



## **Influence of season on gastrointestinal parasites in pigs with an emphasis on *Balantioides coli* in a city with a tropical climate<sup>1</sup>**

Camila S.C. Class<sup>2</sup>, Breno T. da Silva<sup>2</sup>, Ingrid S. Reis<sup>2</sup>, Laís L. Corrêa<sup>2</sup>,  
Fabiana B. Knackfuss<sup>3</sup>, Daniel C. Trindade<sup>4</sup>, Roberto Júnio P. Dias<sup>5</sup>,  
Alynnne S. Barbosa<sup>2\*</sup>

**ABSTRACT.**- Class CSC, da Silva BT, Reis IS, Corrêa LL, Knackfuss FB, Trindade DC, Dias RJP, Barbosa AS. **Influence of season on gastrointestinal parasites in pigs with an emphasis on *Balantioides coli* in a city with a tropical climate.** *Pesquisa Veterinária Brasileira* 45:e07697, 2025. Laboratório de Parasitologia, Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Niterói, RJ 24020-141, Brazil. E-mail: [alynnedsb@gmail.com](mailto:alynnedsb@gmail.com), [alynnebarbosa@id.uff.br](mailto:alynnebarbosa@id.uff.br)

Rio de Janeiro is a state with a hot and humid climate throughout the year. This characteristic can generate parasitic dynamics that are poorly described in the literature, particularly in pigs, which have low tolerance for high temperatures. This study reports the diagnosis of gastrointestinal parasites in pigs in Rio de Janeiro and characterizes the effects of seasons on *Balantioides coli*. Samples from 21 pigs on an industrial farm were analyzed across seasons. Parasites were detected by qualitative and quantitative microscopic techniques, and *B. coli* was molecularly characterized in the feces of two pigs. Climatic data were retrieved from the state's meteorological station. A statistically significant difference in the number of animals positive for parasites between the seasons of the year was detected. In addition, significant differences in the quantification of *B. coli* and *Ascaris suum* between seasons were detected, with the highest counts observed in autumn. Through molecular analysis, *B. coli* infection was confirmed in all fecal samples collected across seasons from two pigs, with type A0 being predominant. Genetic variants of *B. coli* differed according to season, highlighting the heterogeneous distribution of genetic material in parasitic cells. Overall, the diagnosis of gastrointestinal parasite infection in pigs of reproductive age kept on one property located in the state of Rio de Janeiro was most common in autumn.

INDEX TERMS: Swine, helminths, protozoa, Rio de Janeiro.

**RESUMO.**- [Influência da estação do ano sobre parasitos gastrointestinais em suínos, com ênfase em *Balantioides coli*, em uma cidade com clima tropical.] O estado do Rio de Janeiro apresenta clima predominantemente quente e

úmido ao longo de todo o ano. Essa condição climática pode favorecer dinâmicas parasitárias ainda pouco descritas na literatura, especialmente em suínos, que apresentam baixa tolerância a altas temperaturas. O presente estudo relata o diagnóstico de parasitos gastrointestinais em suínos no estado do Rio de Janeiro, bem como caracteriza os efeitos dos períodos sazonais sobre *Balantioides coli*. Foram analisadas amostras fecais de 21 suínos provenientes de uma granja tecnificada, ao longo das quatro estações do ano. A detecção dos parasitos foi realizada por meio de técnicas microscópicas qualitativas e quantitativas, e a caracterização molecular de *B. coli* foi conduzida em amostras de fezes de dois animais. Os dados climáticos foram obtidos junto à estação meteorológica estadual. Verificou-se diferença estatisticamente significativa no número de animais positivos para parasitos entre as diferentes estações. Além disso, diferenças significativas na

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<sup>2</sup>Laboratório de Parasitologia, Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense (UFF), Niterói, RJ 24020-141, Brazil. \*Corresponding author: [alynnedsb@gmail.com](mailto:alynnedsb@gmail.com), [alynnebarbosa@id.uff.br](mailto:alynnebarbosa@id.uff.br)

<sup>3</sup>Laboratório de Estatística, Universidade Unigranrio Afya, Duque de Caxias, RJ, Brazil.

<sup>4</sup>Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Campus Pinheiral, Pinheiral, RJ, Brazil.

