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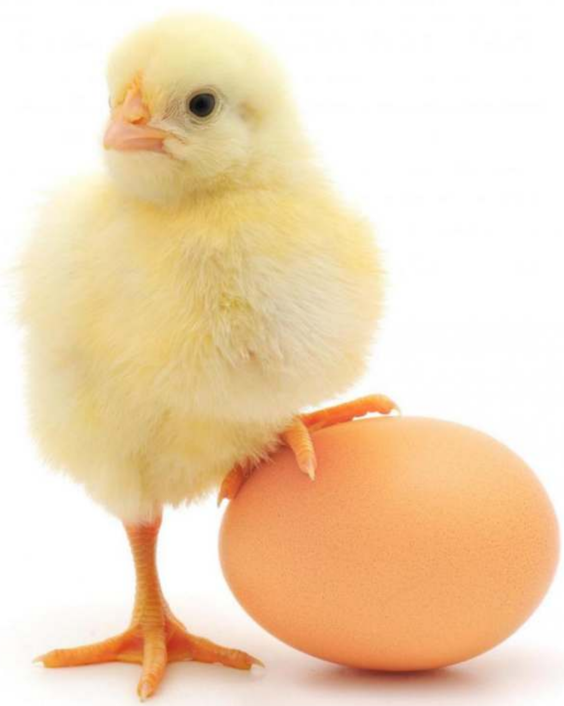

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Salmonella Infantis, a Potential Human Pathogen has an Association with Table Eggs

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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 (Weill 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*., 2006), Argentina (Merino *et al*., 2003), Finland (Pelkonen *et al*., 1994), Australia (Cox *et al*., 2002), India (Patil *et al*., 2012) and Brazil (Fonseca *et al*., 2006). *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* (Ibarra 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 H_2S 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-Forshell and Wierup, 2006; Herikstad *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 Infantis has been identified as a dominant source of dog 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 Heuzenroeder, 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, 1962), 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*., 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, 1969). 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.* (1992) highlighted air born salmonellosis and intra-ocular 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.*, 1950, 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.*, 2006).

Conclusion: *Salmonella* Infantis is a pathogenic bacteria that causes gasteroenteritis 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

Alakomi, H.L. and M. Saarela, 2009. *Salmonella* importance and current status of detection and surveillance methods. Quality Assurance and Safety of Crops and Foods. Retrieved online from: http://onlinelibrary.wiley.com/doi/10.1111/j.1757-837X.2009.00032.x/pdf

Barrow, P.A. and M.A. Lovell, 1991. Experimental infection of egg-laying hens with *Salmonella* Enteritidis phage type 4. Avian Pathol., 20: 335-348.

Barrow, P.A., 1994. The microflora of the alimentary tract and avian pathogens: translocation and vertical transmission. In R. G. Board and R. Fuller (Eds.). Microbiology of the Avian Egg (pp:117-138). London, U.K.: Chapman and Hall.

Berrang, M.E., N.A. Cox, J.F. Frank and R.J. Buhr, 1999. Bacterial penetration of the eggshell and shell membranes of the chicken hatching egg: A Review. J. Applied Poult. Res., 8: 499-504.

Berrang, M.E., J.F. Frank, R.J. Buhr, J.S. Bailey, N.A. Cox and J. Mauldin, 1998. Eggshell characteristics and penetration by *Salmonella* through the productive life of a broiler breeder flock. Poult. Sci., 77: 1446-1450.

Botteldoorn, N., E.V. Coillie, J. Goris, H. Werbrouck, V. Piessens, C. Godard and P. Scheldeman, 2010. Limited genetic diversity and gene expression differences between egg- and non-egg-related *Salmonella* Enteritidis strains. Zoonoses Public Health, 57: 345-357.

Brown, W.E., R.C. Baker and H.B. Naylor, 1965. Comparative susceptibility of chicken, duck and turkey eggs to microbial invasion. Retrieved from onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1965...x/pdf

Bruce, J. and E.M. Drysdale, 1994. Trans-shell transmission. In R.G. Board and R. Fullar (Eds.) Microbiology of the Avian Egg, London, U.K.: Chapman and Hall, pp: 69-91.

Cogan, T.A., F. Jorgensen, H.M. Lappin-Scott, C.E. Benson, M.J. Woodward and T.J. Humphrey, 2004. Flagella and curli fimbriae are important for growth of *Salmonella enterica* serovars in hen eggs. Microbiology, 150: 1063-1071.

