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Infecção experimental de manon (*Lonchura striata domestica*) com *Salmonella* Enteritidis*, S.* Typhimurium*. S.* Pullorum *e S.* Gallinarum*

Experimental infection of manon (*Lonchura striata domestica*) with *Salmonella* Enteritidis, S. Typhimurium, S. Pulloum and S. Gallinarum

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Resumo

Este estudo utilizou os manons (*Lonchura striata domestica*) como modelo experimental para passeriformes, os quais foram desafiados com quatro sorovares de *Salmonella*: Enteritidis (SE), Typhimurium (ST), Gallinarum (SG) e Pullorum, para avaliar o impacto desses sorovares comuns na avicultura industrial em passeriformes. A presença de *Salmonella* foi avaliada na superfície corporal (pés), órgãos internos e excreção fecal, juntamente com avaliação de sinais clínicos, suscetibilidade e lesões histopatológicas. Todos os grupos foram suscetíveis aos sorovares testados. Os sinais clínicos estavam ausentes nas aves infectadas com os sorovares paratifóides, enquanto aquelas que receberam os sorovares tifóides manifestaram prostração, aversão à voar e hesitação em procurar água e alimento. Foi observada mortalidade nas aves infectadas com ST, SP e SG (14,3%, 28,6% e 39,3% respectivamente). Em relação à análise dos órgãos, os cecos exibiram isolamento significativo (p=0,05) de *Salmonella* em todos os sorovares e condições experimentais. O reisolamento de *Salmonella* nos manons variou por sorovar, com os cecos e os pés demonstrando o maior reisolamento bacteriano para ST. Os sorovares Gallinarum e Pullorum apresentaram os maiores índices de contaminação em cecos, baço e fígado, variando de 35,7% a 69,4% das aves infectadas (p=0,05), superando os índices observados em patas (10,7% a 23,2%). As lesões histopatológicas foram semelhantes em todos os sorovares. O modelo experimental utilizado neste estudo evidencia o papel dos Passeriformes como contribuintes substanciais para a contaminação ativa por diversos sorovares de *Salmonella*.

Palavras-chave: passariformes, isolamento, tifo aviário.

Abstract

this study employed the manon (*Lonchura striata domestica*) as an experimental model for Passerine birds, which were challenged with four *Salmonella* serovars: Enteritidis (SE), Typhimurium (ST), Gallinarum (SG), and Pullorum, to assess the impact of these prevalent serovars in industrial poultry farming on Passerines. *Salmonella* presence was assessed on the body surface (feet), internal organs and fecal excretion, along with an evaluation of clinical signs, susceptibility, and histopathological lesions. Manons were susceptible to all four tested *Salmonellan* serovars. Clinical signs were absent in birds infected with paratyphoid serovars, while in those that received the typhoid serovars manifested prostration, aversion to flight, and hesitancy in seeking water and food. Mortality was observed only in birds infected with ST, SP, and SG (rates of 14.3%, 28.6% and 39.3% respectively). Regarding organ analysis, the ceca exhibited significant (p=0.05) *Salmonella* isolation across all serovars and experimental conditions. *Salmonella* reisolation in manons varied by serovar, with the ceca and feet demonstrating the highest bacterial reisolation for ST. Serovars Gallinarum and Pullorum presented the highest contamination indices in ceca, spleen and liver, ranging from 35.7% to 69.4% of infected birds (p=0.05), surpassing the observed indices in feet (10.7% to 23.2%). Histopathological lesions were similar across all serovars. The experiment model utilized in this study demonstrated the role of Passerines as substantial contributors to active contamination by diverse *Salmonella* serovars.

Keywords: passerine, isolation, avian typhoid.

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Introduction

within the realm of major zoonotic diseases, infections caused by *Salmonella enterica* pose a persistent and significant challenge to global poultry production. The free-living Passerines substantially contribute to the persistence and environmental dissemination of *Salmonella* in nature and the incidence of infections in domestic avian species (Santos et al., 2020; Wang et al., 2023).

Within the *Salmonella* genus, more than 2650 serovars have been identified (Issenhuth-Jeanjean et al., 2014). Typhoidal serovars, exemplified by *Salmonella enteric* sub sp, enteric sorovar Gallinarum, biovar Gallinarum (SG) and biovar Pullorum (SP), are host-specific pathogens responsible for avian typhoid and pullorum disease in birds. Paratyphoidal serovars, such as *Salmonella enteric* sorovar Enteritidis (SE) and *Salmonella enteric* serovar Typhimurium (ST), stand out as zoonotic agents responsible for enteritis, severe invasive diseases, and global human fatalities (Marchello et al., 2022). Animal-derived foods, particularly contaminated poultry meat and eggs, represent major transmission sources (Abebe et al., 2020). Additional sources, like Passerines, have also been linked to recent salmonellosis outbreaks and hospitalizations (CDC, 2021). In Brazil, government authorities have implemented official policies for the prevention, monitoring, and specific control of these four serovars (Brasil, 2003ab; Brasil, 2016). Nonetheless, the persistence of these pathogens in poultry establishments requires ongoing epidemiological (Baptista et al., 2023).

