Salmonellosis in Broiler Chickens and it’s Public Health Impacts
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The purpose of this review is to discuss the potential sources of Salmonella, and to aid in the prevention of future disease outbreaks.

Background

To investigate contamination of *Salmonella* spp. and *Campylobacter* spp. in two breeds of pastured broiler chickens, the crop and liver were cultured in 40 out of 160 chickens utilized in a study by Delgadillo et al. Ten chickens from each treatment group and each control group were used for culture. *Salmonella Bareilly* (*Salmonella enterica* subsp. *enterica* serovar Bareilly) was cultured from several of the Cornish Cross broilers. *S. Bareilly* was not isolated in any of the Red Ranger chickens that were cultured. All samples were negative for *Campylobacter* spp.

Non-typhoidal Salmonella is the second most important cause of hospitalizations caused by a foodborne illness in the US (Scallan et al., 2011). Salmonellosis is underreported due to numerous cases of subclinical or mild illness. People become infected through ingestion of undercooked poultry and eggs, contaminated produce, spices, and water. Salmonellosis in humans typically runs a short course of under a week, with symptoms including diarrhea, vomiting, fever, and abdominal cramping.

*S. Bareilly* is a common serovar associated with human salmonellosis. Outbreaks with *S. Bareilly* have been reported including in the US, UK, Korea, Czech Republic, and Slovakia. Sources included bean sprouts, raw yellowfin tuna, egg shells, egg belts, chicken feces, and dust in egg processing facilities. (Hoffmann et al., 2016, Cleary et al., 2010, Im at al., 2015, Labská et al.,2017)

Types of Salmonella in Chickens
Chickens are susceptible to several types of Salmonella infections. *S. Pullorum* and *S. Gallinarum* are both host-adapted and cause high mortality in chickens. Clinical illness in chickens infected with *S. Pullorum* is known as pullorum disease. Pullorum disease has a high mortality rate which can reach up to 100%. Transovarian transmission is the primary mode of transmission. Mortality is the highest in chicks up to 4 weeks of age. Adult carriers are asymptomatic. The important clinical signs of pullorum disease are white diarrhea, pasty vent, and anorexia. *S. Gallinarum*, known as fowl typhoid, is very similar to *S. Pullorum*. A notable difference is that chicks older than 4 weeks are clinically affected, although young birds are most frequently affected clinically. Semi mature birds that are affected clinically may have pale, shrunken combs and wattles, in addition to diarrhea. Both of the host adapted serovars are effectively controlled through the National Poultry Improvement Plan.

The non-host adapted salmonellae are a major public health concern. People become infected from improper handling of poultry products and through contamination. There are many serotypes that infect chickens. Some of the most common include *S. Enteritidis*, *S. Typhimurium*, *S. Kentucky* and *S. Heidelberg*. Horizontal transmission is the most important mode of transmission with the exception of *S. Enteritidis*, which can also be transmitted transovarially. Chickens infected with non-host adapted salmonellae are often subclinical. Younger birds are more likely to exhibit clinical signs, which include diarrhea, feather ruffling, anorexia, and lethargy.

**Diagnosis and Prevention of Salmonella in Chickens**

Diagnosing Salmonella serovars is done by first performing aerobic culture for Salmonella on selective media. When a pure colony is cultured, it is isolated into enrichment media (triple sugar iron agar slant) for serum agglutination testing. Several serum agglutination tests are
performed, and are possible due to the O and H antigens, which vary between serovars. Serovar identification by PCR is also an option.

Live and killed salmonella vaccines are available for chickens, and are an important component of poultry management. Vaccines for the host-adapted serotypes protect against infection and disease (Barrow 2007). However, vaccines designed to protect against non-host adapted strains are variably efficacious. While they help to reduce shedding of salmonellae, they are not preventative for paratyphoid infection.

There are several problems associated with Salmonella vaccines in chickens. Although Salmonella vaccines are effective in reducing transmission of Salmonella, they do not eliminate Salmonella from flocks. Chicks are the most susceptible to infection after hatching, but vaccination series are not complete until 2-6 weeks of age, depending on the vaccine used (Jia et al., 2020). Vaccines were developed to mount immune responses towards prevalent serovars including \textit{S. Typhimurium} (serotype B) and \textit{S. Enteritidis} (serotype D). However, certain serotypes such as C1 are not covered with vaccination (Dorea et al., 2010). This is notable because \textit{Salmonella Bareilly} is a C1 serotype. Fuche et al. showed that the C serogroup had the highest frequency of human salmonella infections compared to any other serogroup of nontyphoidal Salmonella in 2012, which was the final year of data collection. This study highlights the importance of developing a vaccine that reduces transmission of the C serogroup.

