al., 2004). Our data indicated that aerobic organisms and *Enterobacteriaceae* (family of bacteria that includes coliforms, fecal coliforms, *Escherichia coli*,

Salmonella, and other genera) (Kornacki and Johnson, 2001) can be recovered from most egg contact and non-contact equipment surfaces. It has not been determined if these bacterial populations on shell egg surfaces or membranes fluctuate throughout processing. In some cases, base-line information gathered in the current study will be critical as researchers and industry focus their attention on process steps that significantly contribute to cross-contamination during processing. Just as importantly, these data could provide information to debate unnecessary regulatory action at stages of processing that do not affect product safety.

HACCP implementation costs for the broiler industry have been estimated to be over 100 million dollars a year (USDA, 1996). The egg industry functions under a narrow profit margin, at times operating at a loss (American Egg Board, 2004). As a result, most egg processors do not have the resources to conduct the type of background work needed to develop a HACCP plan on their own. The man hours, equipment, and materials required to complete this project are considerable and some egg companies will not have the expertise or equipment needed to gather the needed microbiological data. Base-line information gained by monitoring important indicator populations during processing may help researchers and industry decision makers determine where to focus their attention in terms of sanitation control and in writing sanitation standard operating procedures. Scientific information on pathogen and indicator bacterial population fluctuations during commercial operations will be useful in determining important areas for process control and in designing effective HACCP plans.

Many of the studies that have characterized egg shell microorganisms have focused on spoilage flora (Mayes and Takeballi, 1995). Other studies have concentrated solely on identifying serovars of *Salmonella* (Jones et al., 1995). While both types of information are

useful, it is also important to know more about other species that contaminate the shell before and after commercial processing.

Methods determine the quality and quantity of microbiological information obtained from shell eggs. It has been demonstrated that there are many adequate methods for the recovery of microorganisms from eggshells (Moats, 1978). However, stage of commercial shell egg processing has not been previously considered for more than one population. While slight modifications in sampling and methods may not affect recovery for large populations, occasional organisms may be missed if a less appropriate method has been employed.

CHAPTER 2

LITERATURE REVIEW

Introduction to the United States Shell Egg Industry

Eggs, a nutritious and inexpensive food, are consumed by people all over the world (Anderson, 2003; McNamara, 2003). Though the eggs of other avian species are popular in many countries, chicken (*Gallus domesticus*) eggs are the focus of the United States egg industry (Stadelman, 1995). For this reason, all references in this text will refer to chicken eggs unless otherwise specified.

Since 1900, there have been many changes in the U.S. egg industry (Bell, 1995). From 1898 to the late 1950s, Midwestern Corn Belt states (Iowa, Missouri, Ohio, and Illinois) were the leading egg producers. California, Pennsylvania, and Indiana became important egg producers as well. Development of better, cheaper transportation for broiler industry feeds shifted egg industry growth to the Southeastern United States during the 1960s. Increases in contract production and low cost land were also factors. In the last 25 years, egg industry growth has shifted back to the Midwest and California (Bell, 1995; Bell, 2002). In 2003, the top ten egg producing states were Iowa, Ohio, Indiana, Pennsylvania, California, Texas, Nebraska, Georgia, Florida, and Minnesota (American Egg Board, 2004).

In the early 1900s, 90% of U.S. commercial eggs were produced on multi-purpose farms by 100-300 birds that roamed freely, fed with waste grain, insects and forage (Bell, 2002). Eggs were hand-collected by the family who owned the farm. By the end of World War II, farms with over 1,000-10,000 bird flocks and managed in specialized flocks became more prevalent. In 1949, there were ~ 2.5 million farms producing eggs. By 1964, there were 527,000, but less than 200,000 by 1974. Family farm numbers dwindled as commercialization of the egg industry

expanded. By 1981, 47 egg production companies owned 37% of U.S. egg layers (Stadelman et al., 1988; Stadelman, 1995). Today, 260 egg producers have flocks of ≥ 75,000 hens while just 17 years ago there were ~2,500 operations just with flocks of this size. These 260 producers represent 95% of all layer flocks in this country. There are 65 egg producing companies with ≥ 1 million layers and 9 companies that have over 5 million layers (American Egg Board, 2004).

As farms became modernized, flock sizes have increased to take greatest advantage of efficient systems (Bell, 2002). Multiple-tier cages are common and come equipped with automated transport belts for gathering eggs. More than 80% of U.S. eggs are gathered by this method. Mechanical feed and watering devices are present in 90% of layer houses. Temperature, humidity, feed intake, water consumption and all other mechanical operations are electronically monitored. These conditions allow egg companies to employ only 15 persons per million hens. Prior to environmentally controlled housing, eggs were only produced in the spring and summer months. After eggs were separated according to cleanliness and size, washed and clean eggs were stored until needed for retail. Today, eggs are now transported to retail outlets almost as soon as they are packaged (Stadelman, 1995; Bell, 2002; Zeidler, 2002).

Another important influence on the size of flocks and egg processing complexes are the eggs per hour capacity of washing and packaging equipment. Modern equipment for washing, candling, sizing, and packaging can handle over 90,000 eggs per hour. Facilities may also contract for processing and packaging eggs laid and collected off-site in order to take full use of equipment capacity. This type of operation is known as “off-line” (Klippen, 1990; Zeidler, 2002).

Per capita egg consumption has also undergone a great deal of change. Highest per capita egg consumption was in 1945 at 402 while the nadir occurred in 1991 at 233.9 eggs. Health

concerns associated with egg-related outbreaks of salmonellosis caused by *Salmonella* Enteritidis and a desire to reduce cholesterol intake are regarded as the principle reasons for the decline. Recently, nutritionists and medical doctors have made new recommendations that include daily egg consumption. In 2003, per capita egg consumption was 254.1 (American Egg Board, 2004).

As late as the 1960s, many eggs were obtained directly from farms or home delivered by milk companies (Bell, 1995). Today, most eggs are sold in supermarkets. Once size (pee wee to jumbo), color (brown or white egg shells), and quality (AA, A, B, or ungraded) were the only egg choices consumers could make. Now, specialty eggs comprise ~ 5% of the market. Examples include, organic, vegetarian fed, free range, cage free, or fertile. Some egg types boast higher contents of nutrients such as vitamin E or omega-3 polyunsaturated fatty acids while one type claims 25% less cholesterol than traditional eggs. Even generic eggs provide a number of essential fatty acids, vitamins, and minerals. Human milk is the only food source with a higher biological protein value for people (Anderson, 2003).

In 2003, 59.5% of eggs went to retail, 30.6% were further processed, 9.4% went to foodservice use, and 0.8% of eggs were exported. Top foreign markets for shell eggs were Canada, Hong Kong, and the European Union while Canada and Japan were the top importers of egg products. Superior egg quality allows the U.S. to out compete competitors such as China, even though they are able to produce eggs more cheaply (American Egg Board, 2004).

Processing, grading, and packing equipment have changed a great deal. As late as the 1950's, it took egg producers most of their time to clean, size, and pack eggs. Production was ~1.4 cases/man hour (1 case = 360 eggs). Today's egg washing and packaging equipment can process up to 500 cases/hour (Diamond, 2003). Eggs that are transported to processing and packaging rooms by conveyor from hens housed in buildings attached to the processing facility

are known as “in-line” eggs. Eggs that are transported from remote housing are known as “off-line” eggs. Conveyor systems, mass candling, automatic check (crack) detection, and electronic egg scales with computer controls have allowed for the transformation in production capacity (Zeidler, 2002; Curtis, 2002; Curtis et al., 2004).

Regulations

In 1946, the Agricultural Marketing Act was passed (USDA, 2003). This law gave the Secretary of Agriculture the authority to set standards for agricultural products and on a voluntary basis on the part of producers, to grade and certify conformity of agricultural products to set standards. Inspections are performed by the Agricultural Marketing Service personnel (USDA, 2000). Eggs are judged on external qualities, internal qualities, and weight. Those plants that choose to participate in the program may display the USDA shield and the appropriate grade designation (AA, A, B) and size (peewee, small, medium, large, extra-large, jumbo). This program is voluntary but companies who participate pay for grading and inspection services. Plants that are not under USDA inspection are governed by state laws which are equal to or more stringent than AMS guidelines (Johnson, 2002).

Facilities that elect to participate must file an application for grading and submit to a plant survey. Requirements for buildings, plant facilities, grading room, shell egg cooling and cleaning are described in 7 CFR Part 56 (Regulations governing the voluntary grading of shell eggs). Shell egg cleaning operations (§ 56.76) should be performed following specific guidelines (USDA, 2000; Curtis, 2002):

1. Equipment be kept in good repair and cleaned daily.
2. Wash water must be at least 90^{o}F (32^{o}C) or at least 20^{o}F warmer than eggs to be washed. This temperature must be maintained throughout the cleaning process.

3. Only approved cleaning compounds may be used in the wash water.
4. Wash water must be changed every 4 h or more often if it becomes unsanitary or at least at the end of the shift.
5. Replacement water should be continuously added to the wash water. Rinse water, chlorine or quaternary sanitizing rinse may be used as part of the replacement water, provided they are compatible with the washing compound though iodine sanitizers are prohibited.
6. Wash water must be potable and soluble iron content should not exceed 2 parts per million.
7. Waste wash water must be piped directly to drains.
8. Washing and drying operations should be continuous and completed as rapidly as possible. Eggs should never be soaked or allowed to stand or soak in water.
9. Prewetting shell eggs before washing is allowed by spraying a continuous flow of water over the eggs if water is allowed to drain away and water temperature is kept at levels previously described.
10. Washed eggs should be spray-rinsed with water at a temperature equal to or warmer than the temperature of the wash water and containing an approved sanitizer of 50-200 ppm of available chlorine or its equivalent. Alternatives may be approved by the National Supervisor.
11. Test kits shall be used to determine sanitizing solution strength.
12. During rest periods, eggs should be removed from the washing and rinsing area of the egg washer and from the candling area to avoid extra build up of heat.
13. Washed eggs should be reasonably dry before packaging and casing.

14. Steam and vapors resulting from washing operations should be continuously and directly removed from the building.

Eggs that are washed in plants inspected by AMS may be packed into cartons or cases bearing the USDA grade mark indicating that USDA's sanitation and good manufacturing processes were followed (USDA, 2003).

Additionally, a shell egg surveillance program is administered by AMS (USDA, 2003). This program is designed to assure that all eggs in the marketplace are equal to or of superior quality to U. S. Consumer Grade B. Shell egg handlers and hatcheries are visited quarterly by AMS to enforce compliance.

An Egg Products Inspection Act (EPIA) was passed by Congress in 1970 (USDA, 2000; USDA, 2003). This program is administered by the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA). Public law 91-597 has provided for a mandatory program designed to ensure wholesome shell eggs and egg products are properly labeled and packaged. Marketing of substandard eggs and egg products is controlled by ensuring that wholesome eggs are properly labeled, packaged, and handled. Also, product, premises, and records are inspected quarterly. Producer/packers with less than 3,000 layers are exempt. Specific definitions are given for eggs that are not wholesome (dirties, leakers, checks, rots, incubator reject, bloods, inedibles, losses) and whether or not such eggs are diverted to breakers, processed into animal foods, or discarded as trash. A 1991 amendment to the EPIA stipulates that shell eggs destined for consumers must (1) be held under refrigeration at an ambient temperature of 45^{o}F or less and (2) must display labeling indicating that eggs must be kept refrigerated. There is also a provision that requires imported eggs to be inspected ensuring that they meet the same restricted egg tolerances established for domestic producers.

Largely due to concerns over salmonellosis caused by *S.* Enteritidis, there are now plans for Federal action to address egg safety (USDA, 1998). Federal regulatory responsibility is shared by Food Safety Inspection Service (FSIS) and the FDA. President Bill Clinton's Council of Food Safety identified egg safety as a high priority public health issue (Carson, 2000). Through joint assistance, FSIS and FDA are implementing an action plan to eliminate *Salmonella* Enteritidis (SE) illnesses due to eggs (Eckroade, R.J., 2000; Sharar, 2004). Goals include a 50% reduction of egg-associated illnesses by 2005 and to entirely eliminate them by 2010. Two strategies have been designed towards fulfillment of those goals. Both of these goals include on-farm as well as packing and processing controls (Carson, 2000). FDA has been charged with developing standards for the producer with states providing inspection and on-farm enforcement. Production controls will include biosecurity, use of chicks from SE-monitored breeders, SE-negative feed, cleaning and disinfection of houses and equipment as well as rodent/pest control programs. Under the aegis of FSIS, packing and processing strategies will involve transfer of egg regulations to a HACCP-based system for both shell egg processing and egg products processing. Prerequisite (sanitation) programs will be included. FSIS will provide inspections and enforcement. Refrigeration during transport and at retail will be addressed and the Center for Disease Control (CDC) will monitor human disease in conjunction with FDA. Research and education programs are integral parts of these strategies.

FDA and FSIS are expected to issue separate proposed rules. FDA's egg safety rule is currently being reviewed and should be published in 2004. FSIS issued a proposed Final Rule, effective January 12, 2004 announcing that the FSIS and the AMS are transferring regulations that govern the voluntary inspection of egg products from 7 CFR part 55 to 9 CFR Part 416,

Sanitation, and Part 417, HACCP (USDA, 1996; Reynnells, 2004). Issuance of a proposed rule for shell eggs is imminent (Sharar, 2004).

Egg Shell Structure and Formation

An egg's physical structure is comprised of the yolk, albumen, shell membranes, and the shell (Romanoff and Romanoff, 1949; Board, 1966; Johnson, 1986). Each yolk (ovum) was present within its own follicle in the hen's body when she emerged from the egg as a chick (Johnson, 1986). An ovum, 400 mm in diameter when fully developed, and its vitelline membrane are referred to as a follicle. There is a hierarchy of development within the ovary once a hen reaches sexual maturity resulting in development and release of individual ova in a sequential manner. Depending on the light cycle, an ovary will release a single yolk about once every 24 h. After detaching from the ovary, an ovum passes into the oviduct via the infundibulum. This process takes almost 0.5 h. Inside the magnum, the next portion of the oviduct, three layers of the albumen are secreted and added over a 2.5 h period. Moving through the magnum, the egg rotates spirally. As it twists the chalaze and chalaziferous layer are formed. Primary function of these structures is to stabilize the yolk in the center of the fully formed egg, preventing the nutrient rich and structurally more vulnerable yolk from being in contact with the external environment.

An egg passes from the magnum into the isthmus where the two shell membranes are added as well as water and minerals (Solomon, 1991). This process takes an hour. Eggs pass through the shell gland or uterus, the next portion of the oviduct and the portion in which an egg spends the greatest amount of time (~ 20 h). First the outer thin layer of albumen and water are added, giving the eggs its characteristic shape. Shell formation also occurs within this segment of the oviduct. Calcium carbonate, organic compounds, magnesium carbonate, and phosphate

comprise the shell. Two layers are formed, mammillary and spongy. Knob-like structures made in the mammillary layer provide structure for calcium carbonate. Irregular patterns of calcite crystals comprise the spongy later. Thousands of pores are formed throughout the spongy layer. After it is fully formed, the shell passes through the vagina, the last portion of the oviduct. While passing through this segment, the external surface of the shell is coated with a chitin-like substance known as the cuticle. This material helps seal the pores and prevent bacterial entry, increasing reproductive success.

During oviposition, the vagina everts through the cloaca, depositing the egg outside the hen's body (Mayes and Takeballi, 1983). An egg spends just 0.25 h in the vagina. Within 0.25-0.5 h of oviposition, a new ovum may detach and the process is repeated. As an egg cools, it contracts, pulling the inner shell membrane away from the outer shell membrane. A pocket of air forms between these two membranes, usually at the blunt end of the shell; this is referred to as the air cell.

Antimicrobial Defenses

Physical resistance to bacterial contamination is provided by the cuticle, shell, inner shell membrane, and the outer shell membrane (Mayes and Takeballi, 1983; Solomon, 1991). Sometimes referred to as bloom or shell accessory material, the cuticle is a 0.01 mm thick protein-like substance that coats the outside of the shell. It provides protection in two ways. First, by adding to shell thickness, it increases shell strength. Secondly and most importantly, it prevents flow of water, bacteria, or other materials through the shell pores. Each shell is perforated by numerous pores. There are 7,000- 17,000 pores, many of which are located around the equator or the blunt end of the shell. Pore diameter ranges from 9-35 μ. These openings are wider at the top than the bottom. Some are malformed but many pores run from the outer

surface to the shell membrane. Integrity of these layers are affected by organic material, egg handling (easily damaged immediately after oviposition), length of storage (shrinks, dries, and flakes with time), storage temperature (increases with temperature), fumigation (chemical damage), and cleaning processes (physical and chemical damage). If the cuticle is removed, bacteria gain access to the egg's interior much more easily (Board, 1966; Mayes and Takeballi, 1983; Solomon, 1991; Baker and Bruce, 1995).

Shell thickness, influenced by nutrition, environment, management practices, and genetics, has a significant effect on the ability of bacteria to pass through the shell (Solomon, 1991). This ability has been demonstrated by correlation of bacterial penetration and poor shell quality (Haines and Moran, 1940). Particularly, eggs with cracked shells are invaded more often and with greater numbers of bacteria, including pathogens (Todd, 1996; Ricke et al., 2001). Shell thickness has been measured to be 241-371 μm (Solomon, 1991).

There are two shell membranes that are held closely together except at the blunt end of the egg where the air cell is located (Romanoff and Romanoff, 1949; Solomon, 1991). An inner membrane lies over the albumen and the outer membrane is attached to calciferous shell. Three layers of random fibers make up the outer membrane; two indistinct layers form the inner membrane. Most researchers estimate the outer membrane to be double the thickness of the inner membrane with a combined thickness of approximately 80 μ. These membranes are thought to serve as a bacterial filter (Garibaldi and Stokes, 1958; Kraft et al., 1958). Bacterial penetration is thought to be most effectively prevented by the inner shell membrane, shell, and outer shell membrane. Other researchers believe that the two shell membranes together form the most effective barrier (Board, 1966; Mayes and Takeballi, 1983; Solomon, 1991; Board and Tranter, 1995).

