Salmonella Infantis, a Potential Human Pathogen has an
Association with Table Eggs

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Abstract: Food borne Salmonellosis in human is mainly caused by the consumption of contaminated eggs and other poultry products. Trans-shell route is considered the underlying phenomena leading to the production of Salmonella Infantis contaminated eggs. Salmonella Infantis comes in the top 10 human pathogenic Salmonella serovars, been isolated from human and poultry from diverse group of countries in patients linked to contaminated food. Majority of the Salmonella cases are sporadic, outbreaks occur frequently with a direct or indirect link to contaminated food especially poultry. This review has mainly highlighted the factors affecting Salmonella transmission with a special emphasis on hen eggshell quality.

Key words: Hen eggshell, salmonellosis, transmission route, factors

INTRODUCTION
Non typhoidal salmonellosis is a food borne zoonotic disease of primary concern globally. A variety of animals act as reservoirs for carrying pathogenic Salmonella organism. The human salmonellosis is directly linked to contaminated poultry and poultry products (Parry et al., 2002; Patrick et al., 2004). The incidences of human infection caused by Salmonella Infantis have not been too dramatic as other Salmonella serovars. Salmonella Infantis belong to the 10 main Salmonella serotypes isolated (Well and Grimont, 2005) that causes gastroenteritis in human. Salmonella Infantis have been isolated from food contaminated infections in diverse countries including Japan (Shahada et al., 2008), Argentina (Merino et al., 2003), Finland (Peikonen et al., 1994), Australia (Cox et al., 2002), India (Patil et al., 2012) and Brazil (Fonseca et al., 2008). Salmonella Infantis occur in poultry industry globally (Cox et al., 2002). A report from UK has mentioned 0.3% deaths from Salmonellosis caused by Salmonella Infantis during 1996-2006 (Jones et al., 2008). The percent of naturally Salmonella human cases linked with infected eggs varies in different public health laboratory reports in different countries (De Buck et al., 2004). Non Typhi Salmonella have been reported to be a major cause of mortality and morbidity throughout the world (Graham, 2002). Although some virulence genes are located on plasmid common to many Salmonella serovars, majority of the virulence genes are encoded within Salmonella pathogenicity islands in the chromosome (Marcus et al., 2000). Antibiotic resistance determinants usually are encoded on plasmids but can also be present on the multidrug resistance region of Salmonella Genomic Island 1 (SGI1) (Fluit, 2005).

Salmonella and its taxonomical classification: The Salmonella are facultative, chemoorganotrophic, gram negative rods belong to family Enterobacteriaceae which are relatively small bacteria measuring about 0.5 μm by 2 to 3 μm and most strains are motile with peritrichous flagella (Cox et al., 2000; Alakomi and Saarela, 2009). Salmonella normally resides in the gut of wild and domestic animals (Pang et al., 2011) and this intracellular anaerobe can be found within a variety of phagocytic and non phagocytic cells in vivo (Ibarral and Steele-Mortimer, 2009). Salmonella grow at 7-48°C with an optimum growth temperature at 37°C and at pH 4 to 9.5 with an optimal growth at pH 6.5 to 7.5 (Alakomi and Saarela, 2009).

Taxonomically, Salmonella are divided into two species: Salmonella enterica and Salmonella bongori (subspecies V) and the former is comprised of six subspecies which include Salmonella enterica ssp. Enterica (I), Salmonella enterica ssp. Salamae (II), Salmonella enterica ssp. Arizonae (IIIa), Salmonella enterica ssp. Diarizonae (IIIb), Salmonella enterica ssp. Houtenae (IV) and Salmonella enterica ssp. Indica (VI) (Park et al., 2009; Alakomi and Saarela, 2009; Uzzau et al., 2000; Tinadil et al., 2005). Typical Salmonella can be differentiated from other members of the family by lack of fermentation of lactose, fermentation of glucose with production of gas and production of H2S from thiosulfate (Cox et al., 2000).

