Important Aspects of Salmonella in the Poultry Industry and in Public Health

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1. Introduction

The objective of this review was to discuss relevant issues related to the pathogeny, epidemiology and antimicrobial resistance of Salmonella spp. Due to its economic importance, and because it poses risks to human health, Salmonella spp. is one of the most frequently studied enteropathogens. Nowadays, the disease is considered to be a consequence of interrelated factors, such as food, the environment, vectors, men, utensils and equipments, the production line, animal transit and animal reservoirs.

2. General aspects

Salmonellae are widely distributed in nature. The main reservoir of these bacteria is the intestinal tract of men and warm-and cold-blooded animals (Jakabi et al., 1999), except for fish, mollusks and crustaceans, which may get contaminated after being fished. Among warm-blooded animals, chickens, geese, turkeys and ducks are the most important reservoirs. Domestic animals, such as dogs, cats, turtles and birds may be carriers, and pose great risk, mainly to kids (Franco & Landgraf, 1996).

The natural habitat of Salmonella may be divided into three categories based on the specificity of the host and clinical pattern of the disease: highly adapted to men: Salmonella Typhi and Salmonella Paratyphi A, B and C, agents of typhoid fever; highly adapted to animals: Salmonella Dublin (bovines), Salmonella Choleraesuis and Salmonella Typhisuis (swine), Salmonella Pullorum and Salmonella Gallinarum (birds), responsible for animal paratyphoid. The third category includes most of the serovars that affect men and animals, called zoonotic Salmonella, responsible for worldwide-distributed foodborne diseases, and detected in most species of animals used for human consumption, wild and domestic animals (Gantois et al., 2009).

Salmonellae are short bacilli, 0.7-1.5 x 2.5 µm, Gram-negative, aerobic or facultative anaerobic, positive catalase, negative oxidase; they ferment sugars with gas production, produce H₂S, are nonsporogenic, and are normally motile with peritrichial flagella, except for Salmonella Pullorum and Salmonella Gallinarum, which are nonmotile (Forshell & Wierup, 2006).
Optimal pH for multiplication is around 7.0; pH values above 9.0 or below 4.0 are bactericidal. Ideal temperature is between 35 to 37°C, with minimum of 5°C and maximum of 47°C. As for salt concentration, *Salmonellae* do not survive concentrations over 9% (Franco & Landgraf, 1996).

The first bacteria in the genus *Salmonella* were identified towards the end of the 19th century. *Salmonella Typhi*, the first to be recognized as a pathogen, was found in spleen and lymph nodes of humans in 1880. However, isolation and morphological description were only carried out by Gaffky, in 1884.

In 1885, Salmon and Smith isolated a bacillus from diseased pigs, and called it *Bacterium Suipestifer*. They wrongly considered it the agent of swine fever. This bacterium was later on called *Salmonella Cholerasuis*. In 1888, there was a report on *Salmonella Enteritidis* by Gaëtner; in 1889, Klein identified fowl typhoid in adult birds in England, and in 1892, Loeffler isolated *Salmonella Typhimurium*. In 1899, Retger described pulorosis and differentiated it from the disease that affected pigs. In 1913, Jones used an agglutination test to identify carriers of *Salmonella Pullorum* (Correa & Correa, 1992).

The genus *Salmonella* started to be classified in 1925, with the use of serological methods. *Salmonella Typhimurium*, created by Loeffler (1892), and *Salmonella Paratyphi*, created by Schottimuller (1899), were included in the genus. Later on, several *Salmonella* serotypes were described, and classified according to White (1829) (Correa & Correa, 1992). Popoff et al. (1996) presented a proposal for the reclassification of the genus *Salmonella*, which would have two species: *Salmonella enterica* and *Salmonella bongori*.

In the current classification of the Bergey’s manual, all *Salmonella* serotypes belong to one of two species: *Salmonella bongori*, which has at least 10 extremely rare serotypes; and *Salmonella enterica*, which is phenotypically and genotypically divided into six subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*, differentiated by their biochemical behavior, mainly in terms of sugar and amino acid metabolism (Forshell & Wierup, 2006).

In the current nomenclature, the name of the serovar begins with an uppercase letter, but it is never written in italics. For example in subspecies enterica: *Salmonella enterica* subspecies *enterica* serovar Typhimurium. The short form would be *Salmonella* ser. Typhimurium or *Salmonella* Typhimurium. Other subspecies are designated by the name of the serovar, followed by its antigenic formula, explained below.

Typification of *Salmonella* spp. serovars is based on the antigens found in bacterial cells, somatic (O), flagellar (H) and capsular (Vi) (Selander et al., 1996). Vi antigen is associated with virulence, and is only expressed by serovars Typhi, Paratyphi C and Dublin (Rycroft, 2000; Grimont et al., 2000). H antigen is thermolabile, whereas O and Vi are thermostable, and not destroyed by heating at 100°C for two hours (Franco & Landgraf, 1996). The combination of the antigens O, H1 (flagellar, phase 1) and H2 (flagellar, phase 2) determine the antigenic formula of a serovar. O antigens receive Arabic numerals, whereas H1 antigens are identified by lowercase letters, and H2 antigens by Arabic numerals. For example, *Salmonella enterica* subspp salamae ser. 50: z : e,n,x, or *Salmonella* serotype II 50: z : e,n,x.

Somatic (O) and flagellar (H) antigens, determine different serovars in each subspecies, in a total of 2,610 serovars today, as recognized by Kauffman-White scheme (Grimont & Weill, 2007). Although all of them are considered to be potentially pathogenic to men, only 200 are more frequently related with human disease (Baird-Parker, 1990). Distribution according to species and subspecies is as follows: *Salmonella enterica* subspp. enterica (1,547 serovars); *Salmonella enterica* subspp salamae (513); *Salmonella enterica* subspp arizonae (100); *Salmonella enterica* subspp diarizonae (341); *Salmonella enterica* subsp
houtenae (73); *Salmonella* enterica subsp. indica (13); *Salmonella* bongori (23); the newly proposed species *Salmonella* subterranea was not recognized, and is considered a serovar of the bongori species (Rodrigues, 2011).

Serovars may be further subdivided into biotypes and phagotypes. Biotyping uses different sugar fermentation patterns and assimilation of amino acids among strains of the same serovar, whereas phagotyping is based on the difference in strain susceptibility to a series of bacteriophages (Ward et al., 1987; Grimont et al., 2000; Dunkley et al., 2009).

