

Review on Salmonellosis in Poultry and Its Public Health Importance

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Abstract

Salmonellosis is an important zoonotic disease, which cause a serious illness in animals including birds and humans. The disease is caused by various serotypes of *Salmonella* which are aerobic and facultative anaerobic, gram-negative rods and motile with the exception of *S.pullorum* and *S.Gallinarum*. *Salmonella*, like most Enterobacteriaceae, are motile, nonspore forming, reduce nitrates to nitrites, ferment glucose, and are oxidase negative. The genus *Salmonella* consists of only two species, *Salmonella bongori* and *Salmonella enterica*, with the latter being divided into six subspecies; *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica*. It constitutes a major public health burden and represents a significant cost in many countries. The presence of any serotype of *Salmonella* in food renders that food unfit for human consumption. *Salmonella* are known for its wide range host. It can cause variety of diseases in some hosts while in others, can be asymptomatic. Poultry and eggs are considered as major sources for these pathogenic microorganisms. The disease is transmitted from animal to animal, animal to human and human to human direct or indirect pathway. Among *Salmonella* spp. *Salmonella Enterica* is one of the *Salmonella* serotypes responsible for causing enteric disease in humans.

Keywords: Salmonella, public health, poultry, prevention

1. INTRODUCTION

Eggs and egg products are nutritious foods and they form an important part of the human diet. Consuming eggs, however, has been associated with negative health impacts. Eggs and egg products that are improperly handled can be a source of food-borne diseases, such as salmonellosis. Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported world-wide every year and the disease results in thousands of deaths. ^[11]

Salmonella genus is a member of the Enterobacteriaceae family, comprising Gram-negative rod-shaped non spore-forming bacteria. Their main reservoir is the intestinal tract of humans and animals. ^[4] Among the different serotypes, *Salmonella enterica*, *S. Enteritidis*, and *S. Typhimurium* account for the most non typhoidal *Salmonella* infections in both developed and developing countries. ^[24] These serotypes are regarded as unrestricted, being able to cause infections in animals as well as in humans. Eggs and egg-based products were frequently associated with salmonellosis outbreaks caused by *S. Enteritidis* in the United States of America (U.S.A.), as well as in the European Union (E.U). ^[24] This is a potential consequence of the high frequency at which *S. Enteritidis* colonizes the ovaries of laying hens. Usually this happens without any lesions and furthermore, when egg storage conditions allow it, this foodborne pathogen may be isolated from the shell egg, as it survives in the forming egg. ^[28]

A wide range of foods has been implicated in food-borne illness attributable to *Salmonella enterica*. Foods of animal origin, especially poultry, poultry products and raw eggs, are often implicated in sporadic cases and outbreaks of human salmonellosis. Recent years have seen increases in salmonellosis associated with contaminated fruits and vegetables. Other sources of exposure include water, handling of farm animals and pets, and human person-to-person when hand-mouth contact occurs without proper washing of hands. ^[11]

Human illness by *Salmonella Enteritidis* has increased world-wide in the last two decades, due to ingestion of contaminated eggs, and it is currently considered the primary cause of salmonellosis in the world. In addition, the presence of *S. Enteritidis* in shell eggs constitutes a public health hazard, and poses a considerable economic impact on the poultry and egg industry. ^[58]

It is estimated that in the U.S., *Salmonella* transmission through contaminated shell eggs or egg products results in 700,000 cases of salmonellosis and costs \$1.1 billion annually. ^[58] In many countries, *Salmonella* spp. are controlled in egg production chain. Egg-laying flocks are monitored for *Salmonella* spp., and any flock confirmed with *S. Enteritidis* or *S. Typhimurium* is slaughtered. In addition, both feed materials and compound feeding stuffs for poultry are tested for *Salmonella* in those countries. ^[78]

Despite some attempts to study prevalence of *Salmonella* in Ethiopia, mainly in pig, cattle, poultry meat, minced beef and humans, but the status of the problem in chicken table egg is poorly known. However, studies

made elsewhere indicated that chicken eggs are important sources of *Salmonella* particularly among those raw consumers. One study in kombolcha town indicated that Out of the total 400 chicken table eggs examined for bacteriological status of *Salmonella*, an overall 11.5% prevalence of *Salmonella* was found. Therefore, the objective of this review is to review the occurrence of *Salmonella* spp. in eggs and environment and to highlight the public health importance of *Salmonella*.

2. THE GENUS SALMONELLA

2.1. Characteristics, taxonomy and nomenclature of Salmonella

Salmonella have been known to cause illnesses for more than 100 years when it was discovered by Dr. Daniel Salmon. *Salmonella* are Gram-negative bacilli belonging to the Family Enterobacteriaceae. *Salmonella*, like most Enterobacteriaceae, are motile, non spore forming, and facultative anaerobes that reduce nitrates to nitrites, ferment glucose, and are oxidase negative. The genus *Salmonella* consists of only two species, *Salmonella bongori* and *Salmonella enterica*, with the latter being divided into six subspecies (I – VI); *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI) as shown in Table 1.^[54]

Table 1 Nomenclature for *Salmonella* Enteritidis and associated subspecies,^[10]

family	Genus	Species	Subspecies	Serovar
Enterobacteriaceae	<i>Salmonella</i>	<i>Enterica</i>	<i>Enterica</i> <i>salamae</i> <i>arizonae</i> <i>diarizonae</i> <i>houtenae</i> <i>indica</i>	<i>Enteritidis</i>
		<i>bongori</i>		

Salmonella spp. are bacteria that are widespread in the environment that can be isolated from the intestines of most mammals, reptiles and birds. More than 2,500 serovars of *Salmonella* have been identified. Further studies have shown that *Salmonella* is capable of surviving for approximately 87 days in tap water, 115 days in pond water, 120 days in pasture soil and 280 days in garden soil.^[62] The key factors identified in *Salmonella* survival time in an external environment were temperature, frost, moisture content, humidity, sunlight,

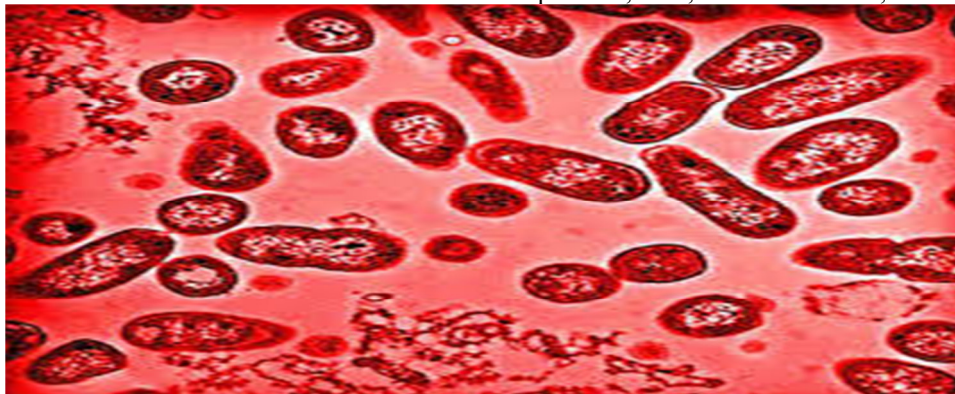


Fig. 1 *Salmonella* bacteria,^[62]

