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Hemoparasites in domestic cats from Uberlândia, Minas Gerais, Brazil: positivity and epidemiological factors

Hemoparasitos em gatos domésticos de Uberlândia, Minas Gerais, Brasil: positividade e fatores epidemiológicos

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Abstract: Hemoparasitosis in cats (*Felis catus*) is caused by protozoan and bacterial agents, primarily transmitted by fleas or ticks. This study was performed to evaluate the presence of hemoparasites in domestic cats from the urban region of Uberlândia (Minas Gerais State, Brazil) and associate positivity with epidemiological variables. Blood samples and data were collected from 300 cats. Blood was obtained from the ear tip, and two blood smears were prepared for each animal. Information on sex, breed, age, origin, habitat, access to external environments, presence of ectoparasites, diet, and region of origin was also gathered. In the blood smears, the positivity rates were 3.66% for *Babesia* spp., 5.33% for *Ehrlichia* spp., and 1.33% for *Mycoplasma* spp. Concomitant infections were observed between *Babesia* spp. and *Ehrlichia* spp. (0.66%) and *Babesia* spp. and *Mycoplasma* spp. (0.33%). Habitat, access to external environments, and region of origin were identified as significant factors for the occurrence of hemoparasitosis. Although most sampled cats lived in houses (66.66%), the majority had free access to outdoor areas (81.00%), increasing their exposure to ectoparasites and, consequently, hemoparasites. Among the city's regions, the highest positivity rate (3.33%) was observed in the east, potentially linked to the lower likelihood of guardians in this area keeping their cats strictly indoors, likely influenced by socioeconomic and cultural factors.

Keywords: *Babesia* spp.; vector-borne diseases; *Ehrlichia* spp.; *Mycoplasma* spp.

Resumo: A hemoparasitose em gatos (*Felis catus*) são causadas por protozoários e agentes bacterianos, transmitidos principalmente por pulgas ou carrapatos. Este estudo foi realizado para avaliar a presença de hemoparasitos em gatos domésticos da região urbana de Uberlândia (Minas Gerais, Brasil) e associar a positividade com variáveis epidemiológicas. Amostras de sangue e dados foram coletados de 300 gatos. O sangue foi obtido da ponta da orelha e duas extensões sanguíneas foram preparados para cada animal. Informações sobre sexo, raça, idade, origem, habitat, acesso a ambientes externos, presença de ectoparasitos, dieta e região de origem também foram coletadas. Nas extensões sanguíneas, as taxas de positividade foram de 3,66% para *Babesia* spp., 5,33% para *Ehrlichia* spp. e 1,33% para *Mycoplasma* spp. Infecções concomitantes foram observadas entre *Babesia* spp. e *Ehrlichia* spp. (0,66%) e *Babesia* spp. e

Mycoplasma spp. (0,33%). Habitat, acesso a ambientes externos e região de origem foram identificados como fatores significativos para a ocorrência de hemoparasitoses. Embora a maioria dos gatos amostrados vivesse em casas (66,66%), a maioria tinha livre acesso a áreas externas (81,00%), aumentando sua exposição a ectoparasitas e, consequentemente, hemoparasitos. Entre as regiões da cidade, a maior taxa de positividade (3,33%) foi observada na região leste, potencialmente ligada à menor probabilidade de os tutores dessa área manterem seus gatos estritamente dentro de casa, provavelmente influenciados por fatores socioeconômicos e culturais.

Palavras-chave: *Babesia* spp.; doenças transmitidas por vetores; *Ehrlichia* spp.; *Mycoplasma* spp.

1. Introduction

Several vector-borne diseases (VBDs) can affect cats in feline veterinary care. Among the primary pathogens are protozoans such as *Babesia* spp., *Cytauxzoon* spp., and *Hepatozoon* spp., as well as bacteria including *Mycoplasma* spp., *Ehrlichia* spp., and *Anaplasma* spp. [1–5]. Notably, cats that are not infested with fleas or ticks can also become infected with VBDs, suggesting alternative routes of transmission such as blood transfusion, ingestion of infected blood during fights, vertical transmission, or ingestion of paratenic hosts [6].

