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Parasitological and microbiological evaluation of sand from beaches in São Luís, Maranhão, Brazil

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Abstract

Brazil has a coastline with large cities and a great flow of human activities, which can lead to contamination by microorganisms and parasites. Given this context, the objective of this study was to evaluate the parasitic and microbiological events in the sand of beaches in São Luís, Maranhão. Therefore, four samples were collected from three beaches, thus totaling 12 samples. Each area was divided into four distinct segments, two dry and two wet, and for each sample, sterile collectors were inserted into the sand at a depth of 20 centimeters. The samples were identified, placed in a Styrofoam box, and taken to the laboratory for analysis. The parasitological analysis was performed through spontaneous sedimentation and centrifugation, while the microbiological analysis used the COLItest® kit to determine total and thermotolerant coliforms (*Escherichia coli*). All the sand samples were positive (100%) for helminths and/or protozoa, and the most frequent helminth and protozoan were *Trichuris trichiura* (100%; n=12) and *Giardia lamblia* (83.3%; n=10), respectively. Microbiological analyses showed that 66.6% (8/12) were contaminated by total coliforms and 58.3% (7/12) by *Escherichia coli*. Collection point 3 of dry sand from São Marcos beach (Sm3S) showed the highest contamination by *Escherichia coli* (4.33×10^2 CFU/mL). The beaches on the seafront called *Litorânea* in São Luís are contaminated by parasites and fecal bacteria from animals or human sources on the beach. Thus, the probability of acquiring infections increases among individuals visiting these environments.

Keywords: Biological contamination. *Escherichia coli* infections. Parasites. Beach sanitation.

INTRODUCTION

Brazil is a country that has a coastline full of large cities and a large flow of human activities. In the summer, there is an increase in population due to the coast receiving many tourists, and the sanitary networks subsequently become burdened. Thus, the sands and waters more easily become contaminated by pathogenic agents, which may cause illnesses among those who visit this environment[1].

Enteroparasitoses are diseases caused by helminths and/or protozoa, whose evolutionary cycle takes place, in part, in the human digestive system. Among the medically relevant protozoa, *Entamoeba coli* and *Giardia lamblia* stand out. Meanwhile, the main species among helminths are *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, and *Necator americanus*. The infection

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caused by these pathogens may or may not present symptoms. When symptoms are present, they may include diarrhea, abdominal pain, fever, intestinal obstruction, and rectal prolapse. They may also interfere with the patient's nutritional status and cause small hemorrhages, favoring the development of anemia and even impaired cognitive function in school-aged children[2].

People acquire *A. lumbricoides* and *T. trichiura* infections by ingesting the infective form (embryonated egg), which may be present on dirty hands, raw or poorly washed food, and by consuming untreated or unfiltered water. An infection with Hookworms happens mainly by the active penetration of the infective larva into the skin or by oral route. This contagion occurs when there is direct contact with the soil contaminated with the larva or by its ingestion with water[3]. Meanwhile, with *Entamoeba coli* and *Endolimax nana*, protozoa contamination occurs mainly through soil, water, and vegetables contaminated with fecal material[4,5].

There are indications that pathogenic bacteria can survive in the sand, even though there are pollutants from different sources in this environment. However, favorable ecosystem factors such as temperature, humidity, pH, and organic matter contribute to the phenomenon of bioaccumulation. Thus, bacterial populations can influence sand contamination, whether these microorganisms are from human feces or warm-blooded animal feces. However, in recent years, the concern about contamination of beach sand has increased due to the inadequate distribution of garbage, untreated domestic sewage, animal waste, and pollution[6].

It is known that simple hygienic habits can prevent contamination by microorganisms and parasites, such as walking with shoes, washing food before consumption, washing hands, and avoiding bathing in contaminated water. Enteroparasitosis and infections caused by bacteria are considered a public health problem, as it affects a large part of the population. Given this context, this study aimed to evaluate the parasitic and microbiological content in the sands of beaches in São Luís, Maranhão.