2.2. Salmonellosis

Salmonellosis is primarily a food-poisoning syndrome, which occurs when ingesting pathogenic *Salmonella* serotypes. The cause of food-borne salmonellosis is the penetration and passage of *Salmonella* organism from the gut lumen into the epithelium of the small intestine where inflammation occurs. There is also evidence that the pathogenesis may involve two toxins; an enterotoxin and a cytotoxin.^[80]

Salmonellosis is an infectious disease in both humans and animals. The infection is manifested in three forms; gastroenteritis, involving nausea, fever, vomiting and diarrhoea, enteric fever (typhoid and paratyphoid) and septicemia, which is usually characterized by fever, anorexia, anaemia and local lesions on the visceral organs. Human infections are usually associated with animal contact and the consumption of contaminated food products such as poultry, meat and other dairy products. *Salmonellosis* is usually considered as an asymptomatic or self-limiting illness, but it can also become invasive and fatal, especially for patients who are young or immunocompromised.^[80]

Non-typhoidal *Salmonella* strains are important causes of infections in both humans and animals, and this disease is caused by *Salmonella* serotypes other than *S. Typhi* and *S. Paratyphi*. It is a major food-borne infection with worldwide distribution. The majority of cases are self-limiting gastroenteritis. The clinical

symptoms usually appear 8 to 72h after contact with the pathogen. The typical symptoms are usually nausea, vomiting, abdominal pain and diarrhea with or without fever. Few (<5%) of the patients develop invasive Salmonella infections or bacteremia and about 10% of those with invasive disease develop localized infections. During the past decade, there had been a significant world-wide increase of non-typhoidal salmonellosis especially in industrialized countries including, the United Kingdom, Germany, France, Austria, Denmark, and the United States of America. In the U.S. 1.3 millions of illnesses and 400 to 600 deaths each year.^[42]

2.3. Salmonella in humans

Among *Salmonella* spp. *Salmonella Enterica* is one of the *Salmonella* serotypes responsible for causing enteric disease in humans.^[12] *Salmonella Enterica* became one of the primary causes for salmonellosis in humans during the late 1970s and early 1980s and continues to be a principal cause in both Europe and North America.^[78] SE continues to persist in populations at relatively stable levels. Data collected from 2007 shows that SE was the primary cause, 25.7% of cases, of salmonellosis of human origin in Canada. It has been suggested that for every case presented to a physician 13 to 37 cases actually occur in the population (BCCDC, 2010).^[3]

The primary signs and symptoms of salmonellosis in humans are: 1) acute enteritis characterized by fever, diarrhoea that may have blood and/or mucus, vomiting and abdominal cramps, all of which last on average 4-7 days; 2) chronic enteritis with chronic diarrhoea and abdominal pains that last for weeks to months; and 3) septicaemia. *Salmonella* does not always cause disease, as the host can destroy the pathogen via immune defence systems or expel the pathogen before damage occurs (Ohland Miller, 2001).^[50]

Non typhoid salmonellosis is usually recorded as a localized enterocolitis. The incubation phase ranges from five hours to seven days but medical signs regularly begin 12 -36 hours after intake of infected food. Shorter incubation periods are usually related to higher doses of the pathogen or highly at risk people. Medical signs include diarrhea, nausea, abdominal pain, fever, chills, vomiting, prostration, anorexia, headache and malaise may also occur. The disease period is most often two to seven days. Systemic infections sometimes happen and frequently involve the very young, old or the immune-compromised hosts. A few numbers of cases may lead to death. The patients have a large numbers of *Salmonella* spp. at the start of disease. Some of the patients become carriers but others have quality to excrete *Salmonella* after 3 months. Nontyphoid salmonellosis can cause chronic diseases with localized infections in particular organs, reactive arthritis, neurological and neuromuscular diseases (Wilson *et al.*, 2003).^[80]

When non-typhoid *Salmonella* spp. causes disease in the gastrointestinal tract they do so by increasing secretions from the intestinal epithelium, invading the intestinal epithelial barrier and recruitment of neutrophils into the intestinal lumen (Ohland Miller, 2001).^[50] This then leads to production of pro-inflammatory cytokines which causes an acute inflammatory response and leads to intestinal fluid secretion and diarrhoea. Thermolabile enterotoxins and cytotoxins are also produced, with the enterotoxin enhancing fluid secretion and the cytotoxin blocking protein synthesis in endothelial cells.^[70] The exact genetic control mechanisms of *Salmonella* spp. virulence factors are unknown. The increased secretion and presence of toxins leads to the combination of diarrhoea and epithelium damage, which subsequently causes dehydration problems.^[70]

In Australia with 210 cases per 100,000 being reported in children under 5 years of age, compared with a total population level of 40 cases per 100,000 (USDA, 2010a).^[15] While young children are not as immune competent as adults, it has been suggested this age group is at a higher risk in large part due to “childish” behavior such as eating dirt, sand and soil, which increases the likelihood of exposure to *Salmonella* and other pathogens. There is also the possibility that numbers are artificially high compared to the population due to the fact that concerned parents are much more likely to seek medical attention for signs such as diarrhoea, whereas most adults would ignore such signs of illness in themselves. Immunocompromised people are also much more likely to become diseased due to *Salmonella* and also to have more severe symptoms due to their inability to fight off the pathogen.^[33]

2.4. Salmonella in chickens

A large number of *Salmonella* serotypes have been associated with poultry meat and egg products and are capable of colonizing and infecting live birds. *Salmonella* contamination has been a persistent problem affecting the poultry industry in the United States, which processes over 9 billion broiler hatching eggs through commercial hatcheries each year (USDA, 2007b).^[72] Contaminated poultry meat and eggs, particularly when the bacterium is present in the egg contents, are important vehicles of *Salmonella* infections. Several factors can affect the susceptibility of poultry to *Salmonella* colonization.^[14] These include 1) age of birds; 2) *Salmonella* serotype and initial challenge dose level; 3) stress, including environmental, transport, and overt or subclinical disease; 4) presence of feed additives, such as antimicrobials and anticoccidials; 5) survival through low pH of the stomach; 6) competition with gut microflora; 7) presence of a compatible colonization site, and 8) host genetic background. There are several potential sources of *Salmonella* contamination in an integrated poultry operation. Environmental

factors such as air, litter and unclean facilities, and vectors, such as insects, humans, and rodents, are responsible for Salmonella contamination in poultry farms. [14]

