

Salmonellas, Poultry House Environments and Feeds: A Review

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Abstract: The gram-negative bacteria and member of the family Enterobacteraceae is one of the most important causes of human food-borne illnesses in recent times. These pathogens may occur naturally in the gastrointestinal tract of poultry and sometimes in eggs through transovarian transmission. A number of factors have contributed to the spread of Salmonella in poultry. Among these are stocking densities of poultry farms, poultry feeds, farming activities, mice, wild animals, transportation of live birds to slaughter houses, slaughtering of live birds and processing of poultry carcasses into processed finished products. Lesser concerned area is the association between Salmonellas, poultry house environments and feeds and the significant role they may play to integrate other factors in contributing to the spread of Salmonella in poultry. Furthermore, techniques for isolating and identifying *Salmonella* species in poultry house environments and feeds are crucial for reliable reporting purposes to reduce the spread of Salmonella by poultry thus the objective of this study.

Key words: Salmonella, poultry house environments, feeds, food-borne illnesses, environment, crucial

INTRODUCTION

Since the isolation and identification of Salmonella in 1885 by Daniel E. Salmon, Salmonella has received much attention and concern by health authorities, researchers, farmers and consumers. Salmonellas (non-typhoidal) are important food-borne pathogens that have emerged to become the 2nd largest cause of food-borne illness after *Campylobacter* (Mead *et al.*, 1999).

They cause Salmonellosis which is a self-limiting food-borne illness although, systemic infections which are detrimental especially in individuals such as infants, pregnant women, the elderly organ recipient individuals cancer and HIV/AIDS patients can occur (Ellermeier and Schlauch, 2006; Sebunya and Kapondorah, 2007; Voetsch *et al.*, 2004).

In healthy persons symptoms such as fever, diarrhoea, abdominal pain, vomiting and occasionally septicaemia occur which self-resolve within 3-4 days (Coburn *et al.*, 2007; Willford *et al.*, 2007). Resistance of *Salmonella* species to multiple drugs is also an area of great concern that has caught the attention of all stakeholders. The ability of Salmonellas to resist multiple drugs will make it cumbersome to treat people with severe systemic Salmonellosis. An example is that *S. typhimurium* have been reported to show multiple drug resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, florfenicol, ciprofloxacin

and tetracycline, commonly used antibiotic for treating patients with Salmonellosis (Aksakal *et al.*, 2009; Coburn *et al.*, 2007; Pan *et al.*, 2010; Willford *et al.*, 2007). Other serovars such as *S. enteritidis*, *S. Newport*, *S. Hader*, *S. java*, *S. heidelberg*, *S. muenchen*, *S. arizonae*, *S. gallinarum* and many have also been reported to show multiple drug resistance (Aksakal *et al.*, 2009; Parry *et al.*, 2002; Sahilah and Son, 2003; Willford *et al.*, 2007).

In recent times poultry has been implicated mostly in the spread of Salmonella (Miller and Pegues, 2000). Other important sources such as pigs, cattle, pets, vegetables, fruits, debris, animal faeces, sewage, irrigation water, reptiles, amphibians newly hatched chicks and many more have been recognised (Amaechi and Ezeronye, 2006; Barak and Liang, 2008; Rahman *et al.*, 2006; Voetsch *et al.*, 2004).

In poultry, Salmonellas may inhabit in the gastrointestinal tracts in the ovary, oviduct or reproductive organs which they can share during defaecation or egg laying. Faeces, soils, litter and water in poultry farms can be important reservoirs for Salmonella and consequently the spread of Salmonella to subsequent flocks.

Salmonellas may also be present in feeds from feed mills as a result of faulty feed formulation and processing procedures. Effective and reliable methods for isolating these pathogens are essential for accurate reporting. This

review briefly looks at Salmonellas isolation methods, Salmonella in poultry house environments and feeds and measures to reduce the spread of Salmonella by these means.