MATERIALS AND METHODS

The study was conducted on the most visited beaches along the shoreline of the city of São Luís, the capital of the state of Maranhão. They are located on the so-called Litorânea, which is formed by the beaches of São Marcos (Sm), Calhau (Ca), and Caolho (Co) (Figure 1). Four samples were collected from each beach, totaling 12 samples, and the collection was carried out in the morning in August 2019.

Each beach was divided into four distinct strips, two dry (S), closer to the restaurants and boardwalk, and two wet (U), closer to the breaking waves. Each collection site was 2 km apart from each other. Furthermore, sterile collectors were used for each sample and were placed into the sand at a depth of 20 centimeters to obtain approximately 50g of sand[7]. The samples were identified and placed in a Styrofoam box with ice and taken to the Laboratory of Biomedical Sciences at Ceuma University, where the analyses were carried out immediately.

Fifty grams (50g) of sand were transferred to a 500mL beaker, mixed for 5 minutes with 300mL of autoclaved water, and filtered to obtain the biological content from the sand washing[8].

For the parasitological analyses, 200mL of sand washing water was processed by spontaneous sedimentation method for 24 hours. Then the precipitate was collected and centrifuged at 3000rpm for 3 minutes. The final pellet was collected and then transferred to the slide, stained with Lugol, and covered with a coverslip for analysis under an optical microscope.

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Finally, the observed parasitic forms were identified by their morphology[9].

For microbiological analyses, approximately 100mL of sand-washing water was used for each sample, which was submitted to microbiological analysis using the COLItest® kit. The test's sensitivity is one colony-forming unit (CFU) per 100mL. In the tubes containing the sand-washing water, COLItest® culture medium was added and homogenized, and the solution was incubated in ovens at 37ºC for 24 hours. For tubes with water samples testing positive for total coliform (change from purple to yellow), the indole test was performed by adding an indole reagent. The formation of a red ring on the surface of the medium was a positive indication of *Escherichia coli*. The negative control bottle contained autoclaved distilled water[10]. An aliquot of positive indole samples was removed and seeded in Petri dishes containing Eosin Blue Methylene Agar (EMB) culture medium and incubated at 37ºC for 24 hours. Finally, the characterization of thermotolerant coliforms (*Escherichia coli*) was evidenced by the growth of colonies with and without blackened centers and a metallic green sheen[11]. The number of colonies that developed on the plates was visually counted, and the number of CFU was determined using calculations referring to each dilution[12].

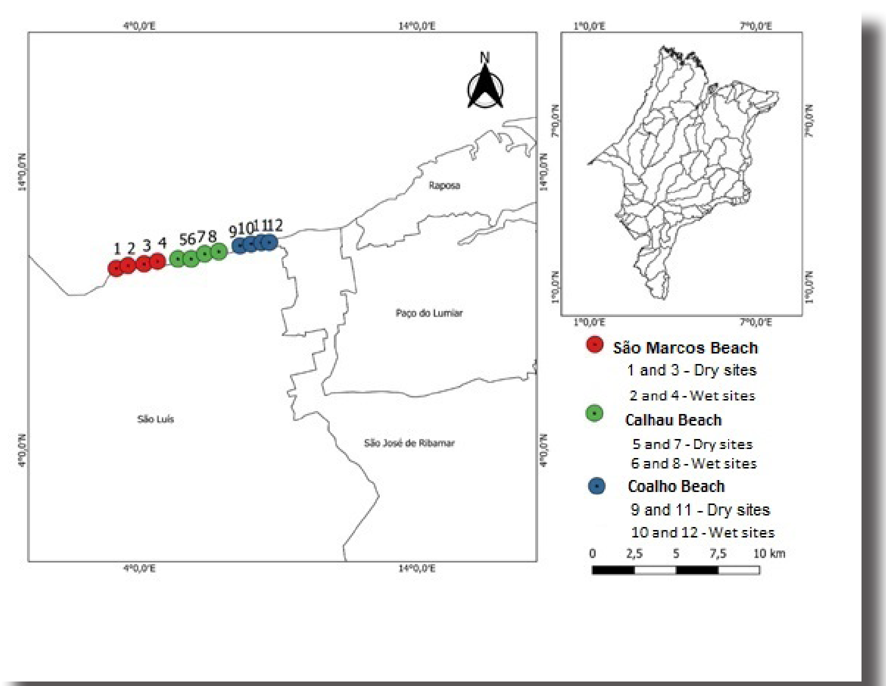

Figure 1 – Location of the study area.

