

## Pathologic and microbiologic aspects of pet psittacine infected by *Escherichia coli* and *Salmonella Typhimurium*<sup>1</sup>

Raul A.S. Siqueira<sup>2</sup>, William C. Maciel<sup>2</sup>, Ruben H. Vasconcelos<sup>2</sup>, Windleyanne G.A. Bezerra<sup>2</sup>, Elisângela S. Lopes<sup>2</sup>, Débora N. Machado<sup>2</sup>, Marcel F. de Lucena<sup>2</sup> and Ricardo B. de Lucena<sup>3</sup>

**ABSTRACT.-** Siqueira R.A.S., Maciel W.C., Vasconcelos R.H., Bezerra W.G.A., Lopes E.S., Machado D.N., Lucena M.F. & Lucena R.B. 2017. **Pathologic and microbiologic aspects of pet psittacine infected by *Escherichia coli* and *Salmonella Typhimurium*.** *Pesquisa Veterinária Brasileira* 37(4):379-384. Laboratório de Estudos Ornitológicos, Universidade Estadual do Ceará, Itaperi, Fortaleza, CE 60740-000, Brazil. Email: [raul\\_spfc15@hotmail.com](mailto:raul_spfc15@hotmail.com)

The role of *Escherichia coli* in healthy microbiota of psittacine is controversial, and the presence of *Salmonella* sp. indicates possible disease. Therefore, this study aimed to identify the presence of *E. coli* and *Salmonella* spp. in a psittacine pet that died in Fortaleza, Brazil, correlating pathogenicity aspects of the isolates through the evaluation of lesions and antimicrobial susceptibility. Psittacine pets sent to the Laboratory of Ornithological Studies, State University of Ceará, that died in 2014 and 2015 were necropsied. Fragments of liver, kidneys, intestine, lung, heart, spleen and brain were collected for microbiological and histopathological analyses. Scores were attributed to lesions and isolated strains submitted to antimicrobial susceptibility test. From the seventy necropsied birds, nineteen were positive for *E. coli* and one for *Salmonella Typhimurium*. Congestive lesions and lymphoplasmocytic inflammatory infiltrate were observed varying from light to moderate and were the main findings. In the analyzed strains, multidrug resistance against different groups of antibiotics was observed. In conclusion, according to the results, *E. coli* strains and the *Salmonella Typhimurium* isolate produced significant lesions in the psittacine pets, and multidrug resistance may hinder treatments with antibiotics used in avian pet medicine.

INDEX TERMS: *Escherichia coli*, *Salmonella Typhimurium*, colibacillosis, salmonellosis, Psittacidae, anatomopathological features, antimicrobial susceptibility test.

**RESUMO.- [Aspectos patológicos e microbiológicos de Psittaciformes de companhia infectados por *Escherichia coli* e *Salmonella Typhimurium*.]** A participação de *Escherichia coli* na microbiota saudável de Psittaciformes e a de *Salmonella* spp. já indica possível doença. O objetivo deste estudo foi pesquisar a presença de *E. coli* e *Salmonella* spp. em psittaciformes de companhia na cidade de Fortaleza/Ceará, traçando os aspectos de patogenicidade destas cepas através das lesões e da sensibilidade antimicrobiana. Foram necropsiados os psittaciformes de companhia encaminha-

dos ao Laboratório de Estudos Ornitológicos da Universidade Estadual do Ceará durante o período de 2014 a 2015. No momento da necropsia foram coletados fragmentos de fígado, rins, intestino, pulmão, coração, baço e encéfalo para posterior processamento microbiológico e histopatológico. As lesões foram graduadas e as cepas isoladas submetidas a antibiograma. Das setenta aves necropsiadas, dezenove foram positivas para *E. coli* e apenas uma para *Salmonella Typhimurium*. As lesões de congestão e infiltrado inflamatório linfoplasmocitário variaram de leve a moderado, e foram as principais lesões encontradas. Nas cepas analisadas foi constatada multiresistência a diferentes grupos de antibióticos testados. De acordo com os achados, pode-se concluir que os isolados de *E. coli* e *Salmonella Typhimurium* produziram lesões significativas em psittaciformes em Fortaleza, Brasil, e a multiresistência pode dificultar o tratamento com antibióticos usados na clínica de aves de companhia.

<sup>1</sup> Received on November 6, 2015.

Accepted for publication on July 13, 2016.

<sup>2</sup> Laboratório de Estudos Ornitológicos, Universidade Estadual do Ceará (UECE), Av. Silas Munguba 1700, Fortaleza, CE 60740-000, Brazil. \*Corresponding author: [raul\\_spfc15@hotmail.com](mailto:raul_spfc15@hotmail.com)

<sup>3</sup> Laboratório de Patologia Animal, Universidade Federal do Rio de Janeiro, Areia, PB 58397-000, Brazil.

