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Salmonella Enteritidis in shell eggs: Current issues and prospects for control

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ABSTRACT

Foodborne illness caused by *Salmonella* spp. is a worldwide problem. In the United States *Salmonella* Enteritidis is the second most commonly isolated serotype from human illness, and is known to be strongly associated with shell eggs and egg containing products. Eggs can become contaminated internally either by penetration through the shell or directly during formation in the reproductive tract. This review begins with a brief account of the physiology of egg production and the various physical and chemical barriers the egg possesses to prevent bacterial contamination. Factors involved in vertical and horizontal transmission of *S.* Enteritidis are examined, as well as the role of forced molt in colonization of the hen. Pre- and post-harvest mitigation strategies are also discussed.

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1. Introduction

In recent decades salmonellosis has been on the rise as a food related illness worldwide. The Centers for Disease Control and Prevention (CDC) estimates 1 million cases of non-typhoidal salmonellosis occur each year in the US (Scallan et al., 2011). Worldwide it is estimated that incidence of nontyphoid *Salmonella* ranges from 200 million to 1.3 billion, with an estimated death toll of 3 million each year (Coburn, Grassi, & Finlay, 2007). In the US it is estimated that 27% of patients require hospitalization and 378 cases lead to death, a death rate of 0.5% (Scallan et al., 2011).

Serotype *Salmonella* Enteritidis is second only to Typhimurium as the most frequently isolated serotype of *Salmonella* from human illness in the US. In 2006, the most recent year for which data is available, *S.* Enteritidis caused almost 17% of reported cases of salmonellosis (CDC, 2010a). The European Union (EU) reported that *S.* Enteritidis was responsible for 60% of all verified outbreaks due to *Salmonella* (EFSA, 2009). *Salmonella* spp., and in particular *S.* Enteritidis, is strongly associated with eggs and egg products (Patrick et al., 2004). Grade A shell eggs or products containing eggs have been the most common vehicle for the transmission of *S.* Enteritidis (Braden, 2006). In 2010 there was an egg associated outbreak that led to nearly 2000 illnesses nationwide in the US (CDC, 2010b).

This review will have a brief description of eggs and egg products and a discussion of the physiology of egg formation. We will outline the natural defenses of the egg, as well as the infection routes of *Salmonella*. Risk factors for flock infection with *S.* Entertidis will be discussed, as well as post-harvest intervention methods.

2. Eggs and egg products

On average, Americans consumed 248 eggs per person in the year 2008 (Evans, 2009). Eggs are used as an inexpensive food source in the form of shell eggs, liquid, frozen, and dried products (Ricke, Birkhold, & Gast, 2001). The US egg industry is the second largest producer of chicken eggs in the world after China (NASS, 2009). According to the 2007 US Census of Agriculture, there were 146,000 US farms with layers in 2007, the majority of which are small-scale producers (USDA, 2009). However, larger producers provide the bulk of commercially produced table eggs. According to United Egg Producers, more than 95% of egg production comes from only 240 egg producing companies that have flocks of more than 75,000 layers per flock. There are six egg producing companies in the US which own from 1 to 5 million layers each, and 12 companies that have more than 5 million layers each.

Not only do eggs and egg products provide a reliable source of nutrition, they also serve a variety of functions in other products. The emulsifying properties of lecithin and cholesterol within the egg yolk make eggs valuable components of mayonnaise and other food systems requiring an emulsifier (Baker & Bruce, 1994). Albumen, or egg white, is noted for its ability to form heat stable foams used in cakes, meringues, and other baked products. Other products involving egg products include noodles, candy and ice creams (Ricke et al., 2001). Eggs used in products with other primary ingredients are referred to as hidden eggs.

Egg products are popular in foodservice operations due to convenience of use, cost savings (for labor and storage) and for

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portion control (Messens, Grijspeerdt, Herman, & Billet, 2002). Egg products include whole eggs, egg whites, and egg yolks in frozen, refrigerated liquid, and dried forms, as well as specialty egg products. Specialty egg products include pre-peeled hard-cooked eggs, omelets, egg patties, quiches, quiche mixes, scrambled eggs, fried eggs, and others. Due to wide spread use of eggs as a food source, the safety of this product is important. Eggs and egg contents can become contaminated by bacteria in a variety of ways, not only after being laid, but also during formation in the ovary as well. Eggs contaminated through transovarian infection may pose a significant hazard to both industry and consumers, and therefore necessitate an understanding of the reproductive tract of the laying hen.

3. The physiology of egg laying

The female reproductive system of the chicken is divided into two separate parts: the ovary and the oviduct. In most chickens, only the left ovary and oviduct are functional, the right typically regresses during development and is non-functional in the adult bird (Okubo, Akachi, & Hatta, 1997). There have been cases, however, where the left ovary and oviduct have been damaged and the right one has developed to replace it.