RESULTS

All samples of sand (100% [12/12]) from the three beaches (100% [3/3]) were positive for the presence of one or more parasitic forms, either protozoan (cyst) or helminths (egg), indepen-

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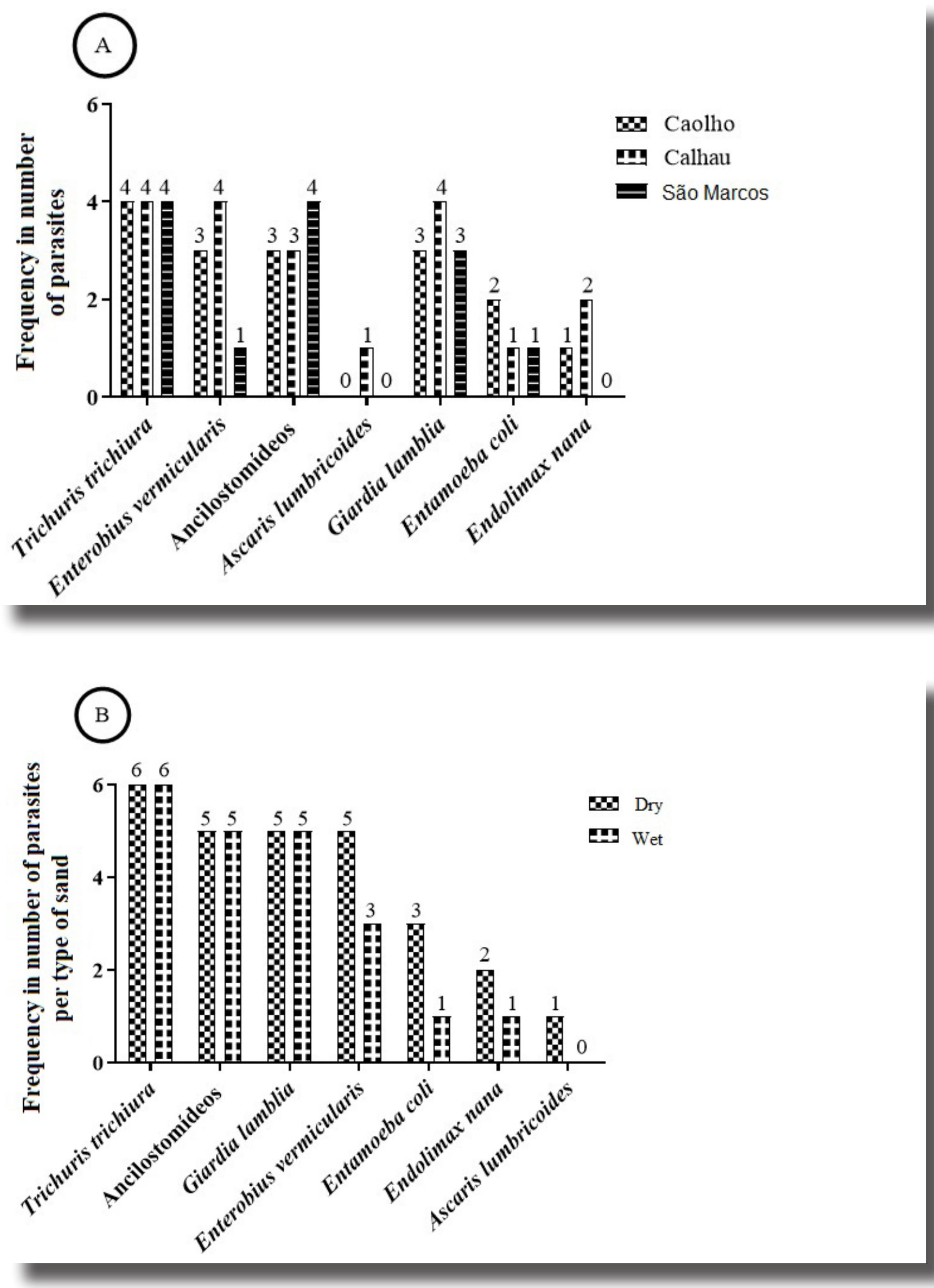
dently of the type of sand, either dry or wet. Four different species of helminths and three species of protozoa were identified, and the most common helminth was *T. trichiura* (100% [12/12]). In contrast, the most common protozoan was *G. lamblia* (83.3% [10/12]) (Figures 2A and 2B).