TERMOS DE INDEXAÇÃO: *Escherichia coli*, *Salmonella* Typhimurium, colibacilose, salmonelose, Psittacidae, características anatopatológicas

## INTRODUCTION

*Escherichia coli* is an anaerobic Gram negative bacillary bacterium, which is a potential pathogen of birds, named *Avian Pathogenic E. coli* (APEC) when are responsible for avian colibacillosis. This disease may develop in different forms, such as: colisepticemia, coligranuloma, cellulitis, aerossaculitis, sinusitis, pericarditis, peritonitis, salpingitis, hepatitis, panofalmitis and osteomyelitis; however respiratory disease followed by septicemia and death is the most frequent process (Janben et al. 2001). The genus *Salmonella* has two species, *S. bongori* and *S. enterica*, which presents six subspecies and 2759 serotypes which cause three distinct diseases: pullorum disease, caused by *Salmonella enterica* serotype Pullorum; Fowl typhoid, caused by *Salmonella enterica* serotype Gallinarum; and paratyphoid infection, caused by any of the remaining serotypes (Seo et al. 2000). Both *Salmonella* sp. and *E. coli* are not regular members of the intestinal microbiota of psittacine and, therefore isolating these bacteria from asymptomatic or immunosuppressed individuals indicates a possibility of disease (Marietto-Gonçalves & Almeida 2010, Marietto-Gonçalves et al. 2010).

Antimicrobial resistance is the mechanism by which bacteria may overcome the activity of antibiotics, which according to WHO (*World Health Organization*) mainly occurs due to the indiscriminate use of these drugs important for human and veterinary medicine (Tortora et al. 2012). There is scarce information about antimicrobial resistance and diseases in pet birds, however there are reports involving free-living birds as potential disseminators of *E. coli* and *Salmonella* sp. resistant to cefalosporins, ampicillin, streptomycin, sulfoxazole and tetracycline isolated from passerines and columbids (Andrés et al. 2013, Poirel et al. 2012, Lopes et al. 2014). This study aimed to perform a survey of *E. coli* and *Salmonella* spp. from pet psittacine that died during one year and to describe the main lesions in the affected organs attributing scores, but also to identify the antimicrobial susceptibility profiles of the isolates.

## MATERIALS AND METHODS

Seventy five psittacine pets from Fortaleza, Brazil delivered dead to or that died in the Laboratory of Ornithological Studies from August 2014 to July 2015 were submitted to necropsy to identify the presence of *E. coli* and *Salmonella* sp. This study was approved by the Ethics Committee of the Use of Animals of the State University of Ceará under protocol number 1586195/2015 and with the consent from all the owners. The species are shown in Table 1.

Fragments were collected from liver, kidneys, intestine, lung, heart, spleen and brain in formaldehyde and submitted to histopathological analysis. In addition, from each organ, samples were collected with aseptic conditions and material close to the Bunsen burner and placed in tubes containing 5mL of buffered peptone water, which were incubated. Then, 0.5mL of this broth was transferred to tubes containing brain heart infusion broth and selenite-cystine broth, which were incubated and aliquots were streaked in Petri dishes with MacConkey agar and brilliant green

**Table 1. Absolute Frequency and Relative Frequency from species of seventy five psittacine birds necropsied**

Species	Frequency of birds	
	AF	RF
AA	13	17,33
AC	1	1,33
AR	2	2,67
AZ	1	1,33
ER	1	1,33
ET	11	14,67
MT	25	33,33
NH	19	25,33
PH	2	2,67

AF = Absolute frequency, RF = Relative frequency, AA = *Amazona aestiva*, AC = *Agapornis roseicollis*, AR = *Ara chloroptera*, AZ = *Amazona amazônica*, ER = *Ectectus rotates*, ET = *Eupsittula cactorum*, MT = *Melopsittacus undulatus*, NH = *Nymphicus hollandicus*, PH = *Psephotus haematonotus*.

agar. After incubation, a single colony with morphological characteristics compatible with *E. coli* was selected and submitted to the following biochemical tests: triple-sugar-iron (TSI) agar, sulfite-indole-motility (SIM) agar, lysine-iron-agar (LIA), citrate, Voges-Proskauer, methyl red, urease broth and malonate. In addition, a single colony from each plate with morphological aspects similar to *Salmonella* sp. was selected and submitted to TSI, urease and LIA, followed by rapid slide agglutination test, whenever the biochemical results indicated the presence of this pathogen. If the serological test was positive, the isolate was submitted to the Enterobacteria Laboratory of the Oswaldo Cruz Institute Foundation (Fiocruz) to be confirmed and serotyped.