Cox, J.M., J.B. Woolcock and A.L. Sartor, 2002. The significance of *Salmonella*, particularly *Salmonella* Infantis, to the Australian egg industry. Report for the Rural Industries Research and Development Corporation Australia.

Cox, N.A., M.E. Berrang and J.A. Cason, 2000. *Salmonella* penetration of eggshells and proliferation in broiler hatching eggs. Poult. Sci., 79: 1571-1574.

De Buck, J., F. Van Immerseel, F. Haesebrouck and R. Ducatelle, 2004. Colonization of the chicken reproductive tract and egg contamination by *Salmonella*. J. Applied Microbiol., 97: 233-245.

De Reu, K., W. Messens, K. Grijspeerdt, M. Heyndrickx, M. Uyttendaele and L. Herman, 2010. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella* Enteritidis. Proceed. Aust. Poult. Sci. Symp., 21: 126-129.

De Reu, K., W. Messens, M. Heyndrickx, T.B. Rodenburg, M. Uyttendaele and L. Herman, 2008. Bacterial contamination of table eggs and the influence of housing systems. World Poult. Sci. J., 64: 5-19.

De Reu, K., K. Grijspeerdt, M. Heyndrickx, M. Uyttendaele, J. Debevere and L. Herman, 2006. Bacterial eggshell contamination in the egg collection chains of different housing systems for laying hens. Br. Poult. Sci., 47: 163-172.

De Vylder, J., J. Dewulf, S. Van Hoorebeke, F. Pasmans, F. Haesebrouck, R. Ducatelle and F. Van Immerseel, 2011. Horizontal transmission of *Salmonella* Enteritidis in groups of experimentally infected laying hens housed in different housing systems. Poult Sci., 90: 1391-1396.

EFSA, 2005. Opinion of the scientific panel on biological hazards on the request from the commission related to the microbiological risks on washing of table eggs. EFSA J., 269: 1-39.

Fluit, A.C., 2005. Towards more virulent and antibiotic-resistant *Salmonella*? Immunol. Med. Microbiol., 43: 1-11.

Foley, S.L., R. Nayak, I.B. Hanning, T.J. Johnson, J. Han and S.C. Ricke, 2011. Population Dynamics of Salmonella enterica serotypes in commercial egg and poultry production. Applied Environ. Microbiol., 77: 4273-4279.

Fonseca, E.L., O.L. Mykytczuk, M.D. Asensi, E.M.F. Reis, L.R. Ferraz, F.L. Paula, L.K. Ng and D.P. Rodrigues, 2006. Clonality and antimicrobial resistance gene profiles of multidrug-resistant *Salmonella enterica* serovar Infantis isolates from four public hospitals in Rio de Janero, Brazil. J. Clin. Microbiol., 44: 2767-2772.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, R. Gast, T.J. Humphrey and F. Van Immerseel, 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiol. Rev., 33: 718-738.

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

Gast, R.K., R. Guraya, J. Guard-Bouldin, P.S. Holt and R.W. Moore, 2007. Colonization of specific regions of the reproductive tract and deposition at different locations inside eggs by hens infected with *Salmonella* Enteritidis or *Salmonella* Heidelberg. Avian Dis., 51: 40-44.

Gentry, R.F. and C.L. Quarles, 1972. The measurement of bacterial contamination on eggshells. Poult. Sci., 51: 930-933.

Gillespie, I.A., S.J. OBrien, G.K. Adak, L.R. Ward and H.R. Smith, 2005. Foodborne general outbreaks of *Salmonella* Enteritidis phage type 4 infection, England and Wales, 1992-2002: where are the risks? Epidemiol. Infect., 133: 795-801.

Graham, S.M., 2002. Salmonellosis in children in developing and developed countries and populations. Curr. Opin. Infect. Dis., 15: 507-512.

Graves, R.C. and D.W. Maclaury, 1962. The effects of temperature, vapor pressure and absolute humidity on bacterial contamination of shell eggs. Poult. Sci., 41: 1219-1225.

Gregory, D.W., 1948. *Salmonella* infections of turkey eggs. Poult. Sci., 27: 359-366.

Guan, J., C. Grenier and B.W. Brooks, 2006. *In vitro* Study of *Salmonella* Enteritidis and *Salmonella* Typhimurium Definitive Type 104: Survival in Egg Albumen and Penetration through the Vitelline Membrane. Poult. Sci., 85: 1678-1681.