A: frequency in numbers of parasites per beach; B: frequency in number of parasites according to the type of sand samples, dry or wet.

Figure 2 – Species of parasites identified in samples of dry or wet sand from beaches in São Luís, Maranhão, Brazil, 2019.

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In Table 1, the microbiological evaluation demonstrated that the Caolho beach sample from collection site 4 of wet sand ($Co4_U$), the Calhau beach samples from collection site 3 of dry sand, and collection site 4 of wet sand ($Ca3_S$ and $Ca4_U$, respectively), and the São Marcos beach sample from collection site 4 of wet sand ($Sm4_U$) did not test positive for the coliform group. Meanwhile, the sample from collection site 1 of wet sand at São Marcos beach ($Sm1_S$) showed no growth of *Escherichia coli*. The site with the highest contamination of *Escherichia coli* was the sample from collection point 3 of dry sand from São Marcos beach ($Sm3_S$) with $4.33x10^2$ CFU/mL.

Table 1 – Population of total coliforms and *Escherichia coli* from sand samples from beaches in São Luís, Maranhão, Brazil, 2019.

		Coliforms	
Beaches	**Samples**	**Total**	***Escherichia coli* (CFU/mL)**
Caolho	$Co1_S$	Present	1.98×10^2
	$Co2_U$	Present	1.16×10^2
	$Co3_S$	Present	1.66×10^2
	$Co4_U$	Absent	Absent
Calhau	$Ca1_S$	Present	0.17×10^2
	$Ca2_U$	Present	2.23×10^2
	$Ca3_S$	Absent	Absent
	$Ca4_U$	Absent	Absent
São Marcos	$Sm1_S$	Present	Absent
	$Sm2_U$	Present	1.58×10^2
	$Sm3_S$	Present	4.33×10^2
	$Sm4_U$	Absent	Absent

Co= Caolho; Ca= Calhau; Sm= São Marcos; 1 to 4= number of samples; S= dry; U= wet; CFU/mL = colony forming unit per milliliter. Source: Authors.

Figure 3 shows the photographic record of Petri dishes with bacterial growth of *Escherichia coli* in some sand samples, and colonies with blackened appearance are characteristic of this species.

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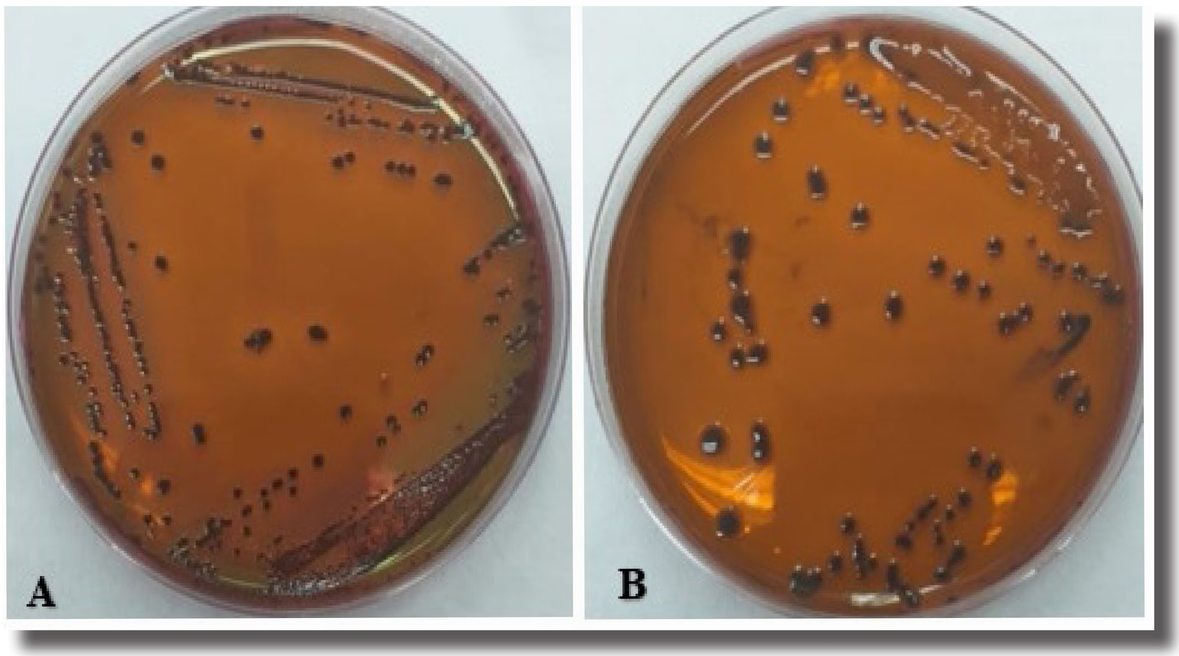

