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VISCERAL NEOPLASMS AND *Chelonid alphaherpesvirus 5* IN GREEN TURTLES WITH FIBROPAPILLOMATOSIS

(*Neoplasias viscerais e Chelonid alphaherpesvirus 5 em tartarugas verdes com fibropapilomatose*)

Silmara Rossi[1,9]http://orcid.org/0000-0003-0281-7236**, Roberta Ramblas Zamana[2], Pedro Paulo de Andrade-Santos[3], Aline da Costa Bomfim[1,4,5,6], Daniel Solon Dias de Farias[1,4,5,6], Augusto Carlos da Boaviagem Freire[4], Rysonely Maclay de Oliveira[4], Marco Aurélio Gattamorta[2,7], Eliana Reiko Matushima[2], Juliana Maia de Lorena Pires[4], Carlos Sacristán[2], Edson Soares da Silva-Júnior[4], Flávio José de Lima Silva[4,8], Simone Almeida Gavilan[1,4,5]**

[1]Universidade Federal do Rio Grande do Norte, Centro de Biociências, Departamento de Morfologia, Laboratório de Morfofisiologia de Vertebrados, Natal/RN, Brazil. [2]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Patologia, Laboratório de Patologia Comparada de Animais Selvagens, São Paulo/SP, Brazil. [3]Universidade Federal do Rio Grande do Norte, Centro de Biociências, Departamento de Morfologia, Natal/RN, Brazil. [4]Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN), Laboratório de Monitoramento de Biota Marinha, Mossoró/RN, Brazil. [5]Centro de Estudos e Monitoramento Ambiental (CEMAM), Areia Branca/RN, Brazil. [6]Programa de Doutorado em Desenvolvimento e Meio Ambiente (DDMA), Universidade Federal do Rio Grande do Norte, Natal/RN, Brazil. [7]Escola de Engenharia e Arquitetura, Faculdades Metropolitanas Unidas, São Paulo/SP, Brazil. [8]Universidade do Estado do Rio Grande do Norte, Departamento de Turismo, Natal/RN, Brazil. [9]Structural and Functional Biology Postgraduate Program - Universidade Federal do Rio Grande do Norte.

*Corresponding author: smara.rossi@gmail.com

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ABSTRACT - Fibropapillomatosis (FP) is a multifactorial, neoplastic and infectious disease that affects all sea turtle species, and *Chelonid alphaherpesvirus 5* (ChHV5) has been singled out as the primary etiological agent. This disease is mainly characterized by cutaneous tumors, but visceral fibromas, myxofibromas and fibrosarcomas have been also reported and can interfere with systemic functions. In spite of previous descriptions of visceral neoplasms in sea turtles from Hawaii and Florida, some of them infected by ChHV5, there are few reports of non-cutaneous tumors in Brazilian sea turtles. In order to fill this gap, we analyzed samples of internal neoplasms from four green turtles (*Chelonia mydas*) by histopathological and molecular techniques. Cutaneous neoplasms were quantified and classified according to their size and tumor score to determine the FP severity, and the presence of internal tumors was confirmed post-mortem via necropsy. Forty-eight cutaneous tumors (7-23 per individual) were found on sampled green turtles, and the FP severity was mild (2 individuals) and moderate (2 individuals). Visceral neoplasms were found in lung (n=4), heart (n=1), intestine (n=2), esophagus (n=1), stomach (n=1), liver (n=1), spleen (n=1), skeletal muscle (n=1) and kidney (n=2) and were classified as fibromas (n=47) and one as renal myxofibroma. We did not detect ChHV5 DNA in the esophageal, skeletal muscle, or hepatic fibromas. Our research brings

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a novel description of renal myxofibroma and ChHV5 infection in visceral neoplasms from green turtles in Brazil, improving our knowledge about the prevalence, anatomic localization, and severity of internal neoplasms associated with FP.
Keywords: fibropapillomatosis; herpesvirus; neoplastic disease; sea turtles.

