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Perihepatic abscess associated with vagal indigestion syndrome in cattle: retrospective study

Abscesso peri-hepático associado à síndrome de indigestão vagal em bovinos: estudo retrospectivo

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Abstract: Vagal indigestion (VI) is a syndrome characterized by changes in the motility of the forestomach and abomasum, which occurs in the event of dysfunctions in the vagus nerve. Several inflammatory conditions are associated with nerve injury, however, the relationship between perihepatic abscesses and this syndrome is poorly documented. Therefore, a retrospective study of the clinical, laboratory, ultrasound and anatomopathological findings of 15 cattle presented with VI due to perihepatic abscess was carried out. Samples from the liver abscesses of three cattle were sent for bacteriological analysis. All were over 24 months old, were dairy cows in the productive phase (86.67%, 13/15) and raised in a semi-intensive system (73.34%, 15/11). The main clinical findings were dehydration, ruminal tympania, ruminal hypermotility and scanty feces. Laboratory tests revealed leukocytosis due to neutrophilia, with a regenerative shift to the left and elevated chloride content in the ruminal fluid. Ultrasonography revealed reticular hypermotility, ruminal distension and large abscesses adjacent to the left hepatic lobe, which were confirmed by pathological examination. Bacteriological evaluation identified Staphylococcus aureus, Enterococcus faecalis and Providencia stuartii. It is concluded that among the causes of IV syndrome, the presence of perihepatic abscesses should be considered when establishing the diagnosis of the disease.

Key-words: Digestive disorder; liver; bacterial isolation; vagus nerve; ruminant.

Resumo: A indigestão vagal (IV) é uma síndrome caracterizada por alterações na motilidade dos pré-estômagos e do abomaso, resultante de disfunções no nervo vago. Embora diversas condições inflamatórias estejam associadas à lesão nervosa, a relação entre abscessos peri-hepáticos e IV ainda é pouco documentada. Diante disto, realizou-se um estudo retrospectivo dos achados clínicos, laboratoriais, ultrassonográficos e anatomopatológicos de 15 bovinos diagnosticados com IV em decorrência de abscesso peri-hepático. Amostras oriundas dos abscessos hepáticos de três bovinos foram encaminhadas para a análise bacteriológica. Todos os animais tinham

idade acima de 24 meses, eram fêmeas de aptidão leiteira em fase produtiva (86,67%, 13/15) e criados em sistema semi-intensivo (73,34%, 11/15). Os principais achados clínicos observados foram desidratação, timpania ruminal, hipermotilidade ruminal e fezes escassas. Os exames laboratoriais revelaram leucocitose por neutrofilia com desvio à esquerda regenerativo, além de elevação do teor de cloretos do fluido ruminal. No exame ultrassonográfico, constataram-se hipermotilidade reticular, distensão ruminal e grandes abscessos adjacentes ao lobo hepático esquerdo, confirmados na avaliação anatomopatológica. Na análise bacteriológica, foram identificados *Staphylococcus aureus, Enterococcus faecalis* e *Providencia stuartii*. Conclui-se que, dentre as causas da síndrome da IV, a presença de abscessos peri-hepáticos deve ser considerada no estabelecimento do diagnóstico da enfermidade.

Palavras-chave: Distúrbio digestivo; fígado; isolamento bacteriano; nervo vago; ruminante.

1. Introduction

Vagal indigestion (VI), also known as Hoflund syndrome, is a syndrome characterized by alterations in the motility of the forestomach and abomasum, due to dysfunctions in the vagus nerve, which is responsible for the sensory and motor innervation of these organs. The syndrome is reported in cattle, buffaloes, and small ruminants of different ages, aptitudes, and breeds, with a predominance of reports in adult dairy cows [1-4]. Depending on the location where the functional disorder occurs in the nerve and the extent of the injury, there are distinct manifestations in the compartments of the digestive system. As a result, the syndrome is classified into types I, II, III, and IV (or gestational) [3-11]. Another type of VI is recognized by some authors as idiopathic. Its occurrence is observed in cattle of the Santa Rosália breed (mini-cattle), which present a clinical picture of chronic ruminal tympanism that can progress to death [2,12].

Dysfunction in the motility of the pre-stomachs and abomasum has severe consequences for the health and productivity of animals, which present apathy, lack of appetite, reduced milk production, dehydration, ruminal dysfunction, and abdominal distension due to the accumulation of gases or poorly digested food. These signs compromise animal welfare and result in substantial economic losses for producers, due to decreased feed efficiency, increased costs for veterinary treatments, and, in most cases, loss of the animal [4,8,11,13].

