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CONCOMITANT INFECTION OF *HAEMOGREGARINA* SP. AND *STAPHYLOCOCCUS AUREUS* IN FREE-LIVING YELLOW-SPOTTED RIVER TURTLE (*PODOCNEMIS UNIFILIS*): CASE REPORT

(Infecção concomitante de Haemogregarina *sp. e* Staphylococcus aureus *em tracajá (*Podocmenis unifilis*) de vida livre: relato de caso)*

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ABSTRACT: A male adult yellow-spotted river turtle *Podocnemis unifilis* (Chordata: Testudines: Podocnemididae) was captured during field collections. Blood sample was collected for haemoparasite screening by conventional light microscopy. Morphometric and morphological analyzes of the parasite developmental stages were performed in the blood extensions, as well as the parasitemia intensity. Gametocytes of hemogregarines and innumerable bacterial cells were observed. To identify the bacteria present, a culture was performed and the presence of *Staphylococcus aureus* was observed. It was concluded that a co-infection of *Staphylococcus aureus* (Firmicutes: Bacillales: Staphylococcaceae) and *Haemogregarina* sp. (Apicomplexa: Adeleina: Haemogregarinidae) was observed in this free-living yellow-spotted river turtle *Podocnemis unifilis.*
Keywords: co-infection; hemogregarines; occurrence; *Podocnemis unifilis*; *Staphylococcus aureus*.

RESUMO: Durante coletas de campo, um macho adulto de tracajá *Podocnemis unifilis* (Chordata: Testudines: Podocnemididae) foi capturado. Amostra de sangue foi colhida para pesquisa de hemoparasitos pela microscopia óptica convencional. Nas extensões sanguíneas foram realizadas análises morfométricas e morfológicas das formas evolutivas encontradas, bem como o cálculo da parasitemia. Foram observados gametócitos de hemogregarinas e inúmeras células bacterianas. Para identificação das bactérias presentes foi realizada cultura, sendo observada a presença de *Staphylococcus aureus.* Conclui-se que havia co-infecção por *Staphylococcus aureus* (Firmicutes: Bacillales: Staphylococcaceae) e *Haemogregarina* sp. (Apicomplexa: Adeleina: Haemogregarinidae) no tracajá *Podocnemis unifilis*.
Palavras-chave: co-infecção; hemogregarinas; ocorrência; *Podocnemis unifilis*; *Staphylococcus aureus*.

INTRODUCTION

The yellow-spotted river turtle *Podocnemis unifilis* Troschel 1848, is a semi-aquatic turtle widely distributed in South America (Arraes and Tavares-Dias, 2014). In the literature, there are few reports of pathogens affecting these turtle species, being the hemogregarines hemoparasites commonly reported (Soares *et al*. 2014; Espinoza *et al*. 2017).

The hemogregarine parasites (Apicomplexa: Adeleina) are characterized as an intracellular protozoan with heteroxenic lifecycle and frequently found in reptiles (Telford, 2009; Soares *et al.,* 2014). The genus *Haemogregarina* (Apicomplexa: Adeleina: Haemogregarinidae) is common in aquatic turtles and has been documented in several countries (Telford, 2009) However in Brazil, there are only a few reports (Soares *et al*., 2014; Picelli *et al*., 2015; Úngari *et al*., 2018). A recent study from Úngari et al. (2018) characterized through morphological, morphometric and molecular analyses, a new species *Haemogregarina podocnemis* in *Podocnemis unifilis*, being the first *Haemogregarina* species described in a Brazilian turtle.

Another pathogen observed in reptiles, including turtles, although less common, is the bacteria *Staphylococcus aureus* (Firmicutes: Bacilllales: Staphylococcaceae). It is present in a wide variety of animal species, including mammals, birds, and reptiles, and may be commensal or pathogenic (Santoro *et al*., 2006). *S. aureus* pathogenesis and, consequently, the host clinical condition are associated with several factors, immunity for example.

Studies in captive sea turtles have demonstrated the association of *S. aureus* with bronchopneumonia, obstructive rhinitis, traumatic ulcer, and ulcerative stomatitis (Glazebrook and Campbell, 1990; Orós *et al*., 2005). Nonetheless, there are no studies associating *Staphylococcus aureus* with parasitic diseases.