Feline VBDs typically occur in domestic cats under 3 years of age, with no predilection for sex or breed [6], and are detected based on clinical findings worldwide. The common clinical signs of ehrlichiosis and anaplasmosis include anorexia, lethargy, dehydration, anemia, thrombocytopenia, leukocytosis/leukopenia, and pancytopenia [2, 7, 8]. Feline babesiosis and cytauxzoonosis are characterized by lethargy, anorexia, anemia, thrombosis, circulatory impairment, systemic inflammatory response, and eventual death [9, 10]. Hepatozoonosis, however, usually presents without specific clinical features [11]. In all these cases, fever is uncommon. When fever is present, however, it often coincides with other concomitant diseases, particularly feline immunodeficiency virus and feline leukemia virus, which predispose cats to infections by other pathogens and exacerbate symptoms [12].

Considering the growing population of cats as companion animals, it is crucial to develop a thorough understanding of the true prevalence of these pathogenic agents [13, 14]. Furthermore, studying the epidemiological aspects of these VBDs is vital for predicting potential risks and their impacts on animal health. Therefore, this study was performed to assess the presence of hemoparasites in blood smears of domestic cats from the urban region of Uberlândia, state of Minas Gerais (Brazil), and to associate positivity with potential epidemiological variables.

2. Material and methods

2.1. Study area and animals

This study was conducted in Uberlândia, located in the Triângulo Mineiro microregion of Minas Gerais, Brazil (18º56’38”S–48º18’39”W). Blood samples and associated information were collected from 300 domestic cats, including both males and females of varying ages (being cats aged ≥8 months classified as young and those older considered adults) and different breeds. The cats were sourced from the urban area and studied between May 2017 and May 2018. The sample size was determined based on data from previous surveys conducted in Brazil and other countries [15–18].

All procedures described in this study were approved by the Ethics Committee on the Use of Animals of the Federal University of Uberlândia (CEUA/UFU, protocol number 011/17). Only cats that appeared clinically healthy (without visible signs of hemoparasitosis) were included in the study. All cats' guardians were informed about the research objectives and procedures, and blood samples were collected only after obtaining their authorization and signed consent forms.

2.2. Blood collection, blood smear preparation and analysis

After the animals were physically restrained according to standard semiological techniques in veterinary medicine for small animals [19], approximately 5 µL of blood was collected from marginal peripheral vessels on the right and left ear tips. This blood was used to prepare blood smears, which were fixed with methanol, stained using the May-Grünwald-Giemsa (MGG) method, and examined along their entire length under an optical microscope at 1000× magnification by two independent parasitologists (D.A.P. and M.M.O.). Following microscopic analysis, the results were communicated to the guardians through detailed reports.

2.3. Epidemiological inquiry

Throughout the questionnaires, we recorded the following information: sex (male or female), breed (defined or undefined), age (juvenile or adult), origin (University Veterinary Hospital - UVH, private clinic, cattery, or Animal Protection Association - APA), habitat (house, apartment, farm, farmhouse, country house or cottage, or street animal), access to sites (restricted or free), presence of ectoparasites (fleas, ticks, both fleas and ticks, or none), type of food (commercial feed, homemade, or mixed food), and region of the municipality to which they belonged (central, north, west, south, or east).

2.4. Statistical analysis

Univariate analyses were initially performed using GraphPad Prism software version 8.0 to evaluate the association between positivity and epidemiological variables. Positivity in blood smears was treated as the dependent variable, dichotomized as 0 (negative) and 1 (positive). Associations were assessed using chi-square tests (X^2), with a 95% confidence interval and a significance level of $p < 0.05$. Subsequently, multivariate analyses were conducted using a logistic regression model. This process involved two steps: a preliminary selection of variables from the bivariate analysis, including those with $p < 0.15$, and the construction of the final model, retaining only variables with a significance level of $p < 0.05$. Multivariate analyses were performed using EpiInfo software version 7.1.3.