Chickens can be infected with many different serovars of Salmonella. Some serovars, such as *S. Pullorum* and *S. Gallinarum*, are host specific for chickens, whereas other serovars, such as *S. Typhimurium*, *S. Enteritidis*, and *S. Heidelberg*, are able to infect a wide range of hosts. There are a number of commonly identified serotypes of Salmonella associated in chickens with the most common serovars being *S. Enteritidis*, *S. Kentucky*, *S. Heidelberg* and *S. Typhimurium* for clinical isolates and *S. Heidelberg*, *S. Kentucky*, *S. Typhimurium*, *S. Senftenberg*, and *S. Enteritidis* for non-clinical isolates. [18] Since 1997, *S. Heidelberg* has been the most prevalent serovar reported, with a peak in 2000 of just over 50% of all isolates reported being *S. Heidelberg*. In the early to mid-1990s, *S. Enteritidis* was the most frequently reported serotype in the United States, as well as in Europe. [14]

Salmonella contamination of poultry in pre-harvest environments can usually be traced to production issues that include contaminated poultry feed or pathogen introduction to the facilities via a wide range of carriers including house pets, wild animals as well as insects (Park *et al.*, 2008). [52]

Many of these environmental sources have been reviewed extensively elsewhere but poultry feed has been discussed in more detail than most other sources (Jones, 2011 [39]; Ricke *et al.*, 2013a [57]). There are several reasons for the extensive focus on poultry feeds as a source of Salmonella. First of all, since one Salmonella organism per gram of feed can colonize in young chicks, low or undetectable numbers of Salmonella represent a high risk for infection in these birds that is further enhanced by the increased feed mixing and incorporation of individual feed ingredients from a multitude of sources. This becomes of particular concern if breeder flock hatchlings are exposed since they represent the starting point for all commercial flocks. In addition, Salmonella can linger in feed for extended time periods with reports of bacterial cells remaining viable for several weeks up to 16 months in dry feed stored at 25 °C. This is further confounded when feeds are treated with antimicrobials such as organic acids where Salmonella either can become acid tolerant or their recovery and/or subsequent enumeration accuracy using conventional plating methods is influenced by carryover of antimicrobial compounds into the media (Carrique-Mas *et al.*, 2007 [8]). Contaminated feed is also regarded as a source of infectious transmission of Salmonella among flocks. This is further accentuated by the larger numbers of birds housed in confinement resulting in an increase in more birds being infected simultaneously via aerosols and other routes (Park *et al.*, 2008). [52]

2.5. Salmonella in table eggs

According to many reports, eggs are the most likely source of Salmonella infections in humans both in outbreaks and in isolated incidences. Eggs may be contaminated externally by feces from hens shedding Salmonella. If the eggs are improperly washed at the egg processing plant, Salmonella is able to persist on the surface and potentially cross-contaminate the liquid portion of the egg when it is cracked for consumption (EFSA, 2010a). [23]

Eggs can be infected by Salmonella via two major routes, vertical and horizontal. Vertical transmission (transovarian infection) occurs when the egg contents are contaminated with Salmonella during the formation of the egg, before this is covered with the shell. Horizontal transmission includes trans-shell infection of the contents of the egg during transit through the cloaca or after oviposition and fecal contamination of the external surface of the shell (EFSA, 2005) [22]. Vertical transmission is common in host restricted *Salmonella* serovars, such as *S. Gallinarum* and *S. Pullorum*, but has also been demonstrated in un-restricted Salmonella, such as *S. Enteritidis*, *S. Typhimurium* and *S. Heidelberg*. Transmission via this route is directly related to the affinity of certain serovars for the reproductive tract of the hens (EFSA, 2010a) [23]. Individual Salmonella strains (within and across serotypes) can show a different ability in colonizing the hen's reproductive tract. This can be dependent on genotypic and phenotypic characteristics of the strain, which can influence its virulence, ability to evade the hen's immune response and persistence in the reproductive tract (Gantoiset *et al.*, 2009). [28]

Various Salmonella serovars can also be found in the egg contents following penetration through the eggshell (trans-shell transmission). This is more likely to happen in the first minutes after oviposition, when the egg's cuticle is immature and offers less protection against the penetration of bacteria into the eggs. Furthermore the positive temperature differential (the egg just laid is warmer than the environment) creates a negative pressure that aids the entrance of bacteria inside the egg if there is a moist environment at the shell surface. Trans-shell contamination of the contents is more likely when the shell quality is poorer for older birds or when there are nutritional problems or certain viral infections. Fecal contamination of the eggshell is normally considerably higher than the contamination of contents, and usually correlates with visible eggshell contamination and with the degree of excretion of Salmonella in feces. Externally contaminated eggs represent a risk in the processing phase, as they could cross-contaminate the egg contents or other foodstuffs (Jones *et al.*, 2002). [38]

In a recent study conducted in France, 150 eggs were collected from the one day production of each of 28 randomly selected Salmonella positive flocks. Eleven of the 28 flocks (39.3%) had at least one positive eggshell. Of the total of eggs tested, the prevalence of Salmonella in the eggshells was 1.05% (Chemaly *et*

al., 2009)[26]. In the egg albumen, *Salmonella* can grow at 20 °C, while it is unable to grow at temperatures below 10 °C. If *Salmonella* reaches the egg yolk, it can grow rapidly, even at room temperature (25 °C) (Gantois *et al.*, 2009) [28]. The age of the egg represents a further risk factor, because the yolk releases iron and nutrients over time. The deterioration of the vitelline membrane leads to the leakage of these nutrients into the albumen and attracts the bacteria towards the yolk, therefore easing the growth of *Salmonella* (Gantois *et al.*, 2009) [28]. Rapid cooling of eggs can be used to reduce the opportunity for bacterial multiplication but lower temperatures can enhance the survival of *Salmonella* on the shells and lead to condensation associated problems. It was shown that condensation can encourage bacterial penetration of the eggshell, but seems to have a smaller impact on whole egg contamination but, cooling eggs rapidly can also lead to damage of the egg shells with an increase of cracked eggs (Jones *et al.*, 2002). [38]