SALMONELLAS

They are gram-negative, nonspore-forming bacillus and facultative intracellular pathogen (Ellermeier and Slauch, 2006) belonging to the genus *Salmonella* and the family *Enterobacteriaceae* (Tindall *et al.*, 2005). Salmonellas have a DNA composition of 50-52 mol% G+C and similar numerical taxonomy and 16 S ssRNA analysis with that of *Escherichia*, *Shigella* and *Citrobacter* (Todar, 2008). Total 2 main species have been recognised after much controversy in classifying these pathogens into the species level. They are *Salmonella enterica* and *Salmonella bongori*. In addition, there are about 2,463 serotypes of Salmonella of which *S. enterica* contains 2,443 serotypes and the rest *S. Bongori* (20 serotypes) (Bhunias, 2008; Ellermeier and Slauch, 2006). Of all the Salmonella serovars *S. typhimurium* and *S. enteritidis* have been the most commonly reported serovars associated with human illnesses (Suresh *et al.*, 2006).

Nonetheless in recent times, other serovars such as *S. Newport*, *S. Hader*, *S. Heidelberg* and *S. Javiana* are reported to be on the increase and have been implicated in a number of food-borne disease outbreaks (Bisbini *et al.*, 2000; Centers for Disease Control and Prevention, 1997; Coburn *et al.*, 2007). The anticipation and fear is that many serovars are more likely to evolve to become more pathogenic, resistant to multiple drugs and be involved in a number of outbreaks and sporadic cases. Salmonellas are also motile (except for *S. pullorum* and *S. gallinarum* which are specifically pathogenic to poultry) and exhibit peritrichous flagella (Bhunias, 2008; Habib-ur-Rehman *et al.*, 2004). They grow in a temperature range between 5-45°C with 35-37°C being the optimum temperature can grow at a pH of 4.4 or 9.4 and generally sensitive to low salt concentrations (Bhunias, 2008). The pathogen lives primarily in the intestinal tract of birds, insects, mice, farm animals, other animals and sometimes in eggs (Coburn *et al.*, 2007; Ellermeier and Slauch, 2006). Furthermore, poultry, egg, meat, dairy products, mice, fruits and vegetables serve as vehicles of transmission (Bhunias, 2008; Ellermeier and Slauch, 2006). Bhunias (2008) showed that Salmonella cause 3 main forms of diseases that is typhoid fever, gastroenteritis and bacteraemia.

Salmonella infection in humans is acquired through the consumption of undercooked or raw contaminated poultry meat, eggs and other poultry products (Centers for Disease Control and Prevention, 2003; De Jong and

Ekdahl, 2006). The infective dose for one to be infested with Salmonellosis has been reported to range from $1-10^9$ cfu g⁻¹ depending on the type of food consumed, ingested amount, immune status of the host and virulence factor of the bacteria (Bhunias, 2008; Sukhadeo and Trinad, 2009). Infectious dose decreases if consumed with liquid food (e.g., milk), foods that neutralizes gastric acid (e.g., cheese), higher number of cells are ingested, immuned challenged individuals and when the pathogen carries a high virulence genes.

ISOLATION AND DETECTION OF SALMONELLA

Efficient methods for isolating and identifying Salmonellas in poultry housing environments, feeds and other sources are essential for clinical and epidemiological purposes. These methods are basically the conventional culture method and several PCR based techniques (e.g., Pulsed Field Gel Electrophoresis (PFGE), Random Amplification of Polymorphic (RAPD), Entobacterial Repetitive Intergenic Consensus PCR-fingerprinting (ERIC) etc.). The conventional culture method involves pre-enrichment in buffered peptone water followed by selective enrichment in 2 or more enrichment broths (e.g., rappaport-vassiliadis, tetrathionate and lactose and selenite cystine broths depending on the kind of food involved) and streaking unto 2 or more selective agars such as xylose lysine desoxycholate, rambach, hektoen enteric, bismuth sulphite, brilliance™ salmonella or rapid salmonella agars. Presumptive Salmonella isolates are purified on MacConkey or nutrient agars observed by Gram staining technique and then confirmed using appropriate biochemical tests and Salmonella polyvalent somatic (O) and flagellar (H) antisera. A more detailed description of the methods for isolation and identification of Salmonellas can be found by Reid (2009) and Wallace and Hammack (2007). Conventional culture techniques for isolating and detecting *Salmonella* species are labour intensive, time consuming, not specific enough and dangerous (Doosti *et al.*, 2008; Lofstrom *et al.*, 2004; Myint *et al.*, 2006). Therefore, several rapid methods based on the antibodies and DNA of Salmonella has been developed to detect and characterize Salmonellas to the species and strain level. Such methods can be categorised into immunological (e.g., latex agglutination test, ELISA), nucleic acid (e.g., Polymerase Chain Reaction (PCR) based methods) and growth-based methods. For instance Salomonsson *et al.* (2005) developed a PCR technique to analyze viable Salmonella from feed samples and scrapings from feed mills. Maciorowski *et al.* (2004) reviewed on the use of PCR to detect Salmonella in animal