RESUMO: A fibropapilomatose (FP) é uma doença multifatorial, neoplásica e infecciosa que afeta todas as espécies de tartarugas marinhas, e o *Chelonid alphaherpesvirus 5* (ChHV5) tem sido apontado como o agente etiológico primário. Essa doença é caracterizada por tumores cutâneos, mas fibromas viscerais, mixofibromas e fibrossarcomas também têm sido relatados e podem interferir nas funções sistêmicas. Apesar de descrições anteriores de neoplasias viscerais em tartarugas marinhas do Havaí e Flórida, algumas infectadas pelo ChHV5, há poucos relatos de tumores não cutâneos em tartarugas marinhas no Brasil. Para preencher essa lacuna, analisamos amostras de neoplasias internas de quatro tartarugas verdes (*Chelonia mydas*) através de técnicas histopatológicas e moleculares. Neoplasias cutâneas foram quantificadas e classificadas de acordo com seus tamanhos e escores para determinar a severidade da FP, e a presença de tumores internos foi confirmada *postmortem* durante necropsia. Quarenta e oito tumores cutâneos (7-23 por indivíduo) foram encontrados nas tartarugas verdes amostradas, e a severidade da FP foi classificada como leve (2 indivíduos) e moderada (2 indivíduos). Neoplasias viscerais foram encontradas em pulmão (n=4), coração (n=1), intestino (n=2), esôfago (n=1), estômago (n=1), fígado (n=1), baço (n=1), músculo esquelético (n=1) e rim (n=2), sendo classificadas como fibromas e uma como mixofibroma renal. Não detectamos DNA do ChHV5 nos fibromas esofágico, muscular esquelético e hepático. Nossa pesquisa relata nova descrição de mixofibroma renal e detecção do ChHV5 em neoplasias viscerais de tartarugas verdes no Brasil, melhorando o conhecimento sobre prevalência, localização anatômica e severidade dos tumores internos associados à FP.
Palavras-chave: doença neoplásica; fibropapilomatose; herpesvírus; tartarugas marinhas.

INTRODUCTION

Fibropapillomatosis (FP) is a neoplastic disease that affects sea turtles and is mainly manifested by cutaneous tumors that can be found anywhere on the turtle's body (Aguirre et al., 1998; Herbst et al., 1999; Santos et al., 2010; Rossi et al., 2016). Internal fibromas, myxofibromas, and fibrosarcomas have been reported in the oral cavity, larynx, pharynx, esophagus, lungs, heart, kidneys, skeletal muscles, liver, spleen, and

gastrointestinal system (Norton et al., 1990; Herbst, 1994; Balazs et al., 1997; Lu et al., 2000; Aguirre et al., 2002; Work et al., 2004; Dutra et al., 2012). Size, anatomic distribution, and number of tumors can hamper mobility, foraging, cause starvation due to impairment of sight, or cause partial or total obstruction of glottis, epiglottis, pharynx, larynx and/or esophagus, contributing to a progressive debilitation (Jacobson et al., 1989; Williams et al., 1994; Herbst et al., 1994; Aguirre et al., 2002; Aguirre and Lutz, 2004). Additionally, internal tumors can interfere with systemic functions (Aguirre and Lutz, 2004).

Chelonid alphaherpesvirus 5 (ChHV5, family *Herpesviridae*) is considered the primary etiological agent of FP (Quackenbush et al., 2001; Patricio et al., 2012; Rodenbusch et al., 2014). Distinct lineages of ChHV5 have been observed according to regional origin of the host; such viral lineages likely diverged long before the FP emerged as panzootic, suggesting environmental or ecological factors play a key role to the global occurrence (Herbst et al., 2004; Patrício et al., 2012; Hargrove et al., 2016).

In Brazil, cutaneous FP tumors have been observed in several sea turtle species, including green, hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*), and olive ridley (*Lepidochelys olivacea*) turtles, although histopathological confirmation was described only in green, hawksbill and loggerhead sea turtles (D'Amato and Moraes-Neto, 2000; Mascarenhas and Iverson, 2008; Zwarg et al., 2014; Rossi et al., 2015; Kroposki et al., 2017; Silva-Junior et al., 2019).

