



Proliferative dermatitis in a loggerhead turtle, *Caretta caretta*, and a green turtle, *Chelonia mydas*, associated with novel papillomaviruses

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Abstract

A subadult loggerhead turtle, *Caretta caretta*, presented with generalized small, white, raised lesions over its neck, shoulders, and all four flippers. A juvenile green turtle, *Chelonia mydas*, recently treated for fibropapillomatosis, presented with four similar localized lesions on one flipper. To diagnose the conditions, biopsies of the lesions were taken for histopathology, electron microscopy, and molecular diagnostics. Histopathologic findings were similar in the two turtles and skin lesions were characterized by multifocal areas of epidermal hyperplasia accompanied by variation and abnormalities in the nuclear morphology of keratinocytes and a few intranuclear inclusions in some cells. Transmission electron microscopy revealed multiple epithelial cells with large intranuclear aggregates of virions consistent in morphology with papillomavirus. Papillomavirus was detected in samples from both turtles by polymerase chain reaction (PCR). Sequence analysis of the partial sequence of the papillomavirus E1 gene revealed two viruses (CcPV and CmPV) that were distinct from each other and from other species in Papillomaviridae, and likely represent two novel species and perhaps a new genus.

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1. Introduction

Papillomaviruses are small, nonenveloped, double-stranded circular DNA viruses. Many species cause epithelial proliferation, and neoplasia may be a sequela. The family Papillomaviridae is divided into

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16 recognized genera (de Villiers et al., 2005), with at least two additional proposed genera (Rector et al., 2005). Papillomaviruses are generally species-specific, although exceptions have been found, such as bovine papillomavirus 1 in domestic horses (Otten et al., 1993). *Thetapapillomavirus* and *Etapapillomavirus* are viruses of avian hosts, and all other currently accepted genera use mammalian hosts (de Villiers et al., 2005). No papillomavirus of reptiles has been characterized beyond the level of the family Papillomaviridae. Papillomavirus-like particles have been identified on electron microscopy in a green lizard, *Lacerta viridis* (Raynaud and Adrian, 1976), twist-necked turtles, *Platemys platycephala* (Jacobson et al., 1982), and a Russian tortoise, *Agrionemys horsfieldii* (Drury et al., 1998). The green lizard also had herpesvirus-like and reovirus-like particles and it is difficult to attribute clinical signs to the papilloma-like virus particles. In the twist-necked turtles, multifocal, raised white oval lesions were seen on the head and regressed over time. Specific lesions were not reported to be associated with the virus in the Russian tortoise. No reptile papillomavirus has been isolated, and no sequence data, serologic assays, or polymerase chain reaction (PCR) diagnostics have previously been available. Here we present a case of generalized proliferative dermatitis in a loggerhead turtle, *Caretta caretta*, and localized proliferative dermatitis in a green turtle, *Chelonia mydas*, associated with papillomaviruses.

2. Materials and methods

2.1. Animals used in the study

2.1.1. Case 1

On 20 December 2006, a subadult loggerhead turtle, straight carapace length 73.0 cm, weight 42.0 kg, was found stranded in northeast Florida (Indialantic, Brevard County), covered in red algae and moderate to heavy barnacle load. This turtle stranded during an epizootic in the area that appeared to involve some toxicosis, although to date, no toxins have been identified. The turtle was originally taken to Volusia County Marine Science Center, Ponce Inlet, FL. On 27 December 2006, due to limited space because of the epizootic, the turtle was transferred to

the Sea Turtle Rehabilitation Hospital (STRH) at Mote Marine Laboratory and Aquarium in Sarasota, FL. Upon admission at STRH, the turtle was still covered with algae and barnacles that were then removed.

Approximately 6 weeks after admission, hundreds of small white cutaneous lesions developed on the head and appendages (Fig. 1A). Three punch skin biopsies were taken under local anesthesia and the turtle was placed in freshwater for 5 days in case there was a parasite involved. One biopsy was placed in 10% neutral-buffered formalin, one in 4% gluteraldehyde, and the other frozen.

2.1.2. Case 2

On 28 April 2007, a juvenile green turtle, straight carapace length 43.2 cm, weight 8.75 kg, was found stranded on the east central coast of Florida (Jupiter Island in Palm Beach County) and was admitted to STRH 2 days later. The turtle was affected over much of its body with fibropapillomatosis (FP). On 7 June, the turtle underwent CO₂ laser surgery for removal of all fibropapillomas, and post-surgical wound healing progressed as expected.

On 18 July four small, white, slightly raised lesions were noted on the anterior surface of the left front flipper. Biopsies for microscopic and EM histopathology and PCR were taken.

2.2. Histopathology

Cutaneous punch biopsies preserved in formalin were bisected and processed by routine methods into paraffin blocks. Paraffin-embedded sections were sectioned at 5- μ m, mounted on glass slides, and stained with hematoxylin and eosin (H&E) and Gomori methenamine silver (GMS).

For transmission electron microscopy, skin biopsies fixed in 4% gluteraldehyde were post-fixed in osmium tetroxide, sectioned at 90 nm, stained with lead citrate and uranyl acetate, and viewed with a Hitachi H-600 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

2.3. PCR amplification and sequencing

DNA was extracted from frozen tissue samples using the DNeasy Kit (Qiagen, Valencia, CA). Herpesvirus detection was attempted using previously

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