Hara-Kudo, Y., Y. Sakakibara, H. Konuma, T. Sawada and S. Kumaga, 2001. Laying season and egg shell cracks on the growth of *Salmonella* Enteritidis in the egg albumen during storage. J. Food Prot., 64: 1134-1137.

Herikstad, H., Y. Motorjemin and R.V. Tauxe, 2002. *Salmonella* surveillance: A global survey of public health serotyping. Epidemiol. Infect., 129: 1-8.

Holt, P.S., R.H. Davies, J. Dewulf, R.K. Gast, J.K. Huwe, D.R. Jones, D. Waltman and K.R. Willian, 2011. The impact of different housing systems on egg safety and quality. Poult. Sci., 90: 251-262.

Humphrey, T.J., A. Baskerville, S. Mawer, B. Rowe and S. Hopper, 1989. *Salmonella* Enteritidis phage type 4 from the contents of intact eggs: A study involving naturally infected eggs. Epidemiol. Infect., 103: 415-423.

Humphrey, T.J., A. Whitehead, A.H.L. Gawler, A. Henley and B. Rowe, 1991. Numbers of *Salmonella* Enteritidis in the contents of naturally contaminated hens' eggs. Epidemiol. Infect., 103: 489-496.

Humphrey, T.J., A. Baskerville, H. Chart, B. Rowe and A. Whitehead, 1992. Infection of laying hens with *Salmonella* Enteritidis PT4 by conjunctival challenge. Vet. Rec., 131: 386-388.

Humphrey, T.J., 1994. Contamination of eggshell and contents with Salmonella Enteritidis: A review. Int. J. Food Microbiol., 21: 31-40.

Ibarra, J.A. and O. Steele-Mortimer, 2009. *Salmonella*-the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. Cell. Microbiol., 11: 1579-1586.

Jones, D.R., M.T. Musgrove, K.E. Anderson and H.S. Thesmar, 2010. Physical quality and composition of retail shell eggs. Poult. Sci., 89: 582-587.

Jones, T.F., L.A. Ingram, P.R. Cieslak, D.J. Vugia, M. Tobin-D'angelo, S. Hurd, C. Medus and A. Cronquist, 2008. Salmonellosis outcomes differ substantially by serotype. J. Infect. Dis., 198: 109-114.

Keller, L.H., C.E. Benson, K. Krotec and R.J. Eckroade, 1995. *Salmonella* Enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. Infect. Immun., 63: 2443-2449.

Kim, C.J., D.A. Emery, H. Rinke, K.V. Nagaraja and D.A. Halvorson, 1989. Effect of time and temperature on growth of *Salmonella* Enteritidis in experimentally inoculated eggs. Avian Dis., 33: 735-745.

Kimura, A.C., V. Reddy, R. Marcus, P.R. Cieslak, J.C. MohleBoetani, H.D. Kassenborg, S.D. Segler, F.P. Hardnett, T. Barrett and D.L. Swerdlow, 2004. Chicken consumption is a newly identified risk factor for sporadic *Salmonella enterica* serotype Enteritidis infections in the United States: A case-control study in FoodNet sites. Clin. Infect. Dis., 38: S244-S252.

Kretzschmar-McCluskey, V., P.A. Curtis, K.E. Anderson, W.D. Berry and L.K. Derth, 2009. Influence of hen age and strain on eggshell exterior, eggshell interior with membranes and egg contents of microflora and on *Salmonella* incidence during a single production cycle. J. Applied Poult. Res., 18: 665-670.

Kraft, A.A., E.H. McNally and A.W. Brant, 1958. Shell quality and bacterial infection of chicken eggs. Poult. Sci., 37: 638-644.

Lapuz, R.R., D.V. Umali, T. Suzuki, K. Shirota and H. Katoh 2012. Comparison of the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities. Avian Dis., 56: 29-34.

Lock, J.L., J. Dolman and R.G. Board, 1992. Observations on the mode of bacterial infection of hen's egg. FEMS Microbiol. Lett., 100: 71-74.

Majowicz, S.E., J. Musto, E. Scallan, F.J. Angulo, M. Kirk, S.J. O'Brien, T.F. Jones, A. Fazil and R.M. Hoekstra, 2010. The Global burden of Nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis., 50: 882-889.

March, B.E., 1969. Bacterial infection of washed and unwashed eggs with reference to *Salmonella*. Appl. Microbiol., 17: 98-101.