<sup>5</sup>Laboratório de Protozoologia, Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora, MG, Brazil.

quantificação de *B. coli* e *Ascaris suum* entre as estações foram detectadas, com os maiores valores de contagem observados no outono. A análise molecular confirmou a infecção por *B. coli* em todas as amostras fecais coletadas nas quatro estações dos dois suínos avaliados, sendo o genótipo A0 o mais prevalente. Variantes genéticas de *B. coli* diferiram de acordo com a estação, indicando uma distribuição desigual do material genético entre as células parasitárias. De forma geral, os resultados indicam que o diagnóstico de infecção por parasitos gastrointestinais em suínos em idade reprodutiva, mantidos em uma propriedade localizada no estado do Rio de Janeiro, foi mais prevalente durante o outono.

TERMOS DE INDEXAÇÃO: Suínos, helmintos, protozoário, Rio de Janeiro.

## INTRODUCTION

Gastrointestinal parasites can cause significant health problems in animals and be a limiting factor in pig production (Class et al. 2022). This issue becomes even more pronounced in tropical countries, which present ideal climatic conditions for the development and maintenance of infective parasitic forms in the environment.

Pigs are considered the main reservoirs of the zoonotic parasite *Balantioides coli*, a protozoan that can infect other host species, including humans, particularly those in close contact with these animals, such as veterinarians and rural producers, who are thus considered a risk group (Soleymani-Mohammadi & Petri Jr 2006, Barbosa et al. 2018, Silva et al. 2021, Class et al. 2022). *Balantioides coli* is considered the only parasitic ciliated protozoan in humans and is transmitted mainly through the ingestion of cysts that contaminate water and food with feces from pigs and other hosts, particularly fruits and vegetables (Soleymani-Mohammadi & Petri Jr 2006, Barbosa et al. 2018). Based on a systematic review, it was found that a considerable number of articles on human balantidiasis were published in South America and Asia, mostly in Brazil and India, respectively. However, in Africa, Ethiopia showed the highest number of infected individuals (Silva et al. 2021).

The presence of zoonotic parasites in pigs is a continuous concern for public health, as these agents can cause serious diseases in humans, especially dysenteric conditions (Silva et al. 2021, Lee et al. 2022, Alegre et al. 2024). It is believed that infected pigs remain asymptomatic (Zaman 1978). However, some authors have noted that these animals may present acute or chronic intestinal clinical manifestations (Schuster & Ramirez-Avila 2008). Death in pigs is rare but can occur one to three weeks after the onset of diarrhea (Sobestiansky et al. 1999).

Several factors can contribute to the prevalence of gastrointestinal parasites in pigs, including farm infrastructure, sanitary management, nutritional management, the genetic characteristics and age range of the animals, geographical location, and climatic factors (D'Alencar et al. 2011, Kumsa & Kifle 2014, Mattos et al. 2020).

In recent years, Brazil has ranked fourth in the world in pig production and third in the export of pork (Embrapa 2024). Despite the importance of pig production in tropical and subtropical countries, the relevance of climatic factors to the incidence of pig parasites is still poorly understood. In

this context, this study reports the dynamics of qualitative and quantitative diagnoses of gastrointestinal parasites in pigs kept on a farm located in a tropical area in Brazil and molecularly characterizes the protozoan *B. coli* across seasons.

## MATERIALS AND METHODS

**Ethics approval.** This study was approved by the Animal Ethics Committee (CEUA) of the Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ) under registration number 03/2022.

**Study location and sampling.** Fecal samples were collected between 2023 to 2024 on an industrial pig farm located in Pinheiral, Rio de Janeiro (Fig. 1). All pigs intended for reproduction were included, comprising 21 sows and four boars, totaling 25 animals from the Choice Genetics lineage. Pigs designated for reproduction were selected because conducting seasonal studies with pigs in other categories is hindered by the short time these animals spend in the nursery (from 21 to 65 days of age) and finishing/fattening phases (from 65 to 150 days of age).

The selected animals were properly identified with numbered ear tags and housed in collective pens accommodating a maximum of three animals, except for the boars, which remained in individual pens. All pens had a roof, cement floor, nipple drinkers, and masonry troughs fixed to the ground. All animals were fed daily with prefabricated feed on the property and had *ad libitum* access to potable water supplied by the municipality. For parasite control, the pigs were treated with an injectable macrolide lactone, administered twice a year to males and after weaning to females. Additionally, once a year, the pigs were vaccinated against paratyphoid, pasteurellosis, colibacillosis, erysipelas, atrophic rhinitis, and leptospirosis; females also received an annual booster against parvovirus.