Salmonella Enteritidis and eggs

Salmonella Enteritidis is the serovar most frequently associated with egg infection (EFSA, 2010a). [23] This is due to two main factors: its unique ability to colonize the ovary and the oviduct of laying hens long term, and its spread and persistence in the parental breeder flock population in most of the world. Despite the high occurrence of SE in laying flocks, the frequency of egg contamination by SE is normally relatively low and depends on the level of contamination of the flock and the time of the production period in which the eggs are laid. Eggs produced soon after the flock was infected with SE, and especially around the onset of lay, are more likely to become internally contaminated (Cogan and Humphrey, 2003). [18]

Salmonella Enteritidis is typically associated with egg-related outbreaks (EFSA, 2010a) [23] and has not been always the most prevalent serovar in human infections, for example in the UK during the late 1970s *Salmonella Typhimurium* was predominant, and *S. Agona* was most common before then (Cogan and Humphrey, 2003). In the UK, a sharp increase of salmonellosis was observed during the 1980s. This was largely due to an epidemic of SE PT4 that, in the United Kingdom, commenced in 1982–1983 and reached its peak in 1993, to start declining only in 1997. In the UK some layer farms subscribe to the British Egg Industry Council that provides a code of practice (Lion Code) on farms' hygiene and welfare standards. Vaccination against *Salmonella* started in layer flocks in 1998 for farms that subscribe to the BEIC Lion Code (Cogan and Humphrey, 2003). [18] These typically larger farms produce more than 80% of retail eggs in the UK. Since the introduction of control measures for *Salmonella* in layers, such as control of the breeding flocks and vaccination, the number of human infections caused by SE, especially PT4, has reduced dramatically (Cogan and Humphrey, 2003). [18]

Salmonella Typhimurium and eggs

Salmonella Typhimurium is often indicated as the prototype un-restricted *Salmonella*, even though it has a number of distinct sub-types that vary in their degree of host adaptation. Few egg related outbreaks of salmonellosis caused by ST are reported in humans in the EU (EFSA, 2010a). [18] Experimental studies have suggested that SE and ST can be equal in their potential to colonize the reproductive tract of hens and to infect forming eggs after a high level artificial challenge, however only SE was isolated from eggs after laying. After intravenous infection of hens with ST, all the eggs laid were negative for ST. It was also demonstrated that ST can persist in the egg albumen during egg formation, and that it could resist lysozyme in the albumen better than SE (Gantois *et al.*, 2009). [28] During the 1990s ST definitive phage type (DT) 104 spread worldwide and is now common in the animal population, including poultry, of many countries. ST DT104 does not appear to frequently infect laying flocks and even when they are infected contamination of eggs or egg handling equipment is very rare. A low capability of ST DT104 to cause egg contamination, however an increased risk of egg contamination was observed if the hens were infected at point of lay. Certain phage types of ST, such as DT2 and DT99, are host-adapted to wild birds and infection in laying flocks with these strains is normally short-lived. ST of wild-bird origin may be found in free-range flocks, or occasionally in enclosed flocks as a result of feed contamination by bird droppings (during the final stages of growth in the field or during storage) (EFSA, 2010a). [18] A phage type (not expressing any phase of the H flagellar antigen) ST in eggs has caused a large outbreak in France in 2009 and ST DT8 contamination of duck eggs has caused significant prolonged outbreaks of salmonellosis in humans in England (AFSSA, 2009). [1]

3. CONTROL AND PREVENTION

The preventive methods for reducing the risk of *Salmonella* contamination of shell eggs and human salmonellosis outbreaks due to their consumption can be either applied as preharvest or as postharvest procedures. Furthermore, they can be either serotype specific or serotype-independent, the latter being considered a more complex approach (Gast, 2007). [30] The environment of the laying hen house can act as reservoir for *Salmonella*, along with the feed that can be already contaminated as it arrives in the farm (Umali *et al.*, 2012). [69]

Due to these various sources of infection for the laying hens, preventive methods are already applied or available at the farm level: flock testing, sanitation and biosecurity; vaccination; passive immunization

(Chalghoumi *et al.*, 2009b);^[16] the use of natural antimicrobial products such as bacteriophages (Toro *et al.*, 2005),^[67] protein and fiber sources (Kassaify and Mine, 2005),^[43] competitive exclusion flora, probiotics, prebiotics, and organic acids (Chalghoumi *et al.*, 2009b),^[16] essential oils (Johny *et al.*, 2008),^[37] and bacteriocins (Dias Paiva *et al.*, 2011).^[20] For postharvest control of Salmonella in shell eggs, the first approach is to maintain an adequate temperature during storage (Gantois *et al.*, 2009).^[28] However, different surface decontamination methods are already applied in the U.S.A. and new ones make the subject of continuous research: egg washing (Caudill *et al.*, 2010);^[9] ultrasounds (Cabeza *et al.*, 2011);^[5] microwaves (Lakins *et al.*, 2008);^[46] irradiation (Cabo Verde *et al.*, 2004);^[6] gas plasma (Ragni *et al.*, 2010);^[56] ultraviolet light;^[58] and pulsed light.^[34] Among all these, the ones authorized in the U.S.A. are the shell washing and irradiation.^[71]

3.1. Pre-harvest methods for reducing the risk of Salmonella contamination of shell eggs

Among the different preventive methods used against Salmonella in poultry, genetic selection may be a promising one in reducing the occurrence of salmonellosis in layers. It has been shown, indeed, that genetic lines of poultry exhibit different resistance levels against *Salmonella spp.*^[28] A genetic correlation between *Salmonella spp.* contamination level in different tissues has been demonstrated. In an investigation concerning the heritability of resistance trait in poultry demonstrated that the genetic correlation (r) between the concentration (log₁₀ CFU/g) of *S. Enteritidis* in the liver and the genital organs was high (0.56).^[28]

Considering the systemic phase of infection, the resistance is partly determined by genetic strains and that, in resistant lines, the microbial load can reach values of up to 1000 times lower, in comparison with susceptible lines. The identification of genes contributing to resistance against this disease may therefore enhance the genetic selection of the resistant lines. Furthermore, in experimental conditions the crossbreeding between different selected lines, for lower or higher propensity to carry *Salmonella spp.*, resulted in a reduction by half of the maximal percentage of contaminated animals. Nevertheless, they were unable to accelerate the extinction of disease.^[55]

3.1.1. Flock management

A series of environmental-related factors may influence the likelihood and outcome of Salmonella infections in poultry. These factors are: litter, dust, mice, flies and the different surfaces from the hen houses or the farm, with which the laying hens may come in contact with. The levels of Salmonella in the litter have been reported to increase with increasing the water-activity levels and the moisture content, mostly due to accidental water leakage. For this, preventive methods are applied, such as maintaining a litter drying environment through a modest and uniformly distributed ventilation rate (100 to 150 ft/min) over the litter surface. The addition of hydrated lime to the litter can markedly reduce *Salmonella Enteritidis* recovery in a relatively short time (less than 24 h), due to the increase in pH of up to 12.57 at an addition of 20% of lime.^[16]