Regarding internal neoplasms, histopathological features and ChHV5 detection of visceral fibromas associated with cutaneous fibropapillomas were reported by previous studies in Brazil and Florida (Dutra et al., 2012; Page-Karjian et al., 2017). However, to the authors' knowledge, in Brazil, visceral neoplasms were only described in four cases (1) three juvenile green turtles with pulmonary, cardiac and renal fibromas, under rehabilitation in the Aquário Municipal de Santos, southeastern Brazil (Dutra et al., 2012); and (2) in a leatherback sea turtle (*Dermochelys coriacea*) from northeastern Brazil with a fibromyxoid pulmonary sarcoma (Díaz-Delgado et al., 2019). Our study presents the histopathological description of visceral neoplasms from four green turtles found in the Potiguar Basin, northeastern Brazil. In addition, we present molecular information on ChHV5 detected in these tumors using formalin-fixed paraffin-embedded (FFPE) samples.

MATERIALS AND METHODS

Monitoring site and green turtles

Since 2010, the Projeto Cetáceos da Costa Branca – Universidade do Estado do Rio Grande do Norte (PCCB-UERN) has conducted the Beach Monitoring Project in the Potiguar Basin, northeastern Brazil, which is a compliance enforced by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) over the activities operated by PETROBRAS (Petróleo Brasileiro S.A., Agreement number 2500.005657510.2). The Potiguar Basin is considered an important habitat for green turtles (Farias et al., 2019), and a study on spatial-temporal analysis of FP revealed a relative frequency of 35.3% (228/646) in 2015 (Silva-Junior et al., 2019) and 26.7% (205/768) in 2017 (PETROBRAS, 2017).

The four examined individuals were found alive in 2015 (Case 1) and 2017 (Cases 2-4): Cases 1 and 4 were found stranded by local residents at Sacutinga and Águas Belas beaches (CE), respectively; Cases 2 and 3 were found by fishermen, the former was afloat, and the latter was entangled in net gear (Figure 1). The staff of PCCB-UERN received the notifications and admitted the individuals in the rehabilitation center in Areia Branca/RN. At the rehabilitation center, the rescued green turtles were clinically examined by the attending veterinarian to verify external FP tumors, hydration status, sarcopenia signs, buoyancy disorder, and eyelid, corneal and cloacal reflexes. Additionally, percussion of the carapace and inguinal palpation were performed. Curved carapace length (CCL) was measured for each examined individual using flexible tape. The green turtles were kept in quarantine with stringent measures to avoid potential FP transmission, housed individually in tanks with independent water supplies (148 cm diameter x 74 cm depth, 25 °C– 26 °C). Ambient light periodicity was approximately 12h, and the diet consisted of algae, seagrass (*Halodule* sp.), and fishes.

Sampling collection and histopathological examination

Cutaneous FP tumors found on the examined turtles were quantified and analyzed according to anatomical distribution, size (A: <1cm, B: 1-4cm, C: >4-10cm, D: >10cm) (Work and Balazs, 1999), and Southwest Atlantic Fibropapillomatosis Score - FPS_{SWA} (mild, moderate, and severe) (Rossi et al., 2016). All individuals died in captivity during rehabilitation, and visceral tumors were confirmed on post-mortem examination. Samples of visceral neoplasms (n=14) from the four turtles were collected for histopathological examination, fixed in 10% formalin, dehydrated, diaphanized, and embedded in paraffin; serial 5 µm sections were prepared and stained with hematoxylin and eosin (H&E). In

addition, we microscopically examined five representative cutaneous tumors (Cases 1-4) in order to confirm the FP diagnosis.