When the cuticle, shell, and shell membranes are unable to prevent bacterial penetration, albumen contamination will occur (Lock et al., 1992). However, there are numerous factors that limit the ability of microorganisms to survive and grow in this material (Mayes and Takeballi, 1983; Board and Tranter, 1995). Firstly, the viscous nature of albumen proteins, particularly when eggs are freshly laid, makes microbial motility difficult. Viscosity coupled with a lack of available water and nutrients make albumen inhospitable to bacterial growth. However, the main impediment to bacterial growth or survival in the albumen is chemical. There are several important naturally occurring antimicrobial compounds within the albumen. Ovotransferrin and conalbumen chelate metal ions particularly iron. Ovomucoid inhibits trypsin. Lysozyme causes hydrolysis of β-1,4-glycosidic bonds in peptidoglycans. Ovoinhibitor inactivates several proteases, ovoflavoprotein chelates riboflavin and avidin binds biotin. In addition, albumen pH is in the alkaline range. Immediately after oviposition, pH ranges from 7.6-7.9 but a gradual increase is observed during storage. As carbon dioxide is lost to the environment, pH increases to greater than 9, beyond the tolerance of most microorganisms. Lysozyme, conalbumen, and pH are considered to be the most important of the antimicrobial factors naturally occurring in albumen (Mayes and Takeballi, 1983).

Microbiology of Eggs

It is estimated that 90% of eggs are microbiologically sterile at oviposition (Board, 1966). Potential sources of contamination include dust, nesting material, and feces (Board, 1966; Mayes and Takeballi, 1983; Board and Tranter, 1995). However, three potential routes of infection have been described. Trans-ovarian infection occurs while the ovum or yolk is still connected to the ovary. Oviducal contamination is an infection of the vitelline membrane or albumen as they

pass through any portion of the oviduct. Trans-shell contamination is when bacteria pass from the outer to the inner surface of the shell (Bruce and Drysdale, 1994).

Trans-ovarian and Oviducal Contamination

Quality and safety may be impacted by trans-ovarian, oviducal, and trans-shell contamination (Mayes and Takeballi, 1983). Of these three, trans-ovarian has been considered the least important. Harry (1963) carefully killed and skinned hens before transferring them to a clean necropsy room. Oviducts, ovaries, ova, and eggs from the oviduct were removed and examined for the presence of various bacterial species. All the ovaries were found to be contaminated with bacteria. Most of the bacteria recovered were determined to be Gram-positive micrococci and lactobacilli though coliform and *Pasteurella haemolytica* bacteria were also infrequently encountered. Of the 28 ova that were examined, 64.3% were found to be contaminated by enrichment but only 26.3% were positive by direct plating. For this reason, it was concluded that bacteria were present in fairly low numbers. Another reason contamination prior to laying has been discounted as a major source of infection is that 90% or more of eggs are sterile at oviposition.

However, for some bacterial species and serotypes, trans-ovarian and oviducal contamination may be very important (Barnhart et al., 1991; Gast et al., 1992; Baumler et al., 2000; Ricke et al., 2001). For most serotypes of *Salmonella*, trans-shell contamination is probably the most important route of egg contamination. In the case of *S.* Enteritidis, this does not appear to be the case. *S.* Enteritidis is recovered from egg contents but not from shells or from hen fecal samples. Mawer et al. (1989) analyzed 360 eggs from a small free-range flock that had been implicated in a salmonellosis outbreak. He recovered *S.* Enteritidis from egg contents but from none of the eggshells. In another study involving an outbreak-implicated flock,

Humphrey et al. (1989) found 194 intact eggs contaminated with *S. enteritidis* but no fecal samples from the flock that laid them. Even when birds are orally challenged with large numbers of organisms, they produce few contaminated eggs (Gast and Beard, 1992). This is not the case with other serotypes (Humphrey et al., 1991). Other researchers have recovered *S. enteritidis* from the reproductive tract but not from the gastrointestinal tract (Lister 1988, Bygrave and Gallagher, 1989).

Campylobacter can be difficult to recover from very dry samples, including egg shells (Cox et al., 2001). Though this organism has been recovered from eggs and implicated in an outbreak involving under-cooked eggs, it has been considered a rare egg contaminant. Recently, Cox et al., (1999, 2000) published molecular evidence of transmission of *Campylobacter* from hens to progeny through the fertile eggs. As a result a frequently over-looked avenue of flock colonization was brought to light. Examination of oviducts from broiler breeder hens revealed infrequent contamination as high as the isthmus with segments closer to the vent yielding a greater number of positives (Buhr et al., 2001). Molecular examination of embryos and hatchery fluff has indicated *Campylobacter* contamination. Perhaps the strongest evidence that trans-ovarian or oviducal contamination of *Campylobacter* occurs was recently uncovered. Immature follicles and mature follicles examined were found to be 11.6% and 25.7% *Campylobacter* contaminated (Cox et al., 2004).

Trans-shell Contamination

Though most eggs are microbiologically sterile at the time of lay, opportunities for contamination abound the instant they leave the oviduct (Board and Tranter, 1995). Eggs are likely to have their first exposure to bacteria as they pass through the cloaca. Egg temperatures are around 42^{o}C, generally warmer than ambient air. Eggs contract as they cool, creating a

negative pressure that can pull material into the pores. As a result, eggs are potentially contaminated by any surface with which they come into contact. Several factors can affect the extent of trans-shell contamination that occurs. Positive correlations exist between shell or environmental moisture, ambient storage temperature, condition of the cuticle, and shell damage (Board et al., 1964; Todd, 1996; Ricke, et al., 2001).

Egg shell bacterial numbers fluctuate widely, from zero to hundreds or even millions (Mayes and Takeballi, 1983; Board and Tranter, 1995). An average number of bacteria per shell is considered to be 100,000 for unwashed or untreated eggs (Board, 1966). Heavily soiled eggs are likely to harbor millions of bacteria while lightly soiled and visibly clean egg bacterial numbers are difficult to predict (Board, 1966; Ricke et al., 2001). This natural variability requires that a great number of eggs need to be collected and sampled to allow adequate statistical data analysis. A recent study indicated that for non-graded eggs (including visibly dirty) approximately 40 eggs were required statistically but that for graded eggs 20 often sufficed (De Reu et al., 2003).

Sources of bacterial shell contaminants can include caging material, nesting materials, water, hands, broken eggs, blood, insects, and transport belting though dust, soil, and feces are probably the most important (Board and Tranter, 1995; Ricke et al., 2001; Davies and Breslin, 2003). There have been several general surveys of the types of bacteria than be recovered from shell eggs prior to washing and packing. In order of prevalence, *Micrococcus*, *Staphylococcus*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, *Cytophaga*, *Escherichia*, *Enterobacter*, *Streptococcus*, *Sarcina*, *Aeromonas*, *Proteus*, and *Serratia* are present on eggshells (Ayres and Taylor; 1956; Board et al., 1963, Board and Tranter, 1995). In addition, *Kurthia*, *Propionibacterium*, *Microbacterium*, *Lactobacillus*, *Moraxella*, *Acetobacter*,

and yeasts are present on washed and unwashed eggs (Moats, 1980). Jones et al. (2004) stored washed and unwashed eggs and plated shell rinses onto violet red bile glucose agar plates for each of six weeks. In a follow-up study, Musgrove et al. (2004) randomly selected presumptive *Enterobacteriaceae* isolates and identified them using biochemical tests. Genera identified from eggs that had not been washed included *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter sakazakii*, *Enterobacter* spp., *Citrobacter youngae*, *Klebsiella pneumoniae*, *Klebsiella* spp., *Serratia odorifera*, *Serratia* spp., *Kluyvera* spp., *Providencia rettgeri*, *Providencia* spp., *Pantoea* spp., *Rahnella aquatilis*, *Salmonella* spp., *Yersinia* spp., *Flavimonas oryzihabitans*, and *Xanthomonas maltophilia*. *Enterobacter amnigenus* and *Salmonella arizonae* were the only identified isolates from washed eggs, recovered from shell rinses at week 5 of storage.

Some bacteria are associated more often with spoilage of eggs, particularly during prolonged storage (Haines, 1938; Forsythe et al., 1953; Ayres and Taylor, 1956; Florian and Trussell, 1956; Board et al., 1963; Board, 1966; Mountney and Day, 1970; Moats, 1979; Moats, 1980; Moats; 1981; Plusquellec, 1995). Recognized spoilage flora include *Pseudomonas fluorescens*, *Pseudomonas putida*, *Alcaligenes*, *Proteus*, *Escherichia*, *Serratia*, *Xanthomonas maltophilia*, *Aeromonas*, *Hafnia*, *Citrobacter*, *Enterobacter*, *Cytophaga*, *Achromobacter*, *Micrococcus*, and *Pseudomonas aeruginosa*. Based on such findings, gram-negative bacteria are considered to be the primary culprits in terms of egg spoilage. A number of rots have been described that render eggs unfit for human consumption. Bacteria with relatively simple nutritional requirements and those that can survive and grow at refrigerated temperatures are also favored. Some species of *Pseudomonas* can breakdown cuticular proteins, allowing for growth of yeasts and molds (Board et al., 1979).

Yeasts and molds, able to withstand harsher environmental stresses, can grow on or in eggs and cause spoilage (Beuchat and Cousin, 2001; Ricke et al., 2001). Particularly when eggs are packed while wet or stored at high relative humidity, molds can be an important quality concern (USDA, 2000). Egg meats readily pick up off odors, including those caused by mold growth. When storage conditions are particularly humid, mycelial growth can cover eggs in what are known in the industry as "whiskers."

Though current regulations and guidelines are geared to address quality concerns, there are pathogenic species of bacteria that are of concern to the egg industry and regulatory agencies (Sharar, 2004). Several serotypes of *Salmonella* have been implicated in egg-borne salmonellosis (Humphrey, 1999; Ricke et al., 2001). *Campylobacter jejuni* has been implicated in at least one outbreak of egg-borne disease (Finch and Blake, 1985). Other organisms, *Listeria monocytogenes, Staphylococcus* and *Yersinia enterocolitica*, are capable of growing in eggs or surviving shell egg and shell egg product processing, or even cooking (Ricke et al., 2001).

Salmonella is the most important pathogen associated with shell eggs and egg products (Fazil et al., 2000; Andrews, et al., 2001; Ricke et al., 2001). Prior to the passage of the Egg Products Inspection Act in 1970, many different serotypes were associated with foodborne illnesses (Gast et al., 1992). Mandatory USDA inspection, removal of cracked or dirty eggs, and pasteurization standards reduced egg-borne illness associated with *Salmonella* (Baker and Bruce, 1994). However, since the 1980's, human salmonellosis caused by *S.* Enteritidis (SE) has increased dramatically (Humphrey, 1999). In 1990, the number of outbreaks in the U. S. peaked but has remained at 45-50 per year for the past several years (Centers for Disease Control, 2003). When outbreaks and sporadic cases are investigated, undercooked and raw eggs are the most common sources of SE infection. Centers for Disease Control uses the *Salmonella* Enteritidis

Outbreak Surveillance System to decipher foods associated with illness caused by this serotype. Each year since the system has been in place, from 55-80% of all SE outbreaks have been somehow linked to shell eggs. Of the 309 confirmed SE outbreaks that occurred from 1990 to 2001, 241 (78.0%) were associated in some way with shell eggs. SE-salmonellosis has also been associated with such foods as juices, salsa, meat, sprouts, fruit, and salads. Most outbreaks, including those considered to be egg-associated, are also linked to an interruption in the cold chain during transport or storage, improper handling, cross-contamination with other foods, or inadequate cooking. A large proportion of SE-illnesses (90%) and deaths (67%) occur in institutional settings where such breakdowns in hygiene or kill steps affect large numbers of people. There were 14,319 cases of illness during this time-span though 72.7% of them occurred from 1990-1995. This overall decrease in SE incidence from 1996-2001 may be attributed in part to implementation of a number of preventative measures and education programs. United Egg Producers (2004) initiated a "5-star" Total Quality Assurance Food Safety Program designed to support the efforts of egg producers, processors, and marketers to establish programs that address food safety concerns. This program's key points include cleaning and disinfection of poultry houses, rodent and pest elimination, proper egg washing, biosecurity, and refrigeration at 7.2^{o}C from the point of packing through delivery. Poultry improvement plans have been implemented in many states. One of the benefits these programs are designed to produce is a reduction of *S.* Enteritidis (and other human or avian pathogens) from the environment of layer houses and breeder farms (Henzler et al., 1998). There is considerable data that indicate that factors such as rodent populations and manure levels increase the likelihood that flocks or their environment will be positive for *S.* Enteritidis. Cleaning and disinfecting hen houses between flocks, following biosecurity measures, monitoring hen mortality, and using SE-free chicks and

pullets are also key in reducing a flock's risk of SE colonization (Sheehan, 2002). A comparison of flocks in Pennsylvania (90% of flocks are in the Pennsylvania Egg Quality Assurance Program) from 1990 to 1998 show a dramatic reduction in the percentage of flocks with SE positive environment (48 v 10) and percent flocks with SE positive egg (26 v 2) (Eckroade, 2000).

Education of consumers and food handlers has been considered to be one of the most important but least emphasized links in the "food chain" approach to food safety (Holmes, 2003). Partnership for Food Safety Education and the FightBAC!® Campaign are two programs designed to focus on consumers, children, and those who prepare food professionally (Foodsafety.gov, 2004). One of the founding members is the American Egg Board, who in cooperation with the Egg Nutrition Center, provide technical and scientific information that help the public decrease the risk of egg-borne disease.

Bringing Eggs and Foodborne Disease into Perspective

Food safety is an important concern world-wide (Holmes, 2003). While there will always be room for improvement, it is important to remember that the U.S. has one of the safest food supplies in the history of the world (Foodsafety.gov, 2004). Numerous advancements in the way our food is produced, harvested, processed, and distributed have contributed to the diminished risk associated with our country's food supply. The food industry is assisted by regulatory agencies and research scientists in maintaining the high standards currently in place. It is estimated that there are 6.5-33 million cases of foodborne illness in the United States annually (Buzby et al., 1996). While most of these cases are mild in nature, it is estimated that up to 9,000 deaths are attributable to food-borne illness (Mead et al., 1999). Fungi, viruses, protozoa, and bacteria are included in the list of >40 microbial foodborne pathogens that cause

these illnesses (Reid and Harris, 1999). Quite often foods of animal origin are implicated as contributing to foodborne illness caused by bacteria though there are often other factors that influence their occurrence. *Salmonella* is one of the most frequent causes of bacterial foodborne illness (Andrews et al., 2001; Bell and Kyriakides, 2002). Table 2.1 lists various foods and a level of risk associated with them (Bell and Kyriakides, 2002). Often these commodities are blamed for disease regardless of how they are handled during transit or preparation. Several factors may contribute to salmonellosis: preparation of food too far in advance, storage of food at ambient temperatures, inadequate cooling or re-heating, contamination of processed foods, undercooking, cross-contamination, consumption of raw food, incorrect handling while foods are warmed, infected food handlers, improper handling of leftovers, or preparation of excessive amounts of food (catering, institutionally) (Bell and Kyriakides, 2002).

In terms of sporadic illness, it is thought that if consumers would handle food properly, 85% of foodborne illnesses would be avoided annually (HHSPHS, 1998). In a case control study of sporadic cases of SE infection in the UK, takeout chicken was the second highest risk factor for infection after dishes containing raw egg (Cowden et al., 1989). This would indicate that poultry meat is also a source of SE. Yet, many consumers, even when they know the importance of washing hands after touching raw meat, fail to do so (Redmond and Griffith, 2003). Surveys of intact shell eggs rarely detect SE contamination (Haque et al., 1989; Jones et al., 1995; Schutze et al., 2002).

While the primary function of the kitchen is storage and preparation of food, it may also serve as a study, laundry, work room, and as housing for pets. As well as foods - people, utensils, water, pets, insects, rodents, and even the air can be a source of bacteria in the home, including the kitchen. However, when an outbreak is reported, seldom is more than the

Table 2.1. Levels of concern for exposure to food-borne salmonellosis for various foods.[1]

Risk Level	Food
Highest	Raw milk ripened soft cheeses, sprouts
High	Raw eggs, salami, dry cured ham, chocolate, infant dried milk powder, raw poultry, cooked poultry, unpasteurized fruit juice
Medium	Prepared salads, sushi, pasteurized milk ripened cheese, Brie, cooked meat, pasteurized milk hard cheese, raw red meats
Low	Raw fish and shellfish, cod, plaice, mussels
Lowest	Chub pate', products cooked in pack

[1]Bell and Kyriakides, 2002

commodity and number of people mentioned, though many other factors may have contributed to the disease occurrence (cross-contamination, improper storage temperatures, inadequate cooking, etc.) (Redmond and Griffith, 2003).

Risk

According to author Theodore Dalrymple (1998), a physician from the United Kingdom, "The United States is the origin of the health scare movement." Often health-related news is reported in an alarmist fashion geared more to entertainment than information. We live in a society that tells us we should be free of risk and are not required to take minimal effort in our own safety. As a result, it is possible for smokers to sue tobacco companies after contracting cancer or for overweight people to sue McDonald's after voluntarily consuming their food. Another of Dr. Dalrymple's aphorisms is "A risk a day keeps common sense away." It's difficult to know how to process information on health risks when so much information is available to us day in and day out. Table 2.2 contains mortality risk comparisons from a variety of causes/activities.

