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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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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 (*Zenaida auriculata*). *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 x 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 x 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 farns) 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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REFERENCES

Andreatti Filho RL, Fernandes SA, Boretti LP, Barros MR, Del Bem SR, Fontana A, Sampaio HM, Savano EM. *Salmonella* serovars isolated from poultry materials from 1994 to 1999. Revista Educação Continuada 2001;4:90-101.

Berchieri Junior A, Fernandes SA, Irino K, Quintana JL, Santos AJ. Salmonella in Brazilian poultry feed. Revista Microbiologia 1993;24:22-25.

Berchieri Junior A, Freitas Neto OC. Salmonellosis. In: Berchieri Junior A, Silva EN, Di Fábio J, Sesti L, Zuanaze MAF, editors. Bird diseases 2nd ed. Campinas: FACTA; 2009. p. 435-454.

Bhatia TR, Mcnabb GD, Wyman H, Nayar GP. *Salmonella* isolation from litter as an indicator of flock infection and carcass contamination. Avian Diseases 1979;23:838-847.

Carrasco AOT, Issakowicz JC, Morais MTGF, Fatoretto LA, Pandolfi JRC, Silva LC, Pinto AA. Serosurvey for *Mycoplasma* spp., *Salmonella* spp., and Newcastle Disease in Free Living Domestic Pigeons (*Columba livia*). Unopar Científica 2011;13:23-27.

Davies RH, Wray C. Persistence of *Salmonella* Enteritidis in poultry units and poultry food. British Poultry Science 1996;37:589-596.

Gama NMSQ, Berchieri Junior A, Fernandes SA. Occurrence of *Salmonella* sp. in laying hens. Brazilian Journal Poultry Science 2003;5:15-21.

Gast RK, Shivaprasad HL, Barrow PA. *Salmonella* Infections. In: Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, editors. Diseases of Poultry. 12th ed. Athens, Georgia: Blackwell Publishing; 2008. p. 619-674.

Hilton AC, Willis RJ, Hickie SJ. Isolation of *Salmonella* from urban wild brown rats (*Rattus norvegicus*) in the West Midlands, UK. International Journal of Environmental Health Research 2002;12:163-168.

Hofer E, Silva Filho SJ, Reis EMF. *Salmonella* serovars isolated from feedstuff and poultry feed in Brazil. Brazilian Journal Veterinary Research 1998;18:21-27.

Joppert AM. Prospective study of the causes of death of free-living Falconiformes and Strigiformes at São Paulo City [thesis]. São Paulo (SP): University of São Paulo; 2007.

Michael GB, Simoneti R, Cardoso MRI, Costa M. *Salmonella* serotypes isolated from a finishing swine farm in southern Brazil. Ciência Rural 2002;32:525-527.

Mutalib A, Mcdonough P, Shin S, Patten V, Lein D. *Salmonella* Enteritidis in commercial layer farms in New York state, environmental survey results and significance of available monitoring tests. Journal Veterinary Diagnostic Investigation 1992;4:416-418.

Oliveira GH, Berchieri Junior A, Fernandes AC. Experimental infection of laying hens with *Salmonella enterica* serovar *Gallinarum*. Brazilian Journal Microbiology 2005;36:51-56.

Pinheiro LAS, Oliveira GH, Berchieri Junior A. Experimental *Salmonella enterica* serovar Pullorum infection in two commercial varieties of laying hens. Avian Pathology 2001;30:129-133.

Ribeiro SAM, Berchieri Junior A, Orsi MA, Mendonça AO, Ferrati AR. Experimental infection by *Salmonella enterica* subsp *enterica* serovar Kottbus in day-old broiler chickens. Brazilian Journal Poultry Science 2005;7:107-112.

Schwarz P, Calveira J, Sella A, Bessa M, Barcellos DESN, Cardoso M. *Salmonella enterica:* isolation and seroprevalence in swine slaughtered in Rio Grande do Sul. Brazilian Journal Veterinary Animal Science 2009;61:1028-1034.

Skov MN, Spencer AG, Hald B, Petersen L, Nauerby B, Carstensen B, Madsen M. The role of litter beetles as potential reservoir for *Salmonella* enterica and thermophilic *Campylobacter* spp. between broiler flocks. Avian Diseases 2004;48: 9-18.

Sousa E, Berchieri Junior A, Pinto AA, Machado RZ, Carrasco AOT, Marciano JA, Werther K. Prevalence of *Salmonella* spp. antibodies to *Toxoplasma gondii* and Newcastle disease virus in feral pigeons (*Columba livia*) in the city of Jaboticabal, Brazil. Journal Zoo and Wildlife Medicine 2010a;41:603-607.

Sousa E, Werther K, Berchieri Junior A. Assessment of Newcastle virus, infectious bronchitis pathogens, and Salmonella spp. in wild birds captured near poultry facilities. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2010b;62:219-223.

Souza LC, Laria ST, Paim GV. Salmonelas e coliformes fecais em águas de bebidas para animais. Revista Saúde Pública 1992;26:321-327.