The prevalence of *Salmonella* spp. in wild birds is relatively variable (Beleza et al., 2020; Santos et al., 2020; Belo et al., 2023) and dependent on the sampling method. Although less explored, analyses of the body surface are of great value, as some species of wild birds may not show infection symptoms but may instead harbor the pathogen in their organisms and carry *Salmonella* spp. across different environments, thus becoming important reservoirs (López-Martín et al., 2011).

In this context, we present an epidemiological and pathological study conducted to assess how the experimental model of a Passerine specie (Manon – *Lonchura striata domestica*) responds to infection by four circulating *Salmonella enteric* serovars prevalent in industrial poultry farming.

Materials and methods

the experimental protocol was approved by the Ethics Committee on Animal Use (Number 239/2012 CEUA) at the School of Veterinary Medicine and Animal Science, São Paulo State University (FMVZ – UNESP), Botucatu, São Paulo, Brazil.

A total of 224 unsexed manon Passerines (*Lonchura striata domestica*) aged up to six months were obtained from commercial aviaries of bird breeders. The birds underwent acclimatization and rigorous quantitative and molecular bacteriological testing, following the protocols outlined by Gonçalves et al. (2011). The birds had *ad libitum* access to water and feed, with the latter comprising a *Salmonella* spp-free blend of millet seeds (*Panicum miliaceum).*

The birds were housed in an isolated room (infectious disease room, of the Ornithopathology Service at FMVZ – UNESP), equipped with three windows protected by internal and external wire meshes and artificial lighting with white bulbs. The lights were kept on from 8 A.M to 3 P.M. The Manons were kept in suspended metal cages, each equipped with an appropriate number of perches, in accordance with the recommendations of Hawkins et al. (2001). For the provision of food and drinking water, sterile containers were randomly positioned on the floor of each cage. The cage floor was covered with craft paper sterilized through UV radiation, and the lining was daily replaced, following the swab collection.

The *S.* Enteritidis*, S.* Typhimurium*, S.* Pullorum *and S.* Gallinarum strains used in this research belong to the bacterial collection of the Ornithopathology Service at FMVZ – UNESP and were isolated through sanitary monitoring of avian-origin materials. The four *Salmonella* strains underwent a process of induced resistance to nalidixic acid (Nal) and rifampicin (Rif) as described by Andreatti Filho et al. (1997).

A culture of each strain was grown in Brain Heart Infusion (BHI) broth containing 100µg/mL of both antibiotics above mentioned in a stove at 37°C for 24 hours. The inocula quantification of each strain was carried out through serial dilutions in Phosphate-Buffered saline (PBS) and subsequently plated in duplicate on BGA also containing 100µg/mL of Nal and Rif. After the incubation process, the plates were read and colonies were counted, and each strain reached the concentration of 10^7CFU/mL. All manons were manually restrained and received the inoculum administration through gavage needle, at dosis of 0.1mL of its respective *Salmonella*.

In the current study, four experiments were independently conducted, each lasting 28 days. The birds were randomly assigned to four experimental groups. The experiment started with 56 manons, initially analyzing eight birds per sampling. The sample size for each experimental group was determined based on an estimated *Salmonella* prevalence of 50%, α=5% and a standard deviation of 10% (Jenicek e Cléroux, 1982).

At intervals of six hours and 3, 5, 7, 14, 21 and 28 days post-challenge, eight birds from each experimental group were euthanized in accordance with the CFMV (2012) guideline.

Clinical signs were observed 30 minutes before each sampling session in all four experiments. Daily mortality records were maintained throughout the experiment. Necropsy and material processing were also performed in birds that died outside scheduled sampling periods.

Aseptic necropsies were performed, and liver, spleen, and cecal fragments were collected to evaluate colonization by different *Salmonella* serovars, as well as for histopathological assessment. Liver, spleen, and cecal fragments fixed in formalin were dissected, dehydrated in ethanol, treated with xylene, and embedded in plastic paraffin. Sections of 5µm were prepared using an automatic microtome (Leica, RM-2145), mounted on glass slides, and stained with hematoxylin-eosin (HE) according to Behmer et al. (1976). The slides were examined by two blinded-pathologists (double blind conditions). The following histopathological aspects were considered: necrosis, vascular congestion, and kind of the inflammatory infiltrate.