Vagus nerve injury is associated with factors such as inflammatory processes, including traumatic reticuloperitonitis and perforated abomasal ulcers, traumatic injuries, compressions, liver abscesses, granulomas, and neoplastic infiltrations, which can injure the nerve at any point along its anatomical path, in the thoracic or abdominal vagal trunks, due to their close anatomical relationship [3,4,13]. Defining the specific cause can be challenging, particularly in cases where the lesion is not evident, such as perihepatic abscesses, whose relationship with vagal indigestion in cattle is poorly documented [14]. In view of this, the objective of the current work was to carry out a retrospective study of cases of VI resulting from perihepatic abscesses in cattle, with emphasis on clinical, laboratory, ultrasound, and anatomopathological findings.

2. Material and methods

A retrospective study was carried out on clinical cases of VI in cattle treated at the Garanhuns Cattle Clinic, Campus of the Federal Rural University of Pernambuco (CBG/UFRPE), to include a period of 11 years, between January 2013 and December 2023. The animals were diagnosed with VI, in its different types [4,5,8], through clinical examination [15] and complementary exams. Screening was carried out in the institution's registry books, and 15 cases of VI due to perihepatic abscess were selected. Clinical information was collected regarding the general condition of the animals (appetite, rectal temperature, degree of dehydration, and respiratory and heart rates) and specific examinations of the digestive system (dynamics of the gastrointestinal tract, abdominal shape, transrectal palpation, and fecal characteristics).

In the 15 animals, blood samples were collected in a tube with EDTA anticoagulant (10%), through jugular venipuncture, to perform a blood count and determine total plasma protein and plasma fibrinogen [16]. Ruminal fluid samples were analyzed [15] in 12 cattle and the concentration of chloride content (Liquiform Chlorides, Labtest) was measured in four of these samples. Clinical and hematological data were tabulated in an electronic spreadsheet and analyzed using descriptive statistics [17].

Ultrasound examinations were performed on 14 animals, according to Braun [18], using a Mode B device (Z6 Vet, Mindray Bio-Medical Eletronics Co. Ltd., Shenzhen China) and a convex transducer, with frequencies of 5.0 MHz (Z6 Vet). Given the severity of the cases, combined with the poor prognosis, one animal evolved to death and six were indicated for euthanasia [19], being sent for anatomopathological examination. The remaining animals were sent for slaughter with conditional use of the carcass.

Abscess samples were collected from three cases for bacteriological analysis. Isolation and phenotypic characterization were performed according to the description and adaptation of Jalalvand et al. [20]. The samples were inoculated on 5% sheep blood agar and MacConkey agar (MC) and cultivated in aerobic and anaerobic conditions (37ºC; 24-48h). The colonies that showed growth on MC were streaked on Salmonella-Shigella agar. After performing the Gram and catalase test, the phenotypic identification of the isolates was carried out using standard biochemical tests and microbiological methods, such as colony types, motility, triple iron sugar, Simmons citrate, and urease.

Subsequently, bacterial DNA was extracted using the PureLink™ Genomic DNA Mini Kit (Invitrogen, USA), according to the manufacturer's instructions. The quantity and quality of DNA were determined by spectrophotometry, using the GENESYS 10S Series spectrophotometer (Thermo Fisher Scientific Inc., USA), and by electrophoresis in 1% agarose gel stained with DSView Nucleic Acid Stain (Sinapse Inc.). Analysis of the 16S rRNA region was performed by the PCR amplification method, using the universal *primers* 27F and 1492R described by Hongoh et al. [21]. The products resulting from the PCR reaction were purified with the Illustra GFX PCR DNA and Gel Band Purification Kit (Cytiva, UK), according to the manufacturer's instructions and the purified DNA samples were sent to the Genetics Department of the

Federal University of Pernambuco (UFPE) for genetic sequencing. Sequence editing and analysis were performed using Bioedit software (Informer Technologies, Inc.) and consensus sequence alignment was performed using the MEGA package (Genetic Analysis of Molecular Evolution; Version 11.0.13).