Growth of *Escherichia coli* on EMB agar after 24 hours of incubation at 37°C. A: sample of dry sand from Caolho beach at site 1; B: sample of wet sand from Caolho beach at site 2.

Figure 3 – Morphological characteristic of the *Escherichia coli* colony on EMB agar, obtained from the isolation of sand from beaches in São Luís, Maranhão, Brazil, 2019.

DISCUSSION

Considering the parasitological results of the sands, 100% were contaminated by helminths and/or protozoa. Ferraz *et al.*[13], in their work in which they analyzed the contamination of beach sand in the municipality of São Lourenço do Sul, RS, observed that 59% of the analyzed sands had some parasitic form, data that differs from ours. However, the contagion of these samples in both studies is worrisome, as it suggests a lack of hygiene among visitors and the presence of animals and sewage in the area. Thus, all these factors alter the environmental quality of the beach[14]. The massive presence of users also greatly contributes to contamination levels[15]. Moreover, the presence of materials such as food packaging, lollipop sticks and popsicles, plastic cups and bottles, food remains, and others[14] may facilitate the presence of animals on the beach.

In our study, the most prevalent parasite was the helminth *T. trichiura* in dry and wet sand types. However, Ferraz *et al.*[16] observed that Hookworms were the most prevalent helminths in their work with contaminated sand of beach Praia do Laranjal, Pelotas, RS. Nonetheless, in the present study, Hookworms were also observed. Pedrosa *et al.*[17] points out that these helminths are the most frequently observed in several studies on Brazilian beaches, being found in up to 75% of the samples. Furthermore, *T. trichiura* and Hookworms are geohelminths, as one of their evolutionary forms necessarily needs to undergo a stage in the soil; therefore, the presence of one of these parasitic agents in the sand with fecal contamination is common[3].

Other parasitic forms of helminths found in the study were *E. vermicularis* and *A. lumbricoides*. These are intestinal parasites, the second

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being a geo-helminth; both represent an extensive public health problem and are related to people's hygiene and socioeconomic level[18].

G. lamblia was the most predominant protozoan in the present study. Graciliano Neto, Farias, and Rocha[19] found similar results concerning the presence of *G. lamblia* in the sand of beaches along the coast of Maceió, AL. This parasite inhabits the small intestine of animals and men[20]; therefore, its presence is common in an environment contaminated by fecal material.

Furthermore, protozoans, *Entamoeba coli* and *E. nana* were observed. However, these parasites are considered commensal agents; the former is a marker of environmental contamination and may represent the presence of other parasitic forms. Contamination occurs mainly through the soil, water, and vegetables contaminated with fecal material[21].

The parasites mentioned above are commonly found on beaches due to the presence of sewage; they support pH differences and temperature changes and can thus contaminate people[3]. However, there is a need to intervene in this process to avoid contamination and, in this way, guarantee a better quality of life for beachgoers, avoiding infections and diseases associated with parasites.