Fecal samples were also collected throughout the first, 7, 14, 21, and 28 days post-inoculation, totaling 10 analyses in each period. These samples were collected using a swab, rubbed at five distinct points on the craft paper placed at the bottom of each cage. Immediately after collection, the swabs were immersed in

5mL of buffered peptone saline solution (PBS, 1%) and stored in a refrigerator at 5°C until incubation. Foot washing was obtained by immersing both bird feet in a test tube containing 5mL of PBS (1%) for five seconds, with the samples being preserved in a refrigerator at 5°C until incubation.

For bacterial isolation, organ fragments were individually stored in sterile microtubes and maintained in a refrigerator at 5°C until processing. Bacterial isolation procedures varied based on the sample type. Swab and foot wash samples, preserved in PBS (1%) were homogenized and incubated at 37°C for 20 hours. Following pre-enrichment, samples underwent selective enrichment in Tetrationate (TT) and Rappaport-Vassiliadis (RVS) broths and dilutions of 1:10 and 1:100, respectively, followed by incubation at 37°C for 24 hours. Subsequently, the samples were cultured on Petri dishes containing Xylose Lysine Deoxycholate (XLD) and Brilliant Green Agar (BGA) with Nal/Rif reaching concentrations of 100µg/mL, and incubated for 24 hours at 37°C. For *Salmonella* spp. isolation from organs, tissue fragments were macerated and directly poured into TT and RVS broths, following the same proportions and conditions described above. Colonies displaying typical *Salmonella* morphology on agar plates were considered positive, confirmed through serological agglutination (SAR) and polymerase chain reaction (PCR). In addition to Nal/Rif supplementation, all positive bacterial colonies underwent SAR procedure with O4, O5 (B), O9 (D1), and polyvalent flagellar antigens. To validate bacterial isolation results, 1mL of TT broth was collected after incubation and immediately frozen at -20°C for subsequent PCR processing.

Bacterial DNA extraction employed a heat treatment method based on the techniques described by Fadl et al. (1995) and by Andreatti Filho et al. (2011). *Samonella* spp. identification was achieved through the standard PCR technique, utilizing the invA primer [521bp (**F:** 5´-TTGTTACGGCTATTTTGACCA-3´; **R:** 5´-CTGACTGCTACCTTGCTGATG-3´)] (Swamy et al., 1996). The PCR reactions and amplification conditions followed the procedures outlined by Gonçalves et al. (2011).

The collected data underwent statistical analysis using the test of the difference between two proportions with normal approximation (one-tailed test), with significance established at p <0.05 (Vieira, 2010).

Results

in the experimental model employed, the animals were susceptible to all tested *Salmonella* serovars. The bacterial prevalence rate varied according to the serovar. The SE serovar had a prevalence rate of 39.3%, while ST had 62.5%, SP had 71.4% and SG had the highest prevalence at 80% (Table 1). There was a significant difference (p<0.05) in the prevalence of SE compared to the other serovars. All recovered samples corresponded to the inoculated serovar, confirmed by SAR and PCR. No clinical signs were observed in Passerines inoculated with the paratyphoids serovars (SE and ST). Three days post inoculation (DPI), all birds infected with SG and SP exhibited ruffled feathers, apathy, lethargy, grouping behavior at the bottom of the cage, anorexia and decreased water consumption. There was no mortality in birds challenged with SE, while those inoculated with ST, SP and SG presented mortality rates of 14.3%, 28.6% and 39.3%, respectively (Table 1), with a significant difference (p<0.05) observed between ST and the other serovars (SG and SP).

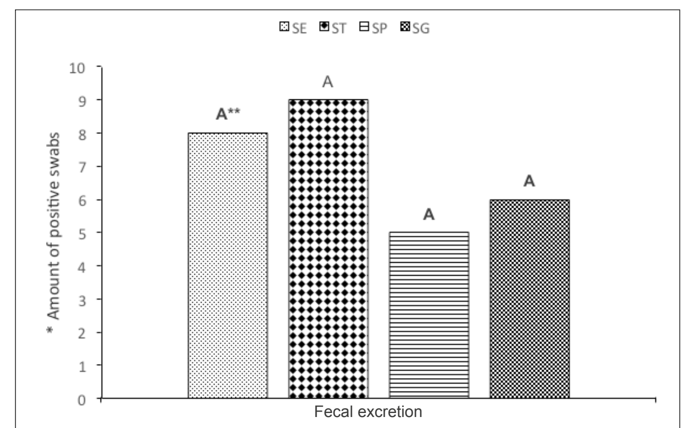
Table 1: Prevalence of *Salmonella* infection and mortality in manon (*Lonchura striata domestica*) following experimental challenge with *Salmonella* Enteritidis (SE), *Salmonella* Typhimurium (ST), *Salmonella* Pullorum (SP), and *Salmonella Gallinarum* (SG).

