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Full Length Research Paper

Occurrence of *Salmonella* sp. and *Escherichia coli* in free-living and captive wild birds from 2010-2013 in Guarapuava, Paraná, Brazil

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Birds are increasingly close to men, and many times are raised as pets. However, many times, these animals may carry and spread enterobacteria that are deleterious to human health. *Salmonella* sp. is considered one of the most common zoonoses in the world, causing important losses to public health. Although, *Escherichia coli* is an important commensal in the gastrointestinal tract of most animals, it may cause disease both in men and animals, depending on the strain and its pathogenicity. Therefore, the objective of this study was to assess the occurrence of *Salmonella* sp. and *Escherichia coli* in free-living and captive wild birds in the city of Guarapuava, PR, Brazil. Animals were divided according to the taxonomic order, as follows: Columbiformes (228), Psittaciformes (128), Passeriformes (63), Piciformes (26), Falconiformes (19), Stringiformes (6), and Accipitriformes (01), in a total of 471 birds. Bacterial isolation was carried out by means of cloacal swabs, with 69.38% birds positive for *E. coli* and 22.32% for *Salmonella* sp. From the total of birds, 143 showed co-infection with *Salmonella* sp. and *E. coli.* Columbiformes showed the greatest occurrence of *E. coli* (82.33%). Falconiformes showed the greatest number of negative birds (57.9%). These results demonstrate that birds that were analyzed may carry and spread these enterobacteria, and preventive measures for human exposure should be determined, as these microorganisms are public health concerns.

Key words: *Escherichia coli*, *Salmonella* sp., zoonosis.

INTRODUCTION

There are 1901 birds species in Brazil, according to the latest data of the CBRO - Brazilian Ornithological Records Committee (Bencke et al., 2010). According to the Abinpet (Brazilian Association of Pet Products Industry), there were 19.1 million possible bird pets, such as cockatiels, parrots, canaries and macaws, in 2012.

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Due to the close contact between these animals and men, the occurrence of zoonoses may be favored.

According to Sick (2001), pigeons are synanthropic birds that are found in large urban areas in Brazil. These birds make their nests in cliffs, and this is the probable reason for their adaptation to urban life, as there are high buildings. Besides, other factors, such as feeding, abundance of shelter and absence of predators enable their disorderly development and reproduction. Therefore, given the close contact between these animals and men, zoonoses may occur in high rates. The same interpretation is valid, in Brazil, for Passeriformes.

Similar to all other vertebrates, birds are susceptible to and may transmit to humans enteropathogenic organisms of zoonotic potential, but there are few comprehensive studies on the issue with wild and domestic birds (Vasconcelos, 2013). Disease studies on the human population worldwide carried out by Jones et al. (2008) showed that emerging infectious diseases are, in most cases, zoonoses (60.3%). From this total, 71.3% are transmitted by wild animals, and 54.3% of these diseases are caused by bacteria and rickettsia, reflecting an increasing number of microorganisms that are resistant to the pharmaceutical drugs available in the market. According to the European Centre for Disease Prevention and Control (2010), salmonellosis and campylobacteriosis are the two most common zoonoses, and were responsible for, respectively, 99,020 and 212,064 cases of human disease in the European Union in 2010 (ECDC, 2012).

Bacteria in the genus *Salmonella* sp., Enterobacteriaceae family, are Gram-negative, facultatively anaerobic, non-sporulating rods (Carvalho, 2006). Nowadays, the genus is divided into two species, *Salmonella enterica* and *Salmonella bongori.* The species *S. enterica*, which is the pathogenic *Salmonella* species, is divided into six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae,* and *indica*), each with several serovars or serotypes. More than 2,500 serotypes are known. Most of them (about 1,500) belong to the subspecies *enterica* and are associated with clinical pictures, both in humans and in animals. This classification in serotypes is based on cell surface structures, such as antigens, flagella lipopolysaccharides, and proteins (Herrera and Jabib, 2015).