All the isolates were maintained in nutrient agar in refrigerator until the antimicrobial susceptibility test, which was performed with the isolates being diluted until a turbidity equivalent to 1.0 in McFarland scale was achieved. Then, the inoculum was streaked in plate with Mueller-Hinton agar, to which discs containing the following antibiotics were added: amoxicillin (10µg), ampicillin (10µg), azithromycin (15µg), ciprofloxacin (5µg), doxycycline (30µg), enrofloxacin (5µg), fosfomicin (200µg), gentamycin (10µg), tetracycline (30µg) and sulfazotrim (25µg). After incubation, inhibition zone diameters were measured and compared to standards previously established (CLSI 2012). Multidrug resistance was considered whenever an isolate was resistant to at least two antibiotics of different groups and in all incubation steps afore mentioned, the conditions in bacteriological incubator were temperature of 37°C and duration 24h.

Histopathological procedure was performed at the Animal Pathology Laboratory of the Federal University of Paraíba, where slides of 5µm were prepared and stained with hematoxylin-eosin to be evaluated for the presence of hemosiderosis (HEMO), inflammatory infiltrate (INFINT), congestion (CNGT) and necrosis (NECRO). Lesions were observed and to each a mild (+), moderate (++) or marked (+++) score was assigned according to the intensity.

## RESULTS

From the seventy five investigated psittacine, nineteen were positive for *Escherichia coli* (nine *Melopsittacus undulatus*, four *Nymphicus hollandicus*, three *Amazona aestiva*, two *Eupsittula cactorum* and one *Psephotus haematonotus*), from which kidneys and liver were the organs with the highest isolation frequency. In addition, from a single *Amazona aestiva*, a strain of *Salmonella enterica* serotype Typhimurium was isolated and the results as well as lesions scores are displayed in Table 2. These histopatholo-

**Table 2. Histopathological and bacteriological findings in twenty pet psittacine from Fortaleza, Brazil, with colibacillosis and salmonellosis**

Organs	Lesions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		AA	AA	AA	NH	NH	NH	AA	MT	MT	MT	PH	ET	MT	ET	MT	MT	MT	MT	NH	MT
Liver	INFINT	++	NO	++	+	NO	++	+++	NO	+	++	+	+	NO	+++	NO	NO	NO	+	NO	NO
	NECR	++	NO	++	++	NO	NO	NO	NO	NO	++	NO	NO	NO	++	NO	NO	NO	+	NO	NO
	CNGT	+++	NO	+++	+++	+++	+++	+++	+	+++	+++	+++	+	+++	+++	++	NO	+++	+	++	+++
	HEMO	+++	NO	NO	+	NO	+	+	NO	+++	NO	+	NO	NO	NO	NO	NO	NO	NO	NO	NO
Lungs	INFINT	NO	NO	NO	NO	NO	NO	NO	NO	+	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	NECR	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	CNGT	+++	+++	+	++	+++	NO	+++	+	+++	+++	+++	++	+++	NO	+	NO	++	++	+++	++
	HEMO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Kidneys	INFINT	NO	NO	NO	NO	++	++	NO	NO	NO	+	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	NECR	++	+	NO	+	+	NO	NO	NO	NO	NO	NO	NO	++	NO	NO	NO	NO	NO	NO	NO
	CNGT	++	+++	+++	+	++	NO	++	NO	+	++	+++	NO	++	NO	+	NO	+	++	+	+
	HEMO	NO	+	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Spleen	INFINT	+	NO	NO	NA	NA	NA	NO	NA	+++	NA	NA	NO	NO	NO	NA	NA	NA	NA	NA	NA
	NECR	NO	NO	NO	NA	NA	NA	NO	NA	NO	NA	NA	NO	NO	NO	NA	NA	NA	NA	NA	NA
	CNGT	NO	NO	+++	NA	NA	NA	+++	NA	+++	NA	NA	NO	NO	+	NA	NA	NA	NA	NA	NA
	HEMO	NO	NO	NO	NA	NA	NA	+	NA	+++	NA	NA	NO	NO	NO	NA	NA	NA	NA	NA	NA
Intestine	INFINT	NO	NO	NO	++	NO	+	NO	NO	NO	+	NO	NO	NO	NO	NO	+++	NO	NO	NO	NO
	NECR	NO	NO	NO	NO	NO	NO	+++	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	CNGT	+++	NO	NO	+	+	NO	NO	NO	+++	NO	NO	NO	NO	NO	NO	+	NO	NO	NO	NO
	HEMO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Encephalus	INFINT	+	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	NECR	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	CNGT	++	NO	NO	+	++	+	+++	+	+	NO	+	NO	NO	+	NO	+	+	NO	+	+
	HEMO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Heart	INFINT	+	NO	NO	NO	NO	NO	+++	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	NECR	NO	NO	NO	NO	NO	NO	+	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
	CNGT	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	no	NO	NO	NO
	HEMO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Bacteriological findings	EC	EC	ST	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC	EC
Other lesions	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No	No

AA = *Amazona aestiva*, NH = *Nymphicus hollandicus*, MT = *Melopsittacus undulatus*, ET = *Eupsittula cactorum*, PH = *Psephotus haematonotus*, INFINT = Inflammatory infiltrate, NECRO = Necrosis, CNGT = Congestion, HEMO = Hemosiderosis, NO = Not observed, NA = Not available, EC = *Escherichia coli*, ST = *Salmonella Typhimurium*. + mild, ++ moderate, +++ severe.

gic lesions were in most cases congestion and inflammatory infiltrates, most frequently in liver and kidneys. The case of salmonellosis presented a multifocal hepatocytes necrosis, accompanied by lymphoplasmocitary and heterophilic inflammatory infiltrates (Fig.1). Hemosiderosis was present only in *E. coli* cases most frequently in liver, followed by spleen and kidneys; however, lymphoplasmocitary infiltrate was also observed varying from light to moderate (Fig.2). Other simultaneous lesions and/or diseases were observed in six birds (one *Amazona aestiva*, two *Aratinga cactorum* and three *Melopsittacus undulatus*) all positive for *E. coli* (Table 3), which consisted of traumas, secondary infections and neoplasms.

The antimicrobial susceptibility tests revealed that sulfonamide was the antibiotic to which the isolates presented the most frequent resistance, which was 75%, followed by sulfazotrim (67%), both from sulfa group, ampicillin (67%), nalidixic acid (58%), tetracycline (50%), norfloxacin (42%) and azithromycin (33%). In three colibacillosis cases, birds were treated with tetracycline, sulfonamide or enrofloxacin, and the respective isolates presented resistance to the same used antibiotics and multidrug resistance. Unfortunately, 66.7% of the cases did not have information about antibiotics use; however, from five of these, the isolated strains presented resistance to at least one of the tested drugs.

**Table 3. Other findings from non-*Escherichia coli* lesions in six pet psittacine from Fortaleza, Brazil, with colibacillosis**

Case	Other lesions or disease	Isolated
1	Granulomatous fungic celomitis	EC
AA		
8	Fusiform sarcoma	EC
MT		
9	Egg retention	EC
MT		
10	Caseous sinusitis	EC
MT		
12	Skin laceration	EC
ET		
14	Limb edema	EC
ET		

AA = *Amazona aestiva*, MT = *Melopsittacus undulatus*, ET = *Eupsittula cactorum*, EC = *Escherichia coli*.

**DISCUSSION**

The role of *Escherichia coli* in as a commensal member of the regular microbiota of healthy psittacine with regular diet (grains, fruits, vegetables and sprouts) is still controversial, however *Salmonella* sp. is not consider as a part of the intestinal microbiota of these birds. Therefore, healthy budgerigars (*Melopsittacus undulatus*) and cockatiels (*Nymphicus hollandicus*) should present an intestinal microbiota composed mostly by Gram positive bacteria Lamb

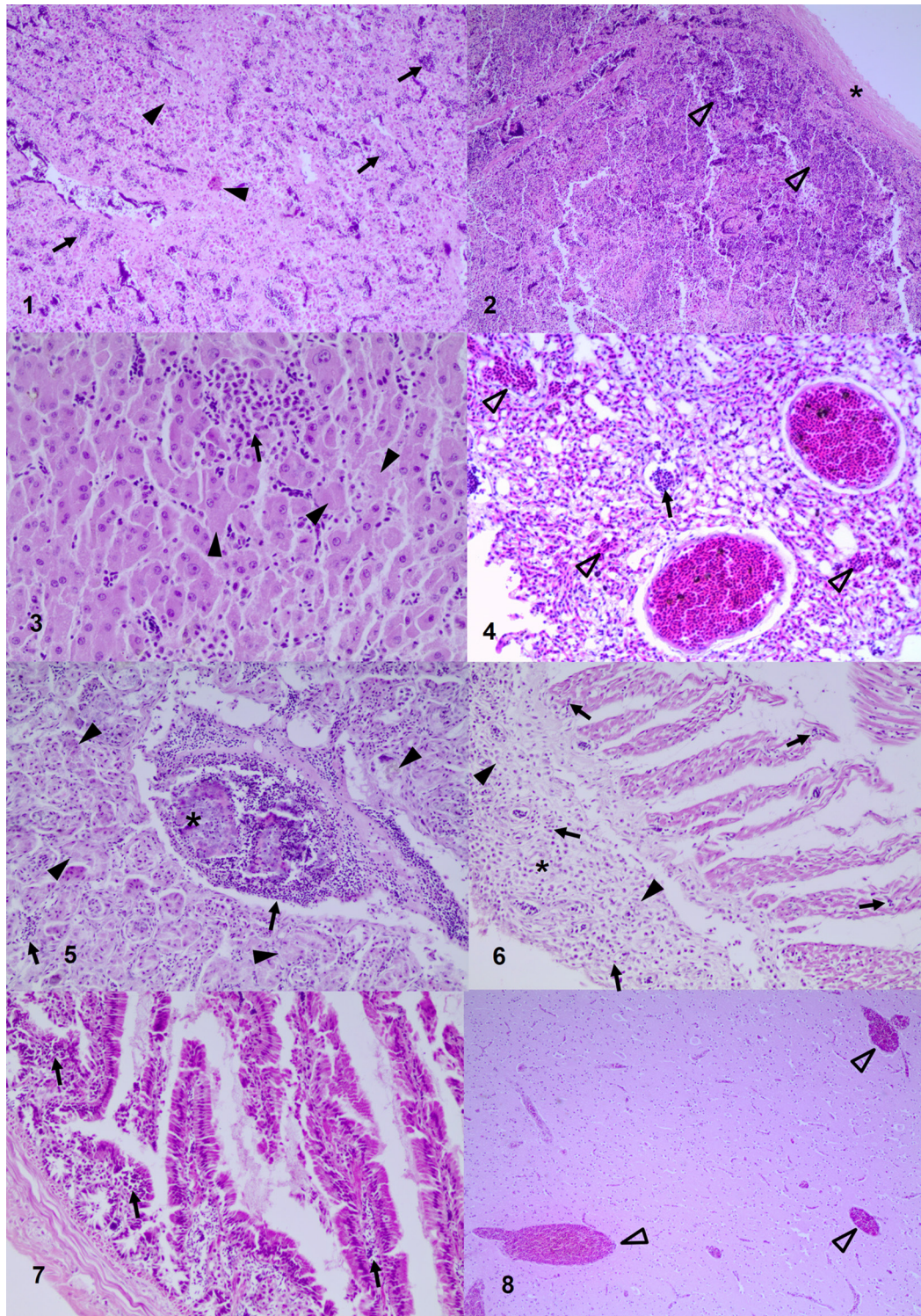


Fig.1-8. Histopathological lesions of salmonellosis and colisepticemia in psittacine pet. (1) Acute hepatitis in *Amazona aestiva* positive for *Salmonella* Typhimurium with multifocal necrotizing hepatitis (arrowhead). (2) Lymphoplasmocitary and heterophilic inflammatory infiltrate in liver (slim arrow) and splenitis with congestion and thick capsule (asterisk). HE, obj.10x. (3) Birds positive for *Escherichia coli*: acute hepatitis in *Melopsittacus undulatus* with random hepatocyte necrosis (arrowhead) and lymphoplasmocitary infiltrate. HE, obj.40x. (4) Pneumonia in *Amazona aestiva* with interstitial pulmonary congestion (unfilled arrow) and mixed infiltrate in the interior of alveoli (slim arrow). HE, obj.20x. (5) Nephritis in *Nymphicus hollandicus* with tubular necrosis (arrowhead), lymphoplasmocitary infiltrate (slim arrow), septic thrombus and basophilic aggregates suggestive of bacterial figures inside vessels (asterisk). HE, obj.20x. (6) Pericarditis in *Amazona aestiva* with inflammatory lymphoplasmocitary infiltrate among the muscle fibers and pericardium (slim arrow), necrosis and cellular debris (arrowhead) with thickened pericardium (asterisk). HE, obj.20x. (7) Enteritis in *Melopsittacus undulatus* with mixed inflammatory infiltrate in lamina propria (slim arrow). HE, obj.40x. (8) Encephalitis in *Amazona aestiva* with marked vessel congestion in the white matter (unfilled arrow). HE, obj.20x.

**Table 4. Antimicrobial susceptibility profiles of *Escherichia coli* and *Salmonella* Typhimurium strains isolated from different organs of dead pet psittacine**

Case and Isolate	Organs	AMP		AZI		CLO		CTF		FOS		GEN		NAL		NOR		POL		SUL		SUT		TET		Previous treatment	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R		
3 AA ST	L	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	-	Tetracycline and Prednisolone
	S	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	-	
4 NH EC	K	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	Tetracycline and Sulfonamide	
5 NH EC	S	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	Not informed	
	I	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-		
6 NH EC	L	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	Not informed	
9 MT EC	L	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-		
	K	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	Ketoprophen	
10 MT EC	L	+	-	-	+	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	-		
12 ET EC	L	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	Not informed	
14 ET EC	K	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+		
15 MT EC	L	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	Not informed	
	I	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+		
16 MT EC	L	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	Not informed	
18 MT EC	I	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+		
20 MT EC	L	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	Not informed	
RF of strains		66,7		33,3		8,3		8,3		8,3		0,0		58,3		41,7		8,3		75,0		66,7		50,0			

AA = *Amazona aestiva*, NH = *Nymphicus hollandicus*, MT = *Melopsittacus undulatus*, ET = *Eupsittula cactorum*, RF = Relative frequency, R = Resistant, S = Susceptible, AMP = Ampicilin, AZI = Azithromycin, CLO = Chlortetracycline, CTF = Ceftiofur, FOS = Fosfomycin, GEN = Gentamicin, NAL= Nalidixic Acid; NOR= Norfloxacin; POL= Polymyxin B, SUT = Sulfazotrim, SUL= Sulfonamide, TET = Tetracycline.

et al. (2014). However, as observed in a study with healthy hispaniolan amazon parrots (*Amazona ventralis*) in captivity, which had the intestinal microbiota assessed with Gram staining and bacterial culture, *E. coli* was the most frequent Gram negative rod (23.8%) (Evans et al. 2014). In addition, a study with three species of *Amazona* (*A. farinosa*, *A. aestiva* and *A. amazônica*) and other psittacine with a considerable health risk due to management conditions of illegal wildlife trade, *E. coli* was isolated from 57.3% individuals (Hidasi et al. 2013).

In birds that died with suggestive colibacillosis lesions (aerossaculitis, cachexy, pericarditis, perihepatitis, hemorrhage in the intestinal mucosa and accumulation of feces around the cloacae) or symptoms, as observed in this study and in a study with red-spectacled amazon (*A. pretrei*) parrots, *E. coli* may be isolated frequently from several distinct organs (Corrêa et al. 2013). Enteropathogenic *E. coli* has been detected from asymptomatic psittacine from several species, among which *Amazona* spp. and *Eupsittula* spp. in captivity (Saidenberg et al. 2012). The frequency of *E. coli* isolation (19%) from psittacine of illegal wildlife trade or in rehabilitation previous to reintroduction in the wild is more frequent than *Salmonella* sp. (1.12%), which was isolated from an *A. aestiva* as previously described, similar to the findings from this study (Marietto-Gonçalves et al. 2010). There are some reports of *Salmonella* sp. being isolated from psittacine of conservationist, breeding and rehabilitation facilities, even in Fortaleza, Brazil from the species *Ara chloroptera*, *Amazona aestiva* and *Melopsittacus undulatus*, however this is the first report in Brazil with a pet psittacine (Marietto-Gonçalves & Almeida 2010, Bezerra et al. 2013, Hidasi et al. 2013, Lopes et al. 2014, Almeida et al. 2015).

Avian colibacillosis aspects were described in a case report with a pet *A. aestiva* (Marietto-Gonçalves et al. 2007),

in which *E. coli* was isolated from several organs and lesions, which consisted of multiple granulomas and heterophilic infiltrate in lungs, congestion in kidneys, liver and brain, which are similar to the findings in this study. The most frequent lesions of colibacillosis are coagulative liver necrosis, aerossaculitis, granulomas, acute lung hemorrhage and congestion, enteritis with fusion of vili, epithelium hypertrophy and exfoliation, cellulitis with intense heterophilic infiltrate, multinuclear giant cells and fibrin-necrotic plaques; salpingitis with intense follicle necrosis and mixed infiltrate (Crespo et al. 2001, Andrade et al. 2006, Seeley et al. 2014).

The most frequent characteristics of avian salmonellosis are poor body condition, muscular atrophy, granulomas varying from multifocal to coalescent, transmural ulcerative necrosis of the gastrointestinal tract with clear presence of bacterial aggregation inside and outside the lesions, necrotizing hepatitis, interstitial pneumonia, myocarditis, epicarditis and necrotizing encephalitis (Madadgar et al. 2009, Giovaninni et al. 2012). In Australia, a flock of budgerigars with mortality caused by *E. coli* presented congestion and hemorrhage (Seeley et al. 2014) as the main findings observed, similar to the results from this study, except the encephalitis present in different species of psittacine. The lesions caused by *Salmonella* Typhimurium in passerines in Switzerland had similar intensity to the ones caused by the strain in this study isolated from the *A. aestiva*, which was also severe necrotizing hepatitis (Giovaninni et al. 2012). The occurrence colibacillosis in birds with other simultaneous diseases or lesions are frequent, which may serve as an entry port for the infection by *E. coli* or even *Salmonella* spp. (Crespo et al. 2001, Seeley et al. 2014).

Elevated rates of resistance to tetracycline or other antibiotics from the same group may occur via transference between microorganisms in the microbiota of birds, which

may be a direct or indirect risk to the human health (Hu et al. 2013). Resistance to tetracycline in *E. coli* strains isolated from pet birds have been reported in Australia (13.9%), from a total of 594 analyzed samples and multidrug resistance was also reported (Blynton et al. 2015). In another study, multidrug resistance to other groups, such as aminoglycosides, quinolones, sulfas and others has been identified in *E. coli* strains isolated from psittacine.

Similar to other studies involving *E. coli* and *Salmonella* sp. isolated from psittacine (Corrêa et al. 2013), antibiotic resistance to tetracycline, sulfonamide and ampicillin presented different results in organs and intestine samples (Case 9 and 14, Table 4). The *Salmonella* Typhimurium strain isolated in this study presented multidrug resistance to six different antibiotic groups, similar to the strain isolated from parrots in rehabilitation prior to reintroduction in the wild that presented resistance to enrofloxacin, ceflaxor, ciprofloxacin and sulfonamide (Marietto-Gonçalves et al. 2010). These findings indicate an indiscriminate use of antibiotics in avian medicine and the occurrence of multidrug resistance involved with failure in antimicrobial therapy.

## CONCLUSIONS

Pet psittacine that died in Fortaleza, Brazil positive for *Escherichia coli* and *Salmonella* Typhimurium had colibacillosis and salmonellosis showed mild to moderate lesions and liver was the most affected organ.

Isolated strains presented multidrug resistance and the most frequent was to tetracycline, followed by ampicillin, sulfonamide, sulfazotrim and nalidixic acid; which suggests an ill use of antimicrobials in pet birds.

## REFERENCES

- Almeida P.M., Otutumi L.K., Gerônimo E., Messa V., Suenaga S.S., Amaral P.F.G.P., Lima E.T., Vendrame A., Gonçalves D.D. & Cestari E.D. 2015. Study of the presence of *Salmonella* spp. and gastrointestinal parasites in excreta from ornamental birds from breeders in the city of Umuarama, Paraná. Afr. J. Microbiol. Res. 9(4):253-257.
- Andrade C.L., Ferreira G.F., Franco R.M., Nascimento E.R. & Tortelly R. 2006. Alterações patológicas e identificação da *Escherichia coli* como agente causal da celulite aviária em frangos de corte inspecionados em um matadouro em São Paulo. Revta Bras. Clín. Vet. 13(3):139-143.
- Andrés S., Vico J.P., Garrido V., Grilló M.J., Samper S., Gavin P., Herrera-León S. & Mainar-Jaime R.C. 2013. Epidemiology of subclinical salmonellosis in wild birds from a area of high prevalence of pig salmonellosis: phenotypic and genetic profiles of *Salmonella* isolates. Zoonoses Publ. Health 60:355-365.
- Bezerra W.G.A., Cardoso W.M., Teixeira R.S.C., Vasconcelos R.H., Machado D.N., Lopes E.S., Albuquerque A.H. & Rocha-e-Silva R.C. 2013. Survey of *Salmonella* sp. in budgerigars (*Melopsittacus undulatus*) in Fortaleza, Brazil. Acta Scient. Vet. 41:1714-1723.
- Blynton M.D.J., Pi H., Vangcchia B., Abraham S., Trott D.J., Johnson J.R. & Gordon D.M. 2015. The genetic structure and antimicrobial resistance of *Escherichia coli* and cryptic clades in birds with diverse human associations. Appl. Environ. Microbiol. 81(15):5123-5133.
- CLSI 2012. Performance standards for antimicrobial disk susceptibility tests: Approved Standards-Eleventh Edition. CLSI document M02-A11. Clinical and Laboratory Standards Institute, Wayne, PA.
- Corrêa I.M.O., Flores F., Schneiders G.H., Pereira L.Q., Brito B.G. & Lovato M. 2013. Detecção de fatores de virulência de *Escherichia coli* e análise de *Salmonella* spp. em psitacídeos. Pesq. Vet. Bras. 33(2):241-246.
- Crespo R., Walker R.L., Nordhausen R., Sawyer S.J. & Manalc R.B. 2001. Salpingitis in Pekin ducks associated with concurrent infection with *Treptachromonas* spp. and *Escherichia coli*. J. Vet. Diagn. Invest. 13:240-245.
- Evans E.E., Mitchell M.A., Whittington J.K. & Tully Jr T.N. 2014. Measuring the level of agrément between cloacal gram's stains and bacterial cultures in Hispaniolan Amazon parrots (*Amazona ventralis*). J. Avian Med. Surg. 28(4):290-296.
- Giovaninni S., Pewsner M., Hüsey D., Hächler H., Degiorgis M.P.R., Hirschheydt J. von & Origgi F.C. 2012. Epidemic of Salmonellosis in passerine birds in Switzerland with spillover to domestic cats. Vet. Pathol. 50(4):597-606.
- Hidasi H.W., Neto J.H., Moraes D.M.C., Linhares G.F.C., Jayme V.S. & Andrade M.A. 2013. Enterobacterial detection and *Escherichia coli* antimicrobial resistance in parrots seized from the illegal wildlife trade. J. Zoo Wildl. Med. 44(1):1-7.
- Hu G.Z., Pan Y.S., Wu H., Han H., Xu R., Yuan L., Liu J.H. & Feng J.K. 2013. Prevalence of tetracycline resistance genes and identification of tet(M) in clinical isolates of *Escherichia coli* from sick ducks in China. J. Med. Microbiol. 62:851-858.
- Janben D.W., Schwarz C., Preikschat P., Voss M., Philipp H.C. & Wieler L.H. 2001. Virulence-associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from intestinal organs of poultry having died from colibacillosis. Int. J. Med. Microbiol. 291(5):371-378.
- Lamb S., Sobczynski A., Starks D. & Sitanas N. 2014. Bacteria isolated from the skin of congo African grey parrots (*Psittacus erithacus*), budgerigars (*Melopsittacus undulatus*), and cockatiels (*Nymphicus hollandicus*). J. Avian Med. Sug. 28(4):275-279.
- Lopes E.S., Cardoso W.M., Albuquerque A.H., Teixeira R.S.C., Salles R.P.R., Bezerra W.G.A., Rocha-e-Silva R.C., Lima S.V.G., Sales R.J.P.F. & Vasconcelos R.H. 2014. Isolation of *Salmonella* spp. em Psitacíformes from zoos and a commercial establishment of Fortaleza, Brazil. Arq. Bras. Med. Vet. Zootec. 66(3):965-968.
- Madadgar O., Zharaei-Salehi T., Ghafari M.M., Tamai A., Madani A.S. & Yahyareyat R. 2009. Study of an unusual paratyphoid epornitic in canaries (*Serinus canarius*). Avian Pathol. 38(6):437-441.
- Marietto-Gonçalves G.A. & Almeida S.M. 2010a. Isolation of *Salmonella* entérica Serovar Enteritidis in Blue-fronted amazona parrot (*Amazona aestiva*). Avian Dis. 54:151-155.
- Marietto-Gonçalves G.A., Almeida S.M., Lima E.T. & Andreatti-Filho R.L. 2010b. Detecção de *Escherichia coli* e *Salmonella* spp. em microbiota intestinal de Psitacíformes em fase de reabilitação para soltura. Brazilian J. Vet. Res. Anim. Sci. 47(3):185-189.
- Marietto-Gonçalves G.A., Lima E.T., Siqueira J.L. & Andreatti-Filho R.L. 2007. Colisepticemia em papagaio-verdadeiro (*Amazona aestiva*). Revta Bras. Saúde Prod. Anim. 8(1):56-60.
- Poirel L., Potron A., de La Cuesta C., Cleary T., Nordmann P. & Munoz-Price S. 2012. Wild coast birds as reservoirs of Broad-Spectrum-β-Lactamase-producing *Enterobacteriaceae* in Miami beach, Florida. Antimicrob. Agents Chemotherapy 56(5):2756-2758.
- Saidenberg A.B., Teixeira R.H.F., Guedes N.M.R., Allgayer M.C., Melville P.N. & Benites N.R. 2012. Molecular detection of Enteropathogenic *Escherichia coli* in asymptomatic captive psittacines. Pesq. Vet. Bras. 32(9):922-926.
- Seeley K.E., Baitchman E., Bartlett S., Debroy C. & Garner M.M. 2014. Investigation and control of an attaching and effacing *Escherichia coli* outbreak in a colony of captive budgerigars (*Melopsittacus undulatus*). J. Zoo Wildl. Med. 45(4):875-882.
- Seo K.H., Holt T.P.S., Gast R.K. & Hofacre C.L. 2000. Elimination of early *Salmonella* Enteritidis infection after treatment with competitive-exclusion culture and enrofloxacin in experimentally infected chicks. Poult. Sci. 79(10):1408-1413.
- Tortora G.J., Funke B.R. & Case C.L. 2012. Microbiologia. 10ª ed. ArtMed, Porto Alegre. 964pp.
- Veldman K., Tulden P.V., Kant A., Testerink J. & Mevius D. 2013. Characteristics of Cefotaxime-Resistant *Escherichia coli* from wild Bird in Netherlands. Appl. Environ. Microbiol. 79(24):7556-7561.