Effects of Processing

Previous discussion has covered the history of early production practices, washing, storage, and research that lead to improvements in processing and appropriate regulations or guidelines, setting the stage for modern processing. In an effort to pinpoint the effects of processing on egg microbiology, empirical data has been collected to chart the disappearance of pathogens from egg shells. Kinner and Moats (1981) inoculated simulated wash water with bacteria previously isolated from shell eggs. Temperature, pH, and detergent affected the survivability of pure cultures of Escherichia coli, Salmonella, Citrobacter, Enterobacter, Proteus, Klebsiella, Alcaligenes, Flavobacterium, and Pseudomonas. Escherichia coli and

Table 2.2. Risk comparisons: Annual risk of dying in the U.S./million persons at risk[1]

Cause of Death	Death per 1,000,000/year
Heart disease	2800
All cancers	2050
Fire fighter; hang glider	800
Lung cancer	590
Pneumonia	320
Diabetes; police officer	230
Motor vehicle accidents; breast cancer	160
Homicide	80
Falls	50
Foodborne bacteria	36
Accidental poisoning (drugs/meds)	30
Fires and burns; drowning	15
Tuberculosis; firearms	5
Choking	4
Lightning; insect bite or sting	0.2

[1] International Food Information Council, 1999

Pseudomonas were almost instantly destroyed. *Staphylococcus aureus* was adversely affected by detergent though protected by 1% egg solids. *Streptococcus faecalis* was the most resistant of the organisms tested, surviving for over 2.5 h.

Catalano and Knabel (1994) analyzed the effects of pH and rapid chilling on *S.* Enteritidis destruction during simulated commercial egg processing. Eggs were immersed in inoculated fecal slurry before being washed at pH 9 or 11 in 37.7^{o}C wash-water followed by rapid or slow chilling. Wash-water pH significantly affected shell surface survival of *Salmonella*. Significant cross-contamination was observed between inoculated eggs and control eggs at wash-water pH 9 (75.0%) but was decreased at pH 11 (8.3%), based on shell surface counts. Slow chilling increased *S.* Enteritidis survivability regardless of wash-water pH. At pH 9, *S.* Enteritidis penetration into egg contents increased.

Leclair et al. (1994) describe a model for inactivation of *Listeria monocytogenes* and *S.* Typhimurium in simulated wash water. Temperature, egg solids, pH, and chlorine were the treatments used to generate the data used in the models. Temperature and egg solids affected survivability of both organisms. *S.* Typhimurium survivability was also significantly affected by pH and chlorine alone. Second order interactions with egg and either pH or chlorine also affected both pathogens. Egg meat reduced the survivability of *L. monocytogenes* but promoted *S.* Typhimurium survivability. Linear equations calculated for each organism were used to estimate washing conditions that would reduce the time for a 4-log reduction in viable counts to a period of less than 30 minutes.

Whiting et al. (2000) described a stochastic model for estimating *S.* Enteritidis growth during shell egg collection, processing, storage, and transportation. Equations for internal egg temperature, vitelline membrane integrity, and *S.* Enteritidis growth rate were included. Monte

Carlo simulations were used to determine that *S*. Enteritidis were unlikely to grow during an average 4.5 d progression from oviposition through transportation. However, parameter fluctuations in this model indicate that ambient air temperature was a key factor. These authors conclude that ensuring refrigeration during transport and cooling eggs as quickly as possible were likely to increase egg safety.

Srikaeo and Hourigan (2002) published a report on the use of statistical process control to enhance validation of critical control points during shell egg washing. Control measures analyzed were pH of wash water (11-13), wash water temperature (32-44^{o}C), rinse water temperature (41-49^{o}C), and chlorine level (100-200 ppm). This model was generated based on literature recommendations for the parameters included. However, pH levels used were higher than typically observed in many shell egg wash water samples and the model does not take into account the effects of egg solids or other organic materials that are always observed in modern operations.

Models and empirical studies are of tremendous value in defining problems and formulating their solutions (Whiting and Buchanan, 1997). However, experimental design and statistical tools are not adequate to fully describe or include all parameters that affect microbial growth or survival. For these reasons, data collected from field or plant situations are important. In fact, data collected during production, processing, and distribution are often required to validate data collected in the laboratory or to test the predictive ability of process models.

Moats (1980) surveyed commercial shell egg washing facilities in Maryland and Pennsylvania. Washed and unwashed eggs, wash water, and equipment surface swabs were collected. An aerobic plating method was employed to enumerate microorganisms and selected isolates were identified to genus. *Aerococcus*, *Streptococcus faecalis*, *Propionibacterium*, and

Lactobacillus populations were reduced by washing. *Escherichia coli* were reduced by 67%. Greater numbers of actinomycetes were found on equipment surfaces than were found on unwashed eggs. *Alcaligenes* and *Moraxella* were the most frequently recovered Gram-negative bacteria from washed or unwashed eggs but 71% of microorganisms recovered from unwashed eggs were Gram-positive cocci.

Moats (1981) collected additional samples from the plants visited in 1980, including wash water, brushes, egg conveyors, washed eggs, and unwashed eggs. A sanitizing rinse was added to operations on sampling days. Chlorinated spray lowered bacterial counts only on conveyor samples though thorough rinsing equipment at the end of daily operations appeared to reduce bacterial counts on equipment surfaces and in wash water. Bacterial counts from washed eggs correlated significantly with equipment surface and wash water counts but not with unwashed eggs. Wash water counts correlated with counts from equipment surfaces but not from unwashed eggs. Moats concluded that the major source of bacteria in the wash water was the equipment rather than the eggs and that the sanitizing rinse was of no benefit. However, it seems likely that the bacteria on the equipment originated from the eggs. This work underscores the value of daily plant sanitation. Sodium hypochlorite works best in a pH range of 6-7 and most egg wash water pH values are greater than 9. This may have contributed to the limited effectiveness of the chlorinated sanitizer on equipment surfaces, particularly if there was wash water present at the time of application.

In a study published in 1979, Moats visited commercial facilities in Maryland and Pennsylvania that used different combinations of washing compounds and sanitizing or water rinses. Counts in plants using sanitizer rinses were very low (<50 cells/shell), significantly lower than one plant using an unsupplemented water rinse. However, when sanitizer rinse was

temporarily cut off in plants that employed this type of rinse, counts did not change. Moats concluded that a lack of significant change in egg shell bacterial numbers indicated that sanitizer rinse was at most an indirect effect. Moats further concluded that due to low correlation between wash water and egg shell data, cross-contamination was minimized by commercial shell egg washing conditions.

Catalano and Knabel (1994) assayed eggs and wash water samples from processing plants in southeastern Pennsylvania for aerobic microorganisms and *Salmonella*. A greater number of microorganisms survived the washing process at relatively low wash water temperature (32.2-35^{o}C) and pH values (9-10). *Salmonella* from serogroup D_1 were recovered from wash water and eggs that operated under those water parameters. When plants operated with higher wash water ranges for pH (11-12.5) and temperature (37.7 – 42.3^{o}C), no *Salmonella* were detected and wash water counts were lower. High detergent concentration and reduced egg solids also contributed to destruction of *Salmonella*.

Jones et al., (1995), collected egg samples from various stages of production from layer houses and processing samples from the adjoining packing plant. Samples collected from the layer house environment (flush water, ventilation fan swabs, egg belt and egg collector swabs) tested 72.0% *Salmonella* positive. Egg shell rinses prior to processing were 7.8% (7/90) *Salmonella* positive while post-processing rinses were only 1.1% (1/90) positive. None of the 180 egg content samples tested positive for the organism. As was the case with the work of Catalano and Knabel (1994), *Salmonella* was detected on shell rinses from an egg collected at a time when the wash water pH was at the lowest measure (10.19). *Salmonella* serotypes recovered from eggshells prior to processing were *S.* Heidelberg and *S.* Montevideo. Production

serotypes were identified as *S.* Agona, *S.* Typhimurium, *S.* Infantis, *S.* Derby, *S.* Heidelberg, *S.* California, S. Montevideo, *S.* Mbandaka, and untypeable.

Murase et al. (2001) surveyed two layer houses and the attached in-line processing facility at a single farm. Samples from the production environment as well as from pre- and post-washed eggs, and the processing environment, were collected over a five year period. Pulsed-field gel electrophoresis (PFGE) patterns from *Salmonella* serotypes collected in the processing plant drains matched serotype patterns consistently recovered from one of the layer houses. Other serotype PFGE patterns (*S.* Cerro, *S.* Mbandaka, and *S.* Montevideo) from production and processing samples also matched. These authors concluded that a single *Salmonella* clone colonized the production facilities and that egg belts are likely the means by which *Salmonella* spread from house to house and to the processing facility.

Davies and Breslin (2003) investigated *Salmonella* contamination at production facilities and farm egg-packing plants. Swabs from the floor, grading tables, conveyor belts or roller, and candlers were often *Salmonella*-positive (23.1-30.8%). Even after disinfection, contamination ranged from 5.0-12.6%. Sterilized eggs passed through facilities showed a contamination rate of 0.3%. These results indicate that cross-contamination may contribute significantly to the external contamination of shell eggs.

De Reu et al. (2003) compared aerobic microbial shell populations on eggs collected from production through retail from cage and organic production systems. Their results indicated that air quality affected shell counts regardless of production system. However, because eggs were collected in Belgium and eggs are not washed in the European Union, it is difficult to make comparisons to eggs processed and packaged in the U.S..

Knape et al., (2002) compared aerobic microbial populations on shell surfaces of eggs from four in-line (IL) and four off-line (OL) commercial processing plants. Eggs were collected from four different sites: accumulator or transfer belt, after washing but before application of sanitizer rinse, immediately after sanitizer rinse, and just before packaging. Five different collection times were observed, spaced equally over the processing day. Wash water temperature, pH, and aerobic microbial populations were also monitored. Though counts from eggs prior to processing (accumulator or transfer belt) were only 0.3 log cfu/ml between IL and OL, by the time eggs were ready to be packaged, OL eggs were 1.4 log cfu/ml greater than IL egg counts. Knape et al. (2002) concluded that OL eggs were more difficult to clean because organic material would have had longer to adhere to shells and therefore be more difficult to remove. OL eggs are stored at 12.8-15.6^{o}C for as long as a week before being processed whereas IL eggs are processed within 24 h of oviposition (Curtis, 2002). As a result, OL internal egg temperatures average 16.7 – 20^{o}C while IL internal egg temperatures typically average 31.1-35.6^{o}C. Average wash water temperatures for all eight plants and all five sampling times were approximately 34^{o}C. If the difference between the wash water temperature and the internal egg temperature is greater than ~7^{o}C, thermal checks and cracks increase (Curtis et al., 2004). Also, OL eggs are transported from a layer house to the processing facility and during this process eggs may be jostled causing minute shell damage that can expand once the eggs encountered warm wash water.

Egg Shell Sampling Methodology

A variety of methods have been developed for the recovery of microorganisms from egg shells. Determination of bacterial numbers on egg shells has been accomplished using surface rinses (Penniston and Hedrick, 1947; Gillespie et al., 1950, Conner et al., 1953; Forsythe et al.,

1953; Gentry, 1970; Gentry and Quarles, 1972; Gunaratne and Spencer, 1973; Moats, 1979; Berrang et al., 1991; Musgrove et al., 2002), shaking crushed shells with glass beads (Haines, 1938), blending egg shells and membranes (Brant and Starr, 1962, Board et al., 1963, March, 1969; Winter et al., 1955), as well as surface swabbing and blending (Penniston and Hedrick, 1947). In 1970, Gentry described a very simple procedure in which an individual egg is placed in 10 ml of a sterile, isotonic buffer in a plastic bag and massaged by hand for 1 min before soaking in the buffer for an additional 5 min. Many shell rinse methods are a variation on Gentry's method. Berrang et al. (1991) described a method in which individual eggs were aseptically cracked, contents discarded, and egg shells were placed into a bag with diluent where they were hand massage for a minute prior to sampling.

Gunaratne and Spencer (1973) recovered more *Pseudomonas* from inoculated eggs by blending than surface rinsing. However, Penniston and Hedrick (1947) found that rinsing and blending methods were equivalent in their ability to recovery bacteria from artificially dirtied but washed eggs. Once eggs were chemically sanitized using chlorine, counts were 3-6 times higher from blended than rinsed samples. Moats (1980, 1981) concluded that whether greater numbers are recovered by surface rinsing/swabbing versus blending the entire shell is dependent on whether bacteria reside on the surface or embedded within the pores or membranes of the shell.

It has been reported (Musgrove et al., 2002) that when evaluating broiler hatching egg disinfectants, method of inoculation and method of microbial recovery greatly affect the interpretation of chemical efficacy. A lenient test of efficacy would result when a method of inoculation less likely to result in sub-surface contamination (droplet) is combined with a shell rinse method. Inoculation by immersion and temperature differential and sampling by

homogenization of shells and membranes would provide the most rigorous test of sanitizer efficacy.

Rinsing is also used for the recovery of microorganisms from poultry carcasses. Lillard (1988) has reported that subsequent rinses (as many as 40 were performed) of poultry carcasses respectively yield bacterial numbers equal to the initial carcass rinse. However, data from multiple rinsing of egg shells do not display the same pattern unless the eggs are extremely dirty. Musgrove (2003) rinsed eggs up to 8 times plating to enumerate aerobes and *Enterobacteriaceae* after the 1st, 2nd, 4th, and 8th rinses. Even the 8th rinse for very dirty eggs recovered the same rate and with similar numbers as the 1st rinse. Unwashed eggs gave lower population levels on subsequent rinses, but the second rinse gave comparable recovery rates to the initial rinse. Washed eggs were different even on the second rinse. This work suggests that a significant portion of shell surface populations will be removed with the initial wash or rinsing of the egg. .

Moats sampled washed and unwashed eggs from commercial shell egg processing plants (1979). He reported that though a great deal of variability was noted, there were generally much lower numbers recovered from washed eggs, particularly those sampled by a surface rinse method. This researcher also compared a whole egg surface rinse technique to blending of the shell and membranes. He concluded that rinsing was more important because the surface bacteria that were recovered would be more likely to contaminate the egg contents when the shell was broken.

Surface rinse methods are easily and rapidly performed. However, surface rinse or swab methods may not be adequate for microbial recovery of washed or previously rinsed eggs. In consideration of the literature, it seems that the more effective the detergent or sanitizer, the more likely it is that a shell homogenization method may be required to recover surviving

microorganisms. Choosing the appropriate method is an important consideration when evaluating the efficacy of washing or sanitizing steps in the processing chain.

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CHAPTER 3

IMPACT OF COMMERCIAL PROCESSING ON THE MICROBIOLOGY OF SHELL EGGS[1]

[1] Musgrove, M. T., D. R. Jones, J. K. Northcutt, M. A. Harrison, and N. A. Cox. To be submitted to *Journal of Food Protection*.

ABSTRACT

Egg shell microbiology has been studied extensively over the years though little of it describes how modern U.S. processing conditions impact microbial populations. As regulations are being drafted for the industry, such information can be important in determining processing steps that are critical to product safety. Five different shell egg surface populations (aerobic, yeasts/molds, *Enterobacteriaceae*, *Escherichia coli*, and *Salmonella*) were monitored at twelve points along the processing line (accumulator, pre-wash rinse, washer one, washer two, sanitizer, dryer, oiler, scales, two packer head lanes, re-wash entrance, re-wash exit). Three commercial facilities were each visited three times allowing for the sampling of 990 eggs and subsequently analyzed by 5,220 microbiological samples. Though variations existed in levels of microorganisms recovered from plant to plant, the patterns of fluctuations for each population were similar at each plant. On average, aerobes, yeasts/molds, *Enterobacteriaceae*, and *E. coli* prevalence were reduced by 30%, 20%, 50% and 30%, respectively, by end of processing. Log_{10} CFU/ml rinse on eggs collected from packer head lanes were decreased by 3.3, 1.3, 1.3, and 0.5, respectively, when compared to rinses from eggs collected at the accumulator. *Salmonella* was recovered from 0–48% of pooled samples in the three repetitions. More *Salmonella* was recovered from pre-processed than in-process or ready to pack eggs. These data demonstrate that current commercial practices decrease microbial contamination of egg shell surfaces.

Hazard Analysis and Critical Control Point (HACCP) management systems are used by the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) and the U.S. Food and Drug Administration (FDA) to address concerns about the safety of meat, poultry, seafood, and other foods (*48*). The effectiveness of HACCP relies heavily upon published scientific data. Currently a voluntary quality-based egg inspection system is administered by the USDA Agricultural Marketing Service (AMS) (*50, 51*). However, FSIS is drafting HACCP documentation for the shell egg processing industry that will mimic regulations already in place for meat and poultry plants (14). A great deal of work has been published on the effect of processing on broiler carcass contamination (*1, 7, 18*). As a result, step-by-step fluctuations in various microbial populations on broiler carcasses have been determined. This has assisted researchers, industry, and regulators in developing HACCP plans in their efforts to decrease contamination of poultry meat with human pathogens (*48*). However, comparable information for shell egg processing facilities is not currently available.

Large scale processing of shell eggs began in the 1940s (*2, 36, 44, 52*). At that time, eggs were often soaked before being scrubbed and stored for long periods of time prior to sale. Many researchers noted that this practice was conducive to microbial cross-contamination and compromised quality and safety. At times, eggs were found to have higher bacterial counts after washing than before. Commercial shell egg production and processing in the U.S. have undergone a great deal of change in the last 60 years; particularly since the Egg Products Inspection Act was enacted in 1970 (*50*). The correlation of wash water temperature, pH, iron levels, sanitizer/detergent selection, and bacterial numbers in wash water has been determined (*2, 36*). Also, larger operations that utilize high speed washing and packing machines are routinely used by the shell egg industry (*52*). Now that effective processing conditions have been

established, a thorough study of their effects on egg quality and safety need to be evaluated. Other researchers in recent years have focused on a single population at a limited number of points along the processing chain (*6, 15, 16, 21, 25, 29, 30, 37, 38, 39*). We conducted a study that has provided in-depth information on how microbiological populations associated with shell egg surfaces fluctuate throughout modern commercial processing. Populations were chosen because of their significance as indicators of quality, process hygiene, or safety (*8, 31, 40, 44, 49*).

MATERIALS AND METHODS

Description of shell egg processing plants.