Salmonella spp. could be isolated as an airborne pathogen, inside the poultry house, and as well in the outside area of the hen house, up to a 40 ft distance (approximately 13 m).^[19] Dust could possibly act as a vector for *S. Enteritidis* spread from infected hens to healthy ones, through a potential airborne transmission. Rats and mice are considered a reservoir of Salmonella, with a high risk of poultry infections. The transmission and shedding patterns of Salmonella in naturally infected wild rats through daily observations and sampling, *S. Enteritidis* was more frequently isolated from the spleen and liver at the end of the study, in comparison to the number of positive cultures from the feces.^[69]

One of the factors that can affect the prevalence of Salmonella on premises is the flock size. A potential connection between the high stocking density of laying hens in conventional cages and the large volume of feces and dust may lead to an increase in the incidence of Salmonella infections in this particular type of housing system. In addition, high stocking densities may indirectly interact with Salmonella infections because of the caused stress.^[35]

3.1.2. Feed withdrawal for molting purposes

Molting induced by feed withdrawal, a common practice destined to increase egg productivity, has been shown to enhance the risk of vertical transmission of *Salmonella spp.*^[32] During the induced molting, due to stress, transient reductions in the number of specific lymphocyte classes appear, which may cause an increased susceptibility to infection. For this reason, new procedures that would avoid feed removal but retain at the same time the economic benefits. For molting purposes, alfalfa could be used as an alternative, resulting in a reduction of *S. Enteritidis* colonization in experimentally challenged laying hens. Furthermore, in order to decrease the population of *Salmonella spp.* in the ceca of laying hens during molting, a combination of alfalfa and an extract of *Lentinus edodes*, also known as the Shiitake mushroom.^[79] The results showed a high decrease, up to 2.72 log CFU/g from the initial *Salmonella spp.* counts, suggesting that this combination might be successfully used as an alternative to feed removal during molting periods. Feeding laying hens with wheat middlings caused a cessation of egg production within 3 to 7 days. The comparison of *S. Enteritidis* levels between unmolted group, molted by feed withdrawal group and wheat middlings feeding group resulted in a difference of 3 to 5 log more *S. Enteritidis* in the feed withdrawal group.^[60]

Whole cottonseed meal (50% of the diet) can also be used when inducing molting, hens voluntarily reducing their feed intake. This type of molting is believed to be equivalent in effectiveness to the one produced by complete feed withdrawal, and with the same consequences on the egg safety, by increasing the risk of *S. Enteritidis* contamination.^[19]

3.1.3. Foodborne *Salmonella* spp. contamination of poultry feeds

Poultry feed can become contaminated with foodborne *Salmonella* either during harvesting, processing at the feed mill or storage. Poultry feeds can also become contaminated with salmonella from animal proteins and other ingredients, or even from the dust present in the feed mills.^[30] Different protein sources and cereals have been identified as contaminated with *Salmonella* spp.; peanut meal, sunflower meal, soybean meal, bran meal, barley, corn, sorghum, and wheat. Animal protein and byproducts destined for obtaining protein meals for animal feed have always been considered a major source of *Salmonella* spp., one cause being the incomplete decontamination of these ingredients during processing.^[48]

Salmonella control principles involve preventing contamination from entering the facility, reducing multiplication within the plant and killing the pathogen. Among the preventive measures to be applied for *Salmonella* feed contamination, the most important are obtaining *Salmonella*-free feed ingredients, controlling the dust, restricting the flow of the personnel, reduction of fat accumulation, controlling rodents and wild birds and maintaining the sanitation of the transport vehicles.^[41]

For feed degradation, shortening storage time to prevent browning and caking of the feed and the supplementation with soybean oil to prevent fat losses would be of first importance, before implementing other prevention methods.^[48] In addition, rapid drying is widely used to preserve raw feed ingredients. Considering the addition of different antimicrobial agents, disinfectants such as acids (mineral acids, short-chain fatty acids), isopropyl alcohol, aldehydes, and trisodium phosphate may reduce the risk of contamination with *Salmonella*, through inactivation of this pathogen during the storage of feed.^[48]

Inactivation of *Salmonella* in feeds may involve pelleting (which consists of thermal processing) and/or chemical addition. The pelleting process consists of 3 major steps: mixing steam with mash feed (also called conditioning), pressing conditioned feed through metal dies (pelleting), and removal of heat and moisture via large volumes of air (cooling).^[39]

3.1.4. Flock testing, sanitation, and biosecurity

Testing is a very important part of the *Salmonella* control programs. Testing is however controversial as its efficacy may be sometimes slow, due to a continuous reintroduction of many serotypes of *Salmonella* in the poultry houses and flocks, from environmental sources. Due to the fact that *Salmonella* fecal shedding is intermittent, testing this kind of samples does not have reliable results.^[30] Nevertheless, environmental sampling has proven to be relatively easy to perform and the testing sensitivity is high, when the appropriate method is chosen,^[2] although it only indirectly reflects the actual probability of the egg contamination. Intensive monitoring for *S. Enteritidis* through the use of drag-swab samples, when sampling from different locations: floors, nest boxes, egg belts, dropping belts, scrapers, fan blades and dust, is considered a very efficient approach and may lead to a high sensitivity detection of *Salmonella*.^[45] Because many of the *Salmonella* serotypes are invasive, different tissues are often collected and further tested for detecting infected birds (liver, spleen, ovary, oviduct, testes, yolk sac, heart, heart blood, kidney, gall bladder, pancreas, synovia, and eye). In the end, egg culturing comes as a confirmatory step in many testing plans, but the detection of *Salmonella* inside eggs is very difficult due to the low incidence at which internal contamination occurs and the very low initial cell densities of salmonella usually found in freshly laid eggs.^[30]

When a flock has been tested positive for *S. Enteritidis* presence in the environment and the eggs, the poultry house in which this flock has resided needs to be cleaned and disinfected through 3 steps: the removal of visible manure, dry cleaning in order to remove dust, feathers and old feed and disinfection with spray, aerosols, fumigation or another appropriate disinfection method. Poultry facilities are often subjected to disinfection using chemical compounds (especially phenolic and quaternary ammonium ones), following the removal of waste materials and cleaning by high-pressure spraying.^[30]

Applying biosecurity measures program, including the limiting of visitors on the farm and in poultry houses, maintaining personnel and equipment practices that will protect against cross contamination from one poultry house to another, preventing stray poultry, wild birds, cats and other animals from entering the poultry houses, and not allowing employees to keep birds at home, to ensure that there is no introduction or transfer of *S. Enteritidis* onto a farm or among poultry houses.^[45]

