Cutaneous Papillomas of Green Turtles: A Morphological and Immunohistochemical Study in Brazilian Specimens

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Eleven juvenile green turtles *(Chelonia mydas)* from the Atlantic Ocean, Brazil with multiple cutaneous papillomatosis were examined. Histologically, the papillomas exhibit stromal hyperplastic proliferation and epithelial proliferation. These lesions exhibit nuclear features suggestive of viral infection. Severe nuclear pleomorphism was also observed in all cases with epithelial proliferation. Nuclear halo of the large type was present in the cases with epithelial proliferation; in these cells, nuclear features are frequently dyscariotic, without inclusion. All fibropapillomas examined were negative for papillomavirus group-specific antigens (BPV) and herpesvirus group-specific antigens (HSVI / HSV2) by the peroxidase-antiperoxidase technique.

Introduction

Fibropapilloma in captured adult green sea turtles was first described by Lucke (1938) and Smith and Coates (1938), over 50 years ago. They observed that papillomas were distributed over the dorsal cervical region, axillary regions of the hindlimbs, eyelids and conjunctivae.

Since then the number of green turtles with papillomas seems to have increased. Of one hundred green turtles captured in the Indian River Lagoon System of east central coastal Florida, U.S.A, before 1982 none displayed this lesion, but 30 of 57 (57%) captured from 1982 to 1986 showed this proliferative arrangement. Similarly, Balazs (pers. comm.) observed that, in 1985 and 1986, tumors were present in 35% of all stranded turtles recovered from the Hawaiian Islands.

Previous work by Sundberg *et al.,* failed to papillomavirus antigens in reptilian species; but virus and fibropapillomas in green turtles; microscopic evaluation (H.E.) revealed areas of ballooning degeneration of epidermal cells associated with eosinophilic intra inclusions. Electron microscopy analysis showed that inclusions consisted of virus-like particles measuring 77 to 90 nm. Envelopment of these particles was observed the nuclear membrane and mature enveloped p measuring 110 to 120 nm were present in the cytoplasm. Morphology, size, and location of the particle compatible with those of the family Herpetoviridae.

The present study focused on features of cut papillomatosis lesions of green sea turtles. Lesions examined morphologically (H.E) and immunohistochemically. An attempt to characterize the etiological agent using a polyclinic antibody for bovine papillomavirus (BP monoclonal antibodies to herpes simplex virus (HSV 2 is also reported.

Materials and Methods

Samples of multiple cutaneous papillomatosis green turtles from the Atlantic ocean were examined Dept. of Pathology of the Veterinary Medicine and / Science Faculty of São Paulo Univ., Brazil, and Pathology Div. of Adolfo Lutz Institute, Brazil.

These turtles, from the coasts of São Paulo, E Santo and Bahia States, were juveniles weighing around 10kg.

Fragments of papillomas were fixed in 10% formalin buffered solution, processed according to routine histological methods, and sections of 5 um thickness were stained with hematoxylin-eosin (H.E.), for light microscopic examination.

Fragments of lesions were fixed in 10% formalin and embedded in paraffin. 4 um sections were submitted for immunohistochemical study. The protocol used was described by Hsu *et al.* (1981). After deparafinization, sections were treated with 3% hydrogen peroxide solution to block endogenous peroxidase activity. Incubation with polyclonal antibodies anti-papillomavirus (DAKO B580) and polyclonal antibodies anti-Herpes Simplex I (DAKO B114) and anti-Herpes Simplex II (DAKO B116), obtained from rabbits, were performed at 4°C for 18 hours. Subsequently, biotinyled goat anti-rabbit immunoglobulin (VECTOR BA1000) was added, and incubated at 37°C for 30 minutes. The amplification of the reaction was obtained with avidin-biotin-peroxidase complex (VECTOR PK4000) and incubated at 37°C for 30 minutes. All incubations were concluded with two PBS washings of 5 minutes each. 50 mg% diaminobenzidine (Sigma D5637) and 0.1 % H_2O_2 in PBS were used as chromogen substrate and Harris Hematoxylin in the counter staining.

Results

Papillomas were distributed over the dorsal cervical region, axillary regions of the hindlegs, eyelids and conjunctivae, and ranged from 0.5 cm to 10 cm in diameter. These formations involved all soft integumentary tissue, but were particularly numerous in the axillary and inguinal soft tissue adjacent to both forelegs and hindlegs (**Fig. 1**).

The smallest recognizable lesions were slightly raised, light-brown in color, oblong in shape and had rough surfaces. In the major lesions, the surfaces were verrucous often ulcerated. Upon gross evaluation all cases exhibited, dispersed on the papillomatosis formations, trematode infections. These appeared, in paraffin sections, as elliptical structures enveloped by a chestnut-brown capsule, surrounded by macrophagic giant cells present in the interstitial area of the proliferative tissue.

The major part of the papillomas exhibit stromal (conjunctive) hyperplastic proliferations, and other epithelial proliferations.

These lesions when compared with that of human papillomavirus lesions exhibit nuclear features suggestive of the viral infection which was observed in all cases with epithelial proliferation. Two cases with stromal hyperplasia showed a discrete epithelial proliferation associated with cytological evidences of the viral infection. Severe nuclear pleomorphism, not seen in all cases with predominantly conjuntive hyperplasia, was also observed in all cases with epithelial proliferation. Nuclear halo of the large type, similar to the human papillomavirus koilocyte, was present in the cases with epithelial proliferation; in these cells, nuclear features are frequently dyscariotic, without inclusion **(Fig.** 2).

All fibropapillomas examined were negative for papillomavirus group-specific antigens (BPV) and herpes virus group-specific antigens (HSV1/HSV2) by the peroxidase-antiperoxidase technique.

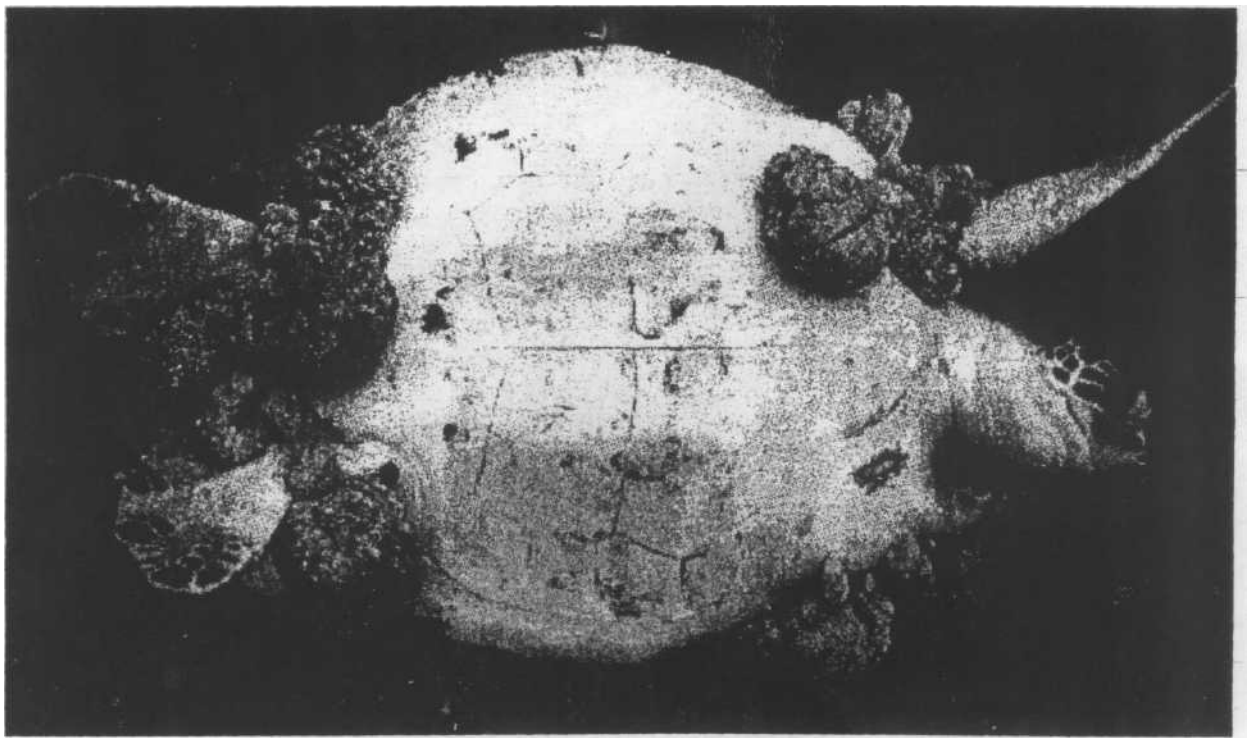

Figure 1. Green turtle fibropapillomas are particularly numerous in the axillary and inguinal soft tissue adjacent to both forelegs and hindlegs.

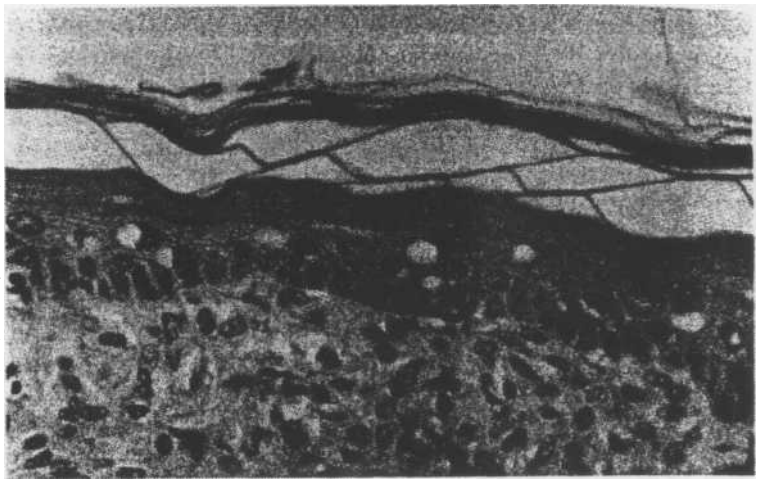

Figure 2. Microscopic lesion of the fibropapilloma (coloration HE and x 165.)

Discussion

The proliferative cutaneous lesions of the green turtles from this report were consistent with previous works which described fibropapillomas of green turtles from Florida (Lucke, 1938; Smith and Coates, 1938). The lesions had some morphological similarities with cutaneous fibropapillomas of mammals, i.e., epithelial hyperplasia marked proliferation of the dermal collagen and koilocytotic-like atypia, a cytopathological feature of productive papillomavirus infection (Koss and Durfee, 1956; Sundberg, 1984). Immunohistochemical and ultrastructural investigations failed to detect the viral particles.

Herpesvirus has also been found in papilloma lesions of a wide variety of vertebrates, including the European green lizard, *Lacerta viridis* (Raynaud and Adrian, 1976), African elephants, *Loxodonta africana* (Jacobson, Sundberg, Gaskin, Kollias and O' Banion, 1986), and green turtle, *Chelonia mydas* (Jacobson, Buergelt, Willians and Harris, 1991). The involvement of herpes virus as a primary agent of these lesions is, until now, uncertain. Except for its demonstration by indirect immunofluorescence or through the observation of intranuclear amphophilic inclusions in paraffin sections, there's no evidence of the activity of this virus in papillomatous lesions.

The occurrence of papillomas in dysjunct populations of green turtles, and the increased numbers of affected turtles in the Atlantic Ocean suggest that it may be caused by an infectious disease or by environmental influences such as chemical or physical pollution.

Further studies are required in order to explain the etiology and biological behavior of these papillomatous lesions.

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