feeds. Lofstrom *et al.* (2004) used a rapid and specific method to detect *Salmonella* species in animal feed samples using PCR after culture enrichment.

Myint *et al.* (2006) determined the sensitivity and specificity of a PCR method to detect *Salmonella* in naturally contaminated poultry feed under varying enrichment protocols and compared it to the conventional culture method. They reported that there was a significant decrease in the sensitivity of the PCR tests when only pre-enrichment (85%) was done compared to when both pre-enrichment and selective enrichment (100%) were used.

A minimum of 12 h pre-enrichment was needed to detect *Salmonella* by PCR at a limit of 100 cfu mL⁻¹ of sample and PCR technique could not detect any positive sample without pre-enrichment. Both conventional and PCR techniques were negative for meat samples that were negative for *Salmonella*. However, feeds either from animal or vegetable source contain certain compounds such as lipids, salt and protein that could inhibit the sensitivity and specificity of PCR techniques (Wilson, 1997).

In addition DNA and cells other than the target organism have also been found to inhibit the performance of PCR analysis (Wegener *et al.*, 2003). Therefore, the use of culture enrichment followed by PCR analysis has been recommended and has been shown to have several advantages over direct detection using only PCR (Sharma and Carlson, 2000).

INCIDENCE OF SALMONELLA IN POULTRY HOUSES AND THEIR ENVIRONMENT

Salmonella transmission and contamination can be aggravated by the situation pertaining in poultry houses. Practises such as overcrowding, unhygienic farming activities, lack of adequate biosecurity measures and movement of birds and equipment from one farm to the other worsens the situation. Mice, wild birds, ants and snakes have been showed by some researchers to be important agents for the transmission of *Salmonella* in among birds, flocks and farms (Angen *et al.*, 1996; Carrique-Mas *et al.*, 2009; Davies *et al.*, 1997).

In addition, farm pertaining samples and their environmental conditions including faeces, soil, crevices, dusts, manure, litter, feeders and/or drinkers will harbour *Salmonellas* and increased the rate of contamination (Mallinson *et al.*, 2000; Wales *et al.*, 2006).

Carrique-Mas *et al.* (2009) sampled 152 laying houses from 42 farms in a 9 year period and recorded an incident rate of 264. Their major findings were a longer persistence of *S. enteritidis* compared to other serovars in farms where rodents were present or absence and houses with

deep pits. They also recorded higher incidences in houses where higher number of rodents were present compared to when lower numbers were present and estimated that the reduction or absence of rodents in laying houses resulted in a the clearance of 42% *S. enteritidis* during laying. The occurrence of the various serovars in the layer houses were *S. enteritidis* (84.9%), *S. agona* (8.6%), *S. typhimurium* (9.9%), *S. agama* (7.9%), *S. mbandaka* (6.6%), *S. livingstone* (5.3%), *S. kedougou* (3.9%) and *S. infantis* (3.9%). Rousi *et al.* (2010) examined 92 laying flocks for *Salmonella* and found that 64.1, 36.5 and 51.1% were positive for the farms houses, faecal and duct samples, respectively.

They also identified 20 different serotypes of which *Salmonella enteritidis* and *Salmonella cerro* were the most dominant ones. Yamane *et al.* (2000) in an attempted to find out the source of *S. enteritidis* in liquid egg samples concluded that the transmission/infection of *S. enteritidis* was due to horizontal infection in the egg-laying farms but not vertical transmission from parental stock, hatcheries, growth or food materials in a 7 years study period.