For optimal protection against Salmonellosis, a combination of live and killed vaccines should be used. Vaccination alone is not sufficient for controlling outbreaks. Monitoring programs should be utilized in hatcheries, breeding stocks, and feed mills. Biosecurity, husbandry protocols, sanitation, and disinfection are all important in controlling outbreaks as well. Insect and rodent populations should be controlled because they are vectors. In addition, no other
species should be permitted on properties housing chickens, and workers ideally should not work around other species off property if possible. Salmonella can be transmitted across species. Low bacterial loads infecting chicks post hatch can lead to long term infection and bacterial shedding (Van Immerseel et al., 2004).

Prevention against *Salmonella spp.* infection at hatcheries involves fumigation of the eggs upon arrival. Formaldehyde is used as the agent to fumigate the eggs. It’s important to note that the efficacy of fumigation with formaldehyde is variable based on bacterial load. Eggs with a high bacterial load ($10^5$ or more bacteria) cannot be entirely decontaminated with formaldehyde, even with prolonged fumigation duration (Cadirci 2009). Eggs with a low bacterial load ($10^4$ and under) can be completely decontaminated if fumigated properly for 30 minutes. The USDA has a document available entitled best management practices with guidelines for hatchery management.

Biosecurity is paramount for control of Salmonella colonization in chickens. Salmonella can spread easily through feces, dust, as well as insect and mammal vectors. Salmonella can penetrate eggs through micro cracks and pores in the shell surface. In addition, *S. Enteritidis* is unique in its ability to colonize the oviduct. Intermittent transovarial transmission occurs upon colonization of the reproductive tract. This makes *S. Enteritidis* particularly problematic, because it may already be present inside eggs, and disinfection of the eggs would not help reduce bacterial load.

Prevention from Salmonella contamination during slaughter is also an important consideration. One important way of reducing broiler meat contamination of Salmonella is to withdraw feed from the broilers prior to slaughter. This can help to reduce fecal output during transport to slaughter facilities, reducing contamination. It can also decrease the volume of intestinal
contents, which is an important consideration during carcass evisceration. However, fasting has been shown to increase Salmonella load within the crop of broiler chickens (Ramirez et al., 1997). The correct amount of time for fasting is important because if fasting is prolonged, the birds become distressed, and post-mortem meat quality is affected (by acidification) (Xue et al., 2021).

Public Health Impacts of Salmonella

Salmonellosis is a widespread public health concern. Non-typhoidal salmonellae are non-species specific, and are a zoonotic concern. They are gram negative, facultative anaerobic, flagellated intracellular bacilli. There are over 2500 identified serotypes, but only a small fraction of these have been associated with human illness. Nontyphoidal salmonellae affect many species, including food production animals such as ruminants (including cows, sheep, and goats), swine and poultry. Equids and camelids may also become carriers and experience clinical illness. Amphibians and reptiles frequently harbor salmonella in the absence of clinical illness. The poultry industry poses an important public health risk for human infections. Meat from broilers can become easily contaminated by crop and intestinal contents during processing. Eggs can also become contaminated externally from feces, and may be contaminated internally as well from translocation through the shell or from infection of the oviduct during egg development. Salmonella survives well in organic matter, and even dust. It can be transmitted by insects and rodents, and it can survive and proliferate in aquatic and terrestrial environments. Salmonella colonizes the epithelial cells within the intestinal tract. Infected individuals may remain asymptomatic or have clinical illness. In either scenario, infected individuals can easily spread salmonellae through feces and fecal contamination. In addition, salmonella can infect macrophages, and has the potential to spread to other organs, primarily the mesenteric lymph nodes, liver and spleen. This is more likely to pose a risk in
individuals with impaired immunity. An estimated 93 million cases of nontyphoidal salmonellosis occurs every year in humans worldwide, with fatalities averaging 155,000 (Gogoi et al. 2019). Approximately 95% of these are cases of gastroenteritis (local infection), and the remaining 5% are caused by disseminated infection.

