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Experimental Infection of One-Day-Old Chicks with *Salmonella* Serotypes Previously Isolated from Poultry Facilities, Wild Birds, and Swine

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■ Keywords

Bacteriology, disease of poultry, diseases of wild birds.

ABSTRACT

In order to maintain the high production and export rates achieved by the Brazilian poultry industry, it is necessary to prevent and control certain disease agents, such as *Salmonella* spp. Using bacterial cultures, the aim of the present study was to investigate the prevalence of *Salmonella* spp. in specimens collected from broiler facilities. Local wild birds were also sampled, as well as the feces of swine housed on the poultry farm. After sample collection, the isolated serotypes were subsequently inoculated into broiler chicks to determine their effects. Positive samples were collected from the following locations in the poultry facilities: poultry litter (*S.* serotype 4,5,12:R:-; *S.* Heidelberg; *S.* Infantis), broiler feces (*S.* Heidelberg; *S.* serotype 6,7:R:-; *S.* serotype 4,5,12:R:-; *S.* Tennessee), water (*S.* Glostrup; *S.* serotype 6,8:d:-;), and lesser mealworms (*Alphitobius diaperinus*) found in the litter (*S.* Tennessee). Among the 36 wild birds captured, *S.* Heidelberg was isolated from one bird's organs and intestinal contents (*Colaptes campestris*), and *S.* Enteritidis was isolated from another bird's intestinal contents (*Zenaidura macroura*). *Salmonella* Panama and *Salmonella* Typhimurium were isolated from swine feces. One-day-old chicks (150) were divided into 10 groups of 15 animals each. Each group was orally inoculated with a previously isolated serotype of *Salmonella*. Soft stools were observed on the cage floor and around the birds' cloaca between 3 and 12 days post-infection (dpi). The different serotypes of *Salmonella* used to inoculate the chicks were re-isolated from the spleen, liver, and cecal content samples of the infected birds on 15 and 21 dpi.

INTRODUCTION

The incidence of *Salmonella* spp. food poisoning has increased in many parts of the world despite the technological advances in food production and the adoption of improved hygienic and sanitary measures (Gast *et al.*, 2008). Many serotypes of the genus *Salmonella* are able to survive for weeks to months in manure, poultry litter, wild bird feces, equipment, empty sheds, soil, dust particles, feeders, and feedstuffs (Davies & Wray, 1996; Berchieri Junior & Freitas Neto, 2009). The ability of this agent to survive for such a significant time favors its transmission and dissemination.

Several studies in the literature have reported the presence of *Salmonella* spp. in poultry facilities. Bacteria have been found inside poultry housing units in the litter (Bhatia *et al.*, 1979), water (Souza *et al.*, 1992), bird feces (Gama *et al.*, 2003), and lesser mealworms (*Alphitobius diaperinus*) (Skov *et al.*, 2004). Equipment and materials kept outside of the housing units and away from the birds have also been tested positive for *Salmonella* spp. (Mutalib *et al.*, 1992). There are also studies reporting the frequent presence of wild birds in poultry



facilities (Sousa *et al.*, 2010a; Sousa *et al.*, 2010b; Carrasco *et al.*, 2011) and of other farm animals, such as pigs (Michael *et al.*, 2002; Schwarz *et al.*, 2009). Other animals are commonly found on the same farm, including synanthropic animals such as rodents (Hilton *et al.*, 2002).

Several researchers have experimentally inoculated one-day-old chicks with *Salmonella* spp. to investigate the pathogenicity of this agent (Pinheiro *et al.*, 2001; Oliveira *et al.*, 2005; Ribeiro *et al.*, 2005).

The aim of the present study was to investigate the presence of *Salmonella* spp. in specimens collected from poultry facilities, local wild birds, and swine reared on the same farm. After the collected bacteria were cultured, broiler chicks were inoculated with the isolated serotypes and evaluated for disease (Pinheiro *et al.*, 2001; Oliveira *et al.*, 2005; Ribeiro *et al.*, 2005).

MATERIAL AND METHODS

All activities related to the capture, sample collection, and experimental infections of animals were in accordance with the requirements set forth by the Ethics Committee of the University. The experiment was also approved by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – authorization no. 14909-1 – registered at the IBAMA under protocol no. 1902993).

This study was conducted in cooperation with a broiler farm located in the state of São Paulo (Jaboticabal). The samples that were used for the culture of *Salmonella* spp. were collected from poultry facilities, wild birds, and swine feces.

Samples from poultry facilities

The samples collected from broilers included carcasses of one-day-old chicks and adult broilers, feces, swabs from chick crates upon arrival to the farm, feeds from silos and feeders, water from the farm water supply and drinkers, litter, lesser mealworms (*Alphitobius diaperinus*) found in the litter, and rodent feces found in the sheds. These samples were collected from six broiler flocks over the 45-day period that each flock spent on the farm.