A survey was of in-line egg processing facilities located in the Southeastern U. S. was conducted. Three plants were selected for sampling on three separate processing days. These plants were designated as X, Y, and Z to protect the anonymity of the participating companies. Plant X was over 20 years old with a 135,000 eggs/h production capacity. Mixed operations (in-line and off-line) were processed though only in-line eggs were being processed during collection. Plant Y was an in-line operation, less than 3 years old, and processed 95,000 eggs/h. Plant Z, with only a 105,000 eggs/h capacity also ran mixed operations but only in-line eggs were being processed when samples were collected.

Shell egg sample collection.

Eggs were collected from commercial plants at the following points of processing: at the accumulator (A) , pre-wash wetting (B), first washer (C), second washer (D), sanitizer spray (E), dryer (F), oiler (G), scales following check detection and candling (H), re-wash belt entrance (I), re-wash belt exit (J), and packaging (at two different packer lane belts, K and L). Each of these sample sites are depicted in Fig. 2.1. Eggs were collected after the line had been operating for at

least two h and during the mid-morning break so as not to interfere with operations. Twelve eggs from each collection site were carefully placed into foam cartons, packed into half-cases and transported back to the laboratory at ambient temperature. Participating plants were chosen based not only on willingness to participate and operational procedures but also on proximity to our facility so that eggs could be collected and analyzed quickly.

Shell egg sampling methodologies.

Ten of the twelve eggs collected at each site were sampled using a shell rinse technique. Each egg was placed into a sterile whirl-pak bag with 10 ml of sterile phosphate buffered saline (PBS) and rinsed by shaking for 1 min. Eggs were room temperature while the diluent had been warmed to 42 C to facilitate recovery of microorganisms from eggshells. After a rinse sample was obtained each egg was removed and transferred to a different sterile bag. Rinsates and intact eggs were then stored at 4^{o}C overnight. On the following morning, each egg was removed from the second bag and cracked open on the edge of a sterile beaker. Egg meats were discarded and the inside of the shell was rinsed using sterile PBS to remove most of the adhering albumen. An effort was made to eliminate as much of this material as possible because of the antimicrobial components of albumen. Shell and membranes from a single egg were crushed in a gloved hand and forced into a sterile 50 ml disposable centrifuge tube. After 20 ml of sterile PBS was added, a sterile glass rod was moved vertically in and out of the tube for 1 min. This allowed for a maceration of shells and membranes as well as a thorough mixing of the sample with the diluent. Rinsate from every egg was then subjected to microbiological analyses.

Direct plating microbiology.

Microbial populations from individual samples described above were enumerated for total aerobes, yeasts and molds, *E. coli*, and *Enterobacteriaceae*. Aerobic populations were

enumerated on plate count agar (PCA) after incubation at 35°C for 48 h. Yeasts and mold counts were determined on dichloran rose bengal chloramphenicol (DRBC) agar plates incubated at 22-25°C for 5 d. *Escherichia coli* were enumerated on Petri-film plates (blue gas producing colonies), incubated at 35-37°C for 18-24 h. *Enterobacteriaceae* were enumerated on violet red bile glucose gar (VRBGA) plates with overlay (purple-red colonies). Plates were incubated at 37°C for 18-24 h. Presumptive colonies were counted and reported as $\log_{10}$ CFU/ml egg rinse or contents.

***Salmonella* enrichment.**

For each of the twelve collection sites, two pooled samples were formed by combining shell egg rinses or crushed shells and membranes from five eggs. Samples were pre-enriched in buffered peptone water at 35°C for 18-24 h, followed by enrichment in TT broth and Rappaport-Vassiliadis broth overnight at 42°C. Enriched samples were plated onto BG Sulfa and XLT-4 agar plates and incubated at 37°C for 24 h. Presumptive positives were inoculated into lysine iron agar (LIA) and triple sugar iron (TSI) slants and incubated at 35°C for 18-24 h. Those samples giving presumptive results on each of these media were confirmed using sero-grouping anti-sera. Confirmed isolates were then streaked for purity and stocked onto agar slants and ceramic beads in cryogenic protective media. A copy of each isolate was provided to the National Veterinary Services Laboratory of the USDA's Animal and Plant Health Inspection Services in Ames, Iowa for serotyping. A sample was recorded as positive if it was confirmed and sero-typed from either of the shell rinse or crushed shell and membrane composite samples.

Statistical analyses.

Population data were analyzed using the general linear model of SAS (*45*). Means were separated with the least-squared means option of the general linear model procedure of the

SAS/STAT program using significance levels of $P \leq 0.05$ (*45*). A comparison of recovery frequency was accomplished by Chi-square test of independence (*45*).

RESULTS

Table 3.1 contains average population level data for all aerobes, yeasts/molds, *Enterobacteriaceae*, and *E. coli* separated by sample site (A-L) and by plant (X-Z). Rinses from shells of eggs collected at the accumulator indicate that populations of yeasts/molds were not significantly different for any of the three plants. For all other populations analyzed from eggs collected at the accumulator, eggs from plant Y were the least contaminated. For aerobic microorganisms and *E. coli*, eggs from plants X and Z were equivalent while eggs from plant Y were contaminated to a significantly lower level of *Enterobacteriaceae*. All the populations surveyed decreased throughout processing in every plant.

Population data collected at each of the twelve processing sites for aerobic microorganisms, yeasts/molds, *Enterobacteriaceae*, and *E. coli* are presented in Table 3.2. Values are averages for all three visits of the three plants sampled. For all populations, greatest numbers of organisms were recovered from shell rinses of eggs collected at the accumulator or the re-wash belt. Pre-wash counts were higher than those obtained from eggs at most other sample collection sites (in-process and post-process).

Prevalence, data for each of the populations directly enumerated is summarized in Figs 3.2, 3.3, 3.4, and 3.5 by stage of processing. Stages of processing were grouped as pre-processing (accumulator, pre-wash, re-wash belts), in-processing (washers, sanitizer rinse, dryer, oiler), or post-processing (scales, packer lanes). Averages were calculated from data collected during three visits at each of the three processing plants. From the pre-processing to post-processing stages, average prevalence of aerobic mesophilic microorganisms, yeasts/molds,

Enterobacteriaceae, and *E. coli* decreased from 100 to 80.6%, 80 to 62%, 60 to 10% and 35 to 2%, respectively.

Salmonella serotypes from composite egg rinse or crushed shell and membrane samples appear in Table 3.3. Eggs collected at the accumulator, pre-wash, or re-wash belts yielded the most *Salmonella* positives though a composite from each of the 12 sites was positive at least once during the 9 plant visits (3 for each of 3 plants). *Salmonella* prevalence based on number of composite samples ranged from 0% (Y1) to 48% (X1). Average prevalence for all three plants and visits was 10.4%. Average per plant was 21.5%, 3.3%, and 4.5%, respectively, for plants X, Y, and Z.

DISCUSSION

Much of the literature concerning the microbiology of shell egg processing was published before the 1970s (*9, 10, 11, 12, 13, 22, 44, 52*). This information was vital to shaping the successful practices currently used today. Recent work has focused on the efficacy of particular detergents, sanitizers, or antimicrobial treatments such as UV radiation (*6, 15, 26, 32*). Other researchers have focused on how processing conditions affect *Salmonella* Enteritidis, the most prevalent serotype associated with foodborne illness in recent years. Many of these studies made use of inoculated eggs. Several researchers have used empirical data to construct useful models for determining the effects of variations in processing parameters on microbial populations associated with shell eggs or wash water (*6, 33, 46, 47*). Models and empirical studies are of tremendous value in defining problems and formulating their solutions. However, experimental design and statistical tools are not adequate to fully describe or include all parameters that affect microbial growth or survival. For these reasons, data collected from field or plant situations are important. Several studies in recent years have described shell processing and distribution

though they tend to focus on production or distribution for a single population (*15, 21, 29, 30 41*). Our study was conducted to provide an intensive analysis of the effects of each stage of processing for five microbial populations that affect shell egg quality or safety.

Data were obtained for external populations only. Processing conditions have been developed to minimize internal contamination (*2, 36, 52*). This is confirmed by published work with commercially processed shell eggs (*28, 29*). In a recent study conducted in our laboratory, even for eggs stored past the "best if used by" date, eggs were rarely internally contaminated (*28*). Jones et al. (*29*) and Haque et al.(*25*) found no *Salmonella* inside commercially processed eggs. Also, intact eggs that are internally contaminated may have become so by transovarian or oviducal routes (*44*). Processing would have no effect on eggs contaminated by these routes. Measures required to ensure that external contamination is eliminated from egg content samples are laborious and time consuming (*9, 44*). For these reasons, evaluation of external contamination was chosen as the best indicator of processing efficacy.

There were some differences in microbial levels recovered from egg shells collected at different plants on different visits (replications). Each plant was visited within two weeks of each other in sequential fashion to prevent a seasonal bias. Prior to processing, aerobic microorganisms, *E. coli*, and yeasts/molds were determined to be less than a $\log_{10}$ CFU/ml rinse different among the plants. Despite differences in age, processing capacity, and water quality (data not reported), all three plants were contaminated at similar levels for yeasts/molds, *Enterobacteriaceae* and *E. coli* at the end of processing. For this reason, most of the data will be discussed as averages among eggs collected from the three plants.

Eggs from plant X were significantly ($P \leq 0.05$) more contaminated with aerobic microorganisms than Y or Z by greater than 1.9 $\log_{10}$ CFU/ml rinse for eggs that were ready to

be packaged. Aerobic plate counts are a gauge of sanitary quality and adherence to good manufacturing practices (*40*). Plant X was the oldest plant with the highest production capacity, lowest average wash water pH (10.0 v. 10.3 and 11.2 for plants Y and Z), and with the least hygienic product flow. For plant X, the off-line eggs cooler (produced by hens housed in remote buildings not connected to the processing plant) is on the other side of the processed egg cooler. This requires these unwashed off-line eggs must be transported past eggs that have been cleaned and packaged. Similarly, trash barrels are carried over belts used to transport packaged eggs to the post-processed egg cooler.

Pre-wash rinsing had less effect for all populations than was observed for the other two plants. For plant X only, none of the directly plated populations decreased by a log until eggs reached the first washer. There was also a great deal of foaming noted during the first visit to plant X. Excessive foaming is one of the wash water parameters recommended in the Agricultural Marketing Service list of guidelines (*51*). Knape et al. (*30*) compared aerobic microbial counts for shell egg surfaces between in-line and off-line operations. They determined that counts were almost a log higher per egg for in-line eggs. All eggs sampled in our study were collected during in-line processing. In-line eggs are those eggs that are produced by hens that are housed in buildings physically connected to the processing plant. Plants X and Z also process off-line eggs (produced by hens housed in buildings not connected to the processing plants) but these eggs were not sampled. Perhaps greater contamination of equipment surfaces, wash water, and plant environment occur at plants where off-line eggs are processed. Based on surveys of commercial shell egg plants, Moats (*39*) concluded that bacteria on equipment surfaces were the most important sources of egg shell contamination. Plant Z maintains the highest pH levels in its washer water (> 11). This may have allowed plant Z to decrease aerobic

microbial levels equivalent with plant Y which had the lowest overall microbial contamination on the unprocessed eggs.

Plants X and Z re-wash sound eggs that have large stains or significant adherent foreign material (egg meats, feces, etc.). When re-washing is incorporated into a plant's processing chain, an egg will either become visibly clean or break. This practice means that a higher proportion of visibly dirty eggs will be passed through the washers. Before parameters known to limit microbial contamination of wash water were determined, it was recommended that dirty eggs not be re-washed. It was thought that re-washing visibly dirty eggs would increase microbial counts in the wash water, increasing chances for cross-contamination, (*13*). Eggs with considerable visible stains or adhering foreign matter are downgraded so re-washing eggs helps to increase profits (*52*). When wash water temperatures are moderate (32-42^{o}C) and pH levels are 9-10, microorganisms are more likely to survive the multiple hurdles presented by commercial shell egg processing. Plant X also had the lowest average temperatures recorded for washer 1 (39.7^{o}C v. 44.1 and 44.5 ^{o}C for plants Y and Z). Even at this plant, all populations were reduced by l $\log_{10}$ CFU/ml except for yeasts/molds.

Chemical oxygen demand (COD) is a measure of organic material in water (*27*). Plant Z had COD values twice those recorded for the other two plants (data not reported). This possibly indicates that there were more eggs breaking in the washers of plant Z. This was the only one of the plants that did not apply oil to eggs following washing and drying procedures. About 30% of commercially processed and graded shell eggs are covered with a thin layer of odorless, tasteless mineral oil to occlude pores and minimize water and gas exchange (*27*). Oiling helps to prolong internal quality and can be very important in warmer climates or for eggs to be imported. Immersing eggs in warm water can decrease shell strength (*23*). Oiling may contribute to shell

strength (*3*). Plant Z had the highest wash water pH (> 11 in both washers) as well as the most buffering capacity. Perhaps very alkaline wash water in combination with no oiling resulted in weaker shells. Egg solids and other organic materials reduce the efficacy of detergents in wash water (*37, 39*). However, counts from plant Z were at the lowest levels recorded for aerobic microorganisms, yeasts/molds, *Enterobacteriaceae*, and *E. coli* despite highest pre-processing levels for all populations but yeasts/molds.

Despite plant differences, the way shell eggs were washed, graded, and sorted was similar. Regardless of plant or microbial population, highest bacterial and fungal counts were observed at the accumulator or the re-wash belts. These are visibly dirty or unwashed eggs. In fact, wash water at plant Z was harsh enough that all populations were decreased by greater than a $\log_{10}$ CFU/ml at the pre-wash rinse. Plant Y achieved the same result except for aerobic microorganisms, which were reduced in washer 1. Plant X achieved a log reduction in washer 1 for the four directly plated populations only after eggs reached the first washer.

Data for each population were averaged for the three plants and separated by sample site. Aerobes reached the lowest levels by the dryer while *Enterobacteriaceae* and *E. coli* were reduced to the lowest levels by washer 1. Yeasts/molds were reduced at pre-wash rinse but increased again at oiling. Oiling follows drying, accomplished by forcing warm air over the eggs as they emerge from the sanitizer rinse. A survey of air quality in shell egg processing plants indicated poorest yeast/mold air quality near the dryers and washers (*42*). De Reu et al. (*21*) compared aerobic shell populations on eggs collected from production through retail from cage and organic production systems. Their results indicated that air quality affected shell counts regardless of production system. However, by the end of the processing chain, all microbial

populations determined in our study were significantly reduced compared to pre-processing levels.

Sanitizing rinse application is just one of the hurdles designed to diminish microbial egg shell contaminants. In a 1979 study, Moats (*37*) visited commercial facilities in Maryland and Pennsylvania that used different combinations of washing compounds and sanitizing or water rinses. Microbial populations on shell eggs in plants using sanitizer rinses were very low (<50 cells/shell), and significantly lower than one plant using an unsupplemented water rinse. However, when sanitizer rinse was temporarily cut off in plants that employed this type of rinse, populations on the shell did not change. Moats (37) concluded that a lack of significant change in egg shell bacterial numbers indicated that sanitizer rinse was at most an indirect effect. In a separate study, Moats (*39*) obtained population data from equipment surfaces, wash water, and eggs. Based on correlations, he concluded that the sanitizer rinse was of no use. Our data offers no sound argument against his conclusion. AMS guidelines specify that sanitizer rinses must be compatible with detergents and of a strength equivalent to 50-200 ppm chlorine. Chlorine compounds perform optimally between pH 6.5-7.5 (*19*), much lower than that measured for wash water in this study. Other compounds have been analyzed to replace chlorine but none has been as effective (*32*).

Prevalence data for individual plants and an average of the three plants were organized by stage of processing. Once eggs were introduced into the washer, microbial populations were reduced and biologically relevant increases were not observed through the remainder of the processing chain. Sanitation affects microbial populations during shell egg processing. Certain sections of the equipment are not water proof (scales), are difficult to reach (re-wash belt), or are

difficult to remove and clean regularly (packer head brushes). However, contact with these surfaces did not result in significant increases in counts.

Upon closer review of analyses for individual sample sites, it was apparent that sample site data separated into the following stages of processing. Pre-processing sample sites were the accumulator, pre-wash rinse, and re-wash belt sites. In-processing samples were those from washer 1 through oiling. Post-processing sites were at the scales and the packer lanes. These figures demonstrate a similar pattern of population reduction as eggs progress through the process. Yeasts/molds were the only group of microorganisms not to decrease from in-processing to post-processing. These organisms are hardier than bacteria under many circumstances (*8*). In-processing prevalence was lower than that observed for post-processing.

Salmonella is considered the most important human enteropathogen associated with shell eggs (*2, 44, 49*). *S*. Enteritidis is the serotype most often implicated in egg-borne outbreaks of salmonellosis though product temperature abuse followed by consumption of raw or undercooked eggs are usually factors. This serotype occurs at a low frequency (1 in 20,000 eggs) even when flocks are known to be *S*. Enteritidis colonized. However, all serotypes of *Salmonella enterica* are potential human pathogens and their presence on eggshells is of interest (*49*).

In our study, we obtained 39 *Salmonella* isolates from egg shell rinses, tap water, and wash water. Individual plant visits yielded 0 – 25 *Salmonella* isolates. Except for X1, 0 – 4 isolates were obtained per plant visit. Between both shell rinse and crush methodologies, 35/396 (8.8%) samples were positive for *Salmonella* following enrichment. Jones et al., (*29*) found 8/180 (4.4%) of egg shell rinses *Salmonella* positive. Prior to processing there were 7.8% (7/90) *Salmonella* positive rinses while post-processing rinses were only 1.1% (1/90) positive. March

(*35*) and Cox and Davis (*17*) did not recover *Salmonella* from 3,995 and 264 individual egg samples, respectively. During X1 sampling 1/3 of the tap water samples were determined to be contaminated with *Salmonella*. Plant X was the oldest plant (> 20 year old) included in the study and unchlorinated well water was used for processing. Potentially some animal (insect, amphibian, reptile, or mammal) may have compromised biosecurity and contaminated the plant's well water or it may have been caused by some other random event. This phenomenon was not observed again at plant X. It was never observed at plants Y and Z. *Salmonella* prevalence at this plant on other visits (X2, X3) was similar to that observed for other plant-visits (Y1-3, Z1-3). *Salmonella* prevalence for X2 and X3 averaged 6.25% (6/96) and 4.0% (10/252) for all other plant-visits, respectively. *Salmonella* was recovered from egg rinses collected during pre-process (10/28) more often than from in-processing (10/42) or post-processing stages (6/27). These data are evidence that commercial processes reduce *Salmonella* contamination of eggshells. Plant-visits in which *Salmonella* was recovered from eggshell rinse samples post-process were X1, Y2, Y3, and Z2.