As for their antigenic profile, *Salmonella* has an antigen common to all species in the Enterobacteriaceae family, called Kunin antigen. The presence of this antigen is not routinely analyzed, once it is not a relevant criterion for the differentiation between genus and species.

Some serovars produce a superficial polysaccharide, or capsular antigen, called “Vi”. It is found outside the cell wall, and prevents detection of the somatic antigen. It is usually found in strains of *Salmonella* Typhi, *Salmonella* Paratyphi C and *Salmonella* Dublin. Vi antigens are thermolabile, and may be destroyed by heating at 100°C for 10-15 minutes.

The somatic antigen, or “O” (Ohne), on the other hand, is specific. It is a lipopolysaccharide, and is resistant to heat and alcohol. It is made up of three parts: a lipid portion, responsible for toxicity and pyrogenic characteristics; a core portion; and the polysaccharide, which confers stability to smooth (S) variants. The “O” antigen is made up of repetitive chains with a definite spatial arrangement. The specificity of “O” antigen is given by this definite nature and the type of bond. The synthesis of this antigen is encoded by about 20 genes (locus rfb). Many somatic antigen factors (67) are recognized and used in the serological identification of *Salmonella*. Although these factors are intimately related, they are not always antigenically identical, and can only be characterized when strains are in the smooth phase. In this phase, colonies show homogenous, shiny surfaces, with regular borders, indicative of the complete "O" antigen. Mutations that affect the core portion of the antigen, or the synthesis of its chain, lead to loss of specificity. In this case, strains are called Rough (R), colonies have irregular borders and surfaces, and it is impossible to recover or recognize their original characteristics. They agglutinate in saline solution, are easily phagocytized, and are sensitive to the action of the complement system. Agglutination of bacterial cells (somatic or “O” antigen) using polyclonal (±7) and specific (65) antisera, which is the laboratory procedure for antigen confirmation, is slow and may form fine granules that are not dissociable by stirring. This occurs because the reaction is based on an interrelationship between the walls of the bacterial cells.

Flagellar antigens, or “H” (Hauch) antigens, are made of a protein called flagellin. Antigenic differences are related to variations in the primary structure or amino acid content of different flagellin molecules. The “H” antigen is thermolabile, may be destroyed at 100°C for 10 minutes, and by slow action of alcohol 50%; but it is resistant to formaldehyde 0.5%. Agglutination of flagellar antigen forms large clumps that are quickly dissociated by stirring. Compared with somatic agglutination, it occurs faster due to the large number of flagella in the cell, and because bacterial cells bind to each other.

Spatial arrangement and intrinsic characteristics of the genus lead to the production of two different types of flagella. In a bacterial population of *Salmonella* spp. strains that produce two different types of flagella, the rate of cell variation among those that present one of the two types or phases is about $10^4$. In most *Salmonella* isolates, two genes encode flagellar antigens: fliC (>50 different alleles), with highly conserved terminal sequences in the genus and which encodes phase 1 antigens; and fljB (±30 alleles), also conserved in the genus,
which encodes phase 2 antigens. These genes are expressed by a phase-variation mechanism, with flfC being found in all *Salmonellae*, and having a homologous gene found in *E. coli*; whereas flfB is located in a region exclusive to the *Salmonella* genome, and is found in four of the six subspecies. In some cases, triphasic strains may be isolated. Besides the other two genes, it was described that these strains presented the flagellin gene (flpA) in a plasmid. The genes that encode flagellin in *Salmonella* spp. are generally highly conserved in extremities 5′ and 3′, whereas the central region is highly variable.

In practical conditions, rapid agglutination with polyclonal antisera (12 polivalent and 85 monovalent antisera) may frequently occur in the absence of expression of one of the phases, preventing the identification of the serovar. This may happen in some serovars, when cell subpopulations, each possessing a given antigen or set of antigens associated with their flagella, are able to produce a third or fourth type of flagellum. Identification, in these cases, requires “immobilization” of one of the phases, in order to characterize the unknown phase, a technique called “phase inversion”. When the phase is not recognized, the serovar will not be conclusively diagnosed, preventing effective control actions. However, considering the complexity of flagellar antigens, if not all monovalent antisera are used, results on antigenic structure may be incorrect, such as g,m; g,t; g,p; g,q; g,p,s; g,z61; m,t.

3. 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 (Tauxe et al., 2010).

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 foodborne disease outbreaks. In 1992, the National Surveillance scheme for general Outbreaks of Infectious Intestinal Disease was introduced in England and Wales to provide comprehensive information on causative agents, sources, vehicles of infection and modes of transmission (Oliveira et al., 2010).

*Salmonella* spp. is an intestinal bacterium responsible for severe foodborne intoxications. It is one of the most important agents involved in outbreaks reported in several counties (Tessari et al., 2003). Salmonellosis is an important socioeconomic problem in several counties, mainly in developing countries, where this etiological agent is reported as the main responsible for foodborne disease outbreaks (Alves et al., 2001). There are reports of foodborne salmonellosis in humans since the 19th century, caused by the ingestion of contaminated bovine meat (Barrow, 1993). 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 (Antunes et al., 2003, Santos et al., 2002), and the significant morbidity and mortality rates (Cardoso et al., 2002).
According to the World Health Organization (WHO), *Salmonella* is the bacterial agent most frequently involved in cases of foodborne disease all over the world. The agent is normally transmitted to humans by means of foods of animal origin, such as meat, eggs and milk (Nascimento et al., 2003). In the past, the main motivations for controlling *Salmonella* spp. infections in poultry were the losses caused by clinical (pullorum disease and fowl typhoid) and subclinical diseases (paratyphoid infections) (Calnek, 1997). Nowadays, due to the public health implications, prevention of foodborne transmission of *Salmonella* spp. is a priority for the poultry sector (Oliveira & Silva, 2000).

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 (Berchieri Jr. & Freitas Neto 2009; Kottwitz, et al., 2010). There is a growing concern about human infections caused by other serovars, such as *Infantis*, *Agona*, *Hadar*, *Heidelberg* and *Virchow* (Freitas Neto et al., 2010).

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 (Colin, 1996; Baxter-Jones, 1996). 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 (Humphrey et al., 1988; International Commission for Microbiological Safety of Foods (ICMSF), 1998). These species replaced *Salmonella Typhimurium*, which was the most common agent of human foodborne infection until the 1980s (Olsen et al., 2003; Jay, 2000).