Marcus, S.L., J.H. Brumell, C.G. Pfeifer and B.B. Finlay, 2000. *Salmonella* pathogenicity islands: big virulence in small packages. Microb. Infect., 2: 145-156.

Merino, L.A, M.C. Ronconi, M.M. Navia, J. Ruiz, J.M. Sierra, N.B. Cech, N.S. Lodeiro and J. Vila, 2003. Analysis of the clonal relationship among clinical isolates of *Salmonella enterica* serovar Infantis by different typing methods. Rev. Inst. Med. Trop. Sao. Paulo., 45: 119-123.

Messens, W., K. Grijspeerdt and L. Herman, 2006. Eggshell penetration of hens eggs by *Salmonella enterica* serovar Enteritidis upon various storage conditions. Br. Poult. Sci., 47: 554-560.

Messens, W., K. Grijspeerdt and L. Herman, 2005. Eggshell characteristics and penetration by *Salmonella enterica* serovar Enteritidis through the production period of a layer flock. Br. Poult. Sci., 46: 694-700.

Miyamoto, T., E. Baba, T. Tanaka, K. Sasai, T. Fukata and A. Arakawa, 1997. *Salmonella* Enteritidis contamination of eggs from hens inoculated by vaginal, cloacal and intravenous routes. Avian Dis., 41: 296-303.

Murase, T., K. Fujimoto, R. Nakayama and K. Otsuki, 2006. Multiplication and motility of *Salmonella enterica* serovars Enteritidis, Infantis and Mentervideo in *in vitro* contamination models of eggs. J. Food Prot., 69: 1012-1016.

Nascimento, V.P. and S.E. Solomon, 1991. The transfer of bacteria (Salmonella Enteritidis) across the eggshell wall of eggs classified as "poor" quality. Animal Technology, 42: 157-166.

Okamura, M., Y. Kamijima, T. Miyamoto, H. Tani, K. Sasai and E. Baba, 2001. Differences among six *Salmonella* serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. Avian Dis., 45: 61-69.

Pang, E., C. Tien-Lin, M. Selvaraj, J. Chang and J. Kwang, 2011. Deletion of the aceEgene (encoding a component of pyruvate dehydrogenase) attenuates *Salmonella enterica* serovar Enteritidis. FEMS Immunol. Med. Microbiol., 63: 108-118.

Parry, S.M., S.R. Palmer, J. Slader, T. Humphrey and the South East Wales Infectious Disease Liaison Group, 2002. Risk factors for *Salmonella* food poisoning in the domestic kitchen: A case control study. Epidemiol. Infect., 129: 277-285.

Park, S.H., H.J. Kim, W.H. Cho, J.H. Kim, M.H. Oh, S.H. Kim, B.K. Lee, S.C. Ricke and H.Y. Kim, 2009. Identification of Salmonella enterica subspecies I, Salmonella enterica serovars Typhimurium, Enteritidis and Typhi using multiplex PCR. FEMS Microbiol. Lett., 301: 137-146.

Patil, S.R., A.Y. Kshirsagar, M.V. Ghorpade and R.V. Shinde, 2012. Cellulitis due to *Salmonella* Infantis. Online J. Health Allied. Sci., 11: 12.

Patrick, M.E., P.M. Adcock, T.M. Gomez, S.F. Altekruse, B.H. Holland, R.V. Tauxe and D.L. Swerdlow, 2004. *Salmonella* Enteritidis infections, United States, 1985-1999. Emerg. Infect. Dis., 10: 1-7.

Pelkonen, S., E.L. Romppanen, A. Siitonen and J. Pelkonen, 1994. Differentiation of *Salmonella* serovar Infantis isolates from human and animal sources by fingerprinting IS200 and 16S rrn loci. J. Clin. Microbiol., 32: 2128-2133.

Plym-Forshell, L. and M. Wierup, 2006. *Salmonella* contamination: A significant challenge to the global marketing of animal food products. OIE Sci. Tec. Rev., 25: 541-554.

Poppe, C., R.P. Johnson, C.M. Forsberg and R.J. Irwin, 1992. *Salmonella* Enteritidis and other *Salmonella* in laying hens and eggs from flocks with *Salmonella* in their environment. Can. J. Vet. Res., 56: 226-232.

Quarles, C.L., R.F. Gentry and G.O. Bressler, 1970. Bacterial contamination in poultry houses and its relationship to egg hatchability. Poult. Sci., 49: 60-66.