Therefore, the aim of this study was to identify the hemogregarines (Apicomplexa: Adeleina) affecting aquatic turtles in their natural habitat, unexpectedly, a concomitant infection by *Staphylococcus aureus* and *Haemogregarina* sp. was reported.

MATERIALS AND METHODS

This study was conducted under the approval of the Ethics Committee for Use of Animals of the University of Uberlândia (CEUA-UFU) (protocol 032/16) and the Biodiversity Information and Authorization System (SISBIO) (protocol 51398-1).

In July 2016, a male adult yellow-spotted river turtle (*Podocnemis unifilis*) was captured during field surveys in Red River, Britânia district, Goiás, Brasil (coordinates 14°57'08,4" South latitude and 51°06'30,7" West longitude). The specimen was submitted to blood collection with disposable and sterile 3 mL syringes and needles (22/19), by puncturing the cervical paravertebral venous sinus (Zippel *et al*., 2001). At the site, three blood smears were performed, and the rest of the material was stored at -4°C, in tubes containing EDTA as anticoagulant, for further analyzes at the Laboratory of Serology and Molecular Biology of Parasites of the Federal University of Uberlândia.

The blood smears were fixed with methanol, stained in 10% Giemsa (Eisen and Schall, 2000) and examined using an optical microscope (Olympus Optical Co) with 40x and 100x magnification.

The hemoparasite parasitemia was estimated according to De Biasi *et al.* (1989) under 100x magnification. The morphometric analysis of the parasite blood developmental stages was performed by measuring the length, width, and area of the body and nucleus of the parasites developmental stages.

For the bacteria analysis, an aliquot of the blood sample was sent to the Laboratory of Research in Bacteriology of the Federal University of Uberlândia. To isolate the bacteria, the blood sample was cultured in 1 mL BHI (Brain Heart Infusion) broth and incubated at 35 ± 2°C, for 24 hours. The culture was then plated on TSA Agar (Trypticase Soy Agar) and Minnitol salt Agar, and the plates were incubated at 35 ± 2°C, for 24 hours. Colonies were subjected to Gram staining, catalase test (using 3% hydrogen peroxide), and coagulase exam (using 0.5 mL of rabbit plasma). To test antimicrobial susceptibility, a bacterial inoculum was prepared, and antimicrobial disks were placed on the agar surface and incubated at 35°C for 16 to 18 hours.

After incubation, the plate was examined under light, and the inhibition halo diameters were compared to the "Clinical and Laboratory Standards Institute©" (CLSI) document to classify as resistant or sensitive (Santoro *et al*., 2006; Walther *et al.*, 2008; Iverson *et al*., 2015).

RESULTS AND DISCUSSION

The occurrence of *Haemogregarina* sp. in turtles is commonly reported in the literature. Jakes et al. (2001) reported a 100% occurrence in the long-necked turtle (*Chelodina longicollis*) and the Brisbane short-necked turtle (*Emydura signata*), the same way as Mihalca et al. (2002) in the lagoon turtle (*Emys orbicularis*). In Brazil, a high occurrence of 98% (Soares *et al*., 2014) and 100% (Úngari *et al*., 2018) were reported for this genus of parasite in free-living *P. unifilis.*

The morphological and morphometric analyses showed gametocytes of different sizes and morphologies. One type of small gametocytes were observed with nuclei not evidenced, similar to a bean shape, with body length, width, and area of the parasite 8.47 ± 1.58 µm, 4.2 ± 1.5 µm, and 15.1 ± 2.4 µm^2, respectively. No measures of the nuclei were performed, since their nuclei were not delimited. The other type of gametocyte were robust, causing deformations in erythrocytes and displacement of cell nuclei, with nuclei of the parasite delimited; the body length, width, and area of the parasite were, respectively, 17.9 ± 1.3 µm, 5.4 ± 0.8 µm, and 39.1 ± 2.9 µm^2 and the nuclei length, width, and area of the parasite were, respectively, 8.25 ± 1.30 µm, 3.59 ± 0.8 µm, and 13.97 ± 1.42 µm^2 (Figure 1).