Wash water parameters that are thought to influence *Salmonella* survival are temperature, pH, organic material, and iron levels. In addition to contaminated tap water, X1 was the plant-visit with the lowest average temperature and one of the only times where wash water pH was ≤10. Jones et al. (*29*) and Catalano and Knabel (*15*), detected *Salmonella* in shell rinses when the wash water pH was at the lowest measure (10.19). However, lowest pH was recorded for X2 (9.1) and Y1 wash water pH was 10. Average wash water temperature for all 9 plant-visits and both washers was 42.6 C. Three of the four plant visits where *Salmonella* was recovered post-process had wash water at or below that temperature. Highest COD values were determined for all plant Z visits and the highest total solids figure was for Z3, iron levels were over 2 ppm for

X3 yet *Salmonella* was only recovered from post-process samples from Z2. As determined from models derived from empirical data, a combination of factors affect whether or not *Salmonella* will survive shell egg processing. Hurdle technology has been built into the AMS guidelines and should be considered when writing HACCP plans for shell egg washing plants (*34*).

Five different serotypes from sero groups B and C were isolated from samples collected in our project. *S.* Enteritidis, of sero group D was not recovered. In a national survey, Garber et al., (*24*) did not isolate this serotype from production or processing samples collected in the southeastern United States. *Salmonella* serotypes recovered by Jones et al. (*29*) from eggshells prior to processing were *S.* Heidelberg and *S.* Montevideo. Production serotypes were identified as *S.* Agona, *S.* Typhimurium, *S.* Infantis, *S.* Derby, *S.* Heidelberg, *S.* California, *S.* Montevideo, *S.* Mbandaka, and untypeable. Poppe (*43*) isolated *S.* Typhimurium and *S.* Heidelberg most often from pools of layer hatching and table eggs. *S.* Heidelberg was a frequent egg belt and fecal contaminant from layer houses in a separate study (*20*). Barnhart et al. (*4, 5*) sampled ovaries from spent laying hens in the southeastern United States. Serotypes most frequently isolated were *S.* Heidelberg, *S.* Agona, *S.* Kentucky, and *S.* Typhimurium.

These data indicate that commercial egg processing significantly reduced levels of aerobic, yeasts/molds, *Enterobacteriaceae* and *E. coli* populations recovered by shell egg rinses. Populations decrease once eggs reach the first washer and remain at low levels through packaging. *Salmonella* was isolated at every sample collection site on at least one of the nine plant-visits. Pre-process shell egg rinse samples were *Salmonella* positive more often than in-process or post-process collected samples. Wash water pH, temperature, and condition (potability, contamination with organic material) seemed to partially account for *Salmonella*'s

ability to survive the commercial process. *S.* Enteritidis was not recovered from any of the samples.

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Table 3.1. Populations (log_{10} cfu/ml) averaged from three repetitions at each of three processing plants (X, Y, Z) for aerobes, yeast/molds, *Enterobacteriaceae*, and *E. coli* recovered from the surface of commercial shell eggs collected at twelve sites in the processing chain.

	A[2]	B	C	D	E	F	G	H	I	J	K	L
Aerobes												
X	4.89[a]	4.61[a]	3.29[a]	2.53[a]	3.46[a]	1.58[a]	1.75[a]	1.60[a]	4.73[a]	5.09[a]	3.20[a]	2.54[a]
Y	3.96[b]	3.33[b]	2.40[b]	1.25[c]	1.18[c]	0.97[b]	0.68[b]	1.62[a]	NS[3]	NS	0.89[b]	0.45[b]
Z	4.97[a]	2.10[c]	1.36[c]	1.91[b]	2.45[b]	0.95[b]	NS	0.48[b]	2.81[b]	2.94[b]	0.68[b]	0.64[b]
Yeasts/molds												
X	1.99	1.51[a]	0.61	0.47	0.59	0.72	1.18[a]	0.70	2.61[a]	2.79[a]	1.21[a]	0.89[a]
Y	1.72	0.48[b]	0.74	0.33	0.87	0.72	0.63[b]	0.85	NS	NS	0.64[b]	0.73[a]
Z	1.58	0.34[b]	0.30	0.72	0.59	0.53	NS	0.58	0.82[b]	1.23[b]	0.44[b]	0.43[b]
Enterobacteriaceae												
X	1.14[b]	1.63	0.19	0.28[a]	0.35[a]	0.24	0.03	0.19[a]	1.70[a]	1.72[a]	0.01	0.04
Y	0.43[c]	0.05	0.12	0.05[b]	0.00[b]	0.00	0.00	0.00[b]	NS	NS	0.01	0.11
Z	2.40[a]	0.06	0.01	0.03[b]	0.18[ab]	0.09	NS	0.01[b]	0.36[b]	0.38[b]	0.11	0.07
Escherichia coli												
X	0.62[a]	0.55[a]	0.11[a]	0.15[a]	0.18	0.09	0.01	0.03	0.29	0.19[a]	0.00	0.04
Y	0.16[b]	0.03[b]	0.01[b]	0.00[b]	0.00	0.01	0.00	0.00	NS	NS	0.00	0.10
Z	0.90[a]	0.00[b]	0.00[b]	0.00[b]	0.15	0.04	NS	0.00	0.15	0.04[b]	0.11	0.01

[1] Values not followed by the same letter within a group (microbial population and sample site) are significantly different from one another ($P \leq 0.05$).

[2] A = accumulator, B = pre-wash rinse, C = washer 1, D = washer 2, E = sanitizer rinse, F = dryer, G = oiler, H = check/detection scales, I = re-wash belt entrance, J = re-wash belt exit, K = packer lane 1, L = packer lane 2.

[3] Eggs were not available for collection from this site because oiling or rewashing of eggs was not observed at a particular plant.

Table 3.2. Mean populations of microorganisms recovered from the surface of commercial shell eggs collected at twelve sites on processing lines in three commercial plants.

	Population (log_{10} cfu/ml)			
Site	Aerobes	Yeasts/molds	*Enterobacteriaceae*	*E. coli*
A Accumulator	4.61[a]	1.76[ab]	1.32[a]	0.56[a]
B Pre-wash	3.34[c]	0.78[cd]	0.58[c]	0.19[b]
C Wash # 1	2.35[d]	0.55[d]	0.10[d]	0.04[c]
D Wash # 2	1.90[e]	0.51[d]	0.12[d]	0.05[c]
E Sanitizer	2.36[d]	0.68[cd]	0.17[d]	0.11[bc]
F Dryer	1.16[g]	0.65[cd]	0.11[d]	0.05[c]
G Oiler	1.22[g]	0.91[c]	0.02[d]	0.00[c]
H Scales/Check	1.24[g]	0.71[cd]	0.07[d]	0.01[c]
I Re-wash enter	3.77[b]	1.69[b]	1.03[b]	0.22[b]
J Re-wash exit	4.03[b]	2.01[a]	1.05[b]	0.11[bc]
K Pack # 1	1.57[f]	0.76[cd]	0.04[d]	0.04[c]
L Pack # 2	1.21[g]	0.68[cd]	0.02[d]	0.05[c]

[a,b,c,d,e,f,,g] Values in the same column that are not followed by the same letter are significantly different ($P \leq 0.001$) for yeasts/molds, *Enterobacteriaceae*, and *E. coli*; ($P \leq$ 0.0001) for aerobes.

Figure 3.1. Diagram of commercial processing plant depicting each of the twelve shell egg sample collection sites.

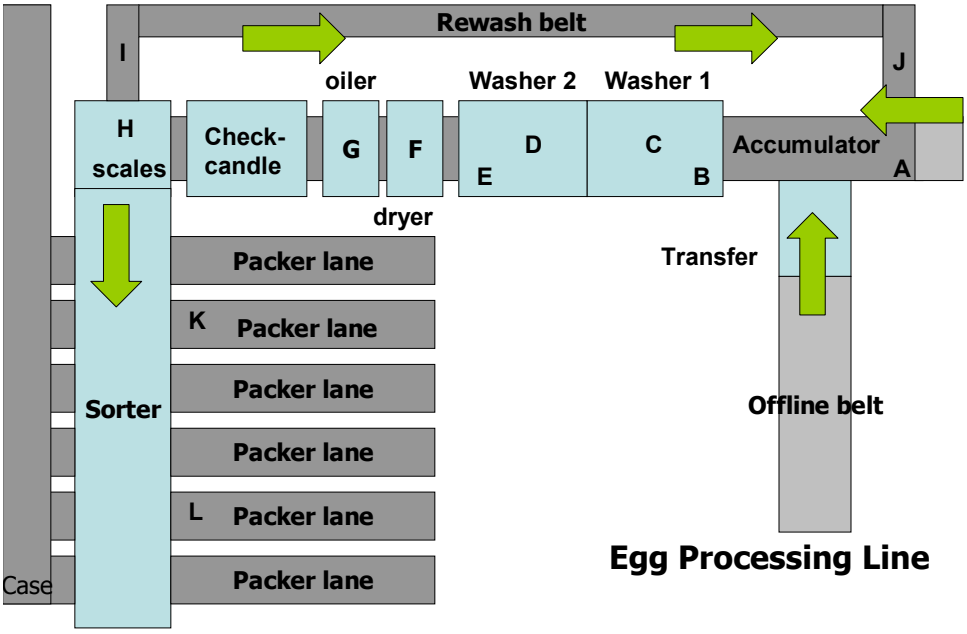

Figure 3.2. Prevalence of aerobic, mesophilic microorganisms recovered from rinses of shell eggs collected during pre-process (PREP), in-process (INPR), or post-process (POST) stages from three commercial shell egg processing plants. (n = 990)

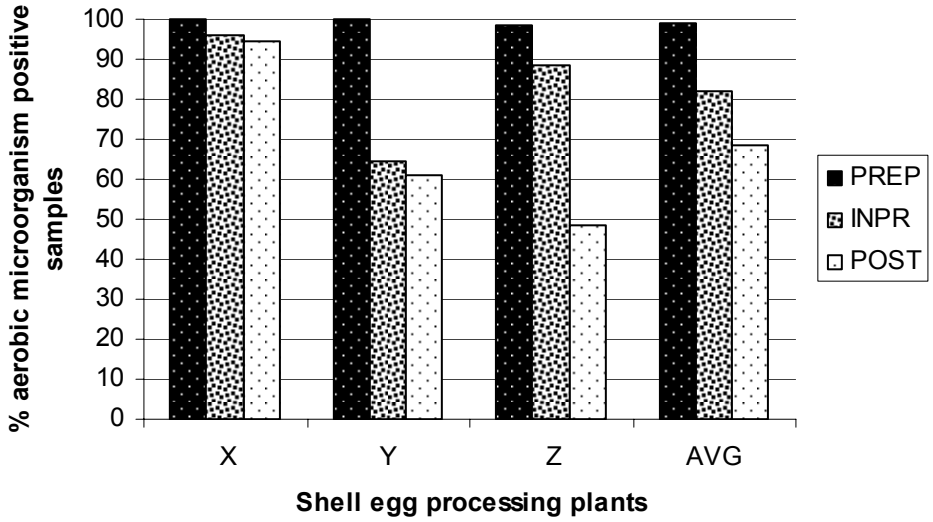

Figure 3.3. Prevalence for yeast/mold recovered from rinses of shell eggs collected during pre-process, in-process, or post-process stages from three commercial shell egg processing plants. (n = 990)

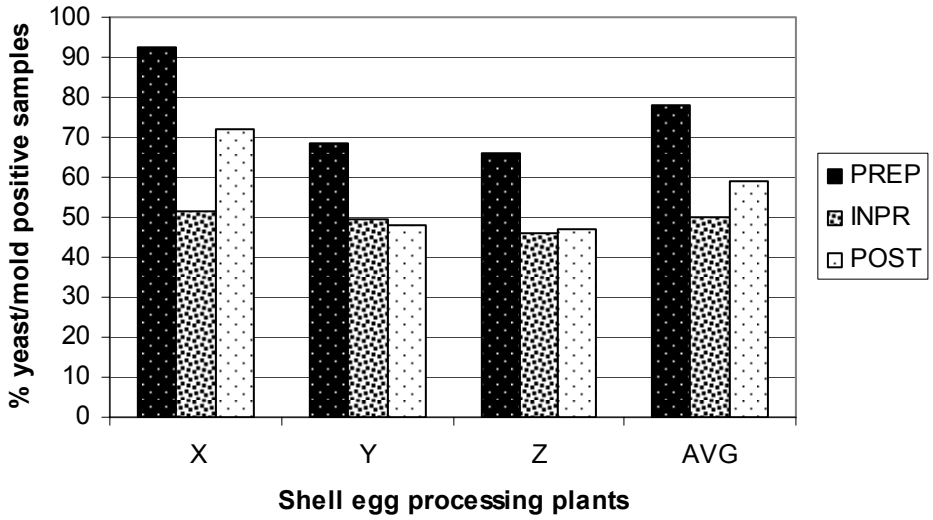

Figure 3.4. Prevalence for *Enterobacteriaceae* recovered from rinses of shell eggs collected during pre-process, in-process, or post-process stages from three commercial shell egg processing plants. (n = 990)

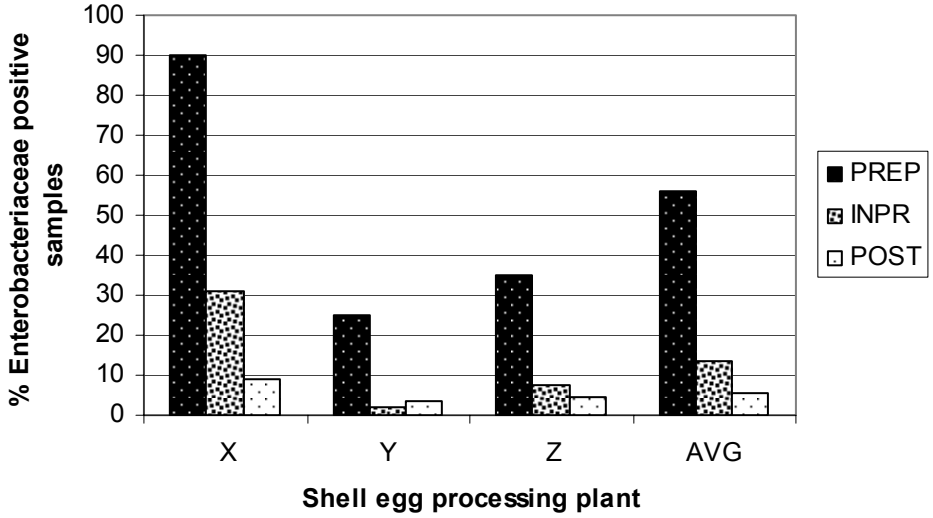

Figure 3.5. Prevalence for *Escherichia coli* recovered from rinses of shell eggs collected during pre-process, in-process, or post-process stages from three commercial shell egg processing plants. (n = 990)

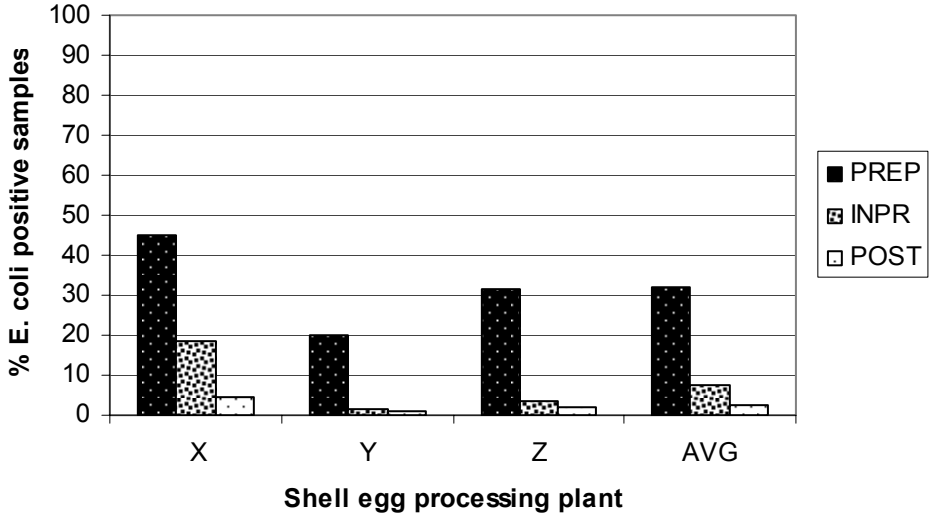

Table 3.3. *Salmonella* serotypes recovered from shell egg rinses or water samples collected from commercial shell egg processing facilities in the Southeastern United States.

Serotype	Number of isolates	Sample type	Plant/visit Recovered[a]
Typhimurium	21	Shell egg rinses	X1, X3, Z2
	1	Tap water	X1
Typhimurium (Copenhagen)	4	Shell egg rinses	X1, Y2, Y3
4-12:i:-monophasic	2	Shell egg rinses	X1, Y2
Heidelberg	9	Shell egg rinses	X2, X3, Z1, Z3
Kentucky	1	Shell egg rinses	Y2
	1	Wash water	Y1
Total	39		

[a] Letter refers to the plant where eggs or water were collected (X, Y, or Z) and the number following refers to the plant visit (replication) when the eggs or water were collected.

