

# Occurrence of Fibropapilloma Tumours on Green Sea Turtles, *Chelonia mydas* in Trinidad, West Indies

Michelle Cazabon-Mannette<sup>1\*</sup> and Ayanna Carla N. Phillips<sup>2</sup>

<sup>1</sup> 38 Papillon Drive, Signature Park, D'Abadie, Trinidad

<sup>2</sup> School of Veterinary Medicine, Faculty of Medical Sciences, UWI, St. Augustine. Trinidad and Tobago

\* Corresponding Author: [mcazabon@gmail.com](mailto:mcazabon@gmail.com)

## ABSTRACT

Fibropapillomatosis, a debilitating disease of marine turtles associated with a herpesvirus, is commonly seen in green sea turtles, *Chelonia mydas* (Chelonia: Cheloniidae) throughout the tropics. It is characterised by the development of external and visceral tumours, which though benign, often obstruct organs or hinder bodily functions that are critical to survival in the wild and can therefore lead to death of the affected animal. Deleterious environmental factors are also thought to contribute significantly to the development of the disease in susceptible animals. A recent upsurge in the incidence of the disease globally has elevated the disease to panzootic status. Several anecdotal reports have suggested that this disease may be present in green turtles that