3. Results

During the eleven-year period considered in the study, 8,612 cattle were treated, of which 1.75% (151) were diagnosed with VI in its different types and, of these, 15 cases were due to perihepatic abscesses. The anamnesis reported reduced appetite, rumination, and milk production, chronic and relapsing bloat, and foul-smelling diarrhea. Most of the animals (86.67%, 13/15) were treated on the property without success. Of the affected animals, all were over 24 months old, 86.67% (13/15) were dairy females in the productive phase, and 73.34% (11/15) were raised in a semi-intensive system. The main clinical findings are presented in Table 1, highlighting dehydration, abdominal distension predominantly in the apple-pear abdominal shape (abdominal distension due to gas in the upper portion and bulging in the ventral portion generally due to accumulation of ingestion), increased abdominal tension, ruminal tympany, undefined ruminal stratifications, ruminal hypermotility, ruminal distension during transrectal palpation and scant feces of pasty to soft consistency.

Table 1. Absolute (n) and relative (%) frequency of clinical findings in cattle affected by vagal indigestion secondary to perihepatic abscess (n=15), treated at the Garanhuns Cattle Clinic, UFRPE.

Variable	Category	n	%
Appetite	Present	09	60.0
	Decreased	05	33.3
	Absent	01	06.6
Temperature (°C)	Physiological (37.0-38.9)	09	60.0
	Hypothermia (<37.0)	0	0
	Hyperthermia (39.0-39.4)	04	26.6
	Fever (>39.4)	02	13.4
Dehydration	Absent	02	13.4
	Light (6%)	02	13.4
	Moderate (8%)	10	66.6
	Severe (10%)	01	06.6
HR (bpm)	Physiological (60-80)	09	60.0
	Bradycardia (<60)	02	13.4
	Tachycardia (>80)	04	26.6
RR (rpm)	Physiological (24-36)	12	80.0
	Bradypnea (<24)	0	0
	Tachypnea (>36)	03	20.0
Ruminal Motility	Physiological	01	06.6
	Hypomotility	02	13.4
	Hypermotility	12	80.0
	Atonic	0	0
Ruminal Tympany	Absent	03	20.0
	Present	12	80.0
Rumen stratification	Defined	04	26.6

	Undefined	11	73.3
Abdomen shape	Physiological	03	20.0
	Unilaterally distended	01	06.6
	Bilaterally distended	01	06.6
	Apple-pear	10	66.6
Abdominal tension	Physiological	05	33.3
	Increased	10	66.6
Transrectal palpation	No alterations	03	20.0
	Rumen distension (L-shaped)	12	80.0
Feces	Present	02	13.4
	Scarce	10	66.6
	Absent	03	20.0
Fecal characteristics	Well digested	04	26.6
	Poorly digested	02	13.4
	Excessively digested	06	40.0

The hematological findings are shown in Table 2, demonstrating leukocytosis due to neutrophilia with a regenerative left shift. Analysis of the ruminal fluid revealed impairment of the ruminal microbiota and elevated chloride levels (>30 mEq/L) in the affected animals.

Table 2. Mean and standard deviation of hematological variables of cattle affected by vagal indigestion secondary to perihepatic abscess (n=15), treated at the Garanhuns Cattle Clinic, UFRPE

Parameter	Mean	Standard Deviation	Reference valuee
Red blood cells (x 10^6/µL)	6.44	0.88	5.00 –10.00
Hematocrit (%)	28	3	24 – 46
Hemoglobin (g/dL)	9.3	1.2	8.0 – 15.0
MCV (ƒL)[a]	43.10	6.28	40.00 – 60.00
MCHC (%)[b]	33.09	1.81	30.00 – 36.00
TPP[c]	8.1	0.9	7.0 – 8.5
PF[d]	633	235	300 – 700
Leukocytes (/µL)	15413	7017	4000 – 12000
Lymphocytes (/µL)	6996	2741	2500 – 7500
Monocytes (/µL)	71	121	25 – 840
Basophils (/µL)	7	29	0 – 200
Eosinophils (/µL)	194	343	0 – 2400
Segmented (/µL)	7631	5424	600 – 4000
Rods (/µL)	480	1235	0 – 120

a: Mean corpuscular volume; b: Mean corpuscular hemoglobin concentration; c: Total plasma proteins; d: Plasma fibrinogen; e: Kramer[22].