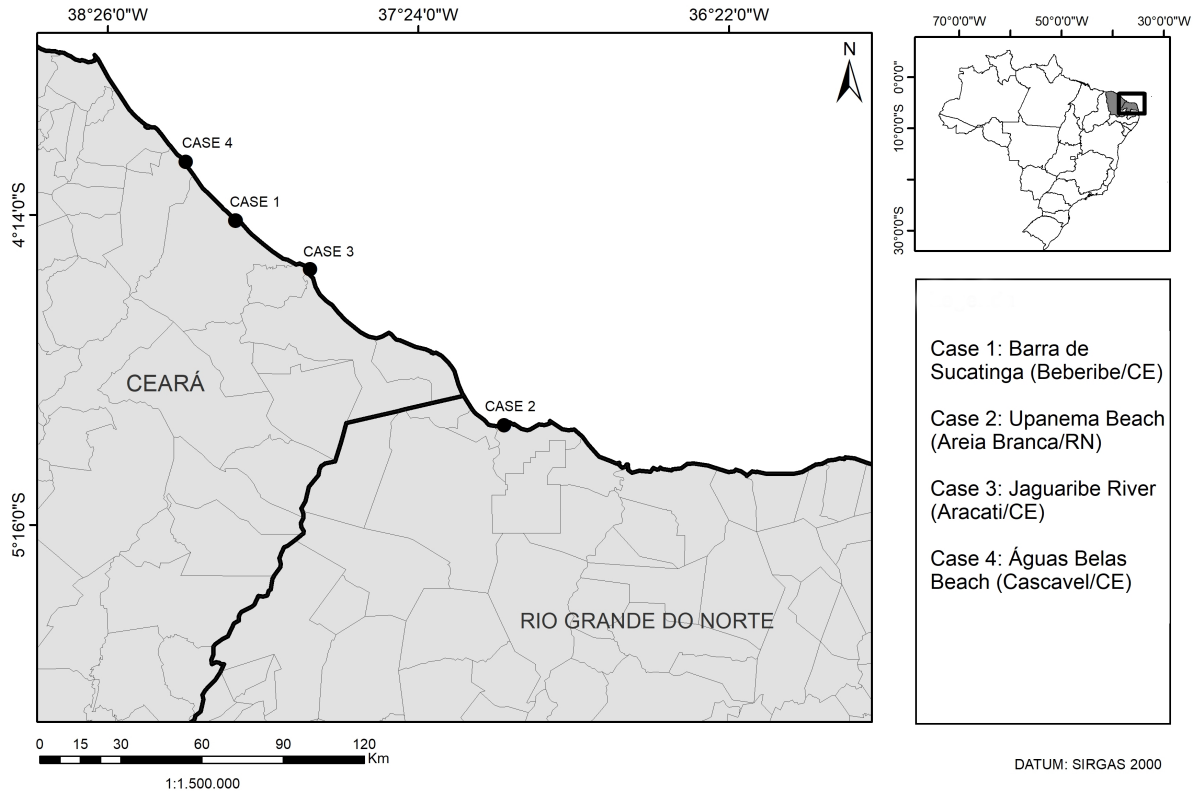

Figure 1 - Locations where the green sea turtles (*Chelonia mydas*, Cases 1-4) were rescued by the Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte.

Molecular analyzes

Considering our histopathological findings, fourteen formalin-fixed paraffin-embedded (FFPE) visceral neoplasm samples (Cases 1-4) were selected for molecular analyzes. DNA isolation from FFPE tissues has been employed by retrospective pathology studies (Akanbi et al., 2017; Navas-Suárez et al., 2018), and FFPE is an alternative when samples cannot be stored frozen after collection due to logistical constraints. Deparaffinization was achieve by consecutive xylol and pure ethanol, followed by 95% and 70% ethanol washes (of twenty 10 µm-sections from each obtained FFPE tissue). Total DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen®, Valencia, CA, USA). Subsequently, extracted DNA was quantified using an Epoch microplate spectrophotometer (Biotek®, Winooski, VT, USA).

The gene ß-actin was selected as an endogenous control and partially amplified (232 bp) by end-point PCR (Page Karjian et al., 2015) using the primers F: 5′-TGGTACAGTCTCCCATTCCA-3′, and R: 5′-AGGCATACAGGGACAACACA-3′). In order to detect ChHV5, a combination of previously published and custom primers was used to amplify the partial ChHV5 genes UL18 (capsid protein, 140 bp) and UL27 (Glycoprotein B, 143 bp). Amplification of UL18 (F: 5′-GTGGAACCCCGCCGGGTAAT-3′, R: 5′-TGATCCGGGCCGAGTAGCGG-3′) and UL27 (F: 5′-CTAGATACATACTGGCCRTGCTCGTC-3′, R: 5′-GCCAGCGACCATCCGGAG-3′) were performed according to Alfaro-Núñez and Gilbert (2014). PCR products were visualized after electrophoresis in 2% agarose gels stained with SYBR Safe (Invitrogen, Carlsbad, CA, USA). Amplicons of the expected size were excised and purified using the GFX gel extraction Kit (GE Healthcare, Buckinghamshire, UK), and confirmed by direct Sanger sequencing. After a BLAST search, our consensus sequences were aligned with similar sequences available at GenBank (NCBI) using the CLUSTAL/W method, and p-distance analysis was performed with the MEGA 7 program (Kumar et al., 2016) to determine the identity percentage ([1 – p distance] x 100).