Most of these bacterial infections are caused by water or food contaminated with feces of infected animals. Similarly, contamination may also be caused by direct contact between susceptible and infected animals (Kuroki et al., 2013). Therefore, birds have an important role in the dissemination of these diseases, although many of them do not show any clinical signs. Birds may also be potential carriers of many other bacteria, besides virus and parasites (Dovc et al., 2004). According to Berchieri et al. (2001) the length of fecal shedding and the level of tissue invasion (pathogenicity) depends on the age of the bird at the moment of infection. Therefore, a bird may infect other animals or human for long periods.

Studies carried out by Sousa et al. (2010) in Jaboticabal, with 126 free-living pigeons (*Columba livia*) in urban environments, showed the isolation of *Salmonella* sp. in 10 animals (7.94%).On the other hand, Silva et al. (2014) analyzed the presence of enterobacteria in domestic birds (*Cairina moschata*) from households in cities of Ceará and did not observe the presence of *Salmonella* sp.

Escherichia coli is considered to be a commensal of the gastrointestinal tract of warm-blooded animals. However, it may also be a pathogenic agent, as it adapts to diverse conditions, which is mainly related to the loss or gain of bacterial genes. Several different *E. coli* strains cause intestinal and extra-intestinal diseases by means of virulence factors that affect a wide variety of cell processes (Kaper et al., 2004). According to Croxen and Finlay (2010), there was an impressive worldwide increase in the number of cases of these diseases, with hundreds of millions of people affected annually. Similar to humans, *E. coli* may also cause diseases in the animals. Colibacillosis is one of the main causes of mortality in birds, and is responsible for significant economic losses all over the world (Schouler et al., 2012). In studies carried out by Trampel et al. (2007) in United States, *E. coli* was isolated from 14 of 15 clinically healthy birds, confirming that these animals carry the bacterium and do not show clinical signs, which may aid disease spreading.

In Brazil, there are few studies on the presence of this bacterium in wild birds. Mattes et al. (2005) evaluated the influence of biosafety measures in the intestinal colonization of Psittacidae by *E. coli*, in the state of São Paulo. Animals studied belonged to different breeding facilities, a conservation facility and a recreational facility. Results show significant differences in the intestinal colonization by *E. coli* in different environments, with more positive birds in the recreational breeding facility. These findings demonstrate the importance of biosafety measures for these animals. Diagnosis of these enterobacteria is based on the isolation of the pathogen in cultures of feces, blood, and urine. Another method used in the detection of enterobacteria is the polymerase chain reaction, PCR (Herrera and Jabib, 2015).

In Brazil, there are screening and research centers that provide veterinary attention to animals that were captured by official organs. These animals were either apprehended or voluntarily handed in to official organs. Many of these animals are highly debilitated and need intensive care to be able to be reintroduced into nature, or even to remain in captivity. Assessment of the sanitary status of the animal, mainly in relation of the presence of pathogenic organisms of zoonotic potential, is an important action that should be carried out together with healthcare measures. This assessment should be done when animals arrive at the wildlife center where they will be housed, making it possible for prophylactic and/or

Table 1. Distribution of the samples according to the bird order and species.

Order	Most common species	Number of animals (%)
Columbiformes	(*Columba livia*, *Zenaida auriculata*)	228 (48.40)
Psittaciformes	(*Ara ararauna*, *Amazona aestiva*)	128 (27.18)
Passeriformes	(*Saltator similis*, *Turdus rufiventris*)	63 (13.37)
Piciformes	(*Rhamphastos dicolorus, R. toco*)	26 (5.53)
Falconiformes	(*Caracara plancus*)	19 (4.03)
Strigiformes	(*Athene cunicularia, Tyto furcata*)	6 (1.28)
Accipitriformes	*(Rupornis magnirostris)*	1 (0.21)
Total		471 (100%)

Table 2. Results (total and percentage) of the *E. coli* and *Salmonella sp.* isolation in the different bird orders.