The main findings observed in the transabdominal and transthoracic ultrasound examination included impaired reticular motility (hypermotility), ruminal distension, and structures delimited by echogenic capsules, with heterogeneous content, located adjacent to the left hepatic lobe, between the pre-stomachs (reticulum and omasum) and thoracic wall, with characteristics of large abscesses (Figure 1).

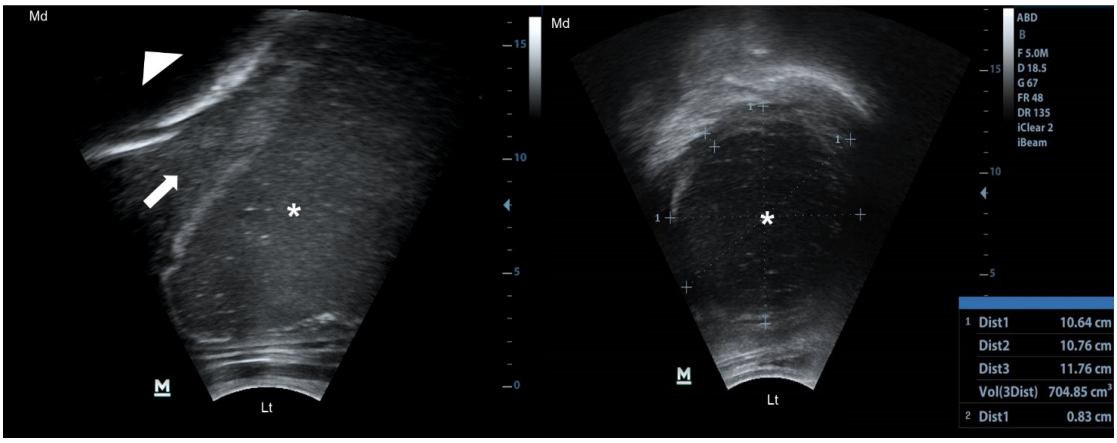

Figure 1. Transthoracic ultrasonography of cattle with VI secondary to perihepatic abscess: (A and B) Structures delimited by an echogenic capsule, with heterogeneous content (asterisks), located adjacent to the liver (arrow), between the rumen (arrowhead) and abdominal wall in the 5th and 8th direct intercostal space, respectively. (M: Cranial; Lt: Lateral and Md: Medial); Source: CBG-UFRPE, 2023.

The definition of types V I resulting from perihepatic abscess was established based on the association of clinical findings and complementary exams, with type II prevailing (86.66%, 13/15), followed by type III (13.34%, 2/15). Among the anatomopathological findings, identification of large perihepatic abscesses located on the diaphragmatic surface of the liver (left lobe) stands out, reaching 40 cm in diameter, surrounded by a thick fibrous capsule, and a purulent content with a consistency varying from fluid to caseous (Figure 2), in addition to extensive perihepatitis, focal peritonitis due to contiguity, and dilation of the pre-stomachs, especially the rumen.