The introduction of *Salmonella Enteritidis* in Brazil probably occurred in the end of the 1980s, by means of breeders acquired from European countries. This may have also facilitated the introduction and dissemination of phagotype PT-4 beginning in 1993 (Irino et al., 1996), the predominant phagotype in Europe at this time (Wall & Ward, 1999).

In the 1990s, there were several reports of foodborne disease outbreaks in humans mainly caused by the ingestion of poultry products (Taunay et al., 1996). Between 1995 and 2011, there were 406 reported outbreaks and 16,304 cases of salmonellosis in Brazil, Chile, Argentina, Peru, Uruguay, Paraguay and Ecuador (Franco et al., 2003). According to the National Health Surveillance Agency in Brazil [ANVISA; *Agência Nacional de Vigilância Sanitária*], among the etiological agents of foodborne 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 (Rodrigues, 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 foodborne disease. According to data of the Brazilian Ministry of Health, 6,602 foodborne 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 (Medeiros, 2011).

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 (EFSA, 2007). 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* (Centers For Diseases Control and Prevention - CDC, 2007).
In Denmark, *Salmonella Infantis* was isolated from samples of pork, which was pointed out as the source human infection (Wegener & Baggesen, 1996). In several industrialized countries, cases of human infection caused by this serovar have been described (Raevuori et al., 1978; Pelkonen et al., 1994). In Finland, *Salmonella Infantis* was described as the third most important serovar; it infects humans, and it is the most frequently isolated serovar in poultry (Pelkonen et al., 1994). In Hungary, the rate of occurrence of *Salmonella Infantis* has increased in the past years both in the poultry industry and in humans (Nógrády et al., 2008).

National and international regulations determine the absence of *Salmonella* spp. in 25 grams of sample, including poultry meat and eggs. In spite the technological development in food production and the adoption of better hygiene measures in the food production and handling, the incidence of human salmonellosis has increased in several parts of the world (Anais de Toxiinfeção Alimentar, 1996).

In the US, there are more than 800,000 notified cases of infections caused by *Salmonella* spp., with an average of 500 deaths a year. Worldwide occurrence of salmonellosis is calculated in 1.3 billion cases and 3 million deaths (Thong et al, 1995). In 1988, there were 4 million cases of foodborne disease in the US and Canada, representing an estimated cost of US$ 4.8 billion, including losses in commercialization, productivity and labor (Todd, 1989). In a five-year period (1985-1989), there were 189 outbreaks in the US caused only by *Salmonella Enteritidis*, with 6,604 people involved, and 43 deaths.

Salmonellosis epidemiology and control are highly complex, and hygienic and sanitary standards vary with the region, based on feeding and cooking habits, and animal raising practices. Control of the disease is a challenge to public health because of the emergence/reemergence of serovars in different areas, both in developing and developed countries.

Carriers are the most important epidemiological factors, because of the lack of symptoms, and the technical difficulty in detecting them before or during the inspection of foods of animal origin. Considering that the main route of transmission is in the food chain, the presence of this microorganism in production animals shows that *Salmonella* is the most incident and relevant etiological agent of intestinal infections. It causes million of dollars in losses to the industry, mainly in cattle, swine and poultry production, both in local and international trade. In some countries, rigid food inspection is a constant need to produce foodstuffs of high quality.

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 (Taitt et al., 2004).

As for fowl salmonellosis, paratyphoid *Salmonellae* are the most important ones in terms of animal and public health (Nascimento et al., 1997). These microorganisms remain in the intestinal tract of the birds, making poultry a possible source of foodborne infection for humans (Berchieri Jr., 1991). Transmission of *Salmonella* to men generally occurs by means of contaminated food and water, although person-to-person transmission may take place, mainly in hospitals. Transmission by contact with infected animals, mainly among veterinarians and farm workers (Trabulsi & Landgraf, 2004), is also possible. It should be emphasized that most serotypes in this genus are pathogenic to men; the differences observed in symptoms may be related to variation in the mechanisms of pathogenicity, age and immune response of the host (Trabulsi & Landgraf, 2004; Hofer et al., 1997).
A large number of Salmonellae have to be ingested to cause gastroenteritis. Generally, the infective dose depends on the serotype, ranging from $2 \times 10^2$ to $1.0 \times 10^6$ CFU/g or mL (Huang, 1999). 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 characteristics symptoms of the disease (Pinto et al., 2004). 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 (Argôlo Filho, 2007). Because of the ability to disseminate and survive for a long period of time in the environment, Salmonella may be isolated from superficial freshwater bodies, from sea water in coastal areas, and from several raw materials used in food production (Jakabi et al., 1999).

According to Nascimento (1996), contamination of poultry products (meat and eggs) destined for human consumption may occur at the slaughterhouse, during food preparation, or by cross-contamination with material from poultry with intestinal and systemic infections. As for poultry meat, even a small number of infected birds may contaminate the whole slaughter line, multiplying the chances of occurrence of foodborne disease. Because of that, slaughterhouses where carcasses are not correctly processed are a threat to public health (Nascimento, 1996); current practices of broiler slaughtering and processing may spread microorganisms from one carcass to another. When consumed, the product may be responsible for human infection (Santos, 2004). 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 (Silva, 2000).

Transmission of Salmonella in birds may occur vertically, via eggs, with the birth of infected chicks; horizontally, by means of ingestion of water, feed, fecal material, contaminated bedding material or dust; or by oral, nasal, conjunctival, cloacal and umbilical routes (Cox et al., 1996; Navarro, 1995; Nascimento, 1996). Many Salmonella serotypes may survive for weeks or months in manure or bedding material, in equipments, in empty sheds, in the dirt around sheds that have been cleaned and disinfected, in feces of wild poultry, in dust particles, and in bird feeders. According to these authors, Salmonella may survive in contaminated feed for 26 months, in feces of infected birds for more than 11 days when inside of sheds, or for 9 days in open spaces. Besides, domestic and wild animals may be carriers of Salmonella, spreading the microorganism in the environment where they live. These bacteria may cause acute and/or chronic disease in susceptible animals. As stated before, the epidemiological complexity of the disease, which involves vertical transmission, fecal excretion, horizontal transmission, environmental contamination and presence of carriers in different species, make salmonellosis control difficult to be achieved (Soncini & Back, 2001).