Order	Positive for *E. coli* (%)	Positive for *Salmonella* sp. (%)	Negative (%)	Total
Columbiformes	191 (82.33)	31 (13.36)	10 (4.31)	232
Psittaciformes	105 (73.94)	14 (9.86)	23 (16.2)	142
Passeriformes	94 (52.22)	84 (46.67)	2 (1.11)	180
Piciformes	20 (71.42)	4 (14.29)	4 (14.29)	28
Falconiformes	8 (42.1)	-	11 (57.9)	19
Strigiformes	7 (58.34)	4 (33.33)	1 (8.33)	12
Accipitriforme	1(100)	-	-	1
Total	426 (69.38%)	137 (22.32)	51 (8.30)	614*****

*From 471 animals analyzed, 143 were positive for both agents.

curative measures to be adopted, if the animal is infected. Given the lack of information on the occurrence of enterobacteria in wild birds, the objective of this study was to evaluate the presence of birds positive for *Salmonella sp.* and *E. coli* among animals seen in wildlife centers.

MATERIALS AND METHODS

Cloacal sterile swabs were collected from 471 wild, clinically healthy birds, as presented in Table 1. No bird showed clinical signs compatible with the presence of *Salmonella* and *E. coli.* The study was carried out between 2010-2013, and bacterial isolation for *Salmonella sp.* and *E. coli* was performed in order to assess the presence of these enterobacteria in wildlife centers located in the cities of Guarapuava and Tijucas do Sul, both in the state of Paraná. Soon after collection, samples were sent to the Laboratory of Infectious and Parasitic Diseases at Universidade Estadual do Centro Oeste – UNICENTRO, Guarapuava, Paraná. For isolation of *Salmonella* and *E. coli* was performed according to Freitas Neto et al. (2009) with modifications. After that, swabs were placed in sterile test tubes containing 6 mL of Selenite broth (Selenite Broth Base, OXOID®) pre-enrichment medium added of Novobiocin 0.4% (1/100 mL, v/v). Tubes were placed in incubators at 37°C for 24 h. After the enrichment period, the content of the tubes was plated onto Brilliant Green (Brilliant Green Agar, KASVI®) and MacConkey agar (Mac Conkey Agar, KASVI®) and incubated again at 37°C, for 24 h. Then, plates were analyzed for colony growth and changes in agar color produced by presence or absence of bacterial fermentation, compared with the positive control was *Salmonella* sp. Colonies suggestive of *Salmonella* were isolated in slants containing (LIA) Lysine Iron Agar (LIA, BIOLOG®) and Triple Sugar Iron agar (TSI, BIOLOG®), which were kept in the incubator at 37°C for 24 h. After this period, new readings were carried out to evaluate positive samples for *Salmonella* sp. and *E. coli.*

RESULTS AND DISCUSSION

The results of the study are shown in Table 2, according to the bird orders. In the present study, a total of 471 samples of seven bird orders were analyzed. From the total of samples, 69.38% were positive for *E. coli*, 22.32% positive for *Salmonella* sp, and 8.30% samples were negative. From the total of birds analyzed, 143 (34.29%) were positive both for *Salmonella* sp. and for *E. coli*. When the bird order is taken into account, Columbiformes showed the greatest occurrence of *E. coli*, with a frequency of 82.33% of the birds. *Salmonella* sp. was more commonly isolated among Passeriformes, in a total of 46.67% of the birds. Falconiformes showed the greatest number of negative birds, with 57.9% of them.