Davies *et al.* (2003) carried out an investigation in a in a layer breeder hatchery, a layer parent rearing farm, a layer parent farm and in a commercial pullet rearing and cage layer farm where mainly *S. enteritidis*-PT6 and to a lesser extent other serovars had become established and were infesting chicks and other poultry samples. They suspected and linked the source of infection to a contract farm that was set up as a breeder site that supplied samples to the affected farms.

Environmental sampling has been reported to be a good indicator for the presence of *Salmonella* in poultry flocks (Davies and Breslin, 2001). Van de Giessen *et al.* (1994) in their modelling research with *S. enteritidis* also suggested that laying flocks were mainly infected from farm environmental samples including improperly cleaned and disinfected poultry houses and infected vermin present on the farm. The frequency of isolating *Salmonella* in environmental samples (dropping boards, faeces, floor spillage under cages/corridors, feeders, egg belts, dust/cages) ranged widely from 7.9-95.7% per flock with a mean of 49.8% (Wales *et al.*, 2006). Altekruise *et al.* (1993) showed that the level of environmental contamination with *Salmonella*, caecal infection, internal egg contamination and human illnesses were associated. Harris *et al.* (1997) reported that *Salmonellas* are ubiquitous in a farm environment.

The underlying principle is that once poultry farms, houses or the rearing environment is infested with *Salmonella* they are more likely to be transmitted to the birds which may subsequently end up in foods exposing humans to the risk of contracting Salmonellosis.

INCIDENCE OF SALMONELLA IN POULTRY FEEDS

Poultry feeds can be sources of Salmonella and consequently serve as an indirect cause of human infection to people consuming poultry meats and meat products. Feeds are contaminated either from feed mills or on farms during feed formulation, feeding or handling and subsequently spread to poultry mostly through ingestion. Salmonellas have the ability to survive under prolonged periods in dry conditions like feeds and may be recycled in all production stages in commercial feed preparation (Whyte *et al.*, 2003).

This makes the impact of reducing Salmonella contamination in feeds and the risks of human infection cumbersome to assess (Davies *et al.*, 2004). Hinton (1988) reported that the incidence of Salmonella carriage in poultry flocks will be underestimated if only cloacal faeces are sampled without sampling poultry feeds. Several factors most especially ingredients used in preparing poultry feeds have been implicated to be the major source of contamination (Bale *et al.*, 2002; Maciorowski *et al.*, 2004; Okoli *et al.*, 2006).

Persistence of the organism in feed mills and feed preparation environments are other predisposing factors. Henken *et al.* (1992) said poultry farms supplied with contaminated poultry feeds are 5.3 times more likely to produce Salmonella positive flocks compared to farms supplied with feeds free from Salmonella.

Lunestad and Borlaug (2009) also said *S. agona* may be found in animal feed and ingredient and feed production facilities occasionally. The incidence of Salmonella in poultry feeds and ingredients varies widely between 0-78% (Veldman *et al.*, 1995; Ward *et al.*, 1996). Okoli *et al.* (2006) reported on the incidence of Salmonella in grower mash (0.0%), layer mash (20.0%), broiler starter 10.4 (40.0%), broiler finisher (25.0%), guinea feed (10.0%), vital feed (11.1%) and top feed (25.0%). About 11.8 and 33.3% of raw feed ingredients and ducts, respectively collected from pre-heated locations in a feed mill were found positive for Salmonella (Whyte *et al.*, 2003). Harris *et al.* (1997) found that 36 out of 1,264 (2.8%) feed ingredients and feed samples from 14 of 30 (46.7%) farms were positive for Salmonella.

In a survey conducted by Boqvist *et al.* (2003) during a 4 year period, the total number of Salmonella positive samples from the feed sector was reported to be 749 which was similar to the previous 5 year period. They isolated 16 *S. Livingstone* from layers which was suggested to be from the feed mill and spread to layers by feed. They also reported that soybean meal, maize and rapeseed were the most frequently important feed raw material contaminated

by Salmonella. Davies *et al.* (1997) monitored broiler breeder farms, hatchery, rendering plant and animal feed and observed a number of cross contamination hazards including the use of processed poultry proteins in feed mill.

