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Fibropapillomatosis Associated with *Chelonid alphaherpesvirus 5* (ChHV5) in a Green Turtle *Chelonia mydas* in Argentine Waters

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ABSTRACT: Fibropapillomatosis is a debilitating neoplastic disease associated with *Chelonid alphaherpesvirus 5* (ChHV5) infection. We detected the Atlantic variant of ChHV5 associated with a fibropapilloma in a green turtle (*Chelonia mydas*) found stranded on the western coast of Rio de la Plata, Argentina. This is the southernmost registered case for the southwestern Atlantic.

Fibropapillomatosis affects all species of sea turtles and has been reported in most of the world's seas (Alfaro-Núñez et al. 2014). This debilitating neoplastic disease is characterized by the formation of multiple skin papillomas, fibromas, and fibropapillomas as well as visceral fibromas. A linear, double-stranded DNA herpesvirus, *Chelonid alphaherpesvirus* 5 (ChHV5), has been associated with this disease (Ene et al. 2005; Rodenbusch et al. 2012). Tumors can be found in the eyes, neck, mouth, flippers, tail, carapace, plastron, or internal organs, and their location may affect the vision, locomotion, feeding, and internal organ function of the turtle (de Deus Santos et al. 2015; Jones et al. 2016; Rossi et al. 2021). The highest prevalences of fibropapillomatosis are generally recorded in nearshore neritic (shallow) areas that tend to suffer greater anthropogenic disturbance, suggesting that disease development also depends on environmental cofactors such as biotoxin-producing algae, chemical carcinogens, and agricultural runoff, among others (Aguirre et al. 2002).

The green turtle (*Chelonia mydas*), leatherback turtle (*Dermochelys coriacea*), loggerhead turtle (*Caretta caretta*), and hawksbill turtle (*Eretmochelys imbricata*) are present in Argentine waters and use the area as a migratory route and feeding grounds during late spring to early fall (González Carman et al. 2011). The main threats to sea turtles in Argentine waters are habitat alteration, incidental capture in fishing nets, and marine pollution with urban solid waste, mainly plastics (Argentina Ministerio de Agricultura, Ganadería y Pesca 2018).

In February 2019, a juvenile green turtle (curved carapace length 44 cm) was found stranded in Quilmes City in the western coast of Rio de la Plata, Argentina (34°43′00″S, 58°16′00″W). It was found in a fluvial environment; thus, it was decided to transfer it to Fundación Mundo Marino rehabilitation center, San Clemente del Tuyú, Argentina. After an admission protocol, it was placed in observation pool at 25 C to evaluate its behavior (swimming, attitude for feed, decompression symptoms, fecal matter, etc.), which appeared normal. The turtle weight 9.4 kg and presented with the plastron slightly sunken; few fat deposits in the shoulders, eyes mildly sunken, neck and prefemoral space sunken, and supraoccipital crest visible. After fluid therapy, the turtle started to eat unaided. The only gross abnormalities observed at the clinical inspection were three cutaneous verrucous growths, one on the lower left eyelid (4-cm diameter) and two on the ventral aspect of the left front flipper (2.5- and 3-cm diameter, respectively; Fig. 1A). The severity of the lesions was classified with a score of 1 on a 0–3 scale (Work and Balazs 1999). The eyelid mass was excised, one portion being placed in 10% buffered formalin for histo-