3. Results

Of the total animals sampled in this study, 64.66% (194/300) were female cats, 96.66% (290/300) were mixed-breed cats, and 65.66% (197/300) were adult cats. Additionally, 81.66% (245/300) of the cats were seen at the Veterinary Hospital of the Federal University of Uberlândia, 66.66% (200/300) were living in houses, and 81.00% (243/300) had free access to external environments (e.g., streets). Furthermore, 52.00% (156/300) showed no infestation by ectoparasites, and 64.66% (194/300) were fed with commercial cat feed (Table 1).

Table 1. Positivity of cats by hemoparasites by microscopy.

Agent	Positive blood smear, n (%)
Babesia spp.	11 (3.66)
Ehrlichia spp.	16 (5.33)
Mycoplasma spp.	4 (1.33)
Babesia spp. + *Ehrlichia* spp.	2 (0.66)
Babesia spp. + *Mycoplasma* spp.	1 (0.33)
Total	34 (11.33)

n: Sample number; %: Frequency.

Out of the 300 cats evaluated, 11.33% (34/300) had positive blood smears, showing intracytoplasmic or peri-erythrocyte inclusions in blood cells (Figure 1). Among these, 3.66% (11/300) tested positive for *Babesia* spp., 5.33% (16/300) for *Ehrlichia* spp., and 1.33% (4/300) for *Mycoplasma* spp. Additionally, concomitant infections were observed in two cats (0.66%) with *Babesia* spp. and *Ehrlichia* spp. and in one cat (0.33%) with *Babesi*a spp. and *Mycoplasma* spp. (Table 1).

In analyzing the associations between the presence of hemoparasites and epidemiological variables, significant differences were observed in three of the nine variables analyzed: habitat ($p < 0.0189$), access sites ($p = 0.0067$), and the region of the city where the animals lived ($p < 0.0045$) (Table 2).