Salmonellae are distributed all over the world. Multiplication outside the body of the host is facilitated by high temperatures and presence of protein (for example, in residual waters). Therefore, the most important points of transmission of Salmonella are tropical and subtropical regions, as well as places where there is a large concentration of animals and people. Salmonella may also be found in products refrigerated at 2°C; the microorganism is able to remain viable in frozen products for long periods.

After entering the digestive system together with contaminated food and water, Salmonellae reach the intestines, where they attach to intestinal cells and multiply. Depending on the host species and age, and on the pathogenicity of the microorganism
and its adaptation to the host, *Salmonellae* 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 (Jay, 1992). 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 (Gomez & Cleary, 1998; Pinto et al., 2004).

Salmonellosis is not limited to intestinal infection and gastroenterocolitis. The microorganism may infect other organs; as *Salmonellae* are able to reach the circulation, they may cause diffuse extraintestinal infections, such as meningitis, osteomyelitis, arthritis, pneumonia, cholecystitis, peritonitis, pyelonephritis, cystitis, endocarditis, pericarditis, vasculitis and other disorders (Gelli, 1995).

*Salmonellae* cross the intestinal epithelium, and reach the *lamina propria* (the layer where epithelial cells are anchored), where they multiply. They are phagocytized by macrophages and monocytes, causing an inflammatory response as a consequence of the hyperactivity of the reticuloendothelial system. Different from what happens in typhoid fever, penetration of *Salmonella* spp. is limited to the *lamina propria* in cases of enterocolitis. In these cases, sepsis or systemic infection are rarely observed, and infection is restricted to the intestinal mucous membrane. Inflammatory response is also related to the release of prostaglandins, which stimulate adenylate cyclase, leading to increased secretion of water and electrolytes and aqueous diarrhea (Franco & Landgraf, 2004).

From 1980 on, human outbreaks caused by *Salmonella* Enteritidis, showed common sources in the US, Great Britain and other European countries (CDC, 2005). 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 (Perales & Audicana, 1989). 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 (Suresh et al., 2006). Changes in the predominance of serotypes reflect changes in animal raising practices and dissemination of new serotypes due to increased international trade. Nowadays, the main concern is the emergence of *Salmonella* serotypes that are resistant to multiple antibiotics (Huang, 1999).

Cases of disease caused by four serovars of subspecies enterica are subject to mandatory reporting, according to regulation number 207 of the Brazilian Agency of Agricultural Defense [SDA; Secretaria da Defesa Agropecuária], reviewed in July 30th, 1995. These serovars are part of the list B of the World Organization for Animal Health (OIE), of diseases that cause regional economic losses. Among them, *Salmonella* Pullorum, Gallinarum, Typhimurium and Enteritidis. About 90 serovars of *Salmonella* spp. are more frequent in cases of human and animal infection (Berchieri Jr. & Freitas Neto, 2009).

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 (Synnott et al., 1998; CDC, 2007). According to Clark et al. (1973), human outbreaks in the US and Europe that occurred around 1970 were caused by poultry meat. Animals were infected by
feed containing contaminated fish meal that came from Peru. This case is an example of the epidemiological complexity of this disease. The intensive breeding system adopted by the poultry industry favors the introduction, establishment, permanence and dissemination of these bacteria (Berchieri Jr. & Freitas Neto, 2009). 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 (Bersot, 2006). Salmonella may affect all segments of poultry production, such as breeder facilities, incubators, commercial raising operations, feed factories, slaughterhouses, transportation systems and commercialization facilities.

Globalization incorporated the sanitary restrictions imposed by the European Community to international traders of foods of animal origin, mainly poultry. The occurrence of cases of foodborne infection linked to Salmonella Enteritidis and Salmonella Typhimurium show the sanitary importance of Brazilian poultry production, in social and economic terms. When the World Trade Organization (WTO) was created, the guidelines and Codex Alimentarius regulations were determined for international trade, and for agreements on sanitary and phytosanitary (SPS) measures and technical barriers to trade (TBT). With these agreements, WTO country members should review, establish and implement internal control systems, that is, adopt the Hazard Analysis and Critical Control Points System (HACCP).

4. Detection methods

Salmonellae are short Gram-negative bacilli, about 0.7-1.5 x 2-5 μm, readily stained, and nonsporulating. Most of them move using peritrichial flagella, although serotypes such as Salmonella Pullorum and Salmonella Gallinarum are nonmotile. They are either aerobic or facultative anaerobic, and grow between 5 and 45°C. Optimum growth occurs at 37°C. Ideal pH for multiplication is 7, but Salmonella survives in pH values between 4 and 9. They grow in culture medium for enterobacteria and in blood agar. Colonies are 2-4 mm in diameter, with smooth and round edges. They are slightly raised in medium containing carbon and nitrogen. Colonies may remain viable for a long time when stored in peptone (Holt et al., 1994; Gast, 1997).

Biochemically, Salmonella strains have the ability to metabolize nutrients, and catabolize D-glucose and other carbohydrates, except lactose and sucrose, with production of acid and gas. They are catalase positive and oxidase negative, as are all genera in the Enterobacteriaceae family. They do not ferment malonate, do not hydrolyze urea, do not produce indol, use citrate as a sole source of carbon, reduce nitrate to nitrite, and may produce hydrogen sulfide (Quinn et al., 2000).

Conventional culture methods for isolating Salmonella spp. in poultry or animal feed or in feed ingredients have been reported in a number of studies, which were summarized by Williams (1981). Although all methods follow the basic strategy of preenrichment followed by selective enrichment, differential plating and biochemical or serological confirmation, there is no single internationally accepted procedure for Salmonella spp. detection.

The Food and Drug Administration (FDA), for example, recommends lactose broth for preenrichment (Andrews et al. 1998), while Wyatt et al. (1993) used buffered peptone water. Cox et al. (1982) reported that preenrichment decreased the recovery of Salmonella spp. from artificially contaminated poultry feed when compared with direct enrichment. Suggested protocols also vary with the substrate: Kafel (1981) suggested the use of anaerobic lactose broth, followed by selection in tetrathionate brilliant green broth and plating on brilliant
green agar, in the analysis of fish meal. Allen et al. (1991) reported that the sensitivity of Rappaport Vassiliadis medium depended on the substrate in the detection of Salmonella spp. in high moisture foods, compared with tetrathionate or selenite cystine broth. Eckner et al. (1992) added novobiocin to tetrathionate selective enrichment and increased the incubation temperature to 42°C.