Association of Salmonella with table egg: Salmonella have been found to be the most common food borne disease in the world (Plym-Forsyth and Wierup, 2006; Henikstad et al., 2002). Salmonellosis is a public health problem of serious magnitude globally (Majowicz et al., 2010). Eggs are prone to microbial attack with subsequent deterioration depending upon the eggshell strength and source of contamination. The most reported source of human salmonellosis is eggs and
egg products (Kimura et al., 2004; Gillespie et al., 2005; Zhang et al., 2006). Among its numerous vectors and reservoirs for transmission, poultry are considered to be a significant reservoir which readily transmit the organism to human (March, 1969; Cox et al., 2000). Salmonella enterica has been identified as a dominant source of egg salmonellosis which is believed to be primarily transmitted by infected eggs (Sato and Kuwamoto, 1999). Most of the Salmonella serovars including Salmonella Enteritidis and Salmonella Infantis are not serious pathogens in the chicken but they pose a potential threat to public health (Lapuz et al., 2012). More than 76 different isolates of Salmonella Infantis associated with poultry have been typed recently by the Australian Salmonella Reference Centre (ASRC) in South Australia (Ross and Heukenroeder, 2008). Salmonella Infantis more intensely colonize the chicken alimentary tract compared to other serovars (Smith and Tucker, 1980) but its presence in the reproductive tract and vertical transmission to the egg is still unclear. Season of the year plays an important role in both environmental and egg microbial level and a higher level of total microbial load in the environment and on the egg shell has been determined in the summer (Hara-Kudo et al., 2001). Differences in the frequency at which they invade internal organs and contaminate eggs have been reported between Salmonella serotypes and even between strains of the same serotype (Gast et al., 2007).

Routes of egg contamination: Increasing consumer awareness of food safety issues has changed the public perception of a “good egg” from shell cleanliness and physical properties to that of microbial integrity (De Reu et al., 2006). Microorganisms can contaminate eggs at different stages, from production through processing to preparation and consumption (De Reu et al., 2006). Chickens are among the avian species that shed Salmonella and other pathogens in the faeces. These bacteria, in turn, attack the egg shell surface and make their way to the internal contents of the egg. Rate of penetration is affected by a number of factors including bacterial load and shell ultrastructural properties. When such infection occurs in hatching eggs, hatchability is reduced while, in commercial eggs, bacteria pose a serious threat to public health (Williams and Dillard, 1973). Eggs can be contaminated in two ways, namely vertical (trans-ovarian) and horizontal (trans-shell) contamination. In trans-ovarian contamination, the egg becomes contaminated prior to oviposition, with the source of contamination originating in the ovary and/or oviduct (Bruce and Drysdale, 1994; De Reu et al., 2008, 2010; Botteldoorn et al., 2010; Keller et al., 1995; Miyamoto et al., 1997; Okamura et al., 2001). The vertical route is considered the most important way of Salmonella Enteritidis and Salmonella Typhimurium transmission (Gast and Beard, 1990; Miyamoto et al., 1997) while Salmonella Infantis is predominantly transmitted through horizontal route (Humphrey, 1994), however these serovars can transmit through either route (Messens et al., 2005; Okamura et al., 2001; De Vylder et al., 2011). In trans-shell contamination, microorganisms gain access to the egg after egg contents enveloped by the shell (De Reu et al., 2010, 2006; Messens et al., 2005; Quarles et al., 1970; Schoeni et al., 1995). Horizontal transmission includes infection of the contents during egg transit through cloaca or after oviposition (EFSA, 2005). Barrow and Lovell (1991) suggest that most of egg contamination is due to horizontal transmission, although others do not agree (Humphrey et al., 1991; Reiber and Conner, 1995). Miyamoto et al. (1997) inoculated hens using different routes and found that intravenous inoculation caused colonization of the ovary and contamination of eggs forming in the oviduct. Their experiments also revealed that intra vaginal inoculation led to the colonization of only the lower parts of the oviduct but internally contaminated eggs were being produced which explains that some internal contamination of eggs may be coming from the lower oviduct through penetration of the egg shell in the oviduct. The role of the infected fowl as a possible vehicle for the transmission of enteropathogens particularly Salmonella is unclear so far but higher bacterial counts were found in the oviduct of birds that were naturally and artificially mated with Salmonella positive fowls than in virgin birds (Reiber and Conner, 1995).