3.1.5. Vaccination

The control of *Salmonella* spp. infection in hen eggs includes various preventive measures, among the most frequently used being vaccination.^[74] Active immunization is achieved by inoculation with microbial pathogens that induce immunity but do not cause disease, or with antigenic components extracted from the pathogens. When it is successful, a subsequent exposure to the pathogenic agent elicits an intensified immune response that will eliminate the pathogen or will prevent the disease mediated by its products. Many of the common vaccines

currently used at a commercial level in poultry consist of inactivated (killed) or live, but attenuated, *Salmonella* spp. strains. Live vaccines generally confer better protection than inactivated ones, the former stimulating both cell-mediated and humoral immunities.^[74]

3.1.6. Passive immunization

Laying hens immunized with antigens from selected microorganisms (for example, *S. Enteritidis* and *S. Typhimurium*) react by producing high quantities of specific antibodies (IgY) which are transported from the blood into the egg yolk. These eggs containing high levels of antigen-specific IgY, called hyper immune eggs, can be administered as a feed additive (usually in the form of whole yolk powder) to other species to provide them with passive immunity. Moreover, they demonstrated that these specific antibodies have a growth inhibitory effect on *Salmonella* spp., in a concentration dependent manner. They also assessed the ability of preventing adhesion of *Salmonella* spp. to intestinal cells by using human epithelial Caco-2 cell lines. The results demonstrated that specific IgY was able to reduce the decrease in trans epithelial electrical resistance of the infected Caco-2 cell monolayers, blocking adhesion of *Salmonella* spp. in a concentration-dependent manner.^[16]

3.1.7. Natural antimicrobial products Bacteriophages

Bacteriophages are bacterial viruses with the ability of using the bacterial cell to multiply. Using a combination of 3 different *Salmonella*-specific bacteriophages to reduce *S. Enteritidis* colonization in the ceca of laying hens resulted in a significant reduction of the incidence, up to 80%. Tailspike protein of bacteriophages is a component of the tail apparatus with the role of mediating the specific recognition of its bacterial host by binding to its surface structures. After oral administration to 1-day-old chickens, it resulted in a significant delay of *Salmonella* spp. growth and colonization and a significant reduction of *Salmonella* spp. counts at the level of the ceca, liver, and spleen, in comparison with control groups.^[77]

Generally regarded as safe, bacteriophages are considered a highly efficient tool for the bio control of pathogens in food products.^[29] Phage therapy can be successfully applied to reduce the *S. Enteritidis* level on poultry carcasses after slaughter.

Protein and fiber sources

Non immunized egg yolk powder could suppress the colonization of *S. Typhimurium* in laying hens. After 2 wks of feeding egg yolk powder at a dose of 10.0% in infected laying hens, *Salmonella* was completely undetected. At a concentration as low as 5% (wt/wt) in the feed, non immunized egg yolk powder eliminated *S. Enteritidis* at the intestinal level after the 1st week, demonstrated by the negative results obtained for the tested fecal samples. This may be explained by the presence of components such as high-density lipoproteins and their derivatives.^[44]

3.1.8. Competitive exclusion flora, probiotics, prebiotics, and organic acids

The use of competitive exclusion flora, probiotics, prebiotics, as well as acid-based products have been widely investigated worldwide as preventive methods for *Salmonella* spp. colonization in poultry. Competitive exclusion products involve the introduction of intestinal bacteria from mature healthy poultry to newly hatched chickens, the concept being defined as “the early establishment of an adult intestinal microflora to prevent subsequent colonization by enteropathogens.” The mechanism used by the bacterial species from the competitive exclusion products to inhibit the proliferation of other bacteria consists of creation of a restrictive physiological environment (for example, bacteriostatic effect of Volatile fatty acid in the ceca).^[75]

The potential mechanisms that allow the exclusion of pathogenic species, among them *S. Enteritidis*, by the probiotics include competition for adhesion sites and nutrients or production of antimicrobial compounds, such as bacteriocins, VFA, or hydrogen peroxide. Besides the inhibition of cecal colonization by pathogens, it has been demonstrated that probiotic bacteria determined an increase of the oxidative burst capacity and degranulation of heterophils isolated from chicks 24 h after probiotic administration. This suggests a possible activation of the innate immune system.^[25] It has been suggested that lactobacilli isolated from either cloaca or vagina of laying hens present *in vitro* inhibitory activity against *S. Enteritidis*, with no differences noticed between those isolated from the cloaca and the ones from the vagina. Lactobacilli isolated from the cloaca and the vagina of laying hens inhibited *Salmonella* growth *in vitro* and decreased *S. Enteritidis* colonization *in vivo*. *Salmonella* inhibition was shown to depend on the species of Lactobacillus, correlated to some extent with the production of lactic acid of each.^[25] Another probiotic with potential use in laying hens is based on an active ingredient consisting of *Bacillus cereus* var. *toyoi* spores. Its efficacy against *S. Enteritidis* has been demonstrated on poultry.^[76]

Another option as a preventive method is the use of pre-biotics. They can be regarded as an integrated approach to an improvement of food safety, starting with the maintenance of a healthy intestinal ecosystem.^[27] Among the beneficial effects of prebiotics these can be mentioned: stimulation of the immune system, reduction of inflammatory reactions, toxin inactivation, modification of the intestinal microbiota, increased production of VFA, and prevention of pathogen colonization.^[59] Prebiotics are not digested or metabolized, or they are metabolized very little, during their passage through the upper portion of the gastrointestinal tract (GIT).

Therefore, they enter the ceca without any change to structure, being fermented by the colonic flora. Through the stimulation of bifido bacteria, they may have the ability to inhibit pathogenic bacteria such as *Salmonella* spp. It is also possible to decrease egg contamination risk by adding organic acids to the feed or drinking water at an appropriate time.^[63] Butyric acid is the most frequently used organic acid as a feed or drinking water additive.

3.2. Postharvest methods for reducing the risk of salmonellosis due to contaminated shell eggs consumption

3.2.1. Shell eggs storage and prevention of growth and multiplication of *Salmonella*

Prompt refrigeration to temperatures capable of restricting microbial growth has been recommended as an approach to reducing the likelihood that contaminated eggs will transmit *S. Enteritidis* to humans. Research in this field has proved that ambient temperatures are not proper for the storage of shell eggs, especially since the risk of *S. Enteritidis* horizontal transmission has increased, and further on, due to its capacity of growth and multiplication inside the shell eggs. The temperature values for shell eggs storage should not exceed 20 °C. In egg albumen, *Salmonella* spp. can grow at 20 °C, while unable to grow at temperatures below 10 °C, therefore showing that a temperature value for optimal storage of eggs should not exceed this last value.^[28]

3.2.2. Egg washing

Egg washing procedure uses water or solutions that involve chemicals (sanitizers) to determine an efficient decontamination. It is believed that different chemicals used to decontaminate the egg shell may interact with its physical barrier components.