MEASURES TO REDUCE SALMONELLA IN POULTRY HOUSING ENVIRONMENTS AND FEEDS

Despite the fact that Salmonellas live primarily in the intestines of birds from where they can be shared during defaecation and subsequently transmission or cross contaminations, the control of Salmonellas in poultry feeds and their rearing environment is the key to eliminate Salmonella infections in birds and humans. Effective control measures will reduce the hazards and risks involved in carrying Salmonellas from feed mills, feeds and poultry rearing environments to the slaughterhouse and finally in poultry meat and meat products. Control of Salmonella has been effective in most developed countries such as United Kingdom, Sweden, Denmark and many more. A number of strategies as stated below can be adapted. There should be frequent monitoring of poultry farms and feed factories for Salmonella. The monitoring should include routine collection of raw materials, feeds, ingredients, cloacal swabs, faeces, soil and many more from critical control points which should be done in agreement and connection with farmers, feed processors and all stakeholders. Farmers should acquire their chicks from certified Salmonella free hatcheries. Chicks, growers, breeding and rearing flocks should be raised in pens, free from rodents, vermins, wild animals and other Salmonella carrying sources. In egg layer farms efforts should be made to store eggs at temperatures (4-8°C) that retard the growth of Salmonella. Dirty and crack eggs should be discarded appropriately where necessary, infected birds should be culled and treated with appropriate antibiotics. It has been shown that improved biosecurity, hygiene and vaccination of commercial laying and broiler birds resulted in a massive reduction of Salmonella in poultry and humans (Anonymous, 2001; Mumma *et al.*, 2004; Wegener *et al.*, 2003).

Cleaning and disinfection of poultry houses with formaldehyde/glutaraldehyde/quaternary ammonium compound disinfectants, followed by fogging with formaldehyde eliminated all *S. enteritidis*-PT6 (Davies *et al.*, 2003). Ghadyanlou *et al.* (2009) also found that formaldehyde destroyed *S. enteritidis* in feeds in a short time. Yamane *et al.* (2000) observed that the administration of Layermune (a bactererin) to egg laying flocks reduced the incidence of *S. enteritidis* in liquid egg samples from egg laying farms. Anonymous (2001)

vaccinated broilers against Salmonella using Salenvac (TM) with success and also suggested the need to slaughter (mandatory slaughter) infected flocks.

Gantois *et al.* (2006) compared vaccination of laying hens (with TAD Salmonella vac E and TAD Salmonella vac T) to unvaccinated birds and found that less eggs, internal egg contents and oviduct contamination in vaccinated birds compared to the unvaccinated ones. They concluded that vaccination of laying hens with these live vaccines could be considered as a valuable tool in controlling internal egg contamination. Mosquito *et al.* (2010) also used bovine lactoferrin against *S. typhimurium* in their study with mice and found that mice given bovine lactoferrin had lower mortality, less symptomatic, few Salmonella positives in blood culture, less inflammation and focal necrosis in the four organs compared to the control.

They concluded that bovine lactoferrin protected mice against *S. typhimurium* infection in mice, reduced the severity, mortality and degree of inflammation of *S. typhimurium* infection. Mice challenged with *S. dublin* and treated with lactic acid bacteria (*Lactobacillus casei* DSPV 318T, *Lactobacillus salivarius* DSPV 315T and *Pediococcus acidilactici* DSPV 006T) had higher survival rate and did not fall ill compared to untreated mice (Frizzo *et al.*, 2010).

The initiation of molting by feeding layers on wheat bran diet reduces the risk of Salmonella in egg producing settings (Murase *et al.*, 2006). Feeds heated to a minimum of 72°C for 12 min and then pelleted will help to destroy Salmonella if already present in any of the raw materials. Manufactured feeds should be transported in cleaned and well maintained vehicles to farms.

CONCLUSION

This review provides relevant information on the incidences, transmissions and contamination of poultry by their rearing environmental samples and feeds isolation methods for Salmonella and measures to reduce the incidence of Salmonella in poultry environments and feeds. A better understanding of these will assist farmers and relevant stakeholders to reduce Salmonella infestation in poultry and thereby reducing the potential hazards and risks involved in transferring Salmonella to humans and consequently contracting human Salmonellosis, an important food-borne disease of public health concern.

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