CHAPTER 4

IDENTIFICATION OF *ENTEROBACTERIACEAE* AND RELATED ORGANISMS FROM RINSES OF EGGS COLLECTED DURING PROCESSING IN COMMERCIAL SHELL EGG PROCESSING PLANTS IN THE SOUTHEASTERN UNITED STATES[1]

[1] Musgrove, M. T., D. R. Jones, J. K. Northcutt, N. A. Cox, and M. A. Harrison. To be submitted to *International Journal of Food Microbiology*.

Abstract

In the United States, shell eggs are washed and graded prior to retail. Since passage of the Egg Inspection Act in 1970, processing guidelines have been set to ensure that external and internal characteristics are improved. However, less is known about safety of commercially processed shell eggs. In order to determine genus or species of enteric bacteria entering plants and persisting throughout processing, eggs were collected from three U.S. commercial shell egg processing plants on three separate visits. On each plant visit, 12 eggs were collected from each of 12 sites along the processing line: accumulator, pre-wash rinse, 1st washer, 2nd washer, sanitizer rinse, dryer, oiler, check detection/scales, 2 egg grader packer head lanes, re-wash belt entrance, and re-wash belt exit. Each egg was sampled by a rinse technique and rinsate was plated onto violet red bile glucose agar with overlay for the detection and enumeration of *Enterobacteriaceae*. From each positive plate, up to five colonies were randomly selected and isolated for further analysis. Using biochemical tests, isolates were identified to the genus or species level. Several genera and species were detected at each of the three plants. Sites from which the greatest numbers of isolates were identified were those collected from eggs during pre-processing (accumulator, pre-wash rinse) or eggs judged as dirty (re-wash belt entrance or exit). Sites yielding the smallest number of isolates were those during or at the end of processing. *Escherichia coli* and *Enterobacter* spp. were isolated from each of the nine plant visits. Other genera isolated from at least one of the three plants included *Cedecea*, *Citrobacter*, *Erwinia*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Morganella*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, and *Serratia*. Non-*Enterobacteriaceae* isolated and identified included *Aeromonas*, *Chryseomonas*, *Listonella*, *Pseudomonas*, *Sphingobacterium*, *Vibrio*, and *Xanthomonas*. As all of the genera and species were recovered less frequently from fully processed eggs than from

unwashed or in process eggs, these data indicate that shell eggs from the Southeastern United States are less contaminated with bacteria of fecal origin as a result of commercial washing procedures currently being used.

Key Words: shell eggs, *Enterobacteriaceae*, commercial processing

1. Introduction

In the United States, Canada, and Japan, shell eggs are washed and graded prior to being packaged for retail (Zeidler, 2002). Though washing eggs was once disallowed in the United States, it is now required for plants that participate in the Agricultural Marketing Service (AMS) voluntary grading program (USDA, 1999). Washing eggs with water colder than the egg, with water heavily contaminated with bacteria, with water containing large amounts of soluble iron, or in machines whose surfaces are contaminated with large numbers of microorganisms are factors determined to increase chances of bacterial cross-contamination during egg washing (Moats, 1978; Baker and Bruce, 1994; Zeidler, 2002; Hutchison et al, 2003). Such conditions are addressed in AMS guidelines (USDA, 2000). Appropriate detergents, sanitizers, sanitizer levels, defoamers, prompt drying of washed eggs, changing of wash water at least every four h, and prohibition of soaking are other washing conditions addressed by the guidelines. When attention is given to these conditions, modern commercial shell egg washing operations result in improved microbiological egg quality (Moats, 1978; Baker and Bruce, 1994). This program guarantees consumers that shell eggs produced by AMS graded facilities will meet quality and size standards (USDA, 2000).

Currently, the Food Safety Inspection Service (FSIS), the United States Department of Agriculture (USDA) regulatory agency for meat and poultry, is drafting safety based policies (Hazard Analysis and Critical Control Point systems) for the shell egg industry (Carson, 2000). Effective Hazard Analysis and Critical Control Point (HACCP) plans require scientific data to be effective and practical (USDA, 1996). Over the years, many microbiological surveys have been conducted in commercial shell egg facilities (Haines, 1938; Florian and Trussell, 1956; Board et al., 1964; Moats, 1980; Davies and Breslin, 2003). However, few of them were designed to

address specific aspects of modern shell egg processes. Our goal for this study was to determine the number of *Enterobacteriaceae* species associated with shell eggs as they progressed through the processing chain. This study was undertaken to characterize *Enterobacteriaceae* species not only with washed and unwashed eggs, but also those microorganisms that persisted during operations in three commercial shell egg washing facilities in the Southeastern United States.

2. Materials and methods

2.1 *Sample collection*

A survey was conducted of in-line egg processing facilities. Three plants were selected for sampling on three separate processing days. These plants were designated as X, Y, and Z to protect the anonymity of the participating companies. Eggs were collected from commercial plants at the following points of processing: at the accumulator, at pre-wash wetting, after the first washer, the second washer, sanitizing, drying, oiling, check detection/weighing, packaging (at two different packer head belts), entrance of the rewash belt, and exit of the rewash belt. Eggs were collected after the line had been operating for at least two h but during the mid-morning break so as not to interfere with processing. This also allowed samples to be taken simultaneously from all sampling sites. Twelve eggs from each collection site were aseptically placed into clean foam cartons, packed into half-cases and transported back to the laboratory. Participating plants were chosen based not only on their operational procedures and willingness to participate but also on proximity to our facility so that eggs could be collected and analyzed expeditiously.

2.2 *Sample preparation*

Upon reaching the laboratory, each egg was aseptically transferred to a sterile zip-lock bag and 10 ml of phosphate buffered saline (PBS) was added. A rinse sample was obtained by

shaking the bag by hand for one min. Rinsates were stored overnight at 4 ^{o}C until microbiological analyses were performed.

2.3 *Cultural techniques*

Enterobacteriaceae were enumerated by duplicate plating of 1 ml aliquots of egg rinsate onto Violet red bile glucose agar (VRBG). Plates were poured with overlay of VRBG to assist in the recovery of injured organisms (Hartman, 1979). Plates were incubated overnight at 37^{o}C and observed for colony formation. Dark red to purple colonies with red-purple haloes were counted and converted to $\log_{10}$ CFU/ml sample. Up to five isolates for each positive sample were randomly selected for further analysis. A numbered circular grid (10 cm dia with 1cm^2 divisions) and random number tables (Steel and Torrie, 1980) were used to select isolates from plates with greater than 20 colonies. Each selected isolate was streaked for purity on plate count agar plates (PCA) and incubated at 37^{o}C overnight. Slants were then stored at 4^{o}C. Using an isolated colony the procedure was repeated twice to ensure purity. An isolate from the third streak plate was saved on brain heart infusion agar slants at 37^{o}C and Protect beads (Technical Service Consultants Ltd., The Ropewalk, Schofield St., Heywood, Lancashire OL10 1DS) at -20^{o}C until further analyses for identification

2.4 *Identification of isolates*

Each stored isolate was streaked onto PCA and incubated overnight at 37^{o}C. A cultural suspension using 5 ml of physiological saline was prepared from each isolate. This material was used to inoculate bioMerieux API 20 E strips (bioMerieux, Marcy-l'Etoile, France). Strips were inoculated, incubated, handled, and analyzed according to manufacturer instructions. Reactions were recorded and identifications were determined using Apilab Plus software (bioMerieux Marcy-l'Etoile, France).

3. Results

Identified isolates were grouped by Plant (X, Y, Z) and by plant visit (replication). For each plant-visit, identified isolates were tabulated alphabetically and arranged into Tables 4.1-4.3. In each of these tables, the number of isolates for a given species is listed for each of the 12 sample sites from which the egg was collected. There are 30 genera in the bacterial family *Enterobacteriaceae* and half of them were recovered at least once from eggs collected at the three shell egg processing plants from which eggs were collected. *Escherichia coli* were the most frequently isolated bacterial species and was recovered from the shells of eggs collected from each of the sites.

Table 4.4 includes genera that were recovered at least once during one of the nine egg processing plant visits. Genera that persisted on eggshells following processing included *Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Listonella*, *Providencia*, and *Salmonella.* Genera that were not recovered from fully processed eggs included *Cedecea, Chryseomonas, Erwinia, Hafnia, Kluyvera, Leclercia, Morganella, Proteus, Pseudomonas, Rahnella, Serratia, Sphingobacterium, Vibrio*, and *Xanthomonas*.

4. Discussion

Since the early 1970's, egg laying operations in the U.S. have predominately shifted to farms with as many as 2 million hens. Automated methods of collection, washing, grading, and packing have allowed for the efficiency of modern egg operations (Zeidler, 2002). Washing eggs under the conditions outlined in USDA guidelines is an integral part of the process of providing clean, safe eggs to domestic and foreign consumers (Baker and Bruce, 1994). Egg washing was once considered to increase the likelihood of microbial contamination leading to rots and other quality problems, particularly for eggs stored for long periods of time. Certain

parameters were eventually identified as contributing to this problem. Microbial quality is improved when eggs are washed soon after lay with clean, warm, low iron content water containing approved sanitizer-detergents, immediately dried, and stored at cooler temperatures after packaging. Modern machinery has been designed to consistently maintain appropriate conditions and eggs are seldom consumed four weeks after packing (Moats, 1978; Baker and Bruce, 1994; Hutchison et al., 2003). However, when 50,000 eggs are being processed each h, there can be failures in maintaining ideal conditions, contributing to the persistence of certain bacterial species on egg shells. Coliform, *Enterobacteriaceae*, and *E. coli* populations can be used as measures of food quality and sanitary processing conditions (Kornacki and Johnson, 2001; Ricke et al., 2001). Though coliform counts have historically been a more common of quality and sanitation in the U.S., we chose to focus on *Enterobacteriaceae* as previously reported byMercuri and Cox (1979). This bacterial family includes coliforms, fecal coliforms, *E. coli* (Holt et al., 2000b), and lactose-negative Gram negative facultatively anaerobic rods (e.g., *Salmonella*) (Mossel, 1978).

Random selection of colonies from VRBG plates allowed us to determine prevalent species on eggshells at the stage of processing during which they were collected. A total of 837 isolates were identified. During the nine visits (three per plant), 549 isolates were identified from plant X, 68 were identified from plant Y, and 220 were identified from plant Z. Plant X was the oldest of the three plants. Plant Y was the only one not to re-wash eggs visually determined to be too dirty or stained for USDA Grade A eggs. Plant Z was the only one that did not oil eggs. It is possible that by diverting dirty eggs to a breaker plant and not re-washing them that less cross-contamination occurred in the washers at plant Y.

For all three plants, a majority of the identified isolates were from shell rinses of eggs collected during pre-processing (accumulator, pre-wash rinse, re-wash entrance, re-wash exit). Eggs from these 4 sites accounted for 70.3% (386/549), 77.9% (53/68), and 83.2% (183/220) of identified isolates for plants X, Y, and Z, respectively. A higher proportion of isolates were identified from eggs collected from in-process sample sites (washer one, washer two, sanitizer rinse, dryer, oiler) for plant X (24.6% [135/549]) than for plant Y (10.3% [7/68]) or plant Z (10.0 % [22/220]). Plant X had lower wash water pH and temperature measurements than the other two plants (data not shown). However, only 5.1% (28/549), 11.8% (8/68), and 6.8% (15/220) of the total isolates from each of the respective plants persisted through the processing chain. This was an indication that far more organisms are being removed by the commercial washing process for shell eggs.

Most of the identified isolates from all three plants were members of the family *Enterobacteriaceae*, though other types were represented. *Chryseomonas*, *Pseudomonas*, *Sphingobacterium*, and *Xanthomonas,* Group 4 Gram-negative aerobic/microaerophilic rods and cocci (Holt et al*.,* 2000a), were occasionally isolated from plants X and Y. These organisms accounted for only 1.4% of all those identified. *Chryseomonas luteola*, *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Sphingobacterium multivorum*, and *Xanthomonas maltophilia* were recovered from plant X samples (first and second visits) while *Pseudomonas cepacia* was the only Group 4 species isolated from plant Y samples (third visit). In both the first and second visit to plant X, wash water samples from the second washer pH values were 9.9 and 9.1, respectively. A wash water sample pH of 10 was recorded for the first and third visit to plant Y though the temperature was lower for the latter (44.3^{o}C compared to 43.9^{o}C). Washer water pH values were greater than 11 for each of the three visits to plant Z.

Kinner and Moats (1981) reported that bacterial counts increased at pH 7 and 8 in simulated wash water at 35, 40, and 45°C. Counts decreased very slowly at pH 9 and 45°C while at pH 10 or 11 counts decreased regardless of temperature. However, this was a laboratory experiment involving synthesized wash water, not actual wash water over an 8 h time period. Excessive foaming, increased solids, and a concomitant rise in chemical oxygen debt caused by eggs breaking during the washing process may have contributed to the ability of these species to survive under field conditions. In fact, Kinner and Moats (1981) showed that adding 1 % suspended whole egg solids increased survivability of *Pseudomonas*, *Flavobacterium*, and *Citrobacter*. None the less, *Chryseomonas*, *Pseudomonas*, *Sphingobacterium*, and *Xanthomonas* were not recovered from the shells of eggs that had completed the processing chain during our study.

Vibronaceae is the second subgroup or family in Group Five (facultatively anaerobic Gram-negative rods) (Holt et al., 2000b). These organisms are found world-wide and often occur aquatically. Several species are pathogenic for humans, fish, and amphibians. Some species of *Vibrio* and *Aeromonas* can cause diarrhea, septicemia, or infect wounds. Once considered *Vibronaceae*, aeromonads have been transferred to the family *Aeromonadaceae*. Members of both of these closely related families were recovered from seven of the nine plant visits and accounted for 4.6% of total isolates identified. *Aeromonas* spp. were recovered from plant X on three visits and from plant Z on the first and third visits. These organisms are ubiquitous in many foods and are thought to play a role in food-borne disease (Isonhood and Drake, 2002). *Listonella damsela*, once classified as *Vibrio damsela*, was first proposed as a genus in 1985 (MacDonnell and Colwell, 1985). Its human clinical significance is limited to necrotizing wounds following sea water exposure. This species was identified from egg shell

rinses from all three plants. *Vibrio* spp., including *Vibrio metschnikovii,* were recovered only at plant Z during the first and third visits, respectively. Some species of *Vibrio* have been implicated in wound infections and diarrheal food-borne disease (Holt et al., 2000b). Aeromonads survived processing on X3 (1/12) and Z3 (7/10) eggs while a single isolate of *L. damsela* survived processing on an X2 egg. An isolate of *V. metschnikovii* survived on a Z3 egg.

Other researchers have reported on genera and species associated with shell eggs (Haines, 1938; Florian and Trussell, 1956; Board et al., 1964; Board, 1966; Moats, 1980). Bacteria from 16 genera were recovered from eggshells in one survey of Gram-positive and Gram-negative species. *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Aerobacter*, *Aeromonas*, *Proteus*, and *Serratia* are organisms mentioned in that survey that we also recovered (Board et al, 1964). *Flavobacterium* and *Aerobacter* are the basonyms of *Sphingobacterium* and *Enterobacter*. In a 1938 study, Haines reported that 38% of eggshell microorganisms are Gram-negative. Moats (1980) reported 39% of the isolates from unwashed shell eggs were Gram negatives. Pseudomonads, *Escherichia*, *Aerobacter*, and *Aeromonas* were isolated from eggs graded as A, B, and C quality. *Flavobacterium* and *Escherichia* were found on shells of washed and unwashed eggs though the latter are enumerated far more often. Board et al (1964) reported *Escherichia*, *Aerobacter*, and *Pseudomonas* were isolated from clean, lightly soiled, and cracked eggs while *Aeromonas* were recovered from clean and cracked eggs. A majority of the isolates that were identified in our study were recovered from shell rinses of eggs collected at one of the pre-processing sampling sites: accumulator, pre-wash rinse, re-wash entrance, and re-wash exit.

In another study recently conducted in our laboratory, *Enterobacteriaceae* were recovered from washed and unwashed shell eggs during six weeks of storage (Jones et al., 2004). In that study, 105 isolates were identified, most of them from unwashed eggs (Musgrove et al.,

2004). Genera identified in the previously published work but not recovered in our present study include *Pantoea* and *Yersinia*. There were many more isolates in the present study and more genera were detected that were absent from the previous study: *Aeromonas*, *Cedecea*, *Chryseomonas*, *Erwinia*, *Hafnia*, *Leclercia*, *Listonella*, *Morganella*, *Proteus*, *Sphingobacterium*, and *Vibrio* (Musgrove et al., 2004). However, the current study involved ten times the number of eggs and isolates and two more plants than were included in the previous study.

E. coli and *Enterobacter* spp. were isolated from every plant and during every visit in the current study and accounted for 55.8% (467/837) of isolates identified. *Escherichia fergusonii* and *Escherichia vulneris* were also isolated but never from fully processed eggs. *Escherichia coli* was isolated more often than any other single species. *Escherichia coli* comprised 25.9% (142/549), 45.6% (31/68), and 26.8% (59/220) of the isolates identified from any sample site at plants X, Y, and Z, respectively. However, only 2.1% (3/142), 12.9% (4/31), and 8.5% (5/59) of them remained on egg shells collected at the end of the processing chain. Mountney and Day (1970) suggest that in some cases *E. coli* may adapt and survive quaternary ammonium detergents though few of them seem to have survived in our study. *Enterobacter* spp. accounted for 24.6% (135/549), 25% (17/68), and 32.7% (72/220) of the isolates identified in plants X, Y, and Z, respectively. Of these isolates, only 5.9% (8/135), 5.9% (1/17) and 0% (0/72) survived processing at each of the plants.

Enterobacter sakazakii, a species that may contaminate soy-based infant formulas (Muytjens et al., 1988), was also isolated from eggshells collected at every processing plant in our study. However, this organism was never isolated from fully processed eggs. Recently, *E. sakazakii* has appeared in fly larvae, food processing plants, and from the home environment (Hamilton et al., 2003; Kandhai et al., 2004). *Salmonella* was presumptively identified in each

of the three plants in our study. A large number of the presumptive *Salmonella* were identified from pre- or in-process isolates during the first visit to plant X. One of the three tap water samples collected during that replication was also found to be *Salmonella* positive. It was not determined how the tap water may have become *Salmonella* positive.