Wild birds

Thirty-six wild birds found inside the poultry houses were captured over a one-year period. Samples obtained from these birds included cloacal swabs and liver, spleen, and gonad fragments. Intestinal

contents were also obtained for bacterial examination. Among the captured birds, there were 14 Smooth-billed Ani (*Crotophaga ani*), nine Ruddy Ground-doves (*Columbina talpacoti*), three Eared Doves (*Zenaida auriculata*), three Campo Flickers (*Colaptes campestris*), two Guira Cuckoos (*Guira guira*), two Plumbeous Pigeons (*Patagioenas plumbea*), two Southern Lapwings (*Vanellus chilensis*), and one Great Kiskadee (*Pitangus sulphuratus*).

Swine

In addition to the broiler houses, fecal samples for bacteriological examination were collected from four sheds on the farm that housed pigs of various ages.

Bacteriological analysis

All collected samples were placed in individual sterile vials containing 1% peptone water (OXOID Brazil LTDA, Rua Arizona 1349, Conjunto 01, Brooklin Novo, 04567-003 São Paulo, SP, Brazil). Samples were incubated at 37°C for 24 hours. From the initial culture, 2 mL was transferred to a tube containing 20 mL selenite/novobiocin (SN) broth (OXOID Brazil). Aliquots of 0.2 mL were placed into tubes containing 20 mL of Rappaport (RP) broth (OXOID Brazil), which were incubated at 37°C for 24 hours. Broth samples were then plated on MacConkey agar (MC) (OXOID CM 115, OXOID Brazil) and brilliant green agar (BG) (OXOID CM263, OXOID Brazil) and incubated at 37°C for 24 hours. Colonies suggestive of *Salmonella* were inoculated into TSI agar (triple sugar and iron) (OXOID CM 277, OXOID Brazil) and LIA agar (agar lysine iron) (OXOID CM 381, OXOID Brazil) and incubated at 37°C for 24 hours. *Salmonella*-positive colonies on TSI and LIA plates were selected to undergo agglutination testing using polyvalent anti-somatic antigen serum (O) (PROBAC Brazil, Rua Martinico Prado, 26 Higienópolis, 01224-010 São Paulo, Brazil) and polyvalent anti-flagellar antigen serum (H) for *Salmonella* (PROBAC Brazil). The Adolfo Lutz Institute of São Paulo, SP, Brazil, confirmed the isolated serotypes.

Experimental infection

All 10 previously isolated serotypes of *Salmonella* were made resistant to nalidixic acid at a concentration of 50 µg/mL and incubated under agitation (100 rpm) at 37°C for 24 hours. The cultures contained 1.2×10^8 colony-forming units/mL (CFU/mL). A commercial line of one-day-old chicks obtained from a hatchery located in the state of São Paulo, Brazil, were used for the experimental infection. These chicks were



derived from breeders vaccinated against *Salmonella* Enteritidis. The experiment was conducted in isolation level 2 biosafety rooms with controlled temperature, artificial light, and ventilation. The birds were kept in battery cages and were offered autoclaved water and feed "*ad libitum*". The diet was based on corn, soybeans, and premix, and no antibiotics were added. As the chicks arrived from the hatchery, samples were taken to assess the presence of *Salmonella* spp. in the feed and at the bottom of the crates (meconium).

Birds were divided into 10 groups of 15 birds each. Each bird received 0.1 mL of culture containing 1.2×10^8 CFU/mL of a *Salmonella* serotype through a tube into their crop. Birds were monitored twice daily for morbidity and mortality.

After the *Salmonella* inoculation, fecal samples were collected from the cloaca of each bird using a sterile swab at time intervals of one, eight, and 15 days post-infection (dpi). Half of the group ($n = 7$) was sacrificed on 15 dpi by cervical dislocation, and the remaining birds ($n = 8$) were sacrificed on 21 dpi. Swabs were taken of the liver and spleen, and cecal contents were removed from each dead bird for incubation in SN broth at 37°C for 24 hours. Broth samples were then plated on BG agar plates containing nalidixic acid (50 µg/mL) and incubated at 37°C for 24 hours. The organs were examined for the presence of gross changes.

RESULTS AND DISCUSSION

Samples from poultry facilities

Two of the 25 water samples were positive for *Salmonella* spp. (*S. Glostrup*; *S. enterica* subsp. *enterica* 6,8:d:-). Among the 36 samples of feces collected from the broilers (one sample corresponding to a pool of 10 fecal swabs), four were positive for *Salmonella* spp. (*S. Heidelberg*; *S. enterica* subsp. *enterica* 6,7:R:-; *S. enterica* subsp. *enterica* 4,5,12:R:-; *S. Tennessee*).