**Sample collection, laboratory processing and climatic information.** Fecal samples were collected from the same animals at specific times across the four seasons. The samples were collected directly from the rectal ampulla using a glycerin-lubricated palpation glove or immediately after defecation. On the same day, the fecal samples were analyzed using direct examination, as well as qualitative coproparasitological techniques, including sedimentation (Lutz 1919) and modified centrifugal flotation (Sheather 1923, Huber et al. 2003). In addition, quantitative analysis was performed using the FLOTAC technique (Cringoli et al. 2010) with a NaCl solution (density = 1.200 g/mL). The microscope slides and FLOTAC chambers were evaluated under an Olympus® BX 41 binocular light microscope at 100x magnification and, if necessary, at 400x magnification for confirmation.

Fecal samples from two randomly chosen animals were analyzed using molecular tools to confirm and characterize the parasite *Balantioides coli* across seasons. DNA was extracted with the QIAamp Fast DNA Stool Mini Kit (Qiagen®). The DNA was subjected to polymerase chain reaction (PCR) using primers described by Ponce-Gordo et al. (2008) that amplify the ITS1.5.8S.ITS2 region. Master Mix Hot Start Platinum (Invitrogen®) was used for the reaction. The PCR-amplified products were purified with the ExoSAP-IT enzyme (Invitrogen®) and sequenced with an ABI 3730xL automatic sequencer on the IOC/Fiocruz platform. Only one amplified DNA sample per animal per season was sequenced.

The analysis and initial editing of the sequences were performed using Chromas Pro software, version 1.7.5, and with BioEdit software, version 7.2.5. Phylogenetic inferences were obtained from maximum likelihood analyses for confirmation with bootstrap support based on 1,000 replications. The best evolutionary model was selected

based on the Akaike information criterion using W-IQ-Tree software<sup>6</sup>. Phylogenetic tree editing and rooting were performed using MEGA-X software, version 11.

Additionally, on the same day as sample collection, climatic information, including minimum and maximum temperature and humidity, was retrieved from the website of the “Instituto Nacional de Meteorologia” (National Institute of Meteorology – INMET) of the “Ministério da Agricultura, Pecuária e Abastecimento” (Ministry of Agriculture and Livestock – MAPA), Brazil, which compiles data from meteorological stations across the state. This methodology was employed to retrieve official data because the property did not have equipment for this purpose. These data were stored in Microsoft Excel<sup>®</sup> spreadsheets.

**Statistical analysis.** The non-parametric Kruskal-Wallis statistical test was used, because the data were neither normally distributed nor homoscedastic, the test was used to compare the number of animals positive for the identified parasitic taxa and quantify parasitic forms across seasons. The Dunn test was used as a *post hoc* test to perform pairwise comparisons of parasite quantification across seasons. The results were considered significant when *p*-values were less than 5% ( $p < 0.05$ ). These analyses were performed with SPSS software version 29.0.

## RESULTS

Of the 25 pigs initially included, four were excluded due to death, uterine prolapse, stroke, or age. Thus, fecal samples were collected from 21 animals across the four seasons. The highest frequency of positivity for all identified parasite taxa, including *Balantioides coli*, coccidia, and *Ascaris suum*, was in autumn. Both forms of the Ciliophora group compatible with *B. coli*, namely cysts and trophozoites, were detected in the fecal samples, with cysts detected in 56 fecal samples, both cysts and trophozoites in two samples, and only trophozoites in one sample. Trophozoites were detected only in spring and summer.

Overall, a statistically significant difference was observed in the quantification of *B. coli* forms and *A. suum* eggs in the fecal samples obtained between the seasons, with the highest count values observed in autumn (Table 1). *Post hoc* analysis revealed significant differences for *B. coli* between autumn and spring ( $p = 0.0124$ ), autumn and summer ( $p = 0.0273$ ), and autumn and winter ( $p = 0.0083$ ), and for *A. suum* in those obtained between autumn and spring ( $p = 0.001$ ) and spring and summer ( $p = 0.036$ ). When pigs were analyzed



Fig. 1. Location map of Pinheiral city where the pig farm is located, Rio de Janeiro state, Brazil. Produced by the authors with QGIS software version 3.40.3.