When Passeriformes were analyzed, there was a large number of birds positive for *E. coli* (52.22%). On the other hand, a study carried out by Vasconcelos (2013) in Ceará with Atlantic canaries (*Serinus canaria*), which belong to the Passeriformes order, showed *E. coli* prevalence equal to 3.62%, with 11 samples of cloacal swabs positive in 487 samples. In the same study, when bacterial isolation was carried out with samples collected in the *post-mortem* examination, 18.97% of them were positive in a total of 19 birds analyzed. Braconaro (2012) evaluated 253 wild Passeriformes in São Paulo and found 10.7% birds positive for *E. coli*. Brittingham et al. (1988) in the analysis of the prevalence of bacteria in Passeriformes and Piciformes birds in the USA found 1% of 387 samples positive for *E. coli*. In the present study, the occurrence of *E. coli* in Psittaciformes showed a result similar to the one observed by Mattes et al. (2005), with 73.94% positive results. These authors analyzed the presence of *E. coli* in 85 samples of Psittaciformes in two breeding facilities in the state of São Paulo. In the first facility, 20% of the birds were positive for *E. coli*, whereas in the second one, 80% of the birds were positive, making this population show a result very similar to the findings of the present study. When Jones and Nisbet (1980) analyzed 271 birds in the London zoo, they found *E. coli* in 180 samples of healthy birds, with 66.42% positive results. Different orders were analyzed in this study, and animals that belonged to the orders Piciformes and Falconiformes were positive in 100% of the cases. In our study, positive results in these two orders were 71.42 and 42.1%, respectively.

Jones and Nisbet (1980) analyzed 26 Columbiformes and showed 81.25% animals positive for *E. coli*. These results are very close to the ones found in the present study, equal to 82.33% in 228 samples.

As for Stringiformes, seven in 12 birds were positive for *E. coli* in the present study, a total of 58.34% positive results. The bacterium was also isolated from one Accipitriforme, which was positive only for *E. coli*.

According to Croxen et al. (2013), *E. coli* may be classified into several serotypes, according to the antigens it presents. There are 173 O antigens, 80 K antigens, and 56 H antigens, yielding countless O:K:H serotypes. However, the number of pathogenic serotypes is limited, with wider occurrence of non-pathogenic strains. There are two main groups of these serotypes: those that cause diarrhea, and those that cause extra-intestinal disease (Orskov and Orskov, 1992). Therefore, even in a bird population with high number of animals positive for *E. coli*, there may be no sick animals, as many serotypes are commensals. However, the possible pathogenicity of these strains to humans cannot be ruled out.

In our evaluation for the presence of *Salmonella* sp. in Passeriformes, we detected a total of 84 (46.67%) positive samples. Brittingham et al. (1988), analyzing the prevalence of enterobacteria in Passeriformes and Piciformes in the USA, did not find the presence of *Salmonella* sp. in these birds. Almeida et al. (2015), in a study with 52 samples of Passeriformes and Psittaciformes in the city of Umuarama in Paraná, did not find birds positive for *Salmonella* sp. Different from the results found in the present study, these authors showed 73.94% Psittaciformes and 52.22% Passeriformes positive for *Salmonella* sp. It should be emphasized that, in the present study, a much larger population of birds was analyzed, with 128 Psittaciformes and 63 Passeriformes.

Padilla et al. (2004) studied some bacteria and parasites of free-living pigeons in the Galapagos and did not find any sample positive for *Salmonella*, different from the samples of the present study, which showed 13.36% *Salmonella* sp. in Columbiformes.

Albuquerque et al. (2013) analyzed the experimental infection caused by *Salmonella* enteritidis in chickens and pigeons and observed that birds shed the bacterium in the feces up to 14 days after the experimental infection, demonstrating that contamination of other birds and animals may take place and cause economic losses, besides posing an important public health risk.

Birds that were analyzed in this study did not show clinical signs of the diseases, similar to the description by Vaz et al. (2015). Many times, affected birds do no show clinical signs, but shed the bacteria in eggs or feces. Bird feces contaminate the environment and, in the case of *Salmonella* sp, may remain there for a long period, depending on the environmental conditions. That is why cleaning procedures, all-in all-out, and rodent control are essential, as these animals may also spread the bacterium.