The conventional technique for the detection of the microorganism includes the following steps: preenrichment, selective enrichment, isolation and selection, biochemical characterization, serological characterization and final identification. This technique requires at least four days for a negative result and six to seven days for the identification and confirmation of positive samples (Soumet et al., 1997). The presence of Salmonella has to be determined in at least 25g or mL of sample.

New methodologies, such as immunological tests, have been proposed as alternatives for direct detection of this pathogen. For example, ELISA (Enzyme-linked Immunosorbent Assay) was used by Loguerio et al. (2002). Immunoenzymatic technology may be combined with other rapid methods in order to decrease total assay time. Luk et al. (1997) combined a digoxigenin-based ELISA with the polymerase chain reaction (PCR) to detect amplified rfbS, a lipopolysaccharide gene of Salmonella spp.; in this case, preenrichment was no longer than 16 hours.

Other types of assays have also been used: techniques based on molecular biology, such as nucleic acid hybridization or PCR, which was used by Flóres et al. (2003); and tests based on metabolism measurements (impedance and radiometry) (Franco & Landgraf, 1996). Ribotyping is the most recent addition to the automated identification of bacteria. The RiboPrinter™ Microbial Characterization System is based on the highly conserved nature of the rRNA operon. Ribotyping provides a reproducible method by which rRNA and polymorphic fragments can be compared with a database for identification of genus, species and strain (Grimont & Grimont, 1986). The system is almost completely automated, requiring only picking up the colonies, suspending them in buffer and submitting them to heat treatment in a special carrier. Once heated, the sample is placed in the device, which automatically lyses the bacteria, releasing DNA; digests it with restriction enzymes; transfers the sample to agarose gel; and separates restricted fragments by electrophoresis. DNA fragments separated by size are then transferred to a nylon membrane, which is hybridized with a chemically-labeled and treated DNA antibody/alkaline phosphatase conjugate.

Resulting stained bands are then photographed, and the image is stored in the computer database and compared with other images in it. The database for this system is less comprehensive than that of other automated systems, but it still adequate for Salmonella spp. The system would, however, be invaluable in epidemiological studies related to (HACCP) incidents.

Serotyping is an important epidemiological tool that complements the identification of Salmonella, making it possible to determine the prevalence/emergence or to show trends of a given serovar in different geographical regions, as well as to identify outbreaks, and discover sources of infection and routes of transmission. Serotyping is based on the Kauffmann & White classification and involves the identification of somatic and flagellar antigens.

The somatic structure is identified based on the recognition of the serovars, which are represented by uppercase letters. For example, group A (O:2), group B (O:4); group C1 (O:6,7), group C2 (O:6,8,20), group D (O:9), group E1 (O:3,10), group E2 (O:3,15), group E4 (O:1,3,19), etc. Some factors identify the antigenic group, for example, O:4, O:9. Other
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factors have little or no discriminatory value, and are normally associated because they represent a complex, such as O:12 (121, 122, 123), with O:2, O:4 and O:9. For example, *Salmonella* Paratyphi A (O:1,2,12), *Salmonella* Typhimurium (O:1,4,5,12) and *Salmonella* Enteritidis (O:1,9,12).

Some antigens appear as a consequence of a change in the structure, such as O:1, which is a result of the insertion of galactose in the polysaccharide; O:5 a results of the acetylation of abequose, found in the repetitive units of the polysaccharide responsible for specificity, such as in serovar *Salmonella* Typhimurium O:4,12 and O:1,4,5,12.

As for the characterization of flagellar antigens, it should be taken into account the fact that some *Salmonella* serovars have only one flagellar phase. They are called monophasic: *Salmonella* Enteritidis (9,12: g,m:-), *Salmonella* Typhi (9,12 [Vi]:d:-); however, most serogroups show two flagellar phases, that is, they are diphasic strains, such as *Salmonella* Typhimurium (1,4,5,12: i: 1,2) and *Salmonella* Hadar (6,8: z10: e,n,x), which express phase 1 (antigens i or z10) and phase 2 antigens (respectively, antigens 1,2 or e,n,x). Nonmotile strains, which have no flagella, have also been recognized (Rodrigues, 2011).

5. Drug resistance

Microbial resistance is related to strains of microorganisms that are able to multiply in the presence of concentrations of antimicrobial compounds even higher than those given as therapeutic doses to humans. Development of resistance is a natural phenomenon that followed the introduction of antimicrobial agents in clinical practice. The irrational and widespread use of these agents has added to the problem, and resistance rates vary from place to place, depending on the local use of antibiotics.

One of the major concerns of the poultry industry is maintaining the sanitary status of the herds. In the incubators where birds are born, there is an attempt to reduce contamination to minimum levels in all phases of the process. Lack of contact with natural biota soon after birth interferes with the normal development of bird intestines (Silva, 2000). Generally, antimicrobial substances (antibiotic or chemotherapeutic agents), called growth promoters, are used in the feed from the first day of life to the moment of slaughter of the birds, respecting the recommended withdrawal period (Mota, 1996). These growth promoters improve performance because they “modulate” intestinal microbiota and improve feed efficiency. Suppliers of growth promoters guarantee that these substances are not absorbed through the intestinal walls and are shed in feces, where they are quickly biodegraded. Thus, they do not leave residues in the animal, and do not pose risks to human health or the environment (Mota, 1996). However, consumers are constantly concerned on the possible risks that antimicrobial resistance poses to human health.

In veterinary medicine, antimicrobial agents are used in therapy, metaphylaxis, prophylaxis, and as growth promoters (Scharwz et al., 2001). The use of subtherapeutic doses of antibiotics as growth promoters is a public health problem, because many resistant microorganisms may transfer resistance to microorganisms found in bird feces. This kind of use may be responsible for selective pressure that generates resistant bacteria, a current, worldwide-spread, public health problem, due to the risk of dissemination of pathogens and transfer of resistance genes, via food chain, to pathogenic and commensal microorganisms of humans, decreasing the treatment options for infections (Medeiros, 2011).

Since antimicrobials started to be widely used by humans at the end of the 1940s, the emergence of resistant strains was observed in most bacterial species, and against all drugs available (Flemming, 2005). The use of antimicrobials, combined with improvements in
sanitation, nutrition and immunization, has lead to a dramatic decrease in deaths and a major gain in human life expectancy (WHO, 2002). However, with the increased use of antimicrobials, antimicrobial resistance has emerged as one of the greatest threats to the safety of human health (WHO, 2007), and as a most pressing problem for public health, animal health and food safety authorities (Tenover, 2006; Marchese & Schito, 2007).