A few of the organisms isolated and presumptively identified in this study are considered to be foodborne pathogens (*Salmonella, E. sakazakii, Vibrio* spp., and *Aeromonas hydrophila*). Others may be opportunistic or rare human pathogens (Holt et al., 2000a; Holt et al., 2000b). A number of the isolates recovered in this study were similar to those recovered in previously published reports. However, this study has provided a comprehensive look at *Enterobacteriaceae* and related organisms as they persist or disappear during commercial operations in three U.S. commercial shell egg plants. Perfecting the effectiveness of the process should always be a goal; however, these data indicate that U.S. commercial washing procedures are successful in removing a majority of the *Enterobacteriaceae* types and related organisms from shell eggs.

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Table 4.1. Identification of isolates randomly selected from violet red bile glucose agar plates inoculated with rinses from shell eggs collected at various stages of processing from U.S. commercial shell egg processing plant X.

	Site from which eggs were collected[a]												
Isolate identification[b]	A	B	C	D	E	F	G	H	I	J	K	L	Total[c]
Aeromonas hydrophila/caviae	-[d]	3	1	-	1	-	-	-	-	-	-	-	5[e]
Aeromonas hydrophila	-	-	-	-	-	1	-	-	-	-	-	-	1
Aeromonas sobria	-	4	-	-	-	-	-	-	-	1	-	1	6
Chryseomonas luteola	-	-	-	-	-	-	-	-	-	1	-	-	1
Citrobacter diversus/amalonaticus	2	-	-	-	-	-	-	-	-	2	-	-	4
Citrobacter freundii	3	3	-	-	-	-	-	-	-	1	-	-	7
Enterobacter aerogenes	2	-	-	1	-	-	-	-	-	-	-	-	3
Enterobacter amnigenus	2	7	2	-	-	-	-	-	4	3	8	-	26
Enterobacter cloacae	13	12	1	8	7	3	-	-	8	7	-	-	59
Enterobacter gergoviae	-	-	-	1	-	-	-	-	-	-	-	-	1
Enterobacter sakazakii	3	3	-	11	10	1	-	-	-	-	-	-	28
Enterobacter taylorae	1	-	-	-	-	-	-	-	5	2	-	-	8
Enterobacter spp.	1	-	1	3	3	-	-	1	2	5	-	1	17
Erwinia spp.	-	2	-	-	-	-	-	-	-	-	-	-	2
Escherichia coli	46	19	14	14	18	4	6	-	17	1	3	-	142
Escherichia fergusonii	-	2	-	-	-	-	-	-	-	-	-	-	2
Escherichia vulneris	-	-	-	-	-	-	-	-	2	2	-	-	4
Hafnia alvei	4	-	-	-	-	-	-	-	3	3	-	-	10
Klebsiella ornithinolytica	1	1	-	-	-	-	-	-	8	8	-	-	18
Klebsiella oxytoca	3	4	-	-	-	-	-	1	48	58	-	1	115
Klebsiella pneumoniae	9	3	-	-	-	-	-	-	-	2	-	-	14
Klebsiella taylorae	-	-	-	-	-	-	-	-	1	-	-	-	1
Kluyvera spp.	-	1	1	-	-	-	-	-	1	-	-	-	3
Leclercia adecarboxylata	2	-	-	-	-	-	-	-	-	-	-	-	2
Listonella damsela	-	2	1	2	-	1	-	1	2	1	-	-	10
Morganella morganii	1	-	-	-	-	-	-	-	-	-	-	-	1
Proteus mirabilis	3	-	-	-	-	-	-	-	-	-	-	-	3

Proteus penneri	-	-	-	-	-	-	-	-	-	1	-	-	1
Proteus vulgaris	-	-	-	-	-	-	-	-	1	2	-	-	3
Providencia alcalifaciens	1	-	-	-	-	-	-	-	-	-	-	-	1
Providencia rettgeri	-	-	1	-	-	-	-	-	-	-	-	-	1
Pseudomonas aeruginosa	-	1	-	-	-	-	-	-	-	-	-	-	1
Pseudomonas cepacia	-	1	-	-	-	-	-	-	-	1	-	-	2
Pseudomonas spp.	1	2	-	-	-	-	-	-	1	-	-	-	4
Salmonella arizonae	1	-	-	-	-	-	-	-	-	-	-	-	1
Salmonella spp.	1	1	-	3	4	8	2	13	2	3	-	-	37
Serratia marcescens	-	-	-	-	-	-	-	-	1	-	-	-	1
Serratia spp.	-	-	-	1	-	-	-	-	-	-	-	-	1
Sphingobacterium multivorum	-	1	-	-	-	-	-	-	-	-	-	-	1
Xanthomonas maltophilia	1	1	-	-	-	-	-	-	-	-	-	-	2
Total	101	73	22	44	43	18	8	16	106	104	11	3	549

[a] Letters in this row designate the site from which eggs were collected: A (accumulator), B (pre-wash rinse), C (washer 1), D (washer 2), E (sanitizer rinse), F (dryer), G (oiler), H (check detection/scales), I (rewash entrance), J, (rewash exit), K (packer head lane), L (packer head lane).

[b] Genus or species as determined by biochemical testing of individual isolates.

[c] Numbers in this column represent the total number of isolates identified to genus or species for all collection sites.

[d] Number of isolates for each genus or species identified from eggs collected at sample sites A-L, a "-" indicates that no isolate was identified for that site.

[e] Numbers in this row represent the total number of isolates identified to any genus or species for each sample collection site.

Table 4.2. Identification of isolates randomly selected from violet red bile glucose agar plates inoculated with rinses from shell eggs collected at various stages of processing from U.S. commercial shell egg processing plant Y.

	Site from which eggs were collected[a]												
Isolate identification[b]	A	B	C	D	E	F	G	H	I	J	K	L	Total[c]
Citrobacter diversus/amalonaticus	1[d]	-	-	-	-	-	-	-	NA[e]	NA	-	2	3[f]
Enterobacter agglomerans	1	-	-	-	-	-	-	-	NA	NA	-	-	1
Enterobacter amnigenus	1	-	-	-	-	-	-	-	NA	NA	-	-	1
Enterobacter cloacae	6	-	-	-	-	-	-	-	NA	NA	-	-	6
Enterobacter sakazakii	4	-	-	-	-	-	-	-	NA	NA	-	-	4
Enterobacter spp.	4	-	-	-	-	-	-	-	NA	NA	1	-	5
Escherichia coli	25	2	-	-	-	-	-	-	NA	NA	-	4	31
Escherichia fergusonii	1	-	-	-	-	-	-	-	NA	NA	-	-	1
Escherichia vulneris	3	-	-	-	-	-	-	-	NA	NA	-	-	3
Hafnia alvei	-	1	-	-	-	-	-	-	NA	NA	-	-	1
Klebsiella ornithinolytica	-	1	-	-	-	-	-	-	NA	NA	-	-	1
Klebsiella pneumoniae	1	1	-	-	-	-	-	-	NA	NA	-	-	2
Listonella damsela	-	-	-	1	-	-	-	-	NA	NA	-	-	1
Morganella morganii	-	-	5	1	-	-	-	-	NA	NA	-	-	6
Pseudomonas cepacia	-	1	-	-	-	-	-	-	NA	NA	-	-	1
Total	47	6	5	2	-	-	-	-	NA	NA	1	6	67

[a] Letters in this row designate the site from which eggs were collected: A (accumulator), B (pre-wash rinse), C (washer 1), D (washer 2), E (sanitizer rinse), F (dryer), G (oiler), H (check detection/scales), I (rewash entrance), J, (rewash exit), K (packer head lane), L (packer head lane).

[b] Genus or species as determined by biochemical testing of individual isolates.

[c] Numbers in this column represent the total number of isolates identified to the genus or species for all collection sites.

[d] Number of isolates for each genus or species identified from eggs collected at sample sites A-L, a "-" indicates that no isolate was identified for that site.

[e] Numbers in this row represent the total number of isolates identified to any genus or species for each sample collection site.

[f] These sample sites were not present in this plant.

Table 4.3. Identification of isolates randomly selected from violet red bile glucose agar plates inoculated with rinses from shell eggs collected at various stages of processing from U.S. commercial shell egg processing plant Z.

	Site from which eggs were collected[a]												
Isolate identification[b]	A	B	C	D	E	F	G	H	I	J	K	L	Total[c]
Aeromonas hydrophila/caviae	-[d]	-	-	-	2	-	NA	-	-	-	-	6	8[e]
Aeromonas sobria	-	-	-	-	-	-	NA	-	-	1	-	1	2
Aeromonas spp.	1	-	-	-	-	-	NA	-	-	-	-	-	1
Cedecea davisae	1	-	-	-	-	-	NA	-	1	-	-	-	2
Citrobacter diversus/amalonaticus	1	-	-	-	-	1	NA	-	-	5	-	-	7
Citrobacter freundii	9	-	-	-	-	-	NA	-	3	-	-	-	12
Enterobacter agglomerans	1	-	-	-	-	-	NA	-	-	-	-	-	1
Enterobacter amnigenus	1	-	-	-	-	-	NA	-	-	-	-	-	1
Enterobacter cloacae	30	1	-	-	-	1	NA	-	2	13	-	-	47
Enterobacter gergoviae	3	-	-	-	-	-	NA	-	-	-	-	-	3
Enterobacter intermedius	1	-	-	-	-	-	NA	-	-	-	-	-	1
Enterobacter sakazakii	4	-	-	-	-	-	NA	-	1	-	-	-	5
Enterobacter taylorae	1	-	-	-	-	-	NA	-	1	-	-	-	2
Enterobacter spp.	10	-	-	-	-	-	NA	-	1	1	-	-	12
Escherichia coli	33	-	-	-	8	5	NA	-	5	3	5	-	59
Escherichia fergusonii	1	-	-	-	-	-	NA	-	-	-	-	-	1
Hafnia alvei	1	-	-	1	-	-	NA	-	-	-	-	-	2
Klebsiella ornithinolytica	1	-	-	-	-	-	NA	-	-	-	-	-	1
Klebsiella oxytoca	4	-	-	-	-	-	NA	-	-	3	-	-	7
Klebsiella pneumoniae	1	-	-	-	1	-	NA	-	-	-	-	-	2
Leclercia adecarboxylata	1	-	-	-	-	-	NA	-	-	2	-	-	3
Listonella damsela	1	1	-	-	-	-	NA	-	-	1	-	-	3
Providencia rettgeri	2	-	-	1	-	-	NA	-	-	-	2	-	5
Pseudomonas aeruginosa	-	1	-	-	-	-	NA	-	-	-	-	-	1
Pseudomonas cepacia	-	1	-	-	-	-	NA	-	-	-	-	-	1
Rahnella aquatilis	1	1	-	-	-	-	NA	-	-	1	-	-	3
Salmonella arizonae	6	-	-	-	1	-	NA	-	1	2	-	-	10
Salmonella spp.	8	-	-	1	-	-	NA	-	1	1	-	-	11

Serratia liquefaciens	-	-	-	-	-	-	NA	-	-	1	-	-	1
Serratia marcescens	-	-	-	-	-	-	NA	-	-	2	-	-	2
Serratia spp.	-	-	-	-	-	-	NA	-	1	-	-	-	1
Vibrio metschnikovii	1	-	-	-	-	-	NA	-	-	-	-	1	2
Vibrio spp.	1	-	-	-	-	-	NA	-	-	-	-	-	1
Total	125	5	-	3	12	7	NA	-	17	36	7	8	220

[a] Letters in this row designate the site from which eggs were collected: A (accumulator), B (pre-wash rinse), C (washer 1), D (washer 2), E (sanitizer rinse), F (dryer), G (oiler), H (check detection/scales), I (rewash entrance), J, (rewash exit), K (packer head lane), L (packer head lane).

[b] Genus or species as determined by biochemical testing of individual isolates.

[c] Numbers in this column represent the total number of isolates identified to genus or species for all collection sites.

[d] Number of isolates for each genus or species identified from eggs collected at sample sites A-L, a "-" indicates that no isolate was identified for that site.

[e] Numbers in this row represent the total number of isolates identified to any genus or species for each sample collection site.

[f] These sample sites were not present in this plant.

Table 4.4. Identification (genus) of isolates randomly selected from violet red bile glucose agar plates of shell egg rinses obtained from eggs collected before, during or after processing at three U.S. egg processing facilities (three visits / plant).

Genus	Before processing	During processing	After processing
Aeromonas	5/9[b]	4/9	2/9
Cedecea	2/9	0/9	0/9
Chryseomonas	1/9	0/9	0.9
Citrobacter	8/9	1/9	1/9
Enterobacter	9/9	3/9	3/9
Erwinia	1/9	0/9	0/9
Escherichia	9/9	5/9	3/9
Hafnia	5/9	1/9	0/9
Klebsiella	8/9	1/9	2/9
Kluyvera	2/9	1/9	0/9
Leclercia	3/9	0/9	0/9
Listonella	6/9	2/9	1/9
Morganella	2/9	1/9	0/9
Proteus	1/9	0/9	0/9
Providencia	5/9	2/9	1/9
Pseudomonas	5/9	0/9	0/9
Rahnella	1/9	0/9	0/9
Salmonella	7/9	3/9	0/9
Serratia	3/9	2/9	0/9
Sphingobacterium	1/9	0/9	0/9
Vibrio	2/9	0/9	1/9
Xanthomonas	2/9	0/9	0/9

[a] Isolates were identified using API biochemical test strip reactions and software.
[b] Number of visits the genus was recovered/number of sampling visits.

CHAPTER 5

SHELL RINSE AND SHELL CRUSH METHODS FOR THE RECOVERY OF AEROBIC MICROORGANISMS AND *ENTEROBACTERIACEAE* FROM TABLE EGGS[1]

[1] Musgrove, M. T., D. R. Jones, J. K. Northcutt, N. A. Cox, and M. A. Harrison. To be submitted to *Journal of Food Protection*.

ABSTRACT

Recovery of bacteria from shell eggs is important for evaluating the efficacy of processing as well as the quality and safety of the final product. Shell rinse (SR) techniques are easy to perform and widely used. An alternative sampling method involves crushing and rubbing the shell (CR). In order to determine the most appropriate method for shell eggs, 358 shell eggs were collected from a commercial egg processor and sampled by SR and CR techniques. Total aerobic mesophiles and *Enterobacteriaceae* were enumerated on plate count and violet red bile glucose agar plates, respectively. Unwashed (PREP), in-process (INPR), and clean eggs (POST) were evaluated in the study. Aerobic microorganism prevalence for eggshells sampled was similar for both methods (~100%) but log CFU/ ml were higher from SR than CR samples (3.2 and 2.2, respectively). Average *Enterobacteriaceae* recovery was similar for both methods (45% SR v. 40% CR) when all eggs were considered together. This population was detected more often by SR when PREP eggs were sampled (90% SR v. 56% CR), equally by SR and CR for INPR eggs (30% SR v. 29.3% CR) but more often by CR for POST eggs (10% SR v. 36% CR). SR was easier to perform and recovered larger numbers of aerobic organisms, particularly for PREP eggs. However, CR was more efficient for recovery of *Enterobacteriaceae* from POST eggs. Stage of shell egg processing may be an important consideration when choosing egg sampling methodology.

Key words: shell eggs, methodology, rinse, *Enterobacteriaceae*, aerobic microorganisms

There are many methods for the recovery of microorganisms from egg shells and membranes. Methods that involve swabbing, blending of shells using mortar and pestle, blenders, and shaking with glass beads have been reported (*1, 2, 3, 4, 6, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20, 22*). One of the simplest and most commonly used methods is the shell rinse (*8*). Some researchers have found that microorganisms within the pores or those embedded in the membranes may not be recovered by rinse methodology (*10, 15, 16*). While more labor intensive, methods that involve crushing shells and membranes together are generally considered the most sensitive, an important consideration when microbial populations are small. Crush methods may allow for the recovery of organisms from the surface of the shell as well as for those that are located inside the pores or the membranes (*3, 17*).

An experiment was conducted in which a shell rinse method was used in conjunction with a technique in which shell and membranes were crushed together. Eggs in various stages of process were sampled. Method efficacy for recovery of aerobic organisms and *Enterobacteriaceae* was determined for both methods. A second experiment was performed to determine if crush efficacy was hampered by first sampling eggs by the rinse method.

MATERIALS AND METHODS

Experiment one: shell egg sample collection.

A single shell egg processing plant was visited on three separate days (six weeks apart) and ten eggs were collected from twelve separate sites along the processing chain before being sampled by two methods (n = 716). Sample sites were at the accumulator, pre-wash wetting, first washer, second washer, sanitizer rinse, dryer, oiler, check detection/scales, packaging (at two different packer lanes), as well as when they enter and exit the rewash belt. Eggs were collected during the mid-morning break so as not to interfere with plant operations. This

occurred about two hours after processing had begun, half-way between the minimum elapsed time before wash water would be completely changed (every four hours). Twelve eggs from each collection site were aseptically placed into clean foam cartons, packed into half-cases, and transported back to the laboratory. Ten eggs per sample site were analyzed. All eggs were collected from the same plant. Eggs were considered as pre-processed (PREP) if they were collected at the accumulator, during the pre-wash rinse, or from the re-wash belts. In-process eggs (INPR) were collected at washer 1, washer 2, sanitizer rinse, drying, and oiling. Post-process eggs (POST) were collected from check detection/scales or packaging sites.

Shell egg sampling.

Each egg was aseptically transferred to a sterile zip-lock bag and 10 ml of phosphate buffered saline (PBS) was added. A rinse sample was obtained by shaking the bag for one min, and then the egg was removed and transferred to a different sterile bag. Rinsates were stored at 4^{o}C overnight until microbiological analyses were performed.