Serovar	Positive birds	Negative birds	Mortality rate
SE	39.3* (22/56)**Cb	60.7 (34/56)***Aa	0 (0/56)****C
ST	62.5 (35/56) Ba	37.5 (21/56) Bb	14.3 (8/56) B
SP	71.4 (40/56) ABa	28.6 (16/56) BCb	28.6 (16/56) A
SG	80.4 (45/56) Aa	19.6 (11/56) Cb	39.3 (22/56) A

*Results presented as percentages (%).
**Number of positive birds/ total number of inoculated birds.
***Number of negative birds/ total number of inoculated birds.
****Number of dead birds/ total number of inoculated birds.
Different uppercase/lowercase letters in the same column/line, respectively indicates statistical difference (p=0.05).

The results of microbiological monitoring using swabs obtained after the *Salmonella* challenge are presented in Figure 1. All experimental groups shed their respective pathogen through feces from the first DPI. It was possible to recover SE from feces up to 14 DPI, while birds infected with ST continued to shed the serovar until 21 DPI. Throughout the experiment, there was intermittent shedding of SP, with birds still shedding the serovar in feces at 14 DPI. Birds infected with SG excreted the serovar until 14 DPI, with no significant difference (p>0.05) in fecal excretion observed among the four serovars.

Figure 1: Fecal shedding of *Salmonella* in Passerines (*Lonchura striata domestica*) experimentally infected with different serovars of *Salmonella* (Enteritidis – SE, Typhimurium – ST, Pullorum – SP and Gallinarum – SG) up to 28 days post infection.



*Mean of the quantity of positive swabs in the four collections conducted (seven, 14, 21 and 28 days post *Salmonella* infection).
**Different uppercase letters indicates statistical difference (p=0.05).

Histopathological findings were similar among the four serovars since the first data collection, with the liver presenting congestion of blood vessels, hepatocyte necrosis, micro-and/or microvacuolar degeneration, and the presence of predominantly lymphocytic mononuclear inflammatory infiltrate, occasionally with heterophils. Splenic congestion and necrosis, mononuclear inflammatory infiltrate, and the presence of nodules (lymphoid germinal centers) in the ceca were also observed. The various lesions are presented in Table 2.

Table 2: Cumulative percentage of the main histopathological changes found in the liver, spleen and ceca of Passerines (*Lonchura striata domestica*) experimentally inoculated with different serovars of *Salmonella* (Enteritidis – SE, Typhimurium – ST, Pullorum – SP and Gallinarum – SG) up to 28 days post infection.

Histopathological lesions	SE	ST	SP	SG
Liver				
Congestion of blood vessesls	98*	100	100	94
Mononuclear inflammatory infiltrate	67	96	97	26
Micro and/or macrovacuolar degeneration	29	52	65	28
Multifocal necrosis of hepatocytes	0	27	35	38
Spleen				
Splenic congestion	57	13	30	51
Necrosis	0	20	6	36
Ceca				
Mononuclear inflamatory infiltrate	90	95	85	92
Lymphoid germinal nodules	0	14	9	42

*Results of the occurence of histopathological changes presented as a percentagem (%).

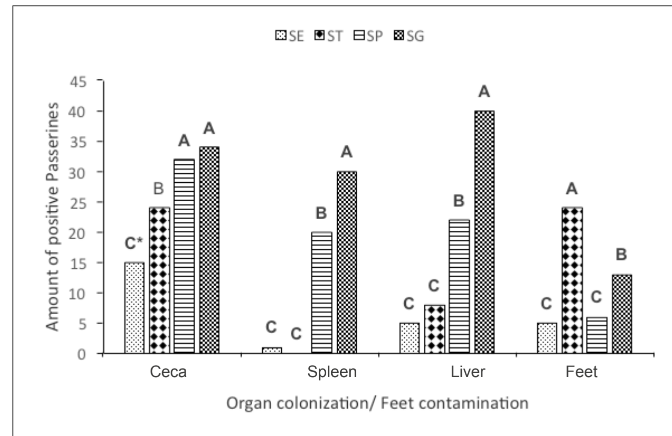
Results from the isolation of all four *Salmonella* serovars corresponded to the characteristic of each one that had been inoculated, being confirmed through SAR and PCR assays. Bacterial isolation differed according to the organ and body surface (Table 3 and Figure 2). There was a significant difference (p<0.05) in organ infection and body surface contamination, with the ceca presenting the highest concentration of SE. There was no significant difference in SE infection in the spleen, liver and foot contamination. ST infection was significantly different (p=0.05) among organs, with the ceca and feet being the sites with the highest bacterial infection, and no ST isolation in the spleen. Exposure to SP resulted in a higher infection rate in the ceca compared to the spleen and liver (p<0.05). The feet had the lowest rates of bacterial recovery compared to other organs. Unlike the other serovars, birds inoculated with SG showed a higher rate (p<0.05) of systemic infection by the serovar, except in the ceca, where same infection potential as SP was observed. When comparing infection between organs, there was no significant difference in the infection of the ceca, liver and spleen, but there was a difference between organs and feet, with feet being the site of the lowest SG reisolation.