RESULTS

Case outcomes and gross findings

The individuals were juvenile males (Cases 1, 2, and 4) and female (Case 3) with CCL between 39.4-61.4 cm. On clinical examination, case 1 was listless and cachectic, Cases 2 and 3 were active, while Case 4 was unable to dive. The time in rehabilitation ranged between 11–85 days (51, 85, 14 and 11 days in Cases 1-4, respectively).

A total of 48 external tumors were counted and analyzed in the four turtles, ranging from 7 to 23 tumors per individual. The forelimbs were the most affected anatomic region (n=21 tumors), followed by the cervical region (11 tumors) and hindlimbs (9 tumors). The majority of the tumors were classified as size B (n=31), followed by A (n=8), and C (n=8); only one tumor in the right forelimb of case 4 was classified as size D. According to the FPS_{SWA} scoring system, the FP severity ranged from mild (Cases 2 and 3) to moderate (Cases 1 and 4). At necropsy, internal masses were found in the lower and upper pulmonary lobes and along the lung parenchyma (Cases 1-4, Figure 2A), on the caudal pole of the kidney (Cases 1 and 3, Figure 2B), on the mucosal surface of the intestines (Cases 1 and 4), and on the heart, esophagus, stomach, liver, spleen, and skeletal muscle (Case 1). The examined visceral neoplasms were light tanned, well-circumscribed and sessile.