The increase in antimicrobial resistance has narrowed the potential uses of antibiotics for the treatment of infections in humans and animals (Angulo et al., 2004). As a striking example, the CDC estimated that the total of methicillin-resistant *Staphylococcus* Infections (MRSI) in US hospitals and communities have increased from 2 % in 1974 to almost 63% in 2004 (CDC, 2010).

In the US, more than 40% of the antibiotics produced are used in animal feed. This non-therapeutic use of antibiotics is a way to promote the selection of a growing number of resistant bacteria (Levy, 1998). As more strains responsible for poultry infections become resistant to therapeutic drugs, these compounds become less available for human treatments. Similarly, with *Salmonella* being an important cause of foodborne diarrheal disease in humans 10/12, the reduction in the number of antibiotics available for effective treatment of *Salmonella*-related infections in humans and animals has become a serious concern (Angulo et al., 2004).

In Europe, besides this concern with resistance, several recent public health episodes were branded on the mind of the consumers. Among them, the connection between eggs and *Salmonella Enteritidis*, BSE/“mad cow disease” and cattle meat and, more recently, avian flu in Asia. Therefore, zoonoses and restricted use of additives and antimicrobials as growth promoters in feeds, together with the occurrence of resistant microorganisms, have become an important challenge in the control of detrimental microorganisms found in the digestive system of birds.

There is a consensus in several countries that the indiscriminate use of antimicrobials in animal production is one of the causes of the increased resistance to antimicrobials. Human infections are more severe when a strain of a given microorganism is resistant to the drug of choice for its treatment. The use of antimicrobials may stimulate the selection of resistant bacteria in this ecosystem. Human pathogens and resistant genes may cross species and ecosystems by contact with, or consumption of contaminated food and water (Kelley et al., 1998). Due to the little knowledge on single, multiple or cross-resistance mechanisms in microorganisms that are highly pathogenic to humans, the WHO has recommended careful use and restrictions to antimicrobials in animal production (WHO, 2001).

Before *Salmonella Enteritidis* outbreaks related to traditional drugs in Europe, different antibiotics – such as nitrofuranzone, furazolidone, novobiocine and tetracyclines - were used in drinking water and in feed offered to poultry. In Brazil, tetracyclines, penicillins, chloramphenicol, sulphonamides, furazolidone, nitrofurazone and avoparcin were banned as additives in animal feed in 1998. However, the use of several other drugs is still allowed: 3-nitro acid, arsanilic acid, avilamycin, colistine sulfate, enramycin, flavomycin, lincomycin, spiramycin, tylosin sulfite and zinc bacitracin.

Extensive use of quinolones in birds was made possible by very flexible prescription regulations, use of generic, lower cost drugs in feed and water, and, without a doubt, because of the efficiency of these agents against *Salmonella*. The use of fluoroquinolones, which have a similar mechanism of action, followed quinolones (Rossi, 2005).

Strains of *Salmonella Enteritidis* may become resistant because of the indiscriminate use of drugs in their country of origin, imports of foodstuffs contaminated with bacteria carrying resistance genes, or infected people returning from international trips. Finnish researchers
Hakanen et al., 2001 observed increased antimicrobial resistance in strains of *Salmonella* Enteritidis isolated from travelers after they came back from Asian countries where quinolones were used indiscriminately. There was an increase from 3.9% to 23.5% in the resistance to fluoroquinolones in samples analyzed between 1995 and 1999 in Finland. These facts, suggest that drug resistance genes may be associated with virulence, or that humans strains have an improved resistance profile compared with *Salmonella* of animal origin, making the whole situation even more concerning from a public health viewpoint.

The frequency and extent of *Salmonella* resistance to antimicrobials vary based on the use of antibiotics in humans and animals, and on ecological differences in the epidemiology of *Salmonella* infections (McDermott, 2006). Globally, *Salmonella* exhibits extensive resistance profiles which have been associated both with higher rates of morbidity and mortality and the use of antimicrobials in food-producing animals (Angulo et al., 2004). Antibiotics suppress normal intestinal microbiota, breaking its protective effect, increasing the competitive advantage of antibiotic-resistant *Salmonella*, and favoring the occurrence of salmonellosis (Eley, 1994).

Salmonellosis surveillance has been described all over the world, specially after the emergence of strains resistant to multiple antibiotics, making control and treatment even more difficult. The WHO observed an alarming increase in the number of strains of *Salmonella* resistant to antibiotics due to the abusive use in intensive animal raising (Eurosurveillance, 1997). This finding is a concern for surveillance and environmental control organisms, once the use of antibiotics in animal feed as growth promoters contributes for the emergence of resistant and pathogenic strains (Pinto, 2000).

Antibiotics may be either bactericidal or bacteriostatic agents. Bactericidal agents cause changes incompatible with bacterial survival, whereas bacteriostatic agents inhibit bacterial growth and reproduction, without immediately killing microorganisms (Tavares, 2001). The mechanism of action of antibiotics is essentially related to interference with cell wall synthesis. Cell wall constitution varies in Gram-positive or Gram-negative bacteria, leading to differences in permeability to drugs. Antibiotics that affect the permeability of the cytoplasmic membrane are similar to cationic detergents, due to the presence of basic groups (NH₃⁺) in a lateral chain of the fatty acid.

Insertion of antibiotic molecules disorganizes the membrane, producing leakage of cell components and death. Antibiotics that interfere with DNA replication generate toxic products that get inserted in the DNA molecule, breaking it up and preventing its synthesis. Others compounds loosen the DNA spiral structure, making it larger and breaking the bacterial cell. Agents that affect protein synthesis act on the ribosome, inhibiting protein synthesis by different mechanisms (Tavares, 2001; Trabulsi & Alterthum, 2008).

Some bacterial species are considered naturally resistant to antibacterial compounds (primary resistance), because only concentrations that would be unviable *in vivo* would affect them. Under continuous exposure to antimicrobials, microorganisms show acquired resistance (secondary) caused by the development of new mechanisms of defense (Fuchs & Wannmacher, 1999).