Table 3: Colonization of organs (ceca, spleen and liver) and body surface (feet) by *Salmonella* in Passerines (*Lonchura striata domestica*), following experimental challenge with different serovars of *Salmonella* (Enteritidis – SE, Typhimurium – ST, Pullorum – SP and Gallinarum – SG) up to 28 days post infection.

Serovar	Isolation site in the birds			
	Ceca	Spleen	Liver	Feet
SE	26.8*(15/56)** Ca	1.8 (1/56) Cb	8.9 (5/56) Cb	8.9 (5/56) Cb
ST	42.9 (24/56) Ba	0 (0/56) Cb	14.3 (8/56) Cb	42.9 (24/56) Aa
SP	57.1 (32/56) Aa	35.7 (20/56) Bb	39.3 (22/56) Bb	10.7 (6/56) Cc
SG	60.7 (34/56) Aa	53.6 (30/56) Aa	69.4 (39/56) Aa	23.2 (13/56) Bb

*Results presented as percentages (%).
**Number of positive birds/ total number of inoculated birds.
Different uppercase/lowercase letters in the same column/line, respectively indicates statistical difference (p=0.05).

Figure 2: Distribution of *Salmonella* colonization in the ceca, spleen, liver and contamination of the feet of Passerines (*Lonchura striata domestica*), following experimental challenge with different serovars of *Salmonella* (Enteritidis – SE, Typhimurium – ST, Pullorum – SP and Gallinarum – SG).



*Different uppercase letters indicates statistical difference (p=0.05).

Discussion

from an epidemiological perspective, this research showed high prevalence rates for different *Salmonella* serovars in Passerines, confirmed by bacterial isolation and identification. Additionally, the recorded mortality rates, especially in birds challenged with typhoid serovars, indicate that manons are sensitive to infection, specially when challenged by SP, SG and ST, given that the mortality rate for these serovars was significantly higher than in birds infected with SE. It is speculated that the virulence and pathogenicity of typhoid serovars in Passerines are higher than paratyphoid serovars, as expected, given that the literature indicates that SG and SP cause severe clinical symptoms and high mortality rates, exceeding 80% in other avian species (Paiva et al., 2009; Rocha-e-Silva et al., 2013; Pinto et al., 2023). In Passerines, multiple outbreaks and epidemics involving ST are frequent, although other paratyphoid serovars like Agona, Bareilly, Dublin, Cerro, Hessarek, Montevideo, Paratyphi, Schleissheim, Saint Paul, Weltevrede and Enteritidis have also been reported **(Brunthaler et al., 2021;** CDC, 2021). The Pullorum and Gallinarum serovars are described as specific pathogens of birds and are reported with frequent occurrence in poultry (Parvej et al., 2016; Zhou et al., 2022). In free-ranging birds, both serovars have been isolated from animals living near poultry farms **(Sousa et al., 2010**).

It is described that some birds can have only the body surface (feet) contaminated without intestinal colonization, and this can be the only source of bacterial isolation (Benskin et al., 2009; Lopéz-Martín et al., 2011). It was observed that fecal excretion also promoted foot contamination, and these birds could act as

sources of infection to contaminate new environments, such as the inside of poultry houses or even other birds. In birds infected with the Enteritidis serovar, the main site of bacterial colonization was the ceca, significantly different from what was observed in the spleen, liver, and feet. This result partially differs from that observed in seagulls, where most isolates were found in feces, feet, and also in the intestine (Lopéz-Martín et al., 2011).

Among the four *Salmonella* serovars analyzed, the ceca were the sites of highest colonization. Gallinarum serovar was the only one that did not differ between organs (ceca, spleen, and liver); however, this same serovar showed a significant potential for colonization and survival when compared to the other serovars. Moreover, the interaction between host, pathogen and the virulence mechanisms of SG concerning birds are likely different from those observed in the other *Salmonella* serovars analyzed.

Histopathological analysis showed that oral inoculation with *Salmonella* serovars was able to determine replication in tissues and cause bacteremia, reaching the liver, spleen and ceca. Pathological changes found in Passerines mostly refer to the Typhimurium serovar. The pathological changes found in the liver are consistent with those observed in sparrows (*Passer domesticus*) and canaries (*Serinus canaria*) infected with ST (Pennycott et al., 2002; Connolly et al., 2006). The vacuolar degeneration and necrosis lesions found in the liver were similar to those described in chickens infected with SG (Pennycott et al., 2002; Connolly et al., 2006). Also, in chickens, Haider et al. (2012), when evaluating lesions accumulated after the challenge with SP, observed that 80% of challenged animals presented focal necrosis and the presence of inflammatory cells in the spleen. The percentages of lesions we observed in this organ were distinct for all inoculated serovar; however, the manons liver presented an inflammatory infiltrate and necrosis in proportions similar to those observed by Freitas Neto et al. (2007) and Haider et al. (2012). Together, these data indicate that the host-pathogen interaction triggered by infection caused changes in the experimental model used in this research.