It is known that there is a density of pathogenic microorganisms in a beach's water and sand, which can lead to repercussions for the environmental quality of that place. Therefore, it is very important to monitor pathogenic bacteria in beach sand because the spread of pollution in bathing areas is directly connected to the health of swimmers, making pollution an important public health problem[22]. Although Brazilian legislation recommends measuring indicators present in sand, this monitoring is done mainly in water in most parts of the country[23]. Still, fecal indicators in seawater suffer inactivation caused by sunlight and are exposed to the actions of bacteriophages, low nutrient content, predation, and competition with native organisms[24]. Therefore, the beach sand constitutes a protective environment for the survival of these bacteria, as they can adhere to sedimentary particles[25].

Regarding the microbiological aspects, eight sites were positive for total coliforms and seven sites for *Escherichia coli* (thermotolerant microorganism) in the present study. Cicero *et al.*[26], in their work on the contamination of beach sand in Brazil by pathological agents, noted that 60% were positive for total coliforms and 10% for *Escherichia coli*. These values are lower than those found in this study and in the work of Tenorio *et al.*[6] on the detection of total coliforms in beach sand in Caraguatatuba, SP, which detected a higher number compared to ours. Despite the methods used differing from that study, it is necessary to understand that several factors are associated with the presence and quantities of these pathogens. As explained by Braga *et al.*[27], different causes, such as the presence of animals, discharge of sanitary sewage, and change of season, have been shown to contribute to the survival and distribution of pathogenic microorganisms in beach sand such as bacteria of the Enterobacteriaceae family. Thus, the contact of people with an environment that has the presence of these microorganisms is worrisome.

Herein, the dry sand had higher contamination than the wet sand, corroborating the study by Panagassi and Catanozi[28] on the beach in the municipality of Estância Balneária de Praia Grande, SP, which reported that dry sand had a higher bacterial density than wet sand. These findings are justified since seawater is washing the adjacent sand (wet/humid), which does not occur with the sand further away (dry). Another hypothesis is that the dry sand is closer to the sidewalk, food vendors, the displacement of pedestrians, dogs, and accumulated garbage. These observations strengthen the argument for more significant microbiological contamination in dry sand[28].

The presence of pathogens in the sand poses a health threat to visitors who may be susceptible to diseases caused by microorga-

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nisms. Increased cases of bacterial infections acquired by people who visit the beaches and use the sands for leisure have been confirmed[6]. When these bacteria are resistant to antibiotic treatment, this health problem becomes much more challenging to solve. There are also reports of resistance to some antibacterial agents by enterobacteria in the literature[29].

CONCLUSION

The sand on the main beaches of São Luís is contaminated by parasites and bacteria from sources that have come into contact with feces, whether from animals or humans. Thus, the probability of acquiring infections among individuals who visit these environments increases, mainly due to the presence of sewers, loose animals, and inadequate hygienic habits of the visitors. Thus, public health policies must be adopted, such as improved basic sanitation, actions to raise public awareness, elimination of vectors, and screening for possible means of transmission.

Author Statement CREdiT

Project Administration: Viana, AL; Bastos, DKL; Firm, WCA. Formal Analysis: Viana, AL; Mosque, AD; Sierra, AKM; I sign WCA. Conceptualization: Viana, AL; Mosque, AD; Sierra, AKM; Bastos, DKL; Firm, WCA. Data Curation: Viana, AL; Bastos, DKL; Firm, WCA. Writing – First Editorial: Viana, AL; Silva, DF; Silva, MRC; Firm, WCA. Writing – Proofreading and Editing: Viana, AL; Mosque, AD; Sierra, AKM; Bastos, DKL; Silva, DF; Silva, MRC; Firm, WCA. Research: Bastos, DKL; Silva, DF; Silva, MRC; Firm, WCA. Methodology: Viana, AL; Bastos, DKL; Firm, WCA. Resources: Silva, DF; Firm, WCA. Supervision: Firm, WCA. Validation: Viana, AL; Mosque, AD; Sierra, AKM; Bastos, DKL; Silva, DF; Silva, MRC; Firm, WCA. Visualization: Viana, AL; Mosque, AD; Sierra, AKM; Bastos, DKL; Silva, DF; Silva, MRC; Firm, WCA.

All authors read and agreed with the published version of the manuscript.

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