Following the rinse procedure, each egg was aseptically removed from the second bag and cracked open on the edge of a sterile beaker. Egg contents were discarded and the inside of the shell was rinsed using sterile PBS to remove most of the adhering albumen. An effort was made to eliminate as much of this material as possible because of the antimicrobial components of albumen. Shell and membrane were crushed in a gloved hand and forced into a sterile 50 ml disposable centrifuge tube. After 20 ml of sterile PBS was added, a sterile glass rod was moved vertically in and out of the tube for 1 min. This allowed for a maceration of shells and membranes as well as a thorough mixing of the sample with the diluent. Samples were stored over night at 4^{o}C prior to microbiological analysis.

Experiment two: shell egg sampling collection.

A second experiment was conducted using eggs collected at only two sampling sites. Large, unwashed eggs were randomly selected as they entered the plant on the accumulator belt before placing into pulp flats. Cracked or excessively dirty eggs were excluded. Washed eggs were collected after they had been packed into pulp cartons. Washed and unwashed eggs were placed into half-cases and transported back to the laboratory at ambient temperature. Upon arrival, all eggs were stored overnight at 4^{o}C until sampling procedures were performed.

Shell egg sampling.

Shell egg sampling procedures were executed as described for experiment one. Three sampling approaches were used: shell rinse (SR), crush method for eggs previously shell rinsed (CRSR), and crush method on eggs not previously rinsed (CR). Twenty eggs per sampling site for each of the three method groups were sampled for each of three replications (n = 360).

Microbiological methodology.

Estimation of the aerobic mesophilic microorganism population levels was determined by duplicate plating of 0.1 ml aliquots of each sample onto plate count agar (PCA). After approximately 48 h of incubation at 35^{o}C, colonies were counted and converted to $\log_{10}$ CFU / ml sample.

Enterobacteriaceae were enumerated by duplicate plating of 1 ml aliquots of sample onto violet red bile glucose agar (VRBG). Plates were poured with VRBG overlay to assist in the recovery of injured organisms (*11*). Plates were incubated overnight at 37^{o}C and observed for colony formation. Following incubation, dark red to purple colonies with red-purple haloes were counted and the counts were converted to $\log_{10}$ CFU / ml sample.

Statistical analyses.

Population level data for the first experiment were analyzed using the general linear model of SAS (*21*). A comparison of recovery frequency was accomplished by Chi-square test of independence (*5*).

RESULTS

Experiment one.

Aerobic mesophilic microorganisms and *Enterobacteriaceae* results for Experiment 1 are displayed in Tables 5.1 and 5.2, respectively. Rinsing eggs yielded 0.9 and 1.4 $\log_{10}$ CFU/ ml more aerobes than crushing for PREP and INPR eggs. Recovery of aerobes from POST eggs using both recovery methods was equivalent. Comparing averages for each method for eggs from all stages of processing, rinsing recovered significantly more aerobes than crushing. Recovery rates were not significantly different for either of the methods at any of the processing stages. Using results from both sampling approaches, aerobic mesophilic microorganisms were recovered from 357/358 eggs.

E*nterobacteriaceae* prevalence results were comparable for both methods (Table 5.2). Overall *Enterobacteriaceae* population level averages were not significantly different for this population, though recovery percentages were. SR recovered significantly more *Enterobacteriaceae* than CR for PREP eggs ($P \leq 0.05$). SR and CR recoveries were equivalent for INPR eggs but CR recovered significantly more *Enterobacteriaceae* than SR ($P \leq 0.05$) for POST egg samples. Using results from both sampling approaches, *Enterobacteriaceae* were recovered from 113/120 (94%) PREP eggs, 72/149 (48%) INPR eggs, and 37/89 (42%) POST eggs.

Experiment two.

Aerobic mesophilic microorganisms and *Enterobacteriaceae* results for experiment two are displayed in Tables 5.3 and 5.4, respectively. For washed eggs, aerobic population levels and recovery rates determined for SR, CRSR, and CR were comparable. However, SR and CRSR each recovered organisms missed by the other. Using both methods detected aerobes more often CR alone. There was no difference in recovery between SR, CRSR, or CR eggs. A different trend was noted for unwashed eggs. SR recovered greater numbers than CR but did not detect them as often. SR recovered greater numbers at an increased rate when compared to CRSR eggs.

Enterobacteriaceae were only occasionally recovered from washed or unwashed eggs in the second experiment. For washed eggs, CRSR and CR were superior to SR for recovery of these organisms. However, with unwashed eggs, SR was superior to CRSR and CR for *Enterobacteriaceae* recovery.

DISCUSSION

Determination of bacterial numbers on egg shells has been accomplished using surface rinses (3*, 8, 9, 10, 15, 16, 18, 19*), shaking crushed shells with glass beads (*1*), blending or crushing egg shells and membranes (*3, 4, 14, 22*), as well as surface swabbing and blending (*19*). In 1970, Gentry (8) described a very simple procedure in which an individual egg is placed in 10 ml of a sterile, isotonic buffer in a plastic bag and massage by hand for 1 min. before soaking in the buffer for an additional 5 min. Many shell rinse methods are a variation on Gentry's method. There have also been many variations on methods involving the blending or crushing of shells and membranes. Berrang (*3*) described a method in which individual eggs were aseptically cracked, contents discarded, and egg shells were placed into a bag with diluent where they were hand massaged for a minute prior to sampling.

Surface rinse methods are easily and rapidly performed. However, we thought that including a shell homogenization method could provide information on bacteria below the shell surface that would be missed by surface rinse methodology. Methods used in this study were loosely based on those two approaches to sampling methodology. In our study, eggs were shaken instead of massaged using a lower volume of diluent and for a shorter amount of time. An attempt was made to adapt Berrang's "crush and rub" procedure by incorporating stomacher blending to replace massaging within the bag. Using a standard speed should reduce variability due to individually massaging samples. However, all bags used were pierced by egg shell fragments during stomacher blending which resulted in sample leaking out of the bag. Even using two bags designed to resist puncture by bone fragments was unsuccessful. Finally, centrifuge tubes and glass rods were chosen because of the availability and sturdiness of the materials.

Rinsing methods are also used for the recovery of microorganisms from poultry carcasses. Lillard (*13*) reported that subsequent rinses (as many as 40 were performed) of poultry carcasses gave bacterial numbers equal to the initial carcass rinse. However, data from multiple rinsing of egg shells does not show the same pattern of recovery unless the eggs are extremely dirty. Musgrove et al. (*18*) rinsed eggs up to 8 times followed by plating to enumerate aerobes and *Enterobacteriaceae* after the 1^{st}, 2^{nd}, 4^{th}, and 8^{th} rinses. Even the 8^{th} rinse for extremely dirty eggs recovered at the same rate and with similar numbers as the 1^{st} rinse. Subsequent rinses (4^{th} and 8^{th}) of unwashed eggs gave lower population levels, but the second rinse gave comparable recovery rates to the initial rinse. With washed eggs, a second rinse recovered fewer cells and less often than the initial rinse. This work suggests that a significant portion of shell surface populations will be removed with the initial wash or rinsing of the egg.

Gunaratne and Spencer (*10*) recovered more *Pseudomonas* from inoculated eggs by blending than surface rinsing. However, Penniston and Hedrick (*19*) found that rinsing and blending methods are equivalent in their ability to recovery bacteria from artificially dirtied eggs that had been washed. Moats (*15, 16*) concludes that whether greater numbers are recovered by surface rinsing/swabbing versus blending the entire shell is dependent on whether bacteria reside on the surface or are embedded within the pores or membranes of the shell. Analysis of the data collected in our study leads to the same conclusions. In our study, rinse and crush methods were effective in recovering aerobes from egg shells regardless of the processing stage from which they were collected. However, shell rinse recovered significantly greater numbers of aerobes than crushing for dirty and in-process eggs. When commercially washed eggs were sampled the methods recovered similar numbers at a comparable rate.

A shell homogenization method may be critical for recovery of organisms present in low numbers, particularly when they are located within the pores or membranes of egg shells. It has been reported (*17*) that when evaluating broiler hatching egg disinfectants that the method of egg inoculation and method of microbial recovery greatly affect the interpretation of chemical efficacy. A lenient test of efficacy is provided when a method of inoculation less likely to result in sub-surface contamination (droplet) is combined with a shell rinse method. Inoculation by immersion and temperature differential followed by shell and membrane homogenization sampling provides the most rigorous test of sanitizer efficacy.

Moats (*15*) sampled washed and unwashed eggs from commercial shell egg processing plants. He reported that though a great deal of variability was noted that there were generally much lower numbers recovered from washed eggs, particularly those sampled by a surface rinse method. This researcher also compared a whole-egg surface rinse technique to blending of the

shell and membranes for washed and unwashed eggs (*16*). Moats' methods involved different volumes of diluent, contact times between eggs and diluents, as well as different means of shell maceration. Moats reports a difference of 0.5 $\log_{10}$ CFU/egg between population levels recovered by the two methods for unwashed eggs. However, for washed eggs the eggshell blending method recovered 1.8 $\log_{10}$ CFU/egg more than did the shell rinse method. In our study, rinsing recovered 1.0 $\log_{10}$ CFU/ml sample more aerobes on average than were recovered by the crush method, when considering all stages of processing. However, there was no difference between bacterial populations recovered for fully processed eggs. These data corroborate Moat's conclusions. Such results indicate that for unwashed eggs, rinsing is a more sensitive sampling approach than crushing. If eggs have been washed or are still visibly dirty, then the reverse is true.

In the second experiment, eggs sampled were either washed (fully processed and packaged) or not yet washed. This work was completed to see how rinsing affected crush efficacy and also to make a fairer comparison between rinsing and crush sampling approaches. In terms of aerobic population recovery rate on washed eggs, shell rinse (SR) crushing after shell rinse (CRSR), and crushing of eggshells without rinsing (CR) were equivalent. However SR or CRSR recovered the most as each of the methods recovered aerobes missed by the other. Results were different for eggs that had been washed. CRSR recovery rate was 30% lower than that observed for SR sampling. CR recovered aerobes at a rate equivalent to SR for washed eggs.

Eggs that had been washed yielded more *Enterobacteriaceae* from CRSR and CR than from SR when washed eggs were sampled. However, for unwashed eggs SR was superior to CRSR or CR as a means of *Enterobacteriaceae* recovery. Data from the second experiment

indicate that for unwashed eggs, comparing CRSR and SR aerobic or *Enterobacteriaceae* results is not valid. Since CRSR and CR are equivalent for washed eggs, SR and CRSR may be compared for either population.

Shell rinse was more easily performed and required less materials than the crush technique. Both the stage of processing and microbial population had an influence on whether rinsing or crushing was the most appropriate method choice. These results indicate that for unwashed eggs, rinsing recovers greater number of aerobes than the crush method employed though recovery rates are similar. Rinsing recovered *Enterobacteriaceae* more often from dirty eggs, the same as crush for in process eggs, but less often than crush for washed eggs. When eggs are unwashed or partially washed, rinsing recovers more aerobic bacteria than the crush method though recovery rates are similar. These methods are equivalent for washed eggs when used for recovering aerobic populations that are present on and in most eggshells. However, for *Enterobacteriaceae* populations, not a contaminant of every egg, sampling methodology that includes shell membranes may be required for recovery.

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Table 5.1. Average aerobic mesophilic population levels (log_{10} CFU/ml) and recovery rates (% positive) obtained from commercial shell eggs sampled by either the shell rinse or shell crush method. Samples were plated on aerobic plate count agar plates for three replicates.

Method	Process Stage	Log_{10} CFU/ml	% positive
Rinse	PREP[a]	4.2	100
Rinse	INPR[b]	3.2	95
Rinse	POST[c]	2.0	96
Rinse	Average	3.3[A]	97
Crush	PREP	3.3	98
Crush	INPR	1.8	95
Crush	POST	1.9	98
Crush	Average	2.3[B]	97

[a] PREP: Eggs collected prior to processing or those that require reprocessing (accumulator, pre-wash rinse, re-wash belt).

[b] INPR: Eggs collected during processing (washer 1, washer 2, sanitizer, dryer, or oiler).

[c] POST: Eggs collected after processing was complete but before packaging (check detection/scales, packer head).

[A,B] Means with different letters in the same column are significantly different ($P \leq 0.05$). Values are means of three replications (n = 720).

Table 5.2. Average *Enterobacteriaceae* population levels (log_{10} CFU/ml) and recovery rates (% positive) obtained from commercial shell eggs sampled by either the shell rinse or shell crush method. Samples were plated on violet red bile glucose agar.

Method	Process Stage	Log_{10} CFU/ml	% positive
Rinse	PREP[a]	1.7	90[A]
Rinse	INPR[b]	0.6	30[A]
Rinse	POST[c]	0.7	10[B]
Rinse	Average	1.3	45
Crush	PREP	1.2	56[B]
Crush	INPR	1.0	30[A]
Crush	POST	0.7	36[A]
Crush	Average	1.2	40

[a] PREP: Eggs collected prior to processing or those that require reprocessing (accumulator, pre-wash rinse, re-wash belt).

[b] INPR: Eggs collected during processing (washer 1, washer 2, sanitizer, dryer, or oiler).

[c] POST: Eggs collected after processing was complete but before packaging (check detection/scales, packer head).

[A,B] Means with different letters from the same stage of processing in the same column for each method are significantly different ($P \leq 0.05$). Values are means of three replications (n = 716).

Table 5.3. Average aerobic mesophilic population levels (log_{10} CFU/ml) and recovery rates (% positive) obtained from commercial shell eggs sampled by either the shell rinse, shell crush of shell rinsed eggs, or shell crush of unrinsed eggs. Samples were plated on aerobic plate count agar. Values are means of three replications (n = 360).

Method	Washed	% positive
SR[a]	Yes	73.3
CRSR[b]	Yes	70.0
SR or CRSR	Yes	91.7
CR[c]	Yes	78.3
SR	No	96.7
CRSR	No	65.0
SR or CRSR	No	98.3
CR	No	91.7

[a] SR: Eggs were sampled by the shell rinse method.
[b] CRSR: Eggs row were sampled by crush after being shell rinsed.
[c] CR: Eggs row were sampled only by crush method.

Table 5.4. Average *Enterobacteriaceae* population levels (log_{10} CFU/ml) and recovery rates (% positive) obtained from commercial shell eggs sampled by either the shell rinse, shell crush of shell rinsed eggs, or shell crush of unrinsed eggs. Samples were plated on aerobic plate count agar. Values are means of three replications (n = 360).

Method	Washed	% positive
SR[a]	Yes	0.0
CRSR[b]	Yes	8.3
SR or CRSR	Yes	8.3
CR[c]	Yes	6.7
SR	No	38.3
CRSR	No	1.7
SR or CRSR	No	38.3
CR	No	18.3

[a] SR: Eggs were sampled by the shell rinse method.
[b] CRSR: Eggs row were sampled by crush after being shell rinsed.
[c] CR: Eggs row were sampled only by crush method.

CHAPTER 6

CONCLUSIONS

Processing conditions observed during commercial shell egg washing and packaging significantly reduced populations of aerobic mesophilic microorganisms, yeasts/molds, *Enterobacteriaceae*, and *Escherichia coli*. Aerobic mesophilic microorganisms reduced populations to the lowest level by the time the eggs reached the dryer. Yeasts/molds were reduced to the lowest level at pre-wash rinsing. *Enterobacteriaceae* and *E. coli* populations were significantly reduced in the first washer. Eggs that were visibly dirty and required to be re-processed were contaminated at levels equal to pre-processed eggs.

Analyses demonstrated that each of the sampling sites could be assigned to one of three stages of processing: pre-process, in-process, and post-process. Prevalence of each microbial group decreased as eggs passed through the processing chain. Greatest decreases were observed for *Enterobacteriaceae* and *E. coli*.

Salmonella was recovered less frequently from eggs that were collected between the first washer and packaging than from unwashed eggs or those that were visibly dirty. Shell egg rinses, crushed eggshells/membranes, and water samples were enriched for the organism and serotypes recovered were *S*. Typhimurium, *S*. Typhimurium (Copenhagen), 4, 12-i:-; S. Heidelberg, and *S.* Kentucky. *S.* Enteritidis, the serotype was often associated with egg-borne outbreaks, was never recovered from any of the three commercial shell egg processing plants. *Salmonella* contamination of eggshells was low but consistent for all three plants. During one visit, *Salmonella* was recovered from one plant's tap water. *Salmonella* was recovered more often from that plant visit than all other plant visits combined.

Temperature and pH of wash water, after potability, were the most important parameters that affected commercial processing efficacy in decreasing microbial populations on shell eggs. However, all populations monitored were decreased on eggs ready for packaging when compared to unwashed eggs. These data were a validation of the effectiveness of the commercial processing chain's ability to decrease microbial populations associated with the surface of shell eggs.

Several genera of *Enterobacteriaceae* and related organisms were recovered from shell egg rinses at the three processing stages. *Escherichia coli* and *Enterobacter* spp. were the most common *Enterobacteriaceae* associated with shell eggs at all stages of processing. *Pseudomonas*, *Sphingobacterium*, and *Xanthomonas* were not detected on processed eggs though *Aeromonas*, *Listonella*, and *Vibrio* were recovered from eggs at all stages of processing. Other genera recovered from processed eggs include *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Providencia*. All genus and species were detected less often after processing than they were prior to processing.

Rinsing and crushing of egg shells were methods used to recover aerobic mesophilic microorganisms, *Enterobacteriaceae*, and *Salmonella*. Overall, these sampling approaches gave similar recovery rates and levels for these populations. However, when data were analyzed by processing stage it was demonstrated that rinsing recovers greater numbers from pre-processed eggs but that crushing resulted in detection more often for post-process eggs. *Salmonella* was recovered only by crushing egg shells regardless of processing stage, except for a plant visit in which tap water was contaminated with *Salmonella*. These data demonstrated that the stage of processing from which eggs were collected should be considered when choosing sampling methodology for microbial recovery.