<sup>6</sup> Accessed on Jan. 23, 2025. <http://iqtree.cibiv.univie.ac.at/>

individually, most animals tested positive, with higher counts of Ciliophora group forms and *A. suum* in autumn. However, for coccidia, fecal samples from Pigs 8 and 21 collected in summer showed markedly higher values compared with samples from the other seasons and animals.

Eight fecal samples collected from pigs in different seasons were subjected to PCR and genetic sequencing, generating nucleotide fragments compatible with *B. coli*. The identity values ranged between 100% and 98.3%, and all generated sequences were deposited in GenBank. The samples that generated the sequences PV105082, PV105084, PV105086, and PV105088 were obtained from female pigs. The sequences PV105083, PV105085, PV105087, and PV105089 were obtained from a male pig. The nucleotide sequences of *B. coli* from the male pig corresponded to the A0 variant. Only one *B. coli* sequence collected from a female in spring was classified as A0, while fragments from this animal's samples collected in other seasons were classified as B0 (Fig. 2).

## DISCUSSION

In this study, the conditions observed in autumn appear to have favorable for the diagnosis of infection with all identified parasite taxa. A similar result was observed on an industrial pig farm in China, where the highest positivity rate for parasites in this animal category was also found in autumn (Lai et al. 2011). In pig farms in Greece and China, autumn was also the season that favored the diagnosis of parasitic infections (Symeonidou et al. 2020). However, on a pig farm in Korea, the highest number of parasitized animals was detected in spring, followed by autumn (Lee et al. 2022).

Overall, most animals appeared to substantially contribute to the detection of forms of the Ciliophora group compatible

with *B. coli* and *A. suum* during the autumn season, indicating that the result was not driven by a single animal sample. The relevance of autumn in favoring the diagnosis of parasites, but mainly in accounting for the parasitic forms of *B. coli* and *A. suum* as evidenced in this study, may be related to (i) the better thermal comfort that this season provides the hosts and consequently to the parasites; (ii) the greater viability of parasites in the autumn environment, since autumn in Rio de Janeiro is still a hot and humid season but does not present temperature extremes like those observed during summer; and (iii) the development of infective forms, especially the development of helminth larvae within their eggs in the environment.

The forms of the Ciliophora group compatible with *B. coli* most commonly detected were cysts, with the diagnosis of trophozoites being less evident. Although this study did not have a clinical character, it can be verified that the fecal samples from the animals did not present a liquefied consistency; all samples maintained a formed structure. This characteristic, associated with the majority diagnosis of the cystic form, highlights a possible subclinical infection of the animals. It is worth noting that the trophozoite forms were detected only by direct examination. Direct examination is the most suitable technique for identifying trophozoites, as coproparasitological techniques, especially those that use flotation solutions, can destroy the trophozoite form, making the detection of the parasite difficult. Furthermore, flotation techniques are not ideal for recovering *B. coli* cysts, as they have low sensitivity and can shrink the cystic form, making its identification difficult. These issues have been previously reported by our research group (Barbosa et al. 2016).

Due to financial limitations, feces from only two pigs positive for Ciliophora group forms were analyzed using

**Table 1. Frequency and quantification of gastrointestinal parasites detected in fecal samples from pigs kept on a farm in Rio de Janeiro from 2023 to 2024, across the four seasons of the year and based on meteorological data**

Seasonal information	Gastrointestinal parasites					
	<i>Balantioides coli</i>		Coccidia		<i>Ascaris suum</i>	
	Frequency (%)	Count Mean ± SD	Frequency (%)	Count Mean ± SD	Frequency (%)	Count Mean ± SD
Spring (September to November) T °C: 24.2 - 27.1 RH: 71 - 79	11 (52.3)	0.4 ± 0.92	11 (52.3)	35.23 ± 130.53	1 (4.7)	0.04 ± 0.21
Summer (December to February) T °C: 22.9 - 23.8 RH: 85 - 93	11 (52.3)	0.61 ± 1.56	14 (66.6)	220.42 ± 878.95	8 (38)	1.095 ± 2.04
Autumn (March to May) T °C: 16.7 - 18.6 RH: 65 - 73	21 (100)	4.095 ± 7.98	19 (90.4)	22.28 ± 56.85	12 (57.1)	1.76 ± 2.34
Winter (June to August) T °C: 17.9 - 19.6 RH: 70 - 76	16 (76.1)	0.47 ± 0.77	11 (52.3)	40.04 ± 68.08	9 (42.8)	0.66 ± 0.88
	0.0019*a	0.0262*b	0.0386*a	0.2391b	0.0089*a	0.0113*b