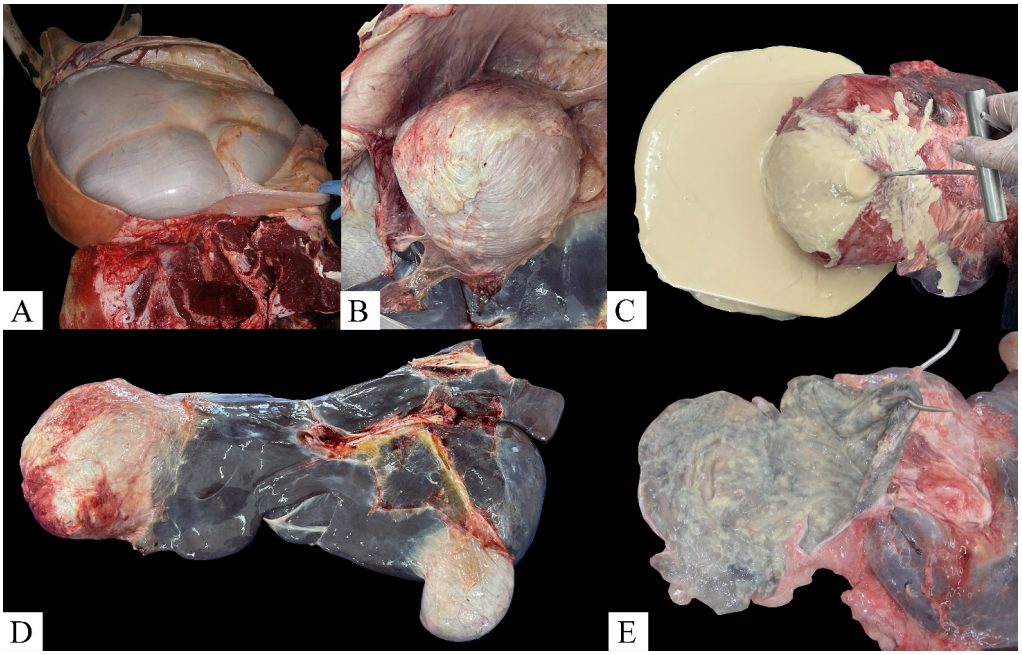

Figure 2. Anatomopathological findings o f c attle w ith V I s econdary t o p erihepatic a bscess: (A) M arked distension of the forestomach, especially the rumen, due to accumulation of ingesta. (B) Abscess located adjacent to the diaphragmatic surface of the liver, between the forestomach and the thoracic wall, measuring 40 cm in diameter. (C) Large volume of liquefied purulent content drained from the abscess. (D) Perihepatic abscess on the left. (E) Thick fibrous capsule of the abscess. Source: CBG-UFRPE, 2023.

In the PCR reaction, the isolates presented an amplicon of approximately 1350bp. Pairwise sequence alignment was performed using the pairwise nucleotide sequence alignment tool accessible on the EzBioCloud web page (https://www.ezbiocloud.net/). From the phenotypic characterization of the isolates and analysis of the 16S gene sequences, *Staphylococcus aureus* (CBG1), *Enterococcus faecalis* (CBG2), and *Providencia stuartii* were identified.

4. Discussion

The percentage of VI cases resulting from perihepatic abscess observed in this study highlights this lesion as responsible for triggering this syndrome in cattle, as previously described by Fubini et al. [14]. VI is a disease with few clinical-epidemiological studies described in the country [13]. However, this disease stands out among the digestive diseases that compromise the productive life of cattle, affecting predominantly adult dairy cows, raised in a semi-intensive system, as in the current study, although no predilection for sex, age, or breed has been reported [23-25].

The origin of vagus nerve injury has been routinely associated with several diseases, such as peritonitis and/or adhesions, resulting mainly from reticulitis and abomasal affections, including ulcers, compaction, and displacement, in addition to liver abscess which, when close to the surface, commonly produce fibrinous inflammation, leading to adhesion to the peritoneum, diaphragm and adjacent viscera [4,14,26,13]. This condition was verified in the current study and confirms perihepatic abscess as the primary cause of VI [14].

Clinical findings observed more frequently in animals, such as abdominal distension predominantly in the apple-pear abdominal shape, increased abdominal tension, chronic moderate ruminal tympany, undefined ruminal stratifications, ruminal hypomotility, ruminal distension during transrectal palpation, and scant feces of pasty to soft consistency, are also reported [10,11,14,27]. These findings are justified by the neuromotor failure of the compartments, which results in the accumulation of gases and digestive contents, especially in the rumen. This accumulation, in turn, causes abdominal distension, often assuming a characteristic apple-pear shape due to the uneven distribution of ruminal stratifications. Furthermore, distension results in an increase in visible and palpable abdominal tension, as well as contributing substantially to the motor dysfunctions characteristic of the disease, since the stimulation of low-tension receptors in the rumen wall decreases the intensity of rumino-reticular contractions, increasing their frequency and, with the progressive increase in distension, there is a decrease in contractions, which may culminate in atony [27,28].

Neutrophilic leukocytosis reflects the response of the inflammatory and/or infectious process, generally associated with the origin of the vagal lesion, due to the perihepatic abscess and focal peritonitis, or even associated with the absorption of toxins due to the alteration of the gastrointestinal motility pattern [7,8,29]. Increased chloride concentration in ruminal fluid may be attributed to reflux of abomasal contents into the rumen due to dysfunction of aboral

pyloric flow [4,27]. The ultrasound findings characterized large perihepatic abscesses, indicating them as causative agents of vagal lesions, in addition to reticular hypermotility and ruminal distension, considered as conditions associated with the consequences of vagal lesions, due to the change in the pattern of contraction, motility, and emptying of the pre-stomachs, as reported by other authors [13,18]. In addition to vagal injury, another possibility for VI signs in animals with perihepatic abscesses is the location of these structures between the liver and the reticulum, which may reflect reticulo-omasal stenosis due to compression [30].