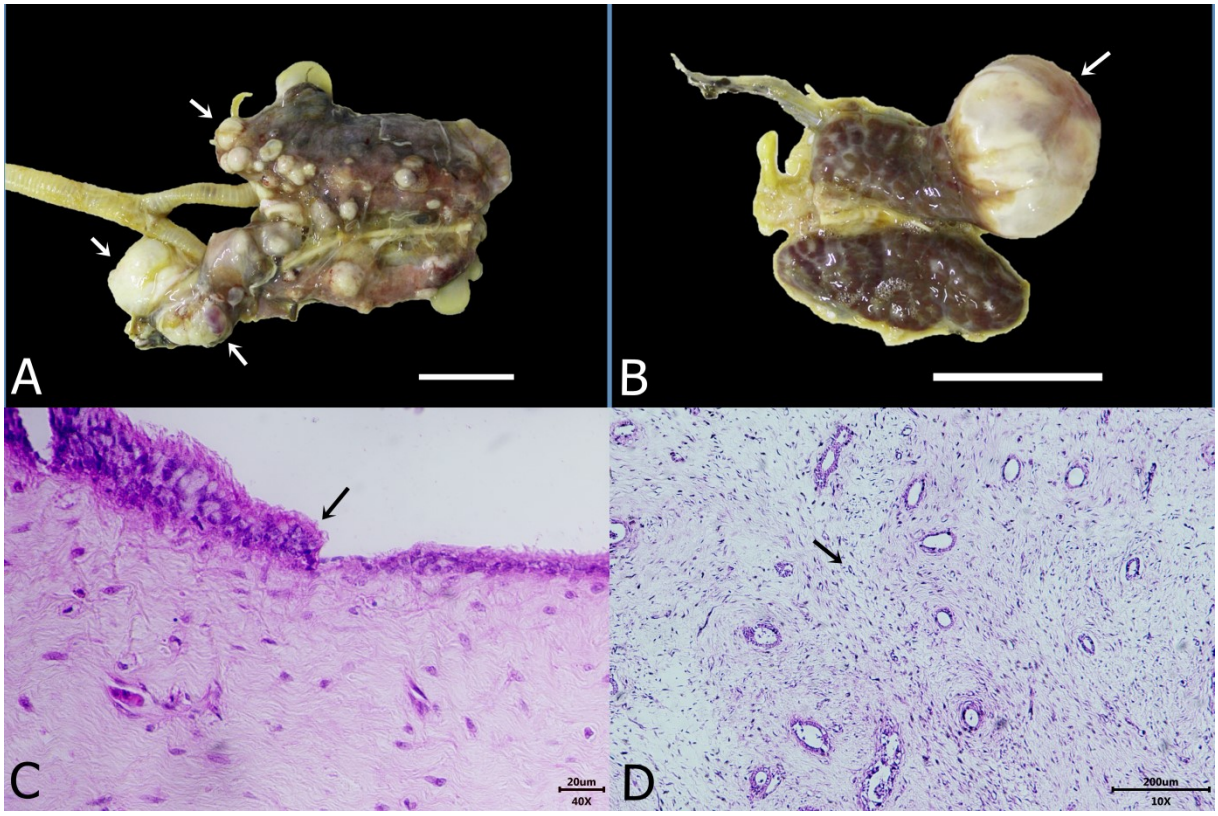

Figure 2 - Visceral neoplasms found in the examined green sea turtles (*Chelonia mydas*). Macroscopic view of pulmonary (A) and renal (B) tumors found in Case 3 (Bar = 5 cm). Histological sections of visceral fibromas: (C) Pulmonary squamous metaplasia (Case 4): note the ciliated pseudostratified columnar epithelium (arrow), (D) Renal myxofibroma (Case 3) with fusiform fibroblasts, and myxoid connective tissue with thin collagen fibers interconnecting fibroblasts (arrow).

Histopathological features

Visceral and skin tumors consisted of loose connective tissue with well-differentiated proliferating fibroblasts arranged among disorganized collagen fibers. The examined skin tumors were classified as fibropapillomas (n=4; Cases 1-4) and one fibroma (Case 1). Well-delimited pulmonary fibromas and focal necrosis were observed in lung samples from Cases 1-4, characterized as areas with dense connective tissue, thickness and in some places destruction of the alveolar septa, fibrosis and focal necrosis. Type II pneumocytes were more numerous than type I, and abundant active fibroblasts exhibiting nuclei with prominent euchromatin and nucleoli were also observed. Case 4 also had epithelial metaplasia with a large amount of mucosal cells (ciliated pseudostratified columnar epithelium) (Figure 2C), without confirmed association with the pulmonary fibroma.

The renal fibroma from Case 1 was well-delimited by a capsule of loose connective tissue, and presented numerous well-differentiated fibroblasts with varied morphology, scarce microvessels in the central portion, proximal and distal tubules at the periphery of the lesion, along with glomerular degeneration. Case 3 had a renal myxofibroma with fusiform fibroblasts, myxoid connective tissue with thin collagen fibers interconnecting fibroblasts within an amorphous extracellular matrix (Figure 2D), with a lymphocytic infiltrate. In some regions of the myxofibroma a higher cell density organizing into a storiform pattern was observed. Additionally, the myxofibroma periphery had renal cortical degeneration with persistent collecting ducts.

The esophageal fibroma (Case 1) was well-vascularized and presented reduction of the esophageal papillae, atrophic epithelium with hydropic degeneration, exocytosis, hypo-orthokeratosis, and areas of hypoparakeratosis. Scarce mononuclear inflammatory infiltrate and lymph vessels were also found. Gastric fibroma (Case 1) was well-delimited by a capsule of loose connective tissue and had lymph and blood vessels in the peripheral region. Intestinal fibromas from Cases 1 and 4 arose from the sub-mucosa, were well-delimited by a capsule of loose connective tissue, and well-vascularized by blood and lymph vessels with areas of lymphocytic inflammatory infiltrate.

The cardiac fibroma (Case 1) was well-delimited, arising from the epicardium, and presented diffuse lymphocytic infiltrate, supported by microvessels. Skeletal muscle fibromas (Case 1) had scarce blood vessels, inflammatory infiltrate, and no muscle degeneration, although muscle fiber separation by expansion of the lesion was noted. The hepatic and splenic fibromas were well-delimited by a capsule of loose connective tissue. Near the hepatic fibroma (Case 1), we noted disorganized hepatic lobules with cords arranged in many directions, periportal fibrosis, sinusoidal capillary dilation with occasional congestion, presence of Kupffer cells, and hemosiderin. The splenic fibroma (Case 1) had an expansive growth of the lesion compressing the splenic parenchyma. The periphery of the lesion was well-vascularized and presented with moderate amounts of lymphocytic inflammatory infiltrate.