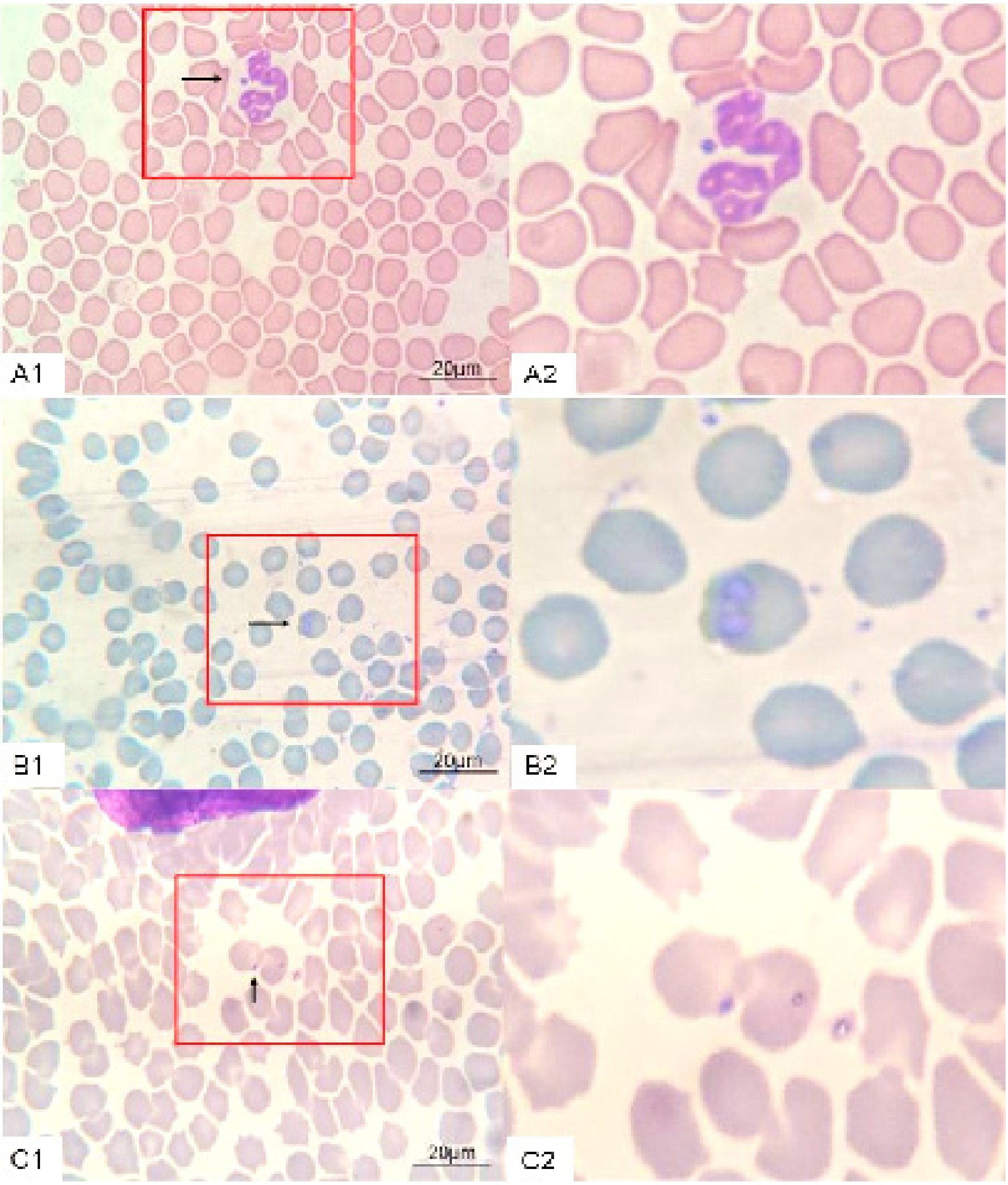

Figure 1. Photomicrograph of hemoparasites in blood smears (ear tip) of domestic cats. Morulae of *Ehrlichia* spp. in neutrophil cytoplasm - A1 and A2; Intraerythrocytic inclusion of *Babesia* spp., showing two merozoites (black arrow) – B1 and B2; Parasitic form of Mycoplasma spp. in the erythrocyte periphery with rounded corpuscle form (black arrow) – C1 and C2. (Stained by the MGG method, 100x objective).

Table 2. Absolute and relative values of the epidemiological variables of all domestic cats sampled in this study.

Variables		Examined cats		$p^{*\text{ or }\#}$
		n (%)	+ (%)	
Sex	Male	106 (35.33)	13 (4.33)	0.8504
	Female	194 (64.67)	21 (7.00)	
Breed	Defined	10 (3.33)	04 (1.33)	0.0460#
	Undefined	290 (96.67)	29 (9.66)	
Age	Juvenile	103 (34.33)	14 (4.66)	0.4280
	Adult	197 (65.67)	20 (6.66)	
Origin	UVH	245 (81.66)	28 (9.33)	0.0433*
	Private clinic	13 (4.33)	-	
	Cattery	21 (7.0)	-	
	APA	21 (7.0)	06 (2.0)	
Habitat	House	200 (66.66)	20 (6.66)	0.0189#
	Apartment	57 (19.00)	03 (1.00)	
	Farm	02 (0.66)	01 (0.33)	
	Farmhouse	05 (1.66)	01 (0.33)	
	Country house	30 (10.00)	08 (2.66)	
	Street animal	06 (2.00)	01 (0.66)	
Access	Restricted	57 (19.00)	15 (5.00)	0.0067#
	Free	243 (81.00)	19 (6.33)	
Ectoparasites	Fleas	133 (44.33)	14 (4.66)	0.5818
	Ticks	04 (1.33)	-	
	Fleas and ticks	07 (2.33)	02 (0.66)	
	Without	156 (52.00)	18 (6.00)	
Food	Cat feed	194 (64.67)	26 (8.66)	0.2652
	Homemade food	17 (5.66)	01 (0.33)	
	Mixed	89 (29.67)	07 (2.33)	
Region	Central	40 (13.33)	2 (0.66)	0.0045*
	North	53 (17.68)	7 (2.33)	
	West	82 (27.33)	9 (3.00)	
	South	55 (18.33)	6 (2.00)	
	East	70 (23.33)	10 (3.33)	