Radkowski, M., 2002. Effect of moisture and temperature on survival of *Salmonella* Enteritidis on shell eggs. Archiv fur Geflugelkunde, 66: 119-123.

Reiber, M.A. and D.E. Conner, 1995. Effect of mating activity on the ability of *Salmonella* Enteritidis to persist in the ovary and oviduct of chickens. Avian Dis., 39: 323-327.

Roberts, J.R., 2004. Factors affecting egg internal quality and eggshell quality in laying hens. J. Poult. Sci., 41: 161-177.

Ross, I.L. and M.W. Heuzenroeder, 2008. A comparison of three molecular typing methods for the discrimination of *Salmonella enterica* serovar Infantis. FEMS Immunol. Med. Microbiol., 53: 375-384.

Sato, Y. and R. Kuwamoto, 1999. A Case of Canine Salmonellosis due to *Salmonella* Infantis. J. Vet. Med. Sci., 61: 71-72.

Sauter, E.A., C.F. Petersen, J.F. Parkinson and E.E. Steele, 1977. Effects of pH on eggshell penetration by *Salmonella*. Poult. Sci., 56: 1754-1755.

Sauter, E.A. and C.F. Petersen, 1974. The effect of eggshell quality on penetration by various *Salmonella*. Poult. Sci., 53: 2159-2162.

Schoeni, J., K.A. Glass, J.L. McDernott and A.C.L. Wong, 1995. Growth and penetration of *Salmonella* Enteritidis, *Salmonella* Heidelberg and *Salmonella* Typhimurium in eggs. Int. J. Food Microbiol., 24: 385-396.

Shahada, F., T. Chuma, T. Tobata, K. Okamoto, M. Sueyoshi and K. Takase, 2006. Molecular epidemiology of antimicrobial resistance among *Salmonella enterica* serovar Infantis from poultry in Kagoshima, Japan. Int. J. Antimicrob. Agents, 28: 302-307.

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

Solomon, S.E., 1991. Egg and shell quality. Published by Wolfe publishing ltd. England, Pages: 149.

Stadelman, W.J., 1994. Contaminants of liquid egg products. In R.G. Board and R. Fuller (Eds.), Microbiology of the Avian Egg, London, U.K.: Chapman and Hall, pp: 139-151.

Thomas, E., A. Bouma and D. Klinkenberg, 2011. A comparison of transmission characteristics of *Salmonella enterica* serovar Enteritidis between pair-housed and group-housed laying hens. Vet. Res., 42: 40-40.

Tindall, B.J., P.A.D. Grimont, G.M. Garrity and J.P. Euzeby, 2005. Nomenclature and taxonomy of the genus *Salmonella*. Int. J. Syst. Evol. Microbiol., 55: 521-524.

Uzzau, S., D.J. Brown, T. Wallis, S. Rubino, G. Leori, S. Bernard, J. Casadesus, D.J. Platt and J.E. Olsen, 2000. Host adapted serotypes of *Salmonella enterica*. Epidemiol. Infect., 125: 229-255.

Vadehra, D.V., R.C. Baker and H.B. Naylor, 1970. Infection routes of bacteria into chicken eggs. J. Food Sci., 61: 61-62.

Vikari, A., 2011. Feed additives role in reducing *Salmonella* in poultry. World poultry, *Salmonella* Special. November, 2011, pp: 10-11.

Walden, C.C., I.V.F. Allen and P.C. Trussell, 1956. The role of the eggshell and shell membranes in restraining the entry of microorganisms. Poult. Sci., 35: 1190-1196.

Weill, F.X. and P. Grimont, 2005. Les salmonelloses en France: données 2001-2003 du Centre National de Référence.www.invs.sante.fr/publications/2005/snmi/pdf/salmonelloses.pdf

Williams, J.E., L.H. Dillard and G.O. Hall, 1968. The penetration patterns of *Salmonella* Typhimurium through the outer structures of chicken eggs. Avian Dis., 12: 445-466.

Williams, J.E. and L.H. Dillard, 1973. The effect of external shell treatments on *Salmonella* penetration of chicken eggs. Poult. Sci., 52: 1084-1089.

Zhang, L., Z. Yan and E.T. Ryser, 2006. Comparison of the reveal test, the U.S. Food and Drug Administration culture method and selective media for recovery of *Salmonella* Enteritidis from commercial egg layer flock environment. J. Food Prot., 69: 2766-2769.