The pathogenesis of perihepatic abscess in cattle is not fully understood, with multiple abscesses in the hepatic parenchyma being more common [14]. It is widely accepted that the main predisposing factor to the formation of liver abscesses in this species is ruminal lactic acidosis, in which there is increased production and accumulation of organic acids, mainly volatile fatty acids and, occasionally, lactic acid, resulting in ruminitis. As a consequence, the protective barrier function of the ruminal wall is interrupted, which allows the translocation of bacteria from the ruminal fluid into the bloodstream, which, through the portal circulation, gain access to the liver and promote the formation of abscesses [31].

The animals in this study were high-production dairy cows, raised in a semi-intensive system, epidemiological conditions that support this hypothesis for the formation of perihepatic abscesses, since in these situations it is common to provide diets with a high energy density (rich in fast-fermenting carbohydrates), generally without prior adaptation, to maintain the beginning of lactation, a condition that favors the occurrence of ruminal acidosis [31].

In addition to these ruminal consequences, high-energy diets increase the rate of passage of ingesta and, consequently, the flow of carbohydrates in the colon and microbial fermentation in this intestinal segment. This results in colic acidosis and the repercussions on microbial diversity, accumulation of toxic products, and inflammation of the wall epithelium are similar to those that occur in ruminal acidosis. However, because it has a simpler epithelial barrier and lower buffering capacity, when compared to the rumen, the colon under acidosis conditions may predispose to bacterial translocation and the formation of liver abscesses [31]. It is important to point out that this hypothesis has not yet been experimentally proven. It is also possible that traumatic reticuloperitonitis, caused by sharp objects, metallic or otherwise, lodged in the reticulum and piercing its wall, may extend the infection to the liver and cause abscesses [14,32].

Bacteria isolated from the contents of perihepatic abscesses (*Staphylococcus aureus*, *Enterococcus faecalis,* and *Providencia stuartii*) in the current study are distinguished from those commonly described, such as *Fusobacterium necrophorum* and *Trueperella pyogenes,* which are frequently isolated bacterial agents with established pathogenesis [33,34]. However, reports demonstrate the variability of the etiological agents associated with these diseases in cattle [35,36]. The bacteria identified in this study are involved in cases of liver and perihepatic abscesses in humans, in which the entry points were lesions in organs where these bacteria are part of the microbiota, especially the skin and digestive system [37-41]. In cattle, there are no reports of the isolation of these agents in perihepatic abscesses, however, these

microorganisms have been associated with inflammation in anatomical sites such as the mammary gland and umbilical vein [42-44], which can act as entry points for bacteremia and subsequent implantation on the hepatic surface.

5. Conclusion

Among the possible causes of vagal indigestion syndrome in cattle, perihepatic abscesses should be considered, highlighting the importance of complementary exams, especially ultrasound examination, in aiding diagnosis. Furthermore, considering its potential as a cause of VI and its distinct characteristics in relation to hepatic abscesses in cattle, additional clinical-epidemiological and anatomopathological studies are needed to deepen the understanding of the origin and pathogenesis of perihepatic abscesses.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

Data will be provided upon request to the corresponding author.

Author contributions

Conceptualization: Conceição AI, Silva TA, Mendonça CL, and Afonso JAB. Data curation: Conceição AI, Silva TA, Mendonça CL, Godoi APS, Mendonça M, Cajueiro JFP, Silva NAA, Souza MI, Souto RJC, and Afonso JAB. Formal analysis: Conceição AI, Silva TA, Mendonça CL, Godoi APS, Mendonça M, and Afonso JAB. Acquisition of funding: Not applicable. Project administration: Mendonça CL, Mendonça M, and Afonso JAB. Methodology: Conceição AI, Silva TA, Mendonça CL, Godoi APS, Mendonça M, Cajueiro JFP, Silva NAA, Souza MI, Souto RJC, and Afonso JAB. Supervisão Afonso JAB. Investigation: Conceição AI, Silva TA, Mendonça CL, Godoi APS, Mendonça M, and Afonso JAB. Preview: Conceição AI, Silva TA, Mendonça CL, and Afonso JAB. Writing (original draft): Conceição AI, Mendonça CL, Mendonça M, and Afonso JAB. Writing (review and editing): Conceição AI, Silva TA, Mendonça CL, and Afonso JAB.

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