Detection of ChHV5

The ß-actin gene was positive in all tested samples (n=14), confirming the presence of amplifiable *C. mydas* DNA. ChHV5-positive amplification was observed for the UL18 and UL27 PCRs. Overall, ChHV5 was identified in at least one sample per individual in the four examined turtles, although the detection sensitivity for ChHV5 DNA varies depending on the PCR assay. UL18 was positive for 78.6% (11/14) of the analyzed

samples and UL27 for only 28.6% (4/14) of them. ChHV5 DNA was detected in all pulmonary fibromas (Cases 1-4), the renal myxofibroma (Case 3), intestinal fibromas (Cases 1 and 4), and renal, cardiac, gastric and splenic fibromas from Case 1. Esophageal, skeletal muscle, and hepatic fibromas (Case 1) were negative for ChHV5 amplification.

Sequences were obtained from all amplified samples. Representative nucleotide sequences of UL18 and UL27 genes were submitted to DDBJ (DNA Data Bank of Japan) under accession numbers LC536650 and LC510263, respectively. The UL18 sequences (LC536650) amplified from all analyzed tissues were identical among them. The same was observed among all our UL27 sequences (LC510263). Our UL18 sequences were identical (100% nucleotide identity) to a sequence found in cutaneous tumor samples from northeastern Brazil, the same region of this study (LC506443). Similarly, our UL27 sequences were identical to sequences previously found in a FP sample from northeastern Brazil (LC509014). The UL27 sequence was also identical to sequences from Puerto Rico (JN580286), Florida (AY390406, AY644454), and Barbados (AY390404).

DISCUSSION

The higher occurrence frequency of FP was on the turtles' forelimbs, as described previously (Page-Karjian et al., 2014; Rossi et al., 2016), and the majority of cutaneous neoplasms were classified as size B (1–4 cm) in accordance with previous descriptions in northeastern Brazil (Silva-Junior et al., 2019). Regarding histopathological examination, the analyzed representative cutaneous tumors presented similar features to those described before (Aguirre et al., 1998; Herbst et al., 1999; Kang et al., 2008). As stated by previous studies, visceral fibromas were characterized by well-differentiated proliferating fibroblasts with varied morphology and disorganized collagen fibers (Herbst et al., 1999; Work et al., 2004; Dutra et al., 2012). Lung fibromas presented areas of fibrosis, also described in other research (Aguirre et al., 1998; Herbst et al., 1999; Dutra et al., 2012). The well-delimited cardiac fibroma (Case 1) arose from the epicardium, similarly to Work et al. (2004), and thrombosis, fibrosis and necrosis described by Aguirre et al. (1998) were not observed. Renal fibromas were well-delimited by a capsule and characterized by glomerular degeneration, as observed in previous studies which reported extensive interstitial fibrosis and atrophy of renal tubules (Herbst et al., 1999; Work et al., 2004). The gastric fibroma was well-delimited by a capsule of loose connective tissue in the sub-mucosal layer, and the intestinal fibroma arose from the sub-mucosa, delimited by a capsule of loose connective tissue. We did not found fibrosis, necrosis and keratin pearls in the collagenous matrix as reported by previous studies (Aguirre et al., 1998; Work et

al., 2004). The hepatic fibroma revealed disorganized hepatic cords arrangement, periportal fibrosis, sinusoidal capillary dilation, Kupffer cells, and hemosiderin. Previous studies described fibroplasia and hepatocellular atrophy in hepatic fibromas related to FP (Herbst et al., 1999; Work et al., 2004). A splenic fibroma was found in one turtle that had visceral tumors in nine organs (Case 1); Work et al. (2004) noted that splenic tumors were more common in Hawaiian green turtles with three or more affected internal organs.