The main advantages of egg washing procedure are: the reduction of microbial load on the shell surface, minimizing the risk associated with the presence of foodborne pathogens, especially *Salmonella* spp., further reduction occurring after washing, since different chemicals may still be present after the washing step, continuing to exert their antibacterial effect, reduced risk of cross-contamination of other foods and reduced risk of contamination of the egg content, provided that the shell itself is not damaged. The main disadvantage comes from the potential damage that this practice can cause to the physical barrier of the egg, especially to the cuticle.^[22]

3.2.3. Electrolyzed water

Electrolyzed oxidizing water (EOW) is produced by passing a diluted salt solution through an electrolytic cell, within which the anode and cathode are separated by a membrane, obtaining an acidic and an alkaline component. Acidic EOW is effective in reducing the populations of pathogenic microorganisms on the surface of shell eggs (aiming at *S. Enteritidis*), but its use is limited when low pH values are observed (less than 2.7), because dissolved Cl₂ gas can be rapidly lost due to volatilization, decreasing the bactericidal activity of the solution with time.^[7]

3.2.4. Microwave technology

Microwaves are oscillating electromagnetic waves with frequencies in the 300 MHz to 300 GHz range. The effects of microwaves on pathogens can be generally expressed in 2 forms: thermal and non-thermal. Thermal inactivation is caused by heating during the microwave application process, involving changes such as denaturation of enzymes, proteins, nucleic acids or other vital components as well as disruption of membranes.^[47]

Non thermal effects have been classified in 4 categories: Selective heating, explained by the fact that microwaves heat solid microorganisms more effectively than by the surrounding medium, causing a more rapid killing of the organism, Electroporation, caused when an electrical potential crosses the membrane of the microorganism, determining the formation of pores in the membrane, and a further leakage of cellular components, Cell membrane rupture, due to the voltage drop across a membrane and Magnetic field coupling, caused by a disruption in internal components of the cell, leading further on to cell lysis.^[47]

3.2.5. Ultraviolet light technology

Ultraviolet (UV) light is lethal to most microorganisms. Among its practical applications may be mentioned: inhibition of microorganisms on surfaces, destruction of microorganisms in the air and sterilization of liquids. UV radiation inactivates microorganisms by inducing a cross-linking between pyrimidine nucleotide bases in the DNA, this resulting in inhibition of DNA transcription and replication mechanisms, leading eventually to microbial cell death. In addition, it has been demonstrated that UV radiation affects cell membrane integrity, inducing protein modifications and inhibiting oxidative phosphorylation.^[58]

3.2.6. Ultrasounds

Ultrasound treatment of food products is a useful tool to minimal processing, due to the fact that the transfer of acoustic energy is instantaneous and distributed throughout the whole volume of the products.^[68] The mechanism of microbial killing by ultrasonic waves is mainly due to the thinning of cell membranes, localized heating and production of free radicals. Micro-mechanical shock waves are created by making and breaking microscopic bubbles induced by fluctuating pressures under the ultrasonication process; these shock waves disrupt cellular structural and functional components and lead to cell lysis.^[68]

3.2.7. The use of plant extracts

The consumers' demand for organic and non processed food products is increasing; therefore the use of plant

extracts for table eggs decontamination may be considered a suitable option, from this point of view. The phenolic compounds are responsible for their bactericidal effects as they interact by permeabilizing the membrane. Their biological activity seems to depend also on the solvent used for extraction. A natural herb extract that has an inhibitory effect on *Salmonella* and other harmful bacteria. [43]

4. Salmonellosis and public health

Growth in international trade and current facilities for traveling increased not only the dissemination of pathogenic agents and contaminants in foodstuffs, but also our vulnerability. Nowadays, the world is interrelated and interdependent. Thus, local foodborne disease outbreaks have become a potential threat for the whole world. Globalization, commercialization and distribution make it possible for a contaminated foodstuff to affect the health of people in several countries at the same time. The identification of only one contaminated food ingredient may lead to the discard of literally tons of food; to considerable economic losses to the production sector; restrictions to trade; and effects on the tourism industry. Therefore, there is an ever growing perception of the need and importance for surveillance systems and adoption of measures to ensure food safety, such as the identification of the foods involved in food borne disease outbreaks. [65]

Salmonella is an intestinal bacterium responsible for severe foodborne intoxications. It is one of the most important agents involved in outbreaks reported in several countries. Salmonellosis is an important socioeconomic problem in several countries, mainly in developing countries, where this etiological agent is reported as the main responsible for foodborne disease outbreaks. It is one of the most problematic zoonosis in terms of public health all over the world because of the high endemicity, but mainly because of the difficulty in controlling it and the significant morbidity and mortality rates. [66]

The CDC regularly reports *Salmonella* outbreaks that are associated with poultry and poultry products, [12] and these food products are generally recognized as a primary source of salmonellosis. Poultry and eggs are considered one of the most important reservoirs from which *Salmonella* is passed through the food chain and ultimately transmitted to humans. With increasing consumption of poultry and poultry products, the number of salmonellosis associated with poultry continues to be a public health issue in the US. Since *Salmonella* is a major causative agent for poultry-associated foodborne illnesses, improving safety of poultry products by early detection of foodborne pathogens would be considered an important component for limiting exposure to *Salmonella* contamination. This monitoring of poultry and other related products for *Salmonella* contamination could be made significantly more effective by employing rapid and sensitive detection systems. Transmission of *Salmonella* to humans typically occurs when ingesting foods that are directly contaminated by animal feces or cross-contaminated by other sources. [49]

Historically, *Salmonella Typhimurium* was the most common agent of the foodborne disease in humans, although in the past decades *Salmonella Enteritidis* has been most frequently involved in salmonellosis outbreaks. There is a growing concern about human infections caused by other serovars, such as *Infantis*, *Agona*, *Hadar*, *Heidelberg* and *Virchow*. [26]

Concerns about the presence of *Salmonella spp.* in foodstuffs of poultry origin increased in the 1980s, when *Salmonella Enteritidis* phagotype 4 was responsible for several outbreaks of foodborne disease in England, caused by the ingestion of foods containing poultry ingredients. The vertical transmission of *Salmonella Enteritidis* in commercial poultry was responsible for the increased number of cases of human infection in Europe, North America and other parts of the world. This species replaced *Salmonella Typhimurium*, which was the most common agent of human foodborne infection until the 1980s. [51]