Case Study:

The study looked at how two breeds of pastured broiler chickens, Red Rangers and Cornish Crosses, were affected by access to hedgerows. 160 chickens total were utilized, and they were split up into four groups. One control group for each breed and one experimental group with access to hedgerow for each breed. The chickens were ordered as day old chicks from Dunlap hatchery in Idaho. These chicks were vaccinated on day one, but were not provided with boosters. Prior to division into treatment and control groups, the chicks were housed within the coops under heat lamps until they were 30 days old. Subsequently, the broilers were randomly divided into treatment and control groups. At this time, they were allowed daily access to pasture, with or without access to hedgerows. The chickens were housed in the coops during the night. The Cornish crosses were given access to food for 12 hours, and the Red Rangers were given 24 hour access to food. The same amount of commercial pelleted feed was provided each day, and weighed each morning and night to calculate intake. The data supported that Red Ranger chickens with access to hedgerows mounted a more robust immune response compared to the control group. This was determined with weekly bacterial killing assays on whole blood. The data also supported that Cornish Cross chickens with hedgerow access had greater rate of weight gain compared to the control group, although both groups consumed the same amount of feed.
At the end of the study, the chickens were culled for analysis of meat quality and for culture of the crop and the liver. Crop and liver aerobic culture was performed in ten chickens from each treatment group. The cultures were performed to isolate Salmonella spp. and Campylobacter spp. *Salmonella Bareilly* (*Salmonella enterica* subsp. *enterica* serovar Bareilly) was cultured from seven out of twenty of the Cornish Cross broilers. Four of the positive cultures were isolated from the treatment group, and the remaining three were isolated from the control group. *S. Bareilly* was not isolated in any of the Red Ranger chickens that were cultured. No other serovars of *Salmonella* were cultured. Campylobacter was not cultured from any of the samples. The results are shown in supplementary table 14 (Delgadillo et al. 2021).

Supplementary table 14: Samples that tested positive for *Salmonella* infection. *Salmonella bareilly* was isolated using standard culture techniques from 7 samples (out of 40 total), all from Cornish Cross samples. None of the Red Rangers tested positive for *Salmonella*. No *Campylobacter* was isolated from any sample.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Source</th>
<th>Isolate</th>
</tr>
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<tbody>
<tr>
<td>Cornish Cross</td>
<td>Hedge</td>
<td>Crop, liver</td>
<td>S. bareilly</td>
</tr>
<tr>
<td>Cornish Cross</td>
<td>Hedge</td>
<td>Liver</td>
<td>S. bareilly</td>
</tr>
<tr>
<td>Cornish Cross</td>
<td>Hedge</td>
<td>Liver</td>
<td>S. bareilly</td>
</tr>
<tr>
<td>Cornish Cross</td>
<td>Pasture</td>
<td>Crop</td>
<td>S. bareilly</td>
</tr>
<tr>
<td>Cornish Cross</td>
<td>Pasture</td>
<td>Liver</td>
<td>S. bareilly</td>
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The cecum would have been a good organ to culture in addition to liver and crop because *Salmonella* spp. readily colonize the cecum (Immerseel et al., 2004.). Gast et al. (2016, 2019) showed liver and spleen cultures are high yield for certain serovars, *S. Enteritidis* and *S. Typhimurium*, but that cecal cultures are higher yield for *S. Heidelberg*. The crop is also useful to culture because it is a common source for contamination with *Salmonella* during processing, as it ruptures easily. Additionally, while pre-slaughter fasting decreases fecal output and carcass contamination during transport for slaughter, pre-slaughter fasting has been shown to increase crop load of *Salmonella* (Ramirez et al., 1997).
The Cornish Cross chickens were most likely infected at the hatchery, with contamination likely originating from the breeding stock. This was likely through egg shell penetration through microcracks on the shells, or from high bacterial load on the shells. The chicks were vaccinated before shipment at the hatchery. However, the chicks were not vaccinated after the initial dose. The current vaccines, as mentioned previously, do not provide adequate cross protection against C1 serotypes including *S. Bareilly*. Cornish Cross chickens create an environment that promotes bacterial growth through increased rates of defecation compared to other breeds. These chickens are bred for rapid growth, and consume large volumes of food and water to support this growth. The prolific production of watery feces from these birds may aid in the transmission and proliferation of *Salmonella* spp..

While ensuring that broilers are fully vaccinated against Salmonella is ideal, completion of the vaccination protocol may not have prevented the proliferation of S. Bareilly because it’s a C1 serotype. Other considerations for decreasing contamination of broilers with Salmonella include breed selection. Cornish Cross broiler are bred for maximizing meat yield, but are also more prone to egg and chick contamination by Salmonella as a result.

References:


