











Pathological aspects of a septicemic salmonellosis outbreak caused by *Salmonella* serotype Typhimurium in captive blue-fronted Amazon parrots (*Amazona aestiva*)¹

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ABSTRACT.- Campos J.A., Guizelini C.C., Pavarini S.P., Azuaga L.B.S., Souza M.L., Leal C.R.B., Ramos C.A.N. & Gomes D.C. 2024. **Pathological aspects of a septicemic salmonellosis outbreak caused by *Salmonella* serotype Typhimurium in captive blue-fronted Amazon parrots (*Amazona aestiva*).** *Pesquisa Veterinária Brasileira* 44:00, 2024. Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Filinto Muller 2443, Bairro Vila Ipiranga, Campo Grande, MS 79074-460, Brazil. E-mail: danilo.gomes@ufms.br

There are reports of septicemic salmonellosis in exotic birds caused by *Salmonella enterica* serotype Typhimurium, although they are scarce and do not address the anatomopathological and immunohistochemical aspects of this condition in blue-fronted Amazon parrots (*Amazona aestiva*). This study aims to report an outbreak of salmonellosis by *S. Typhimurium* in 15 blue-fronted parrots introduced to a rehabilitation center in the state of Mato Grosso do Sul, Brazil. Hepatomegaly and splenomegaly, accompanied by flat white areas in the capsule and parenchyma, were the most frequent gross changes, histologically represented by necrotic and heterophilic hepatitis and splenitis and associated with bacteria in the cytoplasm of macrophages and free in the affected tissue. *S. Typhimurium* was identified from bacterial culture, PCR, and DNA sequencing. Along with immunohistochemistry samples of liver, spleen, kidney, small intestine, pancreas, thyroid, heart, and lung, immunostaining was used for *Salmonella* spp. without previous bacterial isolation. The lethality of this outbreak demonstrates the relevance of salmonellosis in blue-fronted parrots, which are known to be carriers of this bacteria.

INDEX TERMS: One health, carriers, hepatic necrosis, splenitis, immunohistochemistry.

RESUMO.- [Aspectos patológicos de um surto de salmonelose septicêmica causado por *Salmonella* sorotipo Typhimurium em papagaios-verdadeiros (*Amazona aestiva*) de cativeiro.]

Apesar de existirem relatos sobre salmonelose septicêmica em aves silvestres por *Salmonella enterica* sorovar Typhimurium, eles são escassos e não abordam os aspectos anatomopatológicos e imuno-histoquímicos da condição em papagaios-verdadeiros

(*Amazona aestiva*). Este trabalho visou relatar um surto de salmonelose por *S. Typhimurium* em 15 papagaios-verdadeiros introduzidos em um centro de reabilitação localizado no estado de Mato Grosso do Sul, Brasil. Hepatomegalia e esplenomegalia acompanhadas por áreas planas brancacentas na cápsula e no parênquima foram as alterações macroscópicas mais frequentes, representadas histologicamente por hepatite

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e esplenite necróticas e heterofílicas associadas a bactérias no citoplasma de macrófagos e livres no tecido afetado. *S. Typhimurium* foi identificada a partir de cultura bacteriana, PCR e sequenciamento de DNA. Na imuno-histoquímica, as amostras de fígado, baço, rim, intestino delgado, pâncreas, tireoide, coração e pulmão evidenciaram imunomarcção para *Salmonella* spp., mesmo quando não houve isolamento bacteriano prévio. A alta letalidade deste surto demonstra relevância da salmonelose em papagaios-verdadeiros, que são majoritariamente atribuídos como hospedeiros carreadores desta bactéria.

TERMOS DE INDEXAÇÃO: Saúde única, carreadores, necrose hepática, esplenite, imuno-histoquímica.

INTRODUCTION

Salmonellosis is a widespread zoonotic disease in veterinary and human medicine. Bacteria of the genus *Salmonella* are divided per species and, within species, into serotypes. *S. enterica* concentrates more than 1,531 serotypes, including Typhimurium (Sanderson & Nair 2013, Hagedoorn et al. 2023). Typhimurium and Enteritidis, serotypes of *S. enterica*, are the most relevant, causing paratyphoid fever in domestic and wild birds. In wild birds, free-ranging or captive, *S. Typhimurium* is the most frequently isolated serotype (Wilson & MacDonald 1967, Ewen et al. 2007, Madadgar et al. 2009, Pasmans et al. 2013, Siqueira et al. 2017).

Parrots are considered important asymptomatic carriers of *Salmonella* spp. to other species, including humans, constituting a risk to public health (Allgayer et al. 2008, Hidasi et al. 2013, Beleza et al. 2020). In this way, the concern with the “one health” concept is growing, as it is known that blue-fronted parrots (*Amazona aestiva*) are often bred as pets by humans, which promotes their proximity and increases risks of contact with *Salmonella* spp. (Wilson & MacDonald 1967, Saldenberg et al. 2021), reinforcing the need for more information about this in exotic birds.

We report an outbreak of septicemic salmonellosis caused by *Salmonella enterica* serotype Typhimurium in captive blue-fronted Amazon parrots (*Amazona aestiva*), including anatomopathological findings and diagnostic methods to highlight this condition.

MATERIALS AND METHODS

The outbreak occurred at the “Centro de Recuperação de Animais Silvestres” (Wild Bird Rehabilitation Center – CRAS) in Mato Grosso do Sul, Brazil. A total of 15 *Amazona aestiva* birds died and were necropsied at the “Laboratório de Anatomia Patológica” (Laboratory of Pathological Anatomy – LAP) of the “Faculdade de Medicina Veterinária e Zootecnia” (Faculty of Veterinary Medicine and Bird Science – FAMEZ), “Universidade Federal do Mato Grosso do Sul” (UFMS). Clinical and epidemiological history information was provided through interviews with veterinarians of bird management at CRAS.

During necropsy, fragments of several organs were collected for histopathological examination in a 10% formalin solution for 48 hours and then processed for hematoxylin and eosin (HE) staining. Samples of the liver and spleen of one bird were collected, isolated in aseptic Petri dishes, and sent to the “Laboratório de Bacteriologia” (Laboratory of Bacteriology) at FAMEZ-UFMS for isolation and bacterial identification; this was done by morphotintorial characterization

and biochemical reactions in specific media. The samples in which there was growth of *Salmonella* sp. were sent to “Laboratório de Biologia Molecular” (Molecular Biology Laboratory), FAMEZ-UFMS, for serotype identification by PCR (Souza et al. 2018).

Paraffin-embedded liver, heart, kidney, spleen, lung, small intestine, thyroid, and pancreas samples from all necropsied birds were submitted to immunohistochemistry (IHC) for *Salmonella* spp. (Juffo et al. 2017) and *Chlamydia* spp. (Casagrande et al. 2014), carried out by the team of the “Setor de Patologia Veterinária” (Veterinary Pathology Sector) of the “Universidade Federal do Rio Grande do Sul” (UFRGS).

RESULTS

The birds were free-living-ranged in the state of Mato Grosso do Sul (MS), Brazil, and sent to the CRAS in Campo Grande/MS. The birds were introduced to the CRAS at an estimated one-month age and transferred to an enclosure with a 60m² flight structure after quarantine, where they shared space with approximately 250 birds of the same species and similar age. After five months, 15 birds were in the enclosure over three weeks, showing lethargy, diarrhea, dehydration, polydipsia, dyspnoea, and conjunctivitis. All sick birds died; some were found dead without showing clinical signs.

During the necropsy, all 15 birds showed similar lesions. Body status varied from regular (3/15) to cachectic (12/15), with atrophy of the pectoral muscle. There were plumage flaws along the body and, adhering to feathers and cloaca, pasty and white stools were observed. On internal examination, the liver and spleen were enlarged, with rounded edges and random white to slightly yellow areas on the capsular surface and in the parenchyma (Fig.1). In one, there was free yellowish gelatinous material in the pericardial sac, with multifocal petechiae, and hemorrhage in the left thoracic air sac.



Fig.1. Gross changes of septicemic salmonellosis caused by *Salmonella* Typhimurium in blue-fronted Amazon parrots (*Amazona aestiva*). The liver is enlarged and has multiple random and whitish foci on the capsular surface.

Microscopically, in the liver, lung, and white pulp of the spleen of all birds, there were multiple random foci of coagulation to liquefactive necrosis (Fig.2-7), accompanied by cellular debris and inflammatory infiltrate composed of heterophils, macrophages and, to a lesser extent, lymphocytes and multinucleated giant cells, seen only in the liver. Often, there were numerous bacterial aggregates in macrophage cytoplasm and free in the tissue parenchyma (Fig.2-7). In the

spleen, the wall of blood vessels was necrotic. The lumen of affected vessels was partially obstructed by fibrin thrombi (Fig.2-7). The large intestine showed extensive areas of mucosal ulceration covered by amorphous eosinophilic material with bacterial aggregates and viable or degenerated heterophils in one bird. The intensity of lesions in each bird is detailed in Table 1.

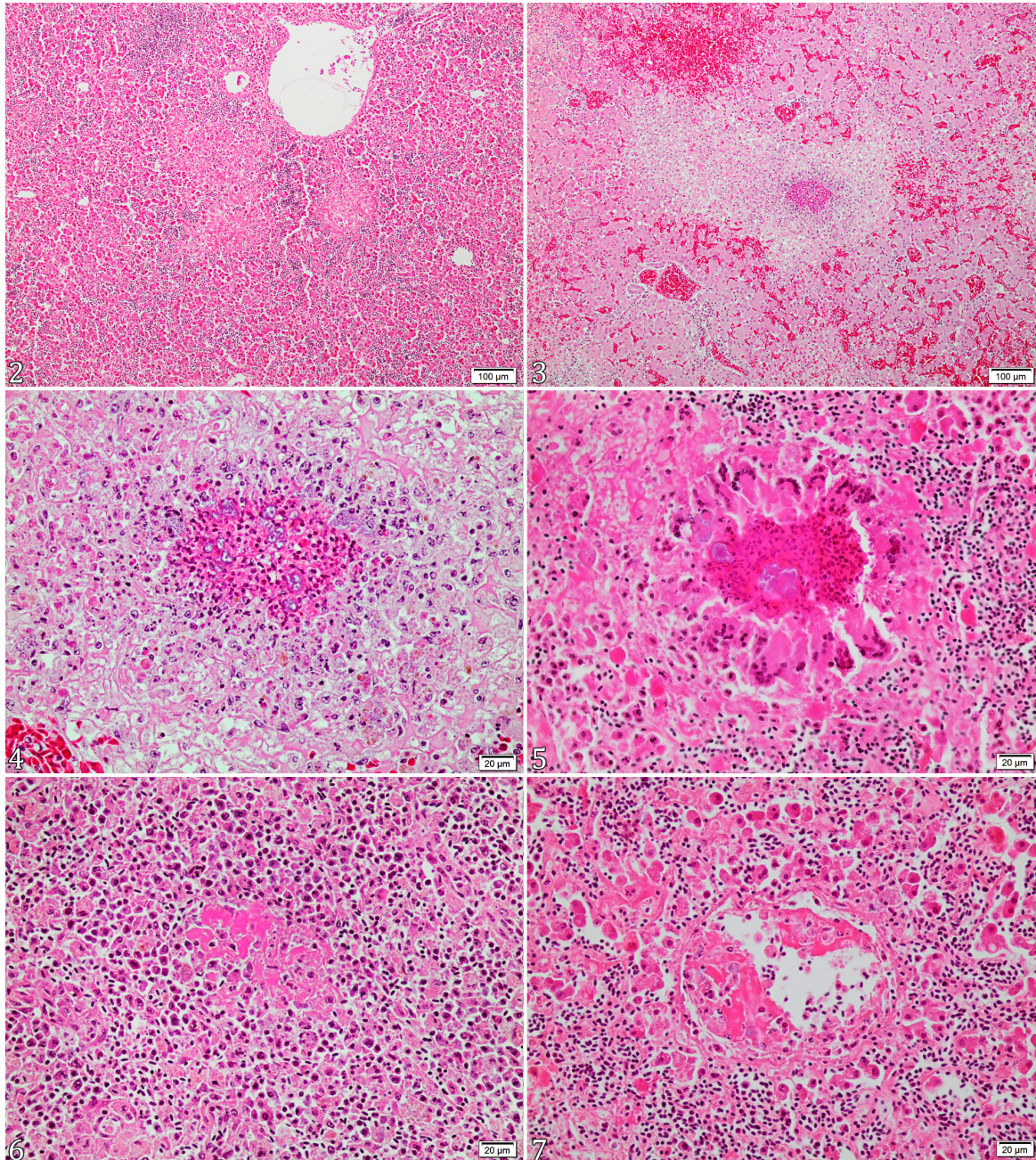


Fig.2-7. Microscopic changes of septicemic salmonellosis caused by *Salmonella* Typhimurium in blue-fronted Amazon parrots (*Amazona aestiva*). (2) Liver. The parenchyma exhibits poorly cellular multifocal and random lytic necrosis. HE, bar = 100µm. (3) Liver. Focally extensive centrilobular lytic necrosis with cellular debris and degenerated heterophils. Multifocal hemorrhage and sinusoidal congestion surround the necrotic area. HE, bar = 100µm. (4) Liver. The parenchyma is interrupted by lytic necrosis, abundant cellular debris, heterophils and macrophages. There are intralosomal and intracytoplasmic basophilic bacterial aggregates in macrophages. HE, bar = 20µm. (5) Liver. Focally extensive lytic necrosis with intralosomal basophilic bacterial aggregates and granulomatous inflammation with multiple multinucleated giant cells. HE, bar = 20µm. (6) Spleen. The parenchyma is interrupted by lytic necrosis and cellular debris. HE, bar = 20µm. (7) Liver. Blood vessels with necrotic walls. The lumen is partially obstructed by fibrin thrombi. HE, bar = 20µm.

Table 1. Histological grade of septicemic lesions caused by *Salmonella* Typhimurium in blue-fronted Amazon parrots (*Amazona aestiva*)

BI ^a	Necrosis			Bacterial aggregates	Inflammatory cell type	
	Liver	Spleen	Lung		Heterophilic	Granulomatous
01	+	-	-	+++ ^b	+	-
02	+++	+++	-	+++	-	++
03	+++	+++	-	+++	++	-
04	+	++	-	+	-	-
05	++	++	-	+++	++	-
06	+	+	-	+	-	-
07	+++	+	+	++	+++	++
08	++	++	-	++	++	-
09	-	+	+	+	+	-
10	++	+	-	+	+	-
11	+	++	-	++	++	-
12	+	-	-	-	-	-
13	++	++	+	++	++	-
14	++	++	-	++	++	++
15	++	+	-	+++	+++	++

^a Bird identification, ^b only in ingluvium; + mild, ++ moderate, +++ accentuated, - absent.

Biochemical reactions in specific media were compatible with the genus *Salmonella* in liver and spleen samples of one bird studied. Confirmation was performed by typing with polyvalent anti-*Salmonella* serum (PROBAC[®]). In PCR, the Typhimurium serotype genome was identified and sequenced. There was immunostaining at IHC for *Salmonella* spp. in the liver, small intestine, spleen, kidney, pancreas (Fig.8-11), thyroid, heart, and lung samples of several birds. No organ was positive for *Chlamydia* spp. Immunostaining of each bird's organs for *Salmonella* spp. is detailed in Table 2.

The sampling and *post mortem* examination of dead birds were approved by the client-owned birds and were performed following the best veterinary care practices.

DISCUSSION

The diagnosis of salmonellosis was based on clinical and pathological findings and confirmed after detection of *Salmonella* spp. in bacterial culture and immunohistochemistry, identifying Typhimurium serotype by PCR and DNA sequencing (Souza et al. 2018). In this outbreak, the affected parrots were approximately 30 days old and, after quarantine, were introduced to an enclosure with 250 birds of the same species. Although it was mentioned that all 250 birds were similar in age, we cannot rule out the possibility that some of them were adult birds. Living indoors with large populations of birds of different ages is an important predisposing factor for young birds, the most susceptible to salmonellosis (Wilson & MacDonald 1967, Pasmans et al. 2013). In rehabilitation centers, the disease has special relevance due to the high resistance of the bacteria in the environment and its multiple forms of transmission, including inhabiting adult and immunocompetent birds, rendering them asymptomatic carriers and possible sources of infection to young birds (Giovannini et al. 2012, Hidasi et al. 2013).