According to the National Health Surveillance Agency in Brazil (ANVISA), among the etiological agents of food borne diseases identified between 1999 and 2004, *Salmonella spp.* was the most prevalent in Brazil, with the predominance of *Salmonella Enteritidis* between 2001 and August 2005. According to the WHO, *Salmonella* is one of the pathogens that causes the greatest impact on population health, and is associated with outbreaks and with sporadic cases of food borne disease. According to data of the Brazilian Ministry of Health, 6,602 food borne disease outbreaks were recorded between 1999 and 2008, and *Salmonella spp.* was associated with 43% of the cases in which the etiological agent was identified. In the European Union, *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Salmonella Infantis*, *Salmonella Hadar* and *Salmonella Virchow* are considered by the European Food Safety Authority the most important serovars in terms of public health. [73] In Japan, between 1999 and 2002, 32% of the cases of foodborne infection were due to *Salmonella*, with *Enteritidis*, *Typhimurium* and *Infantis* as the predominant serovars. In 2005, in the US, the serovars that were most frequently isolated from human sources were *Salmonella Typhimurium*, *Salmonella Enteritidis*, *Salmonella Newport*, *Salmonella Heidelberg* and *Salmonella Javiana*. [15]

Besides the importance of preventive measures against the risk of *Salmonella* infection in humans, control of salmonellosis has a positive economic impact in countries where outbreaks occur. Estimated costs of medical expenses, sick leaves and loss of productivity related to the high incidence of salmonellosis in the US range from US\$1.3 to US\$4.0 billion a year. [64]

A large number of *Salmonella* have to be ingested to cause gastroenteritis. Generally, the infective dose depends on the serotype, ranging from 2.0×10^2 to 1.0×10^6 CFU/g or mL.^[36] Variation in the symptoms is also related to the type of food and the species of *Salmonella* involved, once species that are adapted to men require lower infective doses to cause the same characteristic symptoms of the disease.^[53] *Salmonella* excretion in human and/or animal feces may contaminate the water, soil, other animals and foodstuffs. Animals are infected by direct contact with feces, contaminated water and food. Although broiler carcasses may be contaminated with *Salmonella Enteritidis*, eggs and egg by-products – mainly homemade mayonnaise – are the main products responsible for outbreaks of the disease in humans.^[61]

Depending on the host species and age, and on the pathogenicity of the microorganism and its adaptation to the host, *Salmonella* may cause severe disease, or go unnoticed and remain in the host for months or years. In this case, the host will be a reservoir of the bacteria for susceptible animals. The most common symptoms include diarrhea, abdominal pain, vomit and nausea, and may occur together with prostration, muscle pain, drowsiness and fever. Although symptoms generally disappear after 5 days, the microorganisms may be excreted in the feces for many weeks. Children, mainly those younger than 1 year of age, elderly and immunocompromised patients are much more susceptible to the disease, and may present more severe infections, such as sepsis, which may lead to death.^[53] Since 1980, human outbreaks caused by *Salmonella Enteritidis*, showed common sources in the US, Great Britain and other European countries.^[13] Epidemiological surveys from the CDC identified the consumption of eggs or egg-based foods as responsible for most of the outbreaks involving specific phagotypes (PT) of *Salmonella Enteritidis*; PT-4 in European countries, and PT-8 and PT-13a in the US. The predominant serotypes involved in foodborne diseases changed, in the past decades, from *Salmonella Agona*, *Salmonella Hadar* and *Salmonella Typhimurium* to *Salmonella Enteritidis*, which is the predominant cause of salmonellosis in several countries.^[63]

The typification of serovars is important to track the source of infection. For example, *Salmonella Agona* affected humans in the US, in European countries and in Brazil. The intensive breeding system adopted by the poultry industry favors the introduction, establishment, permanence and dissemination of these bacteria. Therefore, the stage when animals are raised is very important in the dissemination of *Salmonella spp.* among the birds, and consequently, in giving rise to contaminated food products. *Salmonella* may affect all segments of poultry production, such as breeder facilities, incubators, commercial raising operations, feed factories, slaughterhouses, transportation systems and commercialization facilities.^[12]

4. CONCLUSION AND RECOMMENDATIONS

It appears that *Salmonella* is a common or perhaps the leading cause of foodborne bacterial diseases, due to its ubiquitous occurrence in the natural environment and the intensive animal husbandry practices. It is one of the most prevalent foodborne pathogens, its main reservoir being considered the shell egg. As the concerns related to the increasing human salmonellosis cases grow, the need for an application of preventive methods either at the farm level or during the processing steps is crucial for a better control of the foodborne outbreaks due to the consumption of this specific food product. The use of different preventive methods has the effect of reducing the likelihood that eggs become contaminated with *Salmonella spp.*, especially with *S. Enteritidis*. On the farm level, the different preharvest methods may reduce the risk of egg contamination by interfering in the infection process and reducing the likelihood of this foodborne pathogen penetration in the forming egg. Further on, postharvest methods may reduce the risk of human salmonellosis, by respecting the refrigeration step and by different procedures, either chemical or physical. These latter reduce the existing bacterial counts, especially on the eggshell and ensure the microbiological quality of the shell eggs marketed in different parts of the world. However, these postharvest chemical or physical procedures are not worldwide accepted and implemented; as research is still needed on this topic, to ensure that the nutritional quality and properties of shell eggs are maintained, no matter the processing methods applied.

Based on the above conclusion the following recommendations are forwarded:

Eggs are among the most nutritious foods on earth and can be part of a healthy diet. However, they are perishable just like raw meat, poultry, and fish. Unbroken, clean, fresh shell eggs may contain *S. Enteritidis* bacteria that can cause food-borne illness. To be safe, eggs must be properly handled, refrigerated, and cooked.

For producers

- It is essential that all eggs for sale must be candled to remove cracked eggs. Cracked eggs must be disposed of or only sold to businesses to be pasteurized.
- Nests should be kept as clean as possible by removing faeces and broken eggs out of nests and cleaning nest pads.
- Collect eggs daily and more often in the event of increased floor eggs and/ or in the event of hot weather conditions. Dirty or cracked eggs must be separated from clean eggs as soon as possible to minimize contamination.

- Cool all eggs immediately after collection. Cool rooms should be set at 15 °C and be capable of maintaining this temperature.
- Premises and equipment for handling and storage of eggs must be maintained in a sanitized state fit for the production of food for human consumption.

For consumers

- Avoid using cracked eggs as they are more likely to be contaminated and thus present a higher health risk.
- Eating raw or undercooked eggs should be avoided, especially by young children, the elderly and immunocompromised persons.
- Hands, cooking utensils, and food-preparation surfaces should be washed with hot water and soap after contact with raw eggs or foods containing raw eggs.
- Avoid contaminating the egg contents with the outside of the shell when cracking.

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