Several studies have reported internal tumors in green turtles from the Atlantic and Pacific Oceans. In Puerto Rico, lung and kidney were the most commonly affected organs (Williams et al., 1994), while in Florida, neoplasms have been reported in lungs, kidneys, heart, liver, gastrointestinal tract and mesentery (Herbst, 1994; Herbst et al., 1999), while green turtles from Hawaii had neoplasms in the lungs, kidneys, heart, liver, spleen, and stomach (Aguirre et al., 1998; Work et al., 2004). In green turtles from Brazil, Dutra et al. (2012) described pulmonary, cardiac and renal fibromas. Our findings revealed that most of the analyzed visceral tumors were fibromas (observed in lung [n=4], heart [n=1], intestine [n=2], esophagus [n=1], stomach [n=1], liver [n=1], spleen [n=1], skeletal muscle [n=1] and kidney [n=1]), although one mass was diagnosed as a renal myxofibroma. In Hawaii, fibromas were observed predominantly in the lungs, kidneys and musculoskeletal tissues whereas myxofibromas were most common in the spleen and intestines, and fibrosarcomas of low-grade malignancy were mainly found in the heart (Work et al., 2004). This is the first description of a renal myxofibroma associated to FP in sea turtles from South America, and the second worldwide (Norton et al., 1990).

According to Chaves et al. (2017), ChHV5 DNA detection is significantly higher in skin tumors compared with non-cutaneous and swabs samples. However, little information is available regarding ChHV5 presence in internal neoplasms. Herein, we detected ChHV5 in visceral fibromas and a renal myxofibroma, as described previously in lung, heart, kidney, intestine and spleen tumors (Lu et al., 2000; Page-Karjian et al., 2017). Our findings revealed the first detection of ChHV5 DNA in a gastric fibroma (Case 1). The pulmonary neoplasms from the four cases presented here were positive for ChHV5 DNA, similar to that observed by Lu et al. (2000). Interestingly, previous studies also detected ChHV5 in tumor-free pulmonary tissue of green turtles with cutaneous neoplasms (Lu et al., 2000; Quackenbush et al., 2001); therefore, the etiological role of ChHV5 in pulmonary neoplasms should be assessed carefully. The esophageal, skeletal muscle, and hepatic fibroma samples of Case 1 were ChHV5-negative, although that result may be explained

by the reduced PCR sensitivity in FFPE samples and/or a low number of viral copies in these tissues.

Among the ChHV5-positive cases, the UL18 PCR presented the highest detection rate (78.6%, 11/14). Conversely, previous research using the same PCR techniques in frozen samples suggested that UL27 detection was more sensitive than UL18, although no statistics were performed (Alfaro-Núñez and Gilbert, 2014). The UL18 (LC536650) and UL27 (LC510263) gene segments from our study were identical to those previously recorded in sea turtles from northeastern Brazil (LC506443 and LC509014, respectively). Considering the previously observed site fidelity of ChHV5 in foraging grounds (Ariel et al., 2017; Jones et al., 2020), we suggest that the same ChHV5 strain circulates along the Brazilian northeast. In addition, our UL27 sequence is also similar to those circulating in Caribbean waters suggesting that green turtles of similar provenance disperse among feeding grounds along the Caribbean and Brazil. According to our findings, ChHV5 DNA obtained from FFPE extracts, though highly fragmented, can be successfully used to amplify shorter products (up to 250 bp) as described before (Dedhia et al., 2007; Kokkat et al., 2013). This technique could be helpful for retrospective studies using FFPE preserved samples.

All of the visceral neoplasms in this study were diagnosed in juvenile turtles. Based on experimental infectivity trials (Herbst et al., 1999), visceral tumors are thought to develop later (after 2 years) in the clinical course of FP. However, the majority of visceral lesions are observed during postmortem investigations, which could hamper the data available on the occurrence and prevalence of this type of lesion (Jones et al., 2016). Thus, the presence of visceral tumors in early-stage FP development may be underestimated. Because turtles with visceral FP are often euthanized outright, understanding visceral neoplasms associated with cutaneous FP is important for informed decision-making by sea turtle rehabilitators (Page-Karjian et al., 2014, 2019).

CONCLUSION

This is the first report of ChHV5 in visceral tumors of green turtles in Brazil, and to the authors' knowledge, a novel description of renal myxofibroma associated with FP. Our histopathological findings suggest that the visceral tumors contributed to death of the examined individuals (e.g., pulmonary fibromas with thickness/destruction of the alveolar septa, areas of fibrosis and focal necrosis, and glomerular degeneration associated with the renal fibroma). The potential presence of internal neoplasms in sea turtles with FP should be considered by clinicians during triage considerations for rehabilitation efforts.

Furthermore, complete necropsy of all sea turtles that die in captive care is necessary to improve our knowledge about the prevalence, anatomic localization, and severity of visceral neoplasms associated with FP.

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