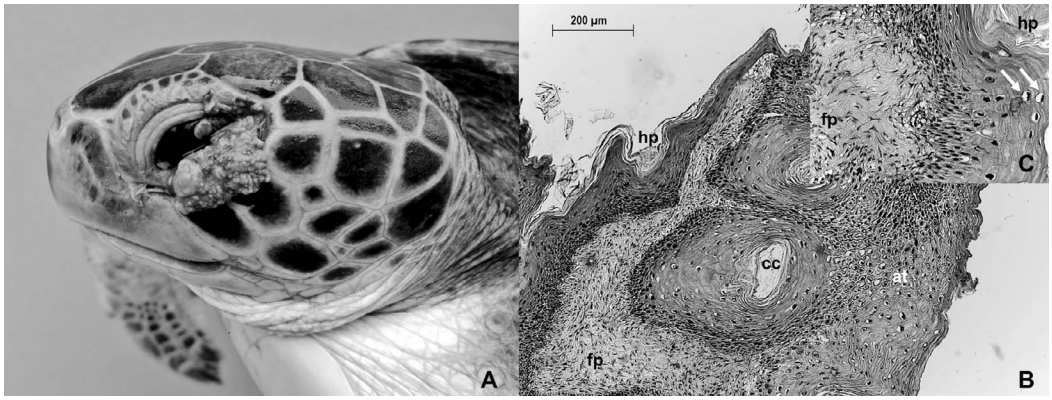

FIGURE 1. (A) Head of a juvenile green turtle, *Chelonia mydas*, found stranded on the western coast of Rio de la Plata, Argentina, showing a fibropapilloma in the lower left eyelid. (B, inset C) Eyelid skin from the same turtle at 10× and 40× magnification, respectively, showing: acanthosis (at) with moderate number of cells with ballooning degeneration (white arrows); cornified cysts (cc); orthokeratotic hyperkeratosis (hp); dermis hyperplasic (fp) with abundant vascularization, fibroblasts, and disorganized collagen fibers. H&E stain.

pathologic studies and another stored at –20 C for molecular studies. Histopathologic examination of H&E-stained sections of the eyelid mass showed a marked papillary hyperplasia of the epidermis, with fibrovascular proliferation in the papillary dermis. In the epidermis, orthokeratotic hyperkeratosis was observed, with some cornified cysts. In several areas there was a marked acanthosis, with a moderate number of ballooning cells, with the nucleus sometimes displaced to the side and transparent cytoplasm. Basal cells were observed with cytoplasmic vacuolization; others were necrotic. The dermis was hyperplasic with abundant vascularization and connective tissue resulting in an increase in dermal thickness. A wide proliferation of fibroblasts was found with a marked predominance in the papillary area. Additionally, the dermis contained a diffuse infiltration of mononuclear cells and a small number of heterophils and melanomacrophages (Fig. 1B).

Detection of viral genome was carried out from total DNA extracted from the eyelid mass using primers specific for a region of the DNA polymerase gene of ChHV5 as previously described (Quackenbush et al. 2001). The haplotype of the turtle was characterized by mitochondrial DNA control region (mtDNA) analysis as described by Abreu-Grobois et al. (2006). Agarose gel electrophoresis of the PCR products revealed amplicons of 483 base pairs (bp; for genomic DNA) and 890 bp (for mtDNA), respectively. The products obtained were subsequently sequenced in the Instituto de Biotecnologia-CICVyA-INTA, Argentina.

A basic local alignment search tool (BLASTn; National Center for Biotechnology Information, September 2022) search was used to analyze both sequences. The viral genomic DNA sample (GenBank accession number ON376265 was confirmed as *Chelonid alphaherpesvirus* 5, with high homology to ChHV5 DNA polymerase sequences stored in GenBank and closely related with the Atlantic variant (Fig. 2). The mtDNA sequence from the turtle (GenBank ON494496) was compared with previously deposited sequences (Archie Carr Center for Sea Turtle Research September 2022; and GenBank https://www.ncbi.nlm.nih.gov/genbank/), showing 100% identity with the reference haplotype sequence CM-A9.1.

Green turtles, mainly juveniles, arrive seasonally and remain from late spring to early fall in coastal waters of Argentina, suggesting that this area of the southwestern Atlantic may be a foraging and developmental ground where young green turtles converge after their initial development period spent in pelagic habitats (González Carman et al.

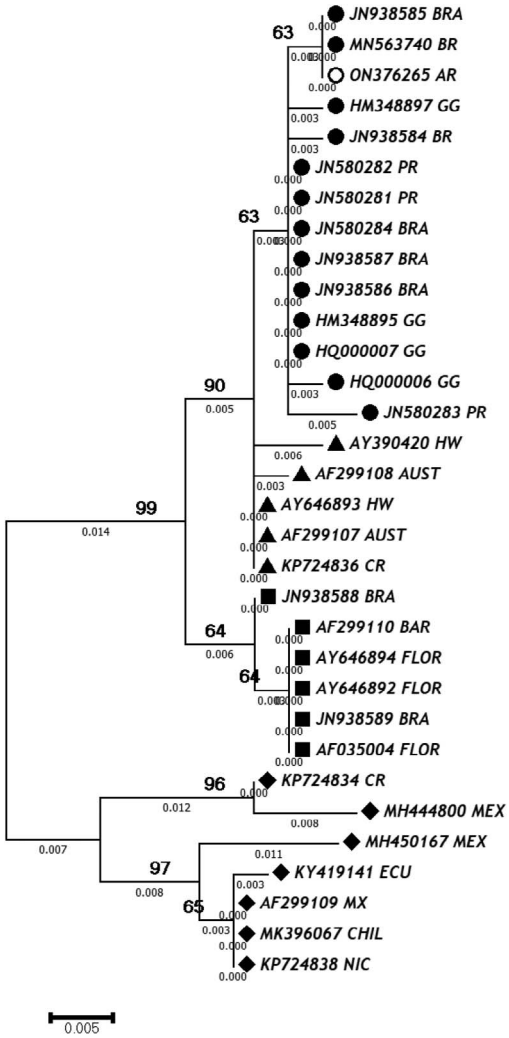

FIGURE 2. Phylogenetic tree created in MEGA7 (Kumar et al. 2016) by comparison of the DNA polymerase gene fragment (about 400 base pairs) from 32 *Chelonid alphaherpesvirus 5*(ChHV5) nucleotide sequences. The sample of ChHV5 from the fibropapilloma on the lower eyelid of a juvenile green turtle, *Chelonia mydas*, found stranded on the western coast of Rio de la Plata, Argentina, GenBank ON376265, is marked with ○. The evolutionary analysis was deduced by the maximum likelihood method based on the TamuraNei model. The tree with the highest log likelihood (762.0096) is shown. Above the branches, the percentage of trees in which the associated taxa cluster is shown. Initial trees for the heuristic search were obtained automatically by applying NeighborJoin and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) and then selecting the topology with superior log likelihood value. The length of the branches is proportional to the number of substitutions per site (below the branches). Strain location: PR (Puerto Rico), AR (Argentina), BR (Brazil), GG (Guinea Gulf), FL (Florida), MX (Mexico), HW (Hawaii), BAR (Barbados), AUST (Australia), ECU (Ecuador), CHIL (Chile), CR (Costa Rica), NIC (Nicaragua). Phylogeographic groups: ◆ eastern Pacific, ■ western Atlantic/eastern Caribbean, ▲ mid-west Pacific, and ● Atlantic (Patricio et al. 2012).

2011). These neritic (shallow) zones are characterized by aggregation of a high density of individuals from various nesting areas and, also, some individuals may circulate between different feeding areas; such aggregation and movements would favor close contact between sick, carrier, and susceptible animals with the consequent transmission of ChHV5 (Ene et al. 2005; Patricio et al. 2012).

Green turtles off the coast of Argentina come mainly from Ascension Island, with fewer numbers from Aves Island, Suriname, and Trinidad Island (Prosdocimi et al. 2012). The haplotype of the turtle found in this study corresponds to that found in nesting areas of Trinidad Island, Ascension Island, Rocas Atoll, and Fernando de Noronha, the first two being the most prolific nesting beaches of the South Atlantic (Proietti et al. 2012). Our ChHV5 sequence was similar to other sequences found along the coast of Brazil (Rodenbusch et al. 2012; Silva-Júnior et al. 2021). The origin of the virus in our case may be the Brazilian sea turtle population.

The macroscopic and microscopic findings were similar to those previously described for green turtle fibropapillomas, and the cranial distribution was similar to that previously recorded on turtles in Brazil (Rodenbusch et al. 2012; de Deus Santos et al. 2015; Rossi et al. 2021; Silva-Júnior et al. 2021).

Fibropapillomatosis has been previously recorded in juvenile green turtles at Cerro Verde Protected Area, Uruguay (33°56′S, 53°30′W; López-Mendilaharsu et al. 2016) and Rio Grande do Sul, Brazil (Rodenbusch et al. 2012), places near our study area. The case we describe is the southernmost registered to date for the southwestern Atlantic area (34°43′00″S, 58°16′00″W) and the first molecular report of ChHV5 in green turtles in

Argentina. Periodic monitoring is needed to obtain data on the dynamics of this disease in the sea turtles that frequent our coasts.

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