Resistance mechanisms may emerge because of changes in bacterial DNA, or biochemical mechanisms of molecule production, reactions and behaviors, which may be transmissible or not to the daughter cells. Resistance is observed when an antibiotic is administered to patients who are carriers of sensitive, mutant strains. Antimicrobials eliminate microorganisms that are sensitive, “selecting” the ones that are resistant. The rate of emergence of mutant strains is highly variable, and the mutation process may occur quickly.
in some cases, and slowly and gradually in other cases, taking years to appear. Some cells may present random genetic changes that may lead to resistance to a given antibiotic (Decamp & Moriarty, 2006). The process is called single resistance when the bacterium is resistant to only one drug; multiple resistance, when it is simultaneously resistant to two or more drugs (Tavares, 2001).

According to Claus (1988), mechanisms of antimicrobial resistance may involve chromosomal DNA, by means of mutations; or may be due to the acquisition of extrachromosomal DNA (by means of gene transduction, transformation or conjugation).

Mutations occur by chromosome swapping. These changes may be random, or caused by physical and/or chemical agents, and the process may be caused or not by exposure to antimicrobial agents. Many microorganisms isolated before the use of antibiotics showed mutations, and were not sensitive to antibiotics when these were discovered. Antimicrobials are not necessarily responsible for mutations, but they have an important role in the selection of resistant strains. Commonly, the genetic change that causes the resistance in a microorganism is generated by genes transported in extrachromosomal plasmids (Claus, 1988).

In the transduction process, a bacteriophage transfers, from a resistant to a sensitive bacterium, extrachromosomal bacterial DNA incorporated in its protein. The previously sensitive bacterium, then, will acquire resistance and transfer it to its daughter cells. This mechanism is easily observed in Staphylococcus aureus strains that acquired resistance to penicillins.

Transformation occurs when bacteria that are sensitive to one substance incorporate the DNA with genes that encode resistance, that are found in the environment. These bacteria, then, become resistant to one or more antimicrobials. Some bacteria, in certain growth phases, are able to excrete DNA to the environment.

Conjugation is caused by a passage of genes (R factors) from a resistant to a sensitive bacterium by attachment to a sex pilus. The R factor may contain resistance information against several antimicrobials. Conjugation and production of the sex pilus requires intervention of another group of genes, called transference factor. Without them, the process is not carried out. The R determinant complex, plus the resistance transfer factor, are known as R factor. R factor is important to Gram-negative bacteria, specially enterobacteria. Escherichia coli, Salmonella, Shigella, Klebsiella and Pseudomonas aeruginosa are among microorganisms capable of transferring this type of resistance to sensitive bacteria. This resistance mechanism has been observed in relation to tetracyclines, chloramphenicol, sulfonamides, penicillins and aminoglycosides.

All these genetic alterations give rise to several biochemical changes in bacterial metabolism. Resistance to antibiotics may be carried out by three basic mechanisms produced by these changes (Strohl et al., 2004): decreased absorption or increase efflux of the antibiotic; change in the target site of the antibiotic, and acquisition of the ability to break or modify the antibiotic.

Acquired resistance to antibiotics is a necessary gain, or temporary or permanent change of bacterial genetic information. Most resistance genes are found in plasmids, which may be swapped with chromosomal elements. Acquired resistance is caused by mutations in the bacterial chromosome (which leads to the emergence of resistance genes in a sensitive bacterium), or by the transfer of resistance genes from one cell to another, with DNA fragments with these genes being inserted in the receptor cell. Both types of resistance, mutation (chromosomal) and transferable (plasmidial) may be found in the same bacterium (Tavares, 2001).
Plasmids are circular, extrachromosomal DNA molecules found in many bacterial species, and in some eukaryotes. They replicate separately or together with the host cell, and are passed on to the daughter cells. Plasmids may removed from the cell by different stress conditions, such as changes in temperature, presence of some stains or lack of certain nutrients. They are not essential to the cell, but may confer some selective advantages: they may have information for the degradation of certain substrates, resistance antibiotics or heavy metals. Plasmids may self-replicate independently of chromosomal replication, and may occur in variable numbers. Sex factors (F factor), antibiotic resistance (R factor), N$_2$-fixation (Trabulsi & Alterthum, 2008) are example of plasmids.

Antimicrobial resistance is one of the most important problems for human and veterinary medicine, and it is recognized by the WHO as an important public health problem (Rossi, 2005).

There was a significant increase in the occurrence of *Salmonella* Enteritidis in poultry carcasses from 2000 to 2005 in the US. Studies in Brazil between 2000 and 2009 show the predominance of this serovar in poultry. More than half of the strains were resistant to multiple antibiotics, and *Salmonella* Enteritidis was the only serovar that showed different degrees of resistance to all antimicrobial compounds. Studies carried out with *Salmonella* Heidelberg demonstrated that all strains showed multiple resistance, including marked resistance to third generation cephalosporins. In the past years in the US, increased resistance to ceftiofur was observed in poultry strains. In 1997, resistance to this antibiotic was 1.6%, and in 2003, 7.4% (Medeiros, 2011).

During decades, ampicillin, chloramphenicol and trimetoprim-sulfametoxazole were the most frequent antimicrobials used in salmonellosis treatment. However, the increase in the number of strains resistant to these drugs reduced their used in medical practice. Consequently, fluoroquinolones became the main antimicrobials used in the treatment of human infections (Souza et al., 2010).

Resistance to *Salmonella* transmitted by contaminated foods of animal origin is undesirable, but it is an inevitable consequence of the use of antimicrobials in animals used in food production (Threlfall et al., 2002). Bacterial resistance is a natural process, but it should and can be prevented with the rational use of antimicrobials in animal production. Therefore, it is very important to follow the evolution of resistance in order to use efficient methods for *Salmonella* control.

### 6. Prevention and control

Prevention and control programs for infections caused by paratyphoid *Salmonellae* aim at protecting the health of the birds, ensure the safety of the consumers, and strengthen the reliability of the poultry production chain. In the case of *Salmonella*, measures recommended for prevention and control are not specific due to the large number of species and their complex epidemiological behavior. Similarly, variability in the implementation of these measures depends on the requisites determined by the international market, or the adaptation of the industry to the chronogram of production.

In the past 10 years, there have been important outbreaks of emerging foodborne diseases all over the world. These outbreaks showed sanitary authorities of the countries affected that there is an increasing need for measures to prevent the risk of transmission. This led the Food and Agriculture Organization (FAO) to create the WTO, which motivated countries to review their innocuousness policies, rules and strategies to ensure that the food consumed...
by the population had appropriate sanitary conditions for international trade (Pan American Health Organization - PAHO, 2001).