In our study, infection with *Salmonella* serovars, especially in birds infected with typhoid serovars, manifested as acute disease, consistent with clinical signs and mortality, typical findings in birds with salmonellosis (Andreatti Filho, 2007; Shivaprasad e Barrow, 2008; Beyaz et al., 2010; Rocha-e-Silva et al., 2013). We emphasize the lack of detailed information on the duration and intensity of bacterial excretion in the environment by *Salmonella*-infected wild birds. All serovars efficiently colonized the birds, promoting environmental contamination through feces up to 28 DPI, with SP excretion being intermittent up to day 15. It is recognized that factors such as the environment, type of challenge, stress and immunity may influence *Salmonella* excretion. Typhimurium serovar excretion persisted for a longer period, considering the maximum fecal excretion period described in the literature (22 days) and considering the end of the experiment (Connolly et al., 2006). It is likely that after this period, excretion may occur intermittently, as suggested by Pennycott et al. (2002). Considering that the experimental model can efficiently disseminate SE, ST, SP and SG, even without clinical infection, as in the cases of SE and ST, alternative sanitary management strategies would be beneficial to prevent *Salmonella* transmission in poultry flocks.

Conclusion

certainly, the experimental model used in this study demonstrates that Passerines are significant active sources of infections by different *Salmonella* serovars. Our study provides information that can support preventive measures and minimize the risks of future epizootic outbreaks of *Salmonella* in commercial poultry farms.

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Referências bibliográficas

ABEBE, E.; GUGSA, G.; AHMED, M. Review on Major Food-Borne Zoonotic Bacterial Pathogens. J. Trop. Med., 4674235. 2020.

ANDREATTI FILHO, R.L.; SILVA E.N.; CURI, P.R. Ácidos orgânicos e microbiota cecal anaeróbia no controle da infecção experimental de frangos por *Salmonella* Typhimurium e *Salmonella* Enteritidis. Arq. Bras. Med. Vet. Zootec.,49: 661-72. 1997.

ANDREATTI FILHO, R.L.; Paratifo aviário. In: Andreatti Filho, R.L. Saúde Aviária e Doenças. São Paulo-SP. Editora Roca Ltda, p.96-111. 2007.

ANDREATTI FILHO, R.L.; GONÇALVES, G.A.M.; OKAMOTO, A.S.; LIMA, E.T. Comparação de métodos para extração de DNA na reação em cadeia da polimerase para detecção de *Salmonellaenterica*sorovarEnteritidis em produtos avícolas. Ciênc. Anim. Bras., 12: 115-119. 2011.

BAPTISTA, D.Q.; BORSOI, A.; REISCHAK, D.; NASCIMENTO, A.C.O.; MONTESINO, L.O.; CAMILLO, S.C.A.; ABREU.D.L.C.; PEREIRA, V.L.A. *Salmonella* Serovars Isolated from Poultry Breeding Flocks under the Brazilian Official Control Programme Between 2016 and 2018. Braz. J. Poul. Sci., 25: 1-12. 2023.

BEHMER, A.O.; TOLOSA, E.M.C.; FREITAS NETO, A.G. Manual de técnicas para histologia normal e patológica. São Paulo: Edusp, 1976, 239p.

BELEZA, A.J.F.; MACIEL, W.C.; LOPES, E.S.; ALBUQUERQUE, A.H.; CARREIRA, A.S.; NOGUEIRA, C.H.G.; BANDEIRA, J.M.; VASCONCELOS, R.H.; TEIXEIRA, R.S.C. Evidence of the role offree-living birds as disseminators of *Salmonella* spp. Arq. Inst. Biol., 87:1-14. 2020.

BELO, N.O.; DE ANGELI DUTRA, D.; RODELLO, D.; CRISPIM, N.P.C.C.; SILVA, A.S.G.; COELHO, H.L.G.; MARTINS, N.R.S.; BRAGA, E.M. Detection of *Salmonella* spp. in wild and domestic birds in an anthropized ecotone between the Cerrado and the Amazon Forest in Brazil. Braz. J. Microbiol.,54: 565-569. 2023.

BENSKIN, C.M.W.H.; WILSON, K.; JONES, K.; HARTLEY, I.R. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. Biol. Rev., 84: 349-373. 2009.