Most *Salmonella* sp. serotypes are pathogenic to humans, and clinical signs of the disease vary according to the serotype. The serotypes *S.* Agona, *S*. Hadar and *S*. Typhimurium were considered the most important causes of foodborne diseases in humans. However, nowadays, *S*. Enteritidis is considered the predominant causing agent of this kind of disease, in several countries. There is great concern, today, about the emergence of serotypes in the genus *Salmonella* that are multiresistant to available antibiotics (Shinohara et al., 2008).

In Brazil, contact between humans and birds is frequented, mainly with Columbiformes, Passeriformes, and Psittaciformes. This fact is easily proven by the number of samples collected from birds of these orders (Table 1). Greater contact with men is due to the fact that these birds may be kept as pets (Passeriformes and Psittaciformes) or their abundant presence in public places, such as parks (Columbiformes and Passeriformes). Therefore, sanitary monitoring measures for these birds are essential, as birds in these orders were those that showed the greatest absolute number of positive samples. After the sanitary monitoring, prophylactic measures may be adopted in order to reduce the dissemination of these pathogens to the human population.

Conclusion

The enterobacteria *Salmonella* sp. and *E. coli* were isolated from feces samples of free-living and captive wild birds, demonstrating that these animals may carry and spread these pathogens. Given their close contact with men, they may transmit the diseases to humans and other animals. Therefore, the typification of the bacteria found in this study is of great importance for the level of pathogenicity of these agents to be assessed.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Albuquerque AH, Cardoso WM, Teixeira RSC, Lopes ES, Sales RJPF, Horn RV, Rocha-E-Silva RC, Bezerra WGA, Gomes-Filho VJR. (2013). Dissemination of *Salmonella* enteritidis by experimentally-infected pigeons. Vet. Bras. Cien. Avic. 15(3):211-215.

Almeida PM, Otutumi LK, Gerônimo E, Messa V, Suenaga SS, Amaral PFGP, Lima ET, Vendrame A, Gonçalves DD, Cestari IED (2015). Study of the presence of *Salmonella* spp. and gastrointestinal parasites in excreta from ornamental birds from breeders in the city of Umuarana, Paraná. Afr. J. Microbiol. Res. 9(4):253-257.

Bencke GA, Dias RA, Bugoni L, Agne CE, Fontana CS, Maurício GN, Machado DB (2010). Revisão e atualização da lista das aves do Rio Grande do Sul, Brasil. Iheringia Sér. Zool. 100 (4): 519-556.

Berchieri A, Wigley P, Page K, Murphy CK, Barrow PA (2001). Further studies on vertical transmission and persistence of *Salmonella enterica* serovar Enteritidis phage type 4 in chickens. Avian Pathol. 30(1):297-310.

Braconaro P (2012). Caracterização das microbiotas bacteriana e fúngica presentes em cloacas de passeriformes silvestres confiscados do tráfico que serão submetidos a programas de soltura. Master Degree Universidade de São Paulo (SP), Brazil. p. 72.

Brittingham MC, Temple AS, Duncan RMA (1988). Survey of the Prevalence of Selected Bacteria in Wild Birds. J. Wildl. Dis. 24 (2): 299-307.

Carvalho VM (2006). Colibacilose e Salmonelose. In: Cubas ZS, Silva, JCR, Catão-Dias JL. Tratado de Animais Selvagens - Medicina Veterinária. São Paulo. Roca. pp. 742-750.

Croxen MA, Finlay BB (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. Nat. Rev. Microbiol. 8 (1): 26-38.

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013). Recent Advances in Understanding Enteric Pathogenic *Escherichia coli*. Clin. Microbiol. Rev. 26(4):822-880.

Dovc A, Zorman-Rojs O, Rataj AV, Bole-Hribovšek V, Krapež U, Dobeic M (2004). Health status of free-living pigeons (Columba Livia Domestica) in the city of Ljubljana. Acta Vet. Hung. 52(2):219-226.

ECDC, European Centre for Diease Prevention and Control. Scientific Report of EFSA and ECDC (2012). EFSA J. 10(3):2597.