Among the 47 litter samples (one sample corresponding to a pool of 10 litter swabs), three were positive for *Salmonella* spp. (*S. enterica* subsp. *enterica* 4,5,12:R:-; *S. Heidelberg*; *S. Infantis*). Regarding lesser mealworms, out of the 25 samples collected (one sample corresponding to a pool of 15 grams of insects), one sample was positive for *Salmonella* spp. (*S. Tennessee*). All 30 samples of rodent feces were negative for *Salmonella* spp., and each sample corresponded to a pool of 20 grams of feces.

Several serotypes of *Salmonella* spp. have been isolated from various poultry farm materials, including

feed, bedding material, and meat meal (Hofer et al., 1998; Andreatti Filho et al., 2001). The intensive rearing system currently used in the poultry industry (high bird density, short downtimes, reuse of litter, and the accumulation of feces) favors the persistence and spread of *Salmonella* among birds and in the environment.

Wild birds

Regarding the presence of *Salmonella* in wild birds, *S. Heidelberg* was isolated from the organs and intestinal contents of a Campo Flicker (*Colaptes campestris*), and *S. Enteritidis* was isolated from the intestinal contents of an Eared Dove (*Zenaida auriculata*). Several other authors have also isolated *Salmonella* from wild birds that visit poultry facilities (Sousa et al., 2010a; Sousa et al., 2010b). The Campo Flicker probably acquired *S. Heidelberg* through direct contact with infected broiler feces and/or contaminated litter, from which this same serotype was isolated. In addition, environmental contamination and/or birds infected with this serotype may be involved in the transmission the disease because birds contaminated with *Salmonella* shed this agent in the feces, contaminating the environment (Davies & Wray, 1996).

When microscopically examining the bodies of birds that were positive for *Salmonella*, moderate diffuse peribronchial anthracosis was observed in Campo Flicker lungs, and anthracosis was found in Eared Dove lungs, in addition to focal inflammatory infiltrate. There was a predominance of mononuclear cells in the infiltrate and a focus of necrosis in the liver. Among the microscopic changes found in the negative birds, bacteria (rods) were found in the duodenum of a Ruddy Ground Dove (*Columbina talpacoti*), and diffuse lipid degeneration in the liver of a Great Kiskadee (*Pitangus sulphuratus*). Among the few studies reported in literature, Joppert (2007) described the isolation of *Salmonella* from two species of owls and associated it with characteristic histopathological findings. In this study, there were no lesions similar to those described by Joppert. Reports of salmonellosis in wild birds are scarce, and few articles describe anatomopathological changes.

Swine

Among the 15 fecal samples obtained from pigs (one sample corresponding to a pool of 10 swabs), four were positive for *Salmonella* spp.: two for *S. Panama* and two for *S. Typhimurium*. The serotype *S.*



Typhimurium has been frequently isolated from swine (Michael *et al.*, 2002; Oliveira *et al.*, 2005). Consistent with this study, Schwarz *et al.* (2009) isolated the serotypes *S. Panama* and *S. Typhimurium* from the mesenteric lymph nodes of swine slaughtered in the state of Rio Grande do Sul, Brazil.

Experimental infection

Clinical signs were observed in infected chicks between 2 and 12 dpi and included soft stools on the floor of the cage and around the bird's cloaca. On 14 dpi, a death occurred in the group infected with *S. enterica* subsp. *enterica* 6,7:R:-. All cloacal swabs collected from the 15 birds in each group on 1 dpi were positive, except for the group infected with *S. Panama*, where two birds were negative for *Salmonella* at the time of sample collection. Except for one bird, the group infected with *S. Tennessee* had not shed the agent by 8 dpi. Some authors have reported that *Salmonella* spp. can be shed in the feces for a long period of time (Pinheiro *et al.*, 2001; Oliveira *et al.*, 2005; Ribeiro *et al.*, 2005).

Half of the birds were sacrificed on 15 dpi, and the remaining birds were sacrificed on 21 dpi. During the autopsy, swabs of the liver, spleen, and cecal contents were aseptically collected for the isolation of *Salmonella* spp. On 15 dpi, *Salmonella* serotypes were present in all 63 (100%) cecal content samples, but they were found in the organs (liver and spleen) of only 34 (25%) birds. On 21 dpi, *Salmonella* serotypes were present in all 72 (100%) cecal content samples, but in the organs of only 16 (11.8%) birds. These results agree with the observations of Ribeiro *et al.* (2005), who investigated *S. Kottbus* in one-day-old chicks.

CONCLUSIONS

The present study confirmed the presence of multiple *Salmonella* spp. serotypes on poultry farms. In addition to broilers tested positive for *Salmonella*, pigs and wild birds (which are often found on poultry farms) were also positive. The experimental infection of one-day-old chicks indicated that inoculation is possible, and that these birds often develop mild to moderate clinical symptoms post-infection. Inoculated birds will often shed viable serotypes of *Salmonella* spp. The diversity of serotypes isolated on one poultry property, together with the shedding of viable *Salmonella* spp. in experimentally-infected chicks, demonstrates the importance of the epidemiology of this pathogen in broilers.

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