BEYAZ, L.; ATASEVER, A.; AYDIN, F.K.; GUMUSSOU, S.; ABAY, S. Pathological and clinical findings and tissue distribution of

Salmonella gallinarum infection in turkey poults. Turk. J. Vet. Anim. Sci., 34: 101-110. 2010.

BRASIL. Ministério da Agricultura, Pecuária e do Abastecimento. Instrução normativa nº 78, de 03 de novembro de 2003. Normas técnicas para controle e certificação de núcleos e estabelecimentos avícolas como livres de *Salmonella* gallinarum e de *Salmonella* pullorum e Livres ou Controlados para *Salmonella* enteritidis e para *Salmonella* typhimurium. Diário Oficial da União, Seção I; 2003a.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento - MAPA. Secretaria de Defesa Agropecuária. Instrução Normativa nº 62, de 26 de agosto de 2003. Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Diário Oficial da União, n.181, seção 1, p.14-51. 2003b.

BRASIL. Ministério da Agricultura, Pecuária e do Abastecimento. Instrução normativa nº 20, de 21 de outubro de 2016. Estabelece o controle e o monitoramento de Salmonella spp. nos estabelecimentos avícolas comerciais de frangos e perus de corte e nos estabelecimentos de abate de frangos, galinhas, perus de corte e reprodução, registrados no Serviço de Inspeção Federal (SIF). Diário Oficial da União, Seção I; 2016.

BRUNTHALER, R.; SPERGSER, J.; WEISSENBOCK, H. Multiple epidermics in Austrian fringillidae caused by aingle variant of *Salmonella* Typhimurium. J. Wild. Dis., 57: 891-899. 2021.

CENTERS FOR DISEASE CONTROL AND PREVENTION. 2021. *Salmonella* outbreak linked to wild songbirds. https://www.cdc.gov/salmonella/typhimurium-04-21/index.html

CONNOLLY, J.H.; ALLEY, M.R.; DUTTON, G.J.; ROGERS, L.E. Infectivity and persistence of an outbreak strain of *Salmone*lla enterica serotype Typhimurium DT160 for house sparrows (*Passer domesticus)* in New Zealand. N. Z. Vet. J., 54: 329-332. 2006.

CONSELHO FEDERAL DE MEDICINA VETERINÁRIA - CFMV. Resolução nº 1000, de 11 de maio de 2012. Procedimentos e métodos de eutanásia em animais. Diário Oficial da União, n.95, seção1, p.124-125. 2012.

FADL, A.A.; NGUYEN, A.V.; KHAN, M.I. Analysis of *Salmonella* enteritidis isolates by arbitrarily primed PCR. J. Clin. Microbiol., 33: 987-989. 1995.

FREITAS NETO, O.C.; ARROYAVE, W., ALESSI, A.C.; FAGLIARI, J.J.; BERCHIERI JÚNIOR, A. Infection of commercial laying hens with *Salmonella* gallinarum: clinical, anatomopathological and haematological studies. Braz. J. Poult. Sci., 9: 133-141. 2007.

GONÇALVES, G.A.M.; LIMA, E.T.; DONATO, T.C.; ROCHA, T.S.; ÁLVAREZ, L.E.C.; SEQUEIRA, J.L.; ANDREATTI FILHO, R.L. Eradication of *Salmonella* Typhimurium in broiler chicks by combined use of P22 bacteriophage and probiotic. Microbiol. Res. 2011; 3: e2. 2011.

HAIDER, M.G.; CHOWDHURY, E.H.; AHMED, A.K.M.; HOSSAIN, M.M. Experimental pathogenesis of Pullorum disease in chicks by local isolate of *Salmonella* pullorum in Bangladesh. J. Bangladesh Agric. Univ., 10: 87-94. 2012.

HAWKINS, P.; MORTON, D.B.; CAMERON, D.; CUTHILL, I.; FRANCIS, R.; FREIRE, R.; GOSLER, A.; HEALY, S.D.; HUDSON, A.; INGLIS, I.R. Laboratory birds: refinements in husbandry and procedures. Lab. Anim., 35: 1-163. 2001.

JENICEK, M.; CLÉROUX, R. Épidémiologie. Principes, Techniques, Applications. Paris: Maloine. 1982.

LÓPEZ-MARTÍN, J.; JUNOD, T.; RIQUELME, F.; CONTRERAS, C.; GONZÁLES-ACUNA, D. Detección de especies de *Salmonella* y *Mycobacterium*en gaviotas dominicanas (*Larusdominicanus*) y gaviotas de Franklin (*Leucophaeuspipixcan*) en la ciudad de Talcahuano, Chile. Rev. Med. Chile., 139: 1496-1502. 2011.