n = Total of 300 cats; +: Positive animals; %: Frequency; UVH: University Veterinary Hospital; APA: Animal Protection Association. #: Chi-square test for association between variables in the total population. *: Logistic regression for multivariate analysis based on the total population.

4. Discussion

Overall, among the 300 cats sampled, 11.33% tested positive for hemoparasites in their blood smears. The main detected parasites were *Babesia* spp. (3.66%), *Ehrlichia* spp. (5.33%), and *Mycoplasma* spp. (1.33%), with some cats presenting concomitant infections, including *Babesia* spp. and *Ehrlichia* spp. (two individuals, 0.66%) and *Babesia* spp. and *Mycoplasma* spp. (one individual, 0.33%). When evaluating associations between positivity and epidemiological variables, significant differences were observed for habitat ($p < 0.189$), access sites ($p = 0.0067$), and the region of the city where the animals lived ($p < 0.0045$). Regarding the origin of the animals, the highest percentage of positive cases (11.43%, 28/245) occurred in cats treated at the University Veterinary Hospital, which contributed most samples (81.66%, 245/300). It is noteworthy that blood sample collection for these cats was performed as part of the spaying/neutering project, which serves animals of all breeds, various age groups, and guardians with lower purchasing power. Animals from the Animal Protection Association also showed a significant positivity rate for hemoparasites (28.57%, 6/21), likely linked to the reception of strictly abandoned animals, some of which could have already been infected upon arrival. Additionally, most sampled animals were mixed breeds (96.67%, 290/300).

To diagnose hemoparasitosis, veterinarians typically rely on a combination of patient history, clinical signs, and laboratory tests [20]. Complementary diagnostic methods, including blood smears, serology, and polymerase chain reaction (PCR), may also be utilized, with PCR being considered the gold standard [9, 20]. Blood smears are valuable tools in the diagnosis and differentiation of hemoparasites because of their speed, ease of use, and cost-effectiveness compared with other methodologies. However, logistical limitations must be acknowledged, such as the quality of staining, the requirement for a trained microscopist, and the availability of microscopes, slides, and dyes [21]. One major challenge in blood smear analysis is differentiating parasitic forms from artifacts [21], which can lead to false-positive results. Additionally, chronic infections and pathogens with cyclic bacteremia pose diagnostic challenges [22, 23], making blood smears generally less sensitive than other diagnostic techniques. Mylonakis et al. [24] highlighted PCR assays as the preferred diagnostic method for accurately identifying hemoparasite species and advocated for their broader use in both epidemiological studies and diagnostic routines. Although PCR offers superior sensitivity and specificity, as noted by Sasaki et al. [25], Mylonakis et al. [24], and André et al. [26], its implementation requires well-equipped laboratories and trained personnel, which may limit its accessibility.

Blood smear positivity rates vary significantly between studies. High positivity rates were reported by De Oliveira et al. [20], who observed 81.25% for *Mycoplasma* spp. (13/16), 12.5% for *Anaplasma* spp. (2/16), and 6.25% for *Babesia* spp. (1/16) in cats from Espírito Santo. By contrast, lower rates were documented by Carvalho et al. [27], who reported a positivity of 1.5% for *Mycoplasma* spp. (2/135) in cats from Goiás. Additionally, Malheiros et al. [28] and De Oliveira et al. [29] did not detect any positive cases in their microscopic analysis. Using PCR, studies in Uberlândia and surrounding areas have reported positivity rates of 12.3% for *Anaplasma* spp., 4.5% for *Ehrlichia* spp., 41.9% for *Cytauxzoon* spp., and 0.6% for *Babesia* spp. [26]. Furthermore, *Cytauxzoon felis* and *Mycoplasma haemofelis* have also been identified in the region via PCR [30].