SD = standard deviation, T = temperature, RH = relative humidity, \* p ≤ 0.05: statistically significant, a = analysis of frequency of positive animals using the Kruskal-Wallis test, b = analysis of quantitative results obtained using the Kruskal-Wallis test.

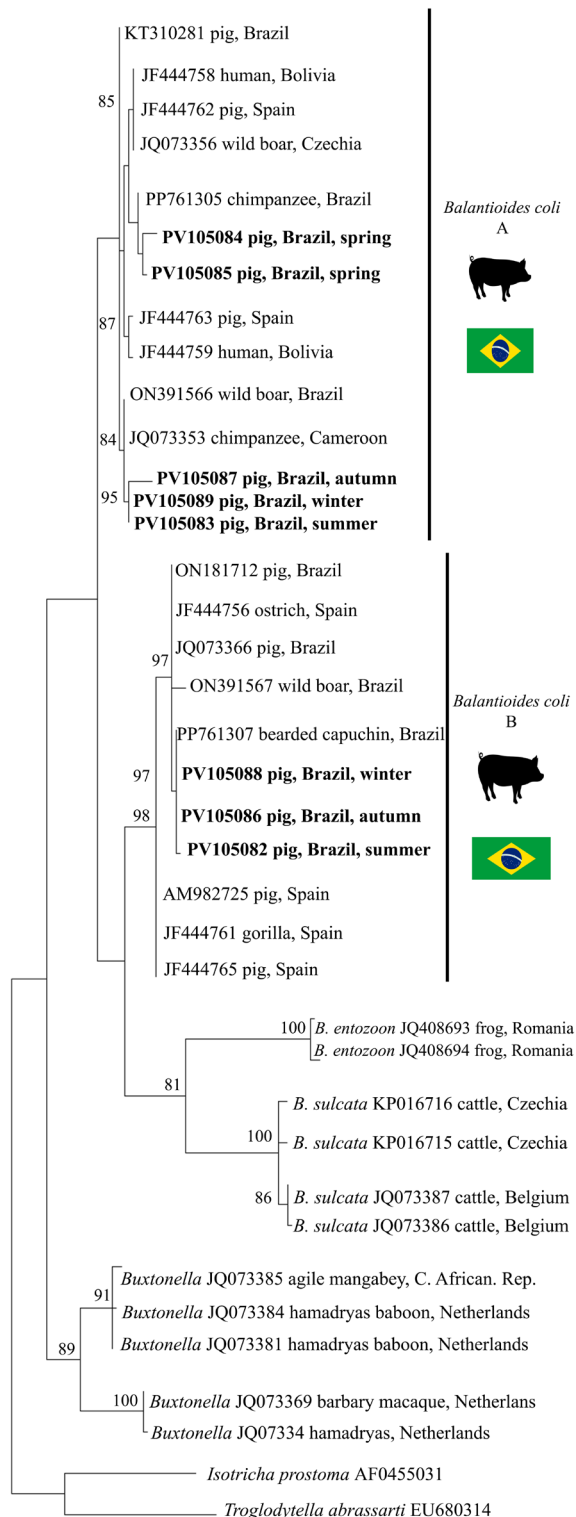


Fig. 2. A phylogenetic tree was constructed based on the alignment of 370 base pairs (bp) of the DNA fragment of the rRNA gene and the ITS1-5.8S rRNA-ITS2 fragment from ciliated protozoa using the maximum likelihood method with the TIM2+F+9 evolutionary model G4. The sequences from this study are highlighted in bold and separated by the genetic variant. The nucleotide sequences of *Balantioides coli* in this study were generated from Ciliophora group forms that were detected in the feces of pigs kept on a farm in Rio de Janeiro, collected from 2023 to 2024.

molecular tools across seasons; nucleotide sequences of *B. coli* were detected in all the samples. The feces obtained from the male pig showed the same type of genetic variant, type A0, in all four collections. In contrast, the genetic sequence of *B. coli* identified in the female varied with the day of collection, with the B0 variant being the predominant variant identified. The A0 and B0 sequences were similar to those reported in other studies conducted in Brazil and other countries (Ponce-Gordo et al. 2011, Pomajbíková et al. 2013, Barbosa et al. 2017, Pinheiro et al. 2023, Class et al. 2024, Dib et al. 2025).