Freitas Neto, OC, Lages SLS, Carrasco AOT, Berchieri A (2009). Search for Salmonella spp. in ostrich productive chain of Brazilian southeast region. Trop. Anim. Health Prod. 41 (1):1607-1614.

Herrera BY, Jabib RL (2015). Salmonelosis, zoonosis de las aves y una patogenia muy particular. Rev. Cient. Elet. Med. Vet. 16(1):1-19.

Jones DM, Nisbet DJ (1980). The gram negative bacterial flora of the avian gut. Avian Pathol. 9(1):33-38.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008). Global trends in emerging infectious diseases. Nature 451(1): 990-993.

Kaper JB, Nataro JP, Mobley HLT (2004). Pathogenic *Escherichia coli.* Nat. Rev. Microbiol. 2(1):123-140.

Kuroki T, Ishihara T, Furukawa I, Okatani AT, Kato Y (2013). Prevalence of Salmonella in Wild Snakes in Japan. Jpn. J. Infect. Dis. 66(1):295-298.

Mattes BR, Consiglio SAS, Almeida BZ, Guido MC, Orsi RB, Silva RM, Costa A, Ferreira AJP Knöbl T (2005). Influência da Biossegurança na Colonização Intestinal por *Escherichia Coli* em Psitacídeo*.* Arq. Inst. Biol. 72(1):13-16.

Orskov F, Orskov I (1992). Escherichia coli serotyping and disease in man and animals. Can. J. Microbiol. 38(7):699-704.

Padilla LR, Alarcon DS, Merkel J, Miller E, Parker PG (2004). Survey for *Haemoproteus* spp., *Trichomonas Gallinae*, *Chlamydophila Psittaci*, and *Salmonella* spp. in Galapagos Islands Columbiformes. J. Zoo Wildl. Med. 35(1): 60-64.

Schouler C, Schaeffer B, Brée A, Mora A, Dahbi G, Biet F, Oswald E, Mainil J, Blanco J, Moulin-Schouleur M (2012). Diagnostic strategy for identifying avian pathogenic Escherichia coli based on four patterns of virulence genes. J. Clin. Microbiol. 50(5): 1673-1678.

Shinohara NKS, Barros VB, Jimenez SMC, Machado ECL, Dutra RAF, Lima Filho JL (2008). *Salmonella* spp., importante agente patogênico veiculado em alimentos. Ciênc. Saúde Colet. 13 (5): 1669-1674.

Sick H (2001). Ornitologia Brasileira. 4 ed. Rio de Janeiro. Nova Fronteira. p. 912.

Silva EE, Lopes ES, Teixeira RSC, Albuquerque AH, Silva RCR, Gomes VJR, Vasconcelos RH, Maciel WC (2014). Pesquisa de enterobactérias em patos domésticos (*Cairina moschata*) de propriedades localizadas em quatro municípios do Ceará, Brasil. Arq. Inst. Biol. 81(1):16-21.

Sousa E, Berchieri A, Pinto AA, Machado RZ, Carrasco AOT, Marciano JA, Werther K (2010). Prevalence of *Salmonella* spp. Antibodies to *Toxoplasma gondii*, and Newcastle Disease Virus in Feral Pigeons (*Columba livia*) in the City of Jaboticabal, Brazil. J. Zoo Wildl. Med. 41(4): 603-607.

Trampel DW, Wannemuehler Y, Nolan LK (2007). Characterization of Escherichia coli isolates from peritonitis lesions in commercial laying hens. Avian Dis. 51(4):840-844.

Vasconcelos RH (2013). Isolamento, Caracterização Bioquímica e Perfil de Resistência a Antimicrobianos de Enterobactérias Isoladas de Canários Belga *(Serinus Canaria*). Master Degree Universidade Estadual do Ceará (CE), Brazil. p. 54.

Vaz AN, Armando AP, Ribeiro AR, Zancan FT, Brisola ML (2015). Pesquisa de *Salmonella* em Mutuns (*Mitu Mitu*) Mantidos em Cativeiro. Cienc. Anim. Braz. 16(1):68-72.