The study by André et al. [26] notably identified the piroplasm sequence obtained as *Babesia vogeli*. This species is commonly transmitted by *Rhipicephalus sanguineus* sensu lato in dogs and has already been detected in 17 naturally infected dogs in Uberlândia [31]. According to Penzhorn and Oosthuizen [9], *B. vogeli* appears to be the most widespread species infecting domestic dogs and cats in Brazil. Similarly, *Ehrlichia* spp. is transmitted by *R. sanguineus* sensu lato [32], while *Mycoplasma* spp. is transmitted by fleas, particularly *Ctenocephalides felis* [33, 34]. A particularly significant relationship was observed between cats with access to external environments and hemoparasite infections. As noted by Tasker [35] and Otranto & Dantas-Torres [36], outdoor access increases cats' exposure to a variety of ectoparasites, making them more vulnerable to parasitic infections. Although most guardians in our study reported that their cats' main habitat was indoors (66.66%, 200/300), most also allowed their cats free access to external environments (81.00%, 243/300). Despite finding no significant differences between ectoparasite infestations and the hemoparasites investigated, an important consideration is cats' meticulous grooming behavior, which helps reduce these infestations. However, hemoparasite transmission depends on the presence of infected vectors rather than high infestation levels [37, 38]. These findings align with previous data by Dos Santos et al. [39], Bergmann & Hartmann [40], and Persichetti et al. [37], particularly regarding the increased risk of hemoparasite infections in cats with outdoor access who are not protected by preventive ectoparasiticide treatments.

Finally, significant differences were observed regarding the region of the study area and positivity rates, with the east region of Uberlândia showing the highest number of positive cats (3.33%, 10/300), followed by the west (3.00%, 9/300), north (2.33%, 7/300), south (2.00%, 6/300), and central region (0.66%, 2/300). The east region comprises 15 integrated neighborhoods and is notable for its territorial and demographic heterogeneity, characterized by intense urban expansion and land value increases driven by natural features. The local population predominantly consists of individuals with a low to medium socioeconomic status, interspersed with small areas catering to higher-income populations. Socioeconomic and cultural factors may contribute to the increased dissemination of pathogens because some guardians may not keep their animals strictly domiciled, increasing their exposure to potential vectors. However, Arruda et al. [41] found no statistical relationship between socioeconomic profiles and practices such as sanitation, parasite knowledge or animal care. These authors also observed that vaccination and deworming were more commonly practiced by dog guardians than cat guardians. This discrepancy is often attributed to the greater challenges associated with managing feline care.

5. Conclusion

All the epidemiological variables explored in this study highlight that controlling hemoparasite infections can be effectively achieved by restricting cats' free access to outdoor environments, implementing ectoparasite control measures, and promoting spaying/neutering among their populations.

Supplementary material

Graphical Abstract (only available in the electronic version).

Conflict of interest statement

The authors declare no conflict of interests.

Data availability statement

The full data set that supports the results of this study was published in the paper itself.

Author contributions

Conceptualization: Pereira, D. A. and Cury, M. C. *Data curation:* Pereira, D. A. and Oliveira, M. M. *Formal analysis:* Pereira, D. A. and Oliveira, M. M. *Funding acquisition*: Cury, M. C. *Project management:* Pereira, D. A. and Oliveira, M. M. *Methodology:* Pereira, D. A. and Oliveira, M. M. *Supervision:* Cury, M. C. *Investigation:* Pereira, D. A., Oliveira, M. M., Miranda, J.S., Pereira, N.A., and de Aguiar, D. M. *Visualization:* Pereira, D. A., Oliveira, M. M., and Cury, M. C. *Writing (original draft)*: Pereira, D. A. and Oliveira, M. M. *Writing (proofreading and editing):* All authors.

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