Factors affecting Salmonella penetration and transmission: Egg is naturally equipped with barriers that help keep microorganisms from penetrating the interior shell, membranes and egg contents (Kretzschmar-Mccluskey et al., 2009). A number of factors like relative humidity (Gregory, 1948), overall shell quality (Sauter and Petersen, 1974; Solomon, 1991; Roberts, 2004), number of shell pores (Walden et al., 1956; Brown et al., 1965), temperature (Graves and Maclaury, 1982), pH (Sauter et al., 1977) and bacterial load (Williams et al., 1968) directly affect microbial penetration across the eggshell. Bacteria can be isolated from shell membranes and egg albumen immediately after a day of artificially eggshell contamination (Humphrey et al., 1991, 1989; Murase et al., 2006).

Eggshell quality: Whole eggs with low specific gravity or low shell quality are more likely to be penetrated by Salmonella (Sauter and Petersen, 1974). Egg weight, specific gravity, conductance and flock age influence penetration of Salmonella with a poor eggshell being penetrated more quickly by Salmonella Berrang et al. (1998). All major food contaminating serovars of Salmonella can penetrate eggshell (Gantois et al., 1997).
2009). The *Salmonella* first penetrate through the cuticle and shell, then colonize the shell membranes from where it moves on to albumen and yolk leading ultimately to whole egg contamination (Lock et al., 1992). Thus, with horizontal transmission, the egg contents are not contaminated until the cuticle, shell and shell membranes fail to prevent microbial invasion and penetration (Berrang et al., 1999). Eggs are most vulnerable to bacterial penetration in the first 30 to 60 sec after lay before the cuticle hardens and effectively caps the pores (Berrang et al., 1999). Shell thickness does not have a significant effect on bacterial penetration but the presence of cuticle plugging the shell pores is more important (William et al., 1968).

Whether microorganisms become localized in the albumen or shell membrane depends largely on whether the infundibulum or the shell gland becomes infected (Barrow, 1994). Shell porosity appeared to be a useful index for determining susceptibility of eggs to bacterial penetration (Kraft et al., 1958). The infection of eggshells is more readily achieved by contaminating the blunt end of the eggs (Vadehra et al., 1970). However, Nascimento and Solomon (1991) reported that bacterial penetration was independent of pore numbers. A 1000 times higher level of bacterial contamination was found in cracked eggs compared to intact one (March, 1989). A number of studies (Messens et al., 2005; De Reu et al., 2010; Williams et al., 1968) have shown the relationship of eggshell quality and *Salmonella* with poor eggshell being highly penetrated.

**Environment:** Normally the prevalence of *Salmonella* in a positive flock varies with the husbandry conditions and *Salmonella* is not always recovered from eggs produced by positive flocks (Cox et al., 2002). Poppe et al. (1992) recovered *Salmonella* from only 2 out of 16000 eggs tested from a *Salmonella* positive environmental flock. Similarly, Humphrey et al. (1991, 1989) found low numbers of *Salmonella* positive eggs from naturally contaminated hens. The shell can already be infected when passing through the vent but many researchers suggest that the main bacterial contamination occurs within a short period after laying due to contact with dirty surfaces (Quarles et al., 1970; Gentry and Quarles, 1972). In the external contamination of eggshell with viable pathogens, the presence of chicken manure and other moist organic materials facilitates the survival and growth of *Salmonella* by providing the required nutrients and a degree of physical protection (Gantois et al., 2009). The quick proliferation rate of *Salmonella* in egg with faeces after artificial contamination suggests that faeces can serve as a nutritional reservoir for *Salmonella* (Schoeni et al., 1995). Humphrey et al. (1982) highlighted air born salmonellosis and intraocular inoculation of about 100 cells of *Salmonella* produced *Salmonella* infection of the ovary and oviduct of laying hens with a positive eggs production. There has been little systematic investigation of *Salmonella* contamination of eggshells from different production systems or on the effects of production system on the internal bacterial contamination of eggs thus shells contaminated by faecal and environmental *Salmonella* can be an important potential source of this organism (Holt et al., 2011). Housing confounding factors greatly affect *Salmonella* transmission and free range flocks are more positive for *Salmonella* compared to conventional cages.