3.1. Follicular hierarchy

In a mature laying hen the ovary is composed of follicles of different size and maturity at any given time, with one follicle being ovulated every 24 to 40 h (Etches, 1990). Immature follicles which do not yet possess large volumes of yolk material contain granulosa cells, a distinct layer of cells releasing a variety of hormones in the more mature follicles (Etches, 1990). The follicular hierarchy of the mature laying hen is composed of 7 to 10 follicles of 10 to 35 mm, 15 to 20 follicles of 1 to 10 mm, and several thousand follicles of less than 1 mm (Williams & Sharp, 1978). The largest, most mature follicle of the hierarchy is referred to as the F1 follicle, and is released into the reproductive tract when maturation is complete. The F1 follicle has the appearance of a fully formed egg yolk, but is still encapsulated in a follicular sack with visible capillaries lining the surface of the follicle, for the purpose of transport and deposition of yolk material (Johnson, 2000). Follicles that are progressively smaller than the F1 follicle and further down the hierarchy of maturity are termed F2, F3, F4, and F5; along with the F1 follicle, these are referred to as the large yellow follicles. Also present are small yellow follicles in which yolk material has begun to deposit, and these move forward in size and volume to become F follicles as more yolk material is carried via the blood stream from the liver into the developing follicle (Johnson, 2000).

3.2. Follicle growth

Maturation and growth of the follicles is divided into three separate phases. First is a slow period of growth which can last over a range of time from a few months up to several years. This slow growth phase is followed by a more rapid period of a few months in which yolk protein is brought into the developing follicle. The last phase of follicle growth is characterized by rapid growth over a period of 6 to 11 days. During these last days before the follicle is released into the reproductive tract a vast amount of yolk material is imported into the follicle (Johnson, 2000). Yolk components are synthesized in the liver and transported via the vascular system to the ova. Precursors for yolk contents, vitellogenin (VTG) and very low density lipoprotein (VLDL) are moved through the granulosa cells by blood capillaries (Johnson, 2000). VTG and VLDL are deposited inside the oocyte and contribute to the observed increase in follicle weight and volume.

3.3. Hormone regulation of the reproductive system

Luteinizing hormone (LH) is a particularly important hormone in the regulation of ovulation in the laying hen. A surge in LH is observed approximately 6 h prior to ovulation of the mature F1 follicle (Wilson & Sharp, 1973). The purpose of LH is to induce ovulation of the largest F follicle in the hierarchy (Etches, 1990). LH also promotes the production of steroids important to the ovulatory cycle of the hen (Etches, 1990). Robinson et al. (1988) demonstrated that androstenedione and estradiol are produced in the small yellow follicles, adrostenedione is secreted by the theca layer, and all are stimulated by LH. Luteinizing hormone also serves to stimulate the production of progesterone from granulosa cells within the follicle. Androgens are released from the theca layer of the follicle until approximately 12 to 36 h prior to ovulation, and are stimulated by LH prior to its major surge (Etches, 1990). Although all large F follicles produce steroids and hormones used throughout the ovulatory cycle, the F1 follicle is far more responsive to the action of LH, accounting for the fact that typically only the largest F follicle is released during a particular ovulation (Robinson & Etches, 1986).

Follicle stimulating hormone (FSH) also plays a major role in the ovulatory cycle, but the actions are less well defined than those of LH (Hammond, Burke, & Hertelendy, 1981). Like LH, FSH stimulates the production and release of progesterone from small yellow follicles. The smaller, preovulatory follicles are apparently the major target of FSH (Imai & Nalbandov, 1971). It is also known that FSH is bound most frequently to all follicles 12 to 16 h prior to ovulation (Etches, Croze, & Duke, 1981).

Progesterone is released from the large F1 follicle of the follicular hierarchy, apparently stimulated by relatively low levels of LH (Etches, MacGregor, Morris, & Willams, 1983). Progesterone release in turn stimulates secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus gland. GnRH is known to induce the large surge of LH which precedes ovulation by 4 to 6 h (Wilson & Sharp, 1973). The subsequent surge in LH accelerates the production of progesterone. Both LH and progesterone have the ability to degrade the follicular sack surrounding the F1 follicle and allow it to drop into the infundibulum, the opening to the reproductive tract (Etches, 1990).

3.4. Descent of the egg in the reproductive tract

The ovulated follicle remains in the infundibulum for 15 to 30 min, and this is where the outer layer of the vitelline membrane and the chalazal layer of the albumin are most likely generated (Burley & Vadehra, 1989). The follicle subsequently descends into the albumen-secreting portion of the oviduct, where it remains for about 3 h (Okubo et al., 1997). The albumen-wrapped egg yolk then moves to the isthmus, where the shell membranes are deposited, a process that takes slightly more than an hour (Okubo et al., 1997). The uterus is a very short portion of the oviduct, but the egg is held there for approximately 21 h to complete the process of calcification (Okubo et al., 1997). The egg passes through the last portion of the oviduct, the vagina, towards an external opening known as a vent. The vent is a common opening for both egg laying and waste elimination, but a chicken cannot perform both functions at the same time. An internal flap known as a cloaca keeps the vaginal canal and the intestinal track separate until either an egg or excrement reach the vent; when a chicken is laying an egg, the cloaca descends and blocks the intestinal track (Koch, 1973).

4. Egg defenses against bacterial contamination

Eggs function primarily as a means of reproduction to avian species, and as such the egg must be able to protect a developing embryo for the period of 21 days during which a chick will mature

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