In the molecular characterization of *B. coli*, no double peaks were detected on the electropherogram. However, differences in genetic variants were observed according to the sample collection date from the same animal, highlighting that the animal was simultaneously parasitized by both A and B variants of *B. coli*. This parasite has at least two rRNA genes present in the same genome (one coding for the type A variant and the other for B) (Ponce-Gordo et al. 2011). The detection of one variant over the other according to the season may be related to the predominance of the variant type evidenced in the amplified DNA product present in the sample that was sequenced. Additionally, the difference in variants may be related to the unequal amount of genetic material of the variant type distributed in the macronucleus of the parasite (Preer et al. 1999). This unequal distribution of genetic material in the macronucleus of ciliates may be related to genetic and epigenetic factors (Preer et al. 1999, Ponce-Gordo et al. 2011). The environmental temperature and relative humidity inherent to the season may be one of the epigenetic factors. However, more studies are needed to evaluate the influence of these factors on parasitology.

It is important to highlight that workers on the farm where the study was conducted performed daily manual removal of feces from the pens using shovels and then washed the area with water and detergent to eliminate excess organic matter. Additionally, during the sanitary break, flame sanitation was performed, and lime was applied to the walls. These sanitary management practices, combined with the routine administration of anthelmintics to the animals, may have influenced the results, particularly those related to helminths. Common pig parasites, such as *Strongyloides ransomi*, strongyles (*Hyostromylus rubidus*, *Trichostrongylus axei*, *Oesophagostomum* spp., *Globocephalus urosubulatus*), and *Trichuris suis*, were not detected. This rigorous management may also explain the low frequency of *A. suum* observed. These results are in line with those observed in previous studies conducted in industrial farms in Brazil, where a low frequency of nematodes was also observed due to the strictness of sanitary management (D'Alencar et al. 2011, Barbosa et al. 2015, Pradella et al. 2020).

Regarding coccidia, the highest number of animals testing positive for this group of parasites was recorded in autumn; however, the highest oocyst counts were observed in summer. The elevated temperatures and humidity typical of the summer season in Rio de Janeiro were not sufficient to hinder these parasitic forms. It is known that coccidian oocysts are highly resistant structures in the environment (Sobestiansky et al. 1999). Furthermore, the possibility cannot be ruled out that the high summer temperatures in Rio de Janeiro may have favored the release of oocysts due to the acceleration of intestinal metabolism. These situations may have contributed to the

higher prevalence of positive cases in autumn, the season immediately following summer. Nonetheless, it appears that a few specific animals disproportionately contributed to the elevated oocyst counts observed during the summer, possibly due to differences in management practices and individual immune responses.

## CONCLUSION

Autumn was identified as the season most conducive to the detection of gastrointestinal parasites in pigs from reproductive categories, specifically sows and boars, with *Balantioides coli* being the most frequently observed parasite. Moreover, it was observed that the genetic variants of *B. coli* differed depending on the day of fecal sample collection from the same pig included in the seasonal study.

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**Conflict of interest statement.**- The authors declare that they have no conflicts of interest.

**Credit author statement.**- Camila S. C. Class and Alynne S. Barbosa – Conceptualization, data curation, methodology, writing the draft manuscript and revising the writing manuscript. Daniel C. Trindade – Data collection. Breno T. da Silva – Data collection, data analysis and processing. Ingrid S. Reis, Laís L. Corrêa and Fabiana B. Knackfuss – Data Analysis and methodology. Roberto Júnio P. Dias – Revising the Writing Manuscript. Alynne S. Barbosa – Funding acquisition.

**Data availability statement.**- No datasets were generated or analyzed in the current study.

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