MARCHELLO, C.S.; BIRKHOLD, M.; CRUMP, J.A.; MARTIN, L.B.; ANSAH, M.O.; BREGHI, G.; CANALS, R.; FIORINO, F.; GORDON, M.A.; KIM, J.H.; HAMALUBA, M.; HANUMUNTHADU, B.; JACOBS, J.; KARIURI, S.; MALVOLTI, S.; MANTEL, C.; MARKS, F.; MEDAGLINI, D.; MOGASALE, V.; MSEFULA, C.L.; MUTHUMBI, E.; NYRENDA, T.S.; ONSARE, R.; OWUSU-DABO, E.; PETTINI, E.; RAMASAMY, M.N.; SOURA, B.A.; SPADAFINA, T.; TACK, B. Complications and mortality of non-typhoidal salmonella invasive disease: a global systematic review and meta-analysis. Lancet Infect Dis., 22: 692-705. 2022.

PAIVA, J.B.; PENHA FILHO, R.A.C.; ARGÜELLO, Y.M.S.; SILVA, M.D.; GARDIN, Y.; RESENDE, F.; BERCHIERI JÚNIOR, A.; SESTI. L. Efficacy of several *Salmonella* vaccination programs against experimental challenge with *Salmonella*Gallinarum in commercial brown layer and broiler breeder hens. Braz. J. Poult. Sci., 11: 65-72, 2009.

PARVEJ, M.S.; NAZIR, K.N.H.; RAHMAN, M.B.; JAHAN, M.; KHAN, M.F.R.; RAHMAN, M. Prevalence and characterization of multi-drug resistant *Salmonella* enterica serovar Gallinarum biovar Pullorum and Gallinarum from chicken. Vet. World. 9: 65-70. 2016.

PENNYCOTT, T.W.; CINDEREY, R.N.; PARK, A.; MATHER, H.A.; FOSTER, G. *Salmonella enterica* subspecies *enterica* serotype Typhimurium and *Escherichia coli* 086 in wild birds at two garden sites in south-west Scotland. Vet. Rec., 151: 563-567. 2002.

PINTO, P.N.; TORRES, A.C.; RODRIGUES, M.P.; OLIVEIRA, L.B.; COSTA, C.S.; ECCO, R.; FREITAS NETO. O.C.; MARTINS, N.R.An outbreak of fatal Pullorum disease (*Salmonella* pullorum) in Guinea fowl keets (*Numida meleagris*). Pesq. Vet. Bras., 43: e07088. 2023.

ROCHA-E-SILVA, R.C.; CARDOSO, W.M.; TEIXEIRA, R.S.C.; ALBUQUERQUE, A.H.; HORN, R.V.; CAVALCANTI, C.M.; LOPES E.S.; GOMES FILHO, V.J.R. *Salmonella* gallinarum virulence in experimentally-infected japanese quails (*Coturnix japonica*). Braz. J. Poult. Sci., 15: 39-46. 2013.

SANTOS, E.J.E.; AZEVEDO, R.P.; LOPES, A.T.S.; ROCHA, J.M.; ALBUQUERQUE, G.R.; WENCESLAU, A.A.; MIRANDA, F.R.; RODRIGUES, D.D.P.; MACIEL, B.M. *Salmonella* spp. in Wild Free-Living Birds from Atlantic Forest Fragments in Southern Bahia, Brazil. BioMed. Res. Int., 7594136. 2020.

SOUSA, E.; WERTHER, K.; BERCHIERI JÚNIOR, A. Assessment of Newcastle and infectious bronchitis pathogens, and *Salmonella* spp. in wild birds captured near poultry facilities. Arq. Bras. Med. Vet. Zootec., 62: 219-223, 2010.

SHIVAPRASAD, H.L.; BARROW, P. *Salmonella* infections. Pullorum disease and fowl typhoid. In: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E., editors. Diseases of Poultry, 12th edition. Blackwell Publishing, Athens, U.S.A., pp.302-345. 2008.

SWAMY, S.C.; BARNHART, H.M.; LEE, M.D.; DREESEN, D.W. Virulence determinants invA and spvC in *Salmonellae* isolated from poultry products, wastewater, and human sources. Appl. Environ. Microbiol., 62: 3768-3771. 1996.

VIEIRA, S. Bioestatística, Tópicos avançados. 3ed. Rio de Janeiro: Elsevier. 2010.

ZHOU, X.; KANG, X.; ZHOU, K.; YUE, M. A global dataset for prevalence of *Salmonella* gallinarum between 1945 and 2021. Sci. Data., 9: 495. 2022.

WANG, J.; VADDU, S.; BHUMANAPALLI, S.; MISHRA, A.; APPLEGATE, T.; SINGH, M.; THIPPAREDDI. H. A systematic review and meta-analysis of the sources of *Salmonella* in poultry production (pre-harvest) and their relative contributions to the microbial risk of poultry meat. Poult.Sci., 102: 102566. 2023.