**Temperature and humidity:** Eggshells can be penetrated by bacteria when water or some other liquid is present, especially if there is a temperature difference between the egg and the liquid (Berrang et al., 1999). Due to the temperature difference between the hen and the environment, the freshly laid warm egg cools rapidly, resulting in egg contents contraction which forms negative pressure within the egg and bacteria present in the environment or on the egg surface, is pulled into and through the eggshell and its membranes (Berrang et al., 1999). The air cell end is the only area where the inner and outer shell membranes do not remain in close contact, so this region may respond more rapidly to a change in temperature than the rest of the egg contents (Berrang et al., 1999). Temperature and number of viable *Salmonella* play a vital role in the growth of bacteria and the growth rate of *Salmonella* markedly increases as the temperature increases above 4°C (Kim et al., 1989) but declined rapidly at temperature above 42°C (Guan et al., 2006). Cooling has a positive effect on the overall quality of egg and the more quickly an egg is brought near the freezing point, the greater the egg quality is maintained (Jones et al., 2010). About 12% of eggshells less than 7 hours old contain bacteria (Wolk et al., 1960, as cited in Stadelman, 1994) and it has been concluded that the penetration and survival of bacteria in eggshells are favoured by elevated holding temperatures. Eggshell penetration studies at 9, 25 and 35°C showed maximum bacterial activity at 25°C (Stadelman, 1994). The survival of *Salmonella* in an environment is encouraged by low temperature (Messens et al., 2006; Radkowski, 2002). Control of the proliferation of *Salmonella* within eggs may be achieved by their storage at lower than ambient temperature which slows down both bacterial growth rates and changes to egg contents which facilitate *Salmonella* multiplication (Cogan et al., 2004).

**Flock health status and sanitation:** Poor sanitation in processing plants, improper handling and preparation of poultry, eggs and their products contribute to the *Salmonella* problem but the basic source of infection
probably lies within the avian population (Foley et al., 2011). In general, the dynamics of Salmonella infection in the flock depend on the susceptibility of hens for colonization, the number of Salmonella shed by colonized hens into the environment and the contact structure between colonized and susceptible hens (Thomas et al., 2011). Generally, aerobic bacterial counts on eggshells are lower from caged (conventional and furnished) than from non caged (aviary and floor) flocks and this difference is very marked when eggs laid outside of the nest boxes in the non cage flocks are included De Reu et al. (2008). Stress factors like re-housing, thermal extremes, transport, initiation of egg lay and molting have all been shown to exacerbate infection susceptibility in poultry (Holt et al., 2011). Certain concurrent diseases like Eimeria infection, infectious bursal disease virus and reticuloendotheliosis virus has been shown to increase the severity and persistence of Salmonella infections (Holt et al., 2011). Salmonella are carried within the gut of the birds and are shed from infected bird through faeces, feather dust and secretions from the eyes and nose contaminating the environment (Vikari, 2011). Increasing numbers of micro-organisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination (Messens et al., 2005; De Reu et al., 2008).

Conclusion: Salmonella Infantis is a pathogenic bacteria that causes gastroenteritis in human. Contaminated table eggs are a high source of Salmonella transmission to human food chain. Eggshell quality, temperature and overall flock health status are the factors that highly affect Salmonella persistence in the poultry and their ultimate transmission to human.

REFERENCES