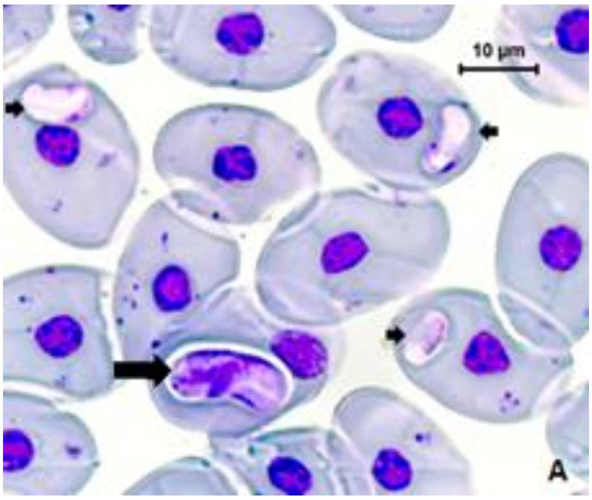

Figure 1 - Intraerithrocytic gametocytes of *Haemogregarina* sp. (Apicomplexa: Adeleina: Haemogregarinidae) in *Podocnemis unilifis*. Hemoparasites are indicated by arrows (Small arrows: immature gametocytes; big arrow: mature gametocytes). Scale: 10 µm.

The same morphologies of gametocytes were observed by Soares *et al.* (2014) and Picelli *et al.* (2015) from Brazilian *P. unifilis* and *P. expansa* turtles, respectively. The authors nominated the small gametocytes as microgametocytes and the robust gametocytes as macrogametocytes. The morphometric values were similar too. Therefore, from the morphological and morphometric analyses of the

hemoparasite blood developmental stages, it was possible to identify *Haemogregarina* sp. (Apicomplexa: Adeleina: Haemogregarinidae).

Regarding the bacteria, infection with *Staphylococcus aureus* was confirmed by the detection of protein A by the latex agglutination test (Staphclin). Moreover, the bacterial sample presenting multidrug resistance, including methicillin-resistant *Staphylococcus aureus* (MRSA), was identified.

Although *S. aureus* lineages are generally regarded as host-specific, some seem to have several hosts, as they can be isolated from both humans and animals (Hasman *et al*., 2010). In a study developed by Espinosa-Gongora *et al*. (2012), the author's analyzed 149 mammals and 21 reptiles kept at the Copenhagen Zoo. *S. aureus* was detected in 15 individuals, all of which were mammals. None of the isolates presented resistance to methicillin. According to these authors, the negative results obtained for the presence of *S. aureus* in reptiles could indicate that these animals are unlikely natural hosts of this species. However, a study by Walther *et al.* (2008) reported the presence of MRSA in exotic animals such as bats, parrots, and turtles.

The contact between wild animals and humans has increased progressively due to population growth, consequently, loss of habitat by wild animals. However, this proximity may be a public health problem since the pathogenic potential of some diseases that affect these animals. In this case report, the yellow-spotted river turtle was in its natural environment, which is bordered by a riverine population, whose sewage is released in the river. In addition, this population has a custom (regional culture) to feed on these turtles. The close contact between humans and turtles can provide the ideal transmission route for MRSA strain, being a possible explanation for the turtle infection observed in this case, since there are not many reports on this pathogen in reptiles, especially in their natural environment.

The specimen analyzed herein was co-infected with *Haemogregarina* sp. and, in the literature, concomitant infections in turtles have been reported usually associated with the quality of the environment in which they live. According to Santoro *et al.* (2006), the quality of water available to these animals influences the composition of the microbiota, and possible stress conditions can affect the host's immune system, making it more susceptible to infection by these opportunistic bacteria, which are normally present in its microbiota. Among these stress conditions, infection with another parasite should be considered. When an individual is parasitized, the immune system tries to fight the invader, most of the time unsuccessfully, as these hemoparasites have evasion mechanisms. The simple act of the organism fighting against this pathogen causes the host to automatically spend energy. This energy loss influences the behavior of the animal in nature, such as hunting, motility, mating, oviposition, among others (Amo *et al*., 2004).

CONCLUSION

From the above information, we can infer that the proximity of humans and wild animals and the frequent environmental contamination imposed by riverine population can directly interferes with the quality of life of wild animals, as well as in their health. In the case reported herein, it is not possible to say which infection led to susceptibility to the other; nevertheless, we can assume that hemogregarine parasite, together with the reduced quality of the river, were crucial for the described situation, since infection with hemoparasites makes individuals more susceptible to other pathologies.

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