General regulations issued all over the world for Salmonella control and prevention are: Proposed Guidelines for the Control Campylobacter and Salmonella in chicken meat, from the Codex Alimentarius; Prevention, Detection and Control of Salmonella in poultry, Chapter 6.5 of the Terrestrial Animal Health Code of 2010, from the World Organization for Animal Health (OIE); Compliance Guideline for Controlling Salmonella and Campylobacter in Poultry, of May 2010, from the Food Safety Inspection Service and United States Department of Agriculture (FSIS/USDA); and the national programs for eradication control and surveillance of some Salmonella serotypes in breeding chickens and broilers, from the Ministry of Environment of Spain.

Together with many other biosafety measures, monitoring of these bacteria, which may be associated with foodborne disease in humans, is one of the great objectives of the poultry industry. Health education actions that emphasize personal hygiene habits, mainly correct hand washing, care in food preparation, handling, storage and distribution, are recommended for food handlers. Main prevention strategies should be: selection of raw materials; carefully cleaning of equipment and utensils; adequate supply of potable water; adequate garbage disposal and sewage treatment; adoption of good manufacturing practices and implementation of the HACCP; removal of asymptomatic carriers from the production area, and adequate methods for transportation and preservation. All these actions are in compliance with the recommendations of public health authorities from all over the world (ICMSF, 2002; Brazil, 2002; Reuben et al., 2003).

Literature information show that one year after the implementation of Salmonella control in Finland, prevalence was below 1% in egg and bovine, swine and poultry meat production, decreasing the occurrence of salmonellosis outbreaks (Maijala et al., 2005). Food hygiene, therefore, is based on the adoption of preventive and control measures. The HACCP system is an efficient tool to remove disease-causing agents. The system provides specific protection against foodborne disease, and leads to reduction in costs and warranties of microbiologically safe foods.

The risk of vertical transmission may be minimized by bacteriological and serological monitoring of breeding chicken lots, resulting in Salmonella-free birds; by purchasing birds more resistant to Salmonella infection (Bumstead, 2000); by culling birds that are carriers of the microorganism; by treatment of eggs that are still in the sheds, and careful incubation of dirty and cracked eggs (Berchieri Jr., 2000).

Biosafety and sanitary management are important to reduce the environmental presence of Salmonella. According to Gast (1997), one of the methods employed to achieve this aim is cleaning and disinfection of the sheds with chemical disinfectants. However, not all disinfectants are efficient and depend, for example, on their behavior in the presence of large amounts of organic material (Berchieri Jr. & Barrow, 1996). Together with this, it is important to control rodents found in bird sheds. These animals have an important role in Salmonella infection by contaminating the environment and transmitting the microorganism to birds and eggs (Henzler & Opitz, 1992).

Specific procedures that aim at controlling Salmonella in bird feed include pelleting and use of organic acids (Silva, 2005). According to Gama (2001), as pelleting is carried out at temperatures over 60°C, the process may eliminate Salmonella from poultry feed, provided that the feed is not recontaminated by handling, rats or insects. Iba & Berchieri Jr. (1995),
observed that a mixture of formic and propionic acids was efficient in controlling *Salmonella Typhimurium* in artificially contaminated feed. Another important tool in *Salmonella* prevention and control is the use of quantitative thresholds. These values vary from country to country and correspond to the measures and control systems that are adequate for local production. These limits should be established based on scientific research and special attention should be paid to the use of antibiotics, detergents, disinfectants and process temperature.

Indiscriminate use of antibiotics and addition of growth promoters in animal feed contributed to the emergence of resistance among strains of *Salmonella* and other bacteria (Berchieri Jr. & Barrow, 1998). Besides, according to Barrow (1999), after the therapeutic agent is removed, there may be a period in which birds may become susceptible to *Salmonella* infection, because their normal microbiota – which would inhibit *Salmonella* naturally – is also affected by the use of the antibiotic. Competitive exclusion is based on oral inoculation of the cecum contents of adult birds in newborn chicks, speeding the establishment of desirable intestinal microbiota (Nurmi & Rantala, 1973). The process attempts to prevent the establishment of pathogenic microorganisms in the intestinal mucous membrane. This is an important method in the control of *Salmonella* infection in birds with immature or debilitated intestinal microbiota (submitted to antibiotic therapy).

Another measure for *Salmonella* control and prevention is vaccination of susceptible birds (Gast, 1997). Nowadays, several studies have been carried out in order to evaluate the efficacy of live (Barrow et al., 1991; Hassan & Curtiss III, 1997) and inactivated vaccines (Timms et al., 1990; Gast et al., 1993; Nakamura et al., 1994; Miyamoto et al., 1999; Woodward et al., 2002). These studies support the use of vaccination, in a safe and efficient manner, as part of the prevention of infection in birds and contamination of eggs by *Salmonella Enteritidis* (Gast et al., 1992).

Notification and epidemiological records are important sources of information for inspection and control agencies, which may estimate which pathogens and foods may possibly be involved in foodborne disease outbreaks. For example, the presence of several *Salmonella* serotypes that did not show high prevalence some years ago, are found now in poultry herds and represent an important public health problem worldwide. Control of salmonellosis cases will be achieved by the adoption of some measures, such as frequent and systematic surveillance of food production and distribution. An efficient program both provides warranties in the production of safe foods and reduces costs.

### 7. Conclusions

It is concluded that salmonellosis outbreaks still occur daily, even when recommended biosafety measures to ensure the health of poultry herds are in place. This may be due to the lack of awareness on animal health issues and due to the difficult control of this microorganism.

Birds may carry *Salmonella* spp. to inside of the industry by means of utensils, men, rodents, and mainly feces. Therefore, the microorganism may be introduced in all facilities and equipments of a slaughterhouse, negatively affecting the quality of final products and by-products destined for human consumption and animal feed. Due to the wide distribution and variety of forms of *Salmonella* transmission, and the large number of foodstuffs involved in salmonellosis outbreaks, programs for guiding and
sensitizing the consumers, the trade, food handlers and breeders of animals, mainly of poultry, should be implemented in order to improve health and hygiene conditions of products and processes, and ensure the health of the final consumer. Resistance of Salmonella strains to antimicrobials normally used in poultry raising may serve as a warning against the indiscriminate use of antibiotics in the treatment of infections. Addition of antibiotics in animal feed as growth promoters may contribute for selecting resistant strains, and may affect human health.

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More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at $2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

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