The clinical course ranged from acute, in which birds were found dead without previous clinical signs, to subacute, accompanied by diarrhea and dyspnoea, as described

in salmonellosis caused by *S. Typhimurium* and several serotypes of other exotic bird species (Wilson & MacDonald 1967, Madadgar et al. 2009, Pasmans et al. 2013). The gross and microscopic lesions in several organs, mainly the liver and spleen, indicating that all necropsied birds developed the septicemic form of the condition. Macroscopically, only one bird had enteritis, but a clinical history of diarrhea was described in several birds. Necrotic esophagitis and ingluvitis are lesions commonly found in birds infected with *Salmonella* sp. (Daoust et al. 2000, Giovannini et al. 2012); however, none of the necropsied birds showed lesions in these organs. The major microscopic alterations included coagulation to lytic necrosis and heterophilic and histiocytic inflammation and, in some cases, granulomatous, with multinucleated giant cells, an alteration observed mainly in subacute to chronic clinical conditions (Madadgar et al. 2009, Pasmans et al. 2013).

The serotype Typhimurium, identified by PCR in the liver and spleen, is the most isolated free-ranging in wild birds kept in domestic environments (Wilson & MacDonald 1967, Ewen et al. 2007, Madadgar et al. 2009, Pasmans et al. 2013, Siqueira et al. 2017) and, as is considered a non-host adapted microorganism, is commonly isolated from healthy adult birds. However, in the parrots of this outbreak, *S. Typhimurium* was responsible for causing acute to subacute septicemia with 100% lethality in the affected population. In bird populations of the order Passeriform (songbirds), *S. Typhimurium* caused septicemia with high mortality rates in Canada, similar to what was described in this report (Daoust et al. 2000).

Among 15 birds, only one was submitted for microbiological culture and typification by DNA analysis; all 14 birds presented autolysis changes, and as such, their samples were not tested for PCR or microbiological culture. Positive immunostaining for *Salmonella* spp. in several organs, including the thyroid and mainly the liver, spleen, and lung, helped to confirm the diagnosis of all birds in this study. This report demonstrated that immunohistochemistry showed high sensitivity and allowed diagnosis confirmation in suspected cases of septicemia by

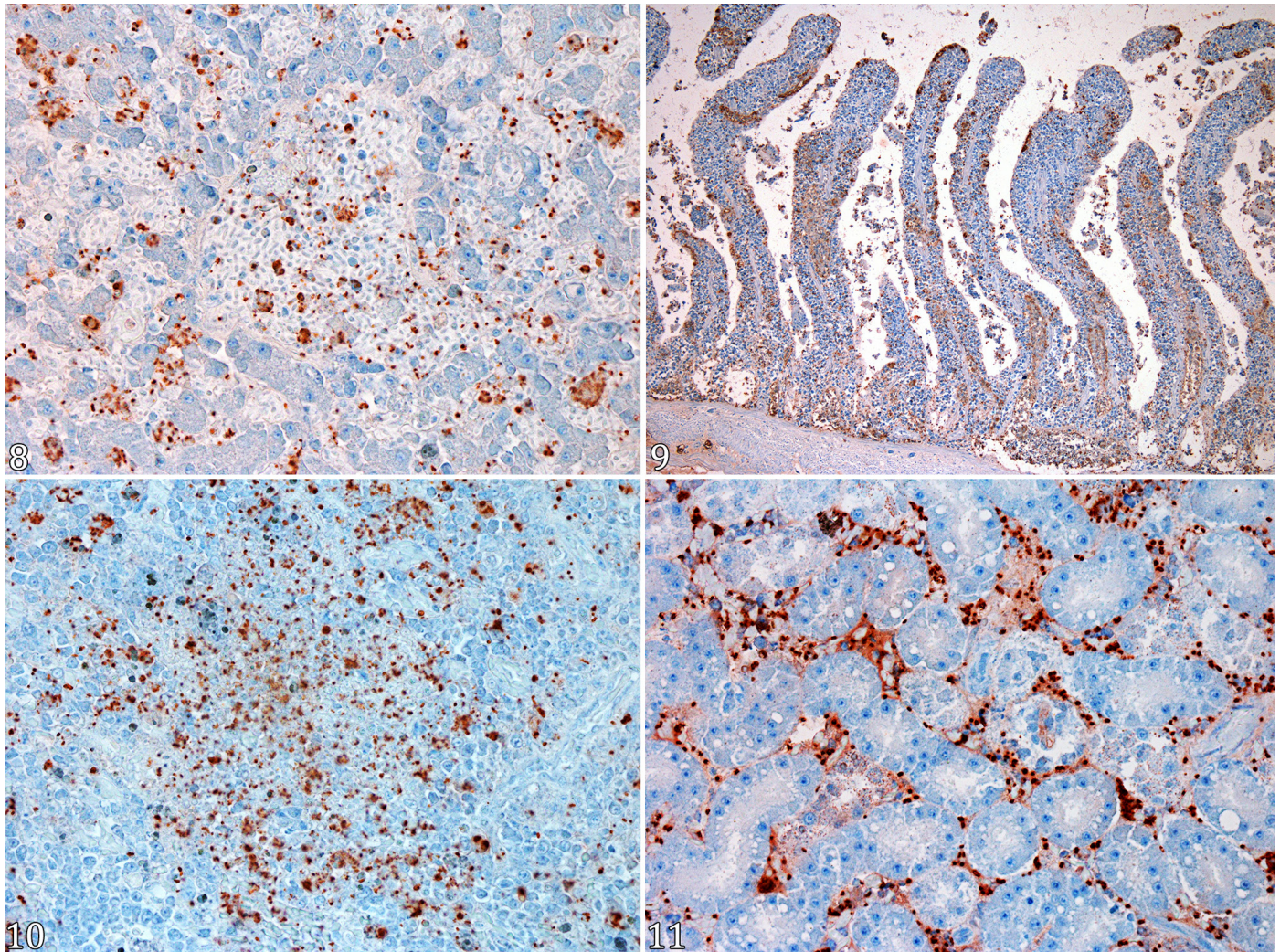


Fig.8-11. Microscopic changes of septicemic salmonellosis caused by *Salmonella* Typhimurium in blue-fronted Amazon parrots (*Amazona aestiva*). Immunohistochemistry for *Salmonella* spp. Necrotic foci and the cytoplasm of macrophages exhibit strongly positive immunostaining in (8) liver, (9) intestine, (10) spleen and (11) pancreas. (8) IHC, obj.40x. (9) IHC, obj.10x. (10) IHC, obj.20x. (11) IHC, obj.40x.

Table 2. Microscopic changes of septicemic salmonellosis caused by *Salmonella* Typhimurium in 15 blue-fronted Amazon parrots (*Amazona aestiva*). Immunostaining for *Salmonella* spp. in samples of several organs

BI ^a	Organ							
	Liver	Spleen	Lung	Heart	Kidney	Intestine	Pancreas	Thyroid
01	-	-	-	-	-	-	-	-
02	+	+	+	+	+	-	-	-
03	+	+	+	-	-	+	-	-
04	+	+	-	-	+	+	-	-
05	+	+	+	-	+	+	-	-
06	+	+	+	-	-	+	-	-
07	-	+	+	-	-	+	-	-
08	+	+	-	-	-	-	-	-
09	+	+	+	+	-	-	-	-
10	+	+	-	+	-	+	-	+
11	+	+	-	-	+	+	+	-
12	+	+	-	-	-	-	-	-
13	+	-	+	-	-	-	-	-
14	+	+	+	-	+	+	+	-
15	+	+	+	-	+	+	-	-

^a Bird identification; + positive, - negative.

Salmonella spp., especially in cases where bacterial culture was not possible.

Diseases of wild fauna, mainly those of zoonotic nature, are included in the ecosystem of the “One Health” concept (Destoumieux-Garzón et al. 2018). Our research shows that in addition to the known importance of hosts as asymptomatic carriers of *S. enterica*, septicemic salmonellosis in bird rehabilitation centers significantly impacts captive bird health, which is fatal in blue-fronted Amazon parrots. Given the system of rehabilitation centers presenting proximity between humans and birds, the importance of surveillance of diseases, such as salmonellosis in such restricted environments, is highlighted here.

CONCLUSION

Salmonella Typhimurium causes outbreaks of septicemia with high lethality in captive blue-fronted Amazon parrots due to necrotic and heterophilic to granulomatous hepatitis and splenitis. Immunohistochemistry for *Salmonella* spp. was an important diagnostic tool when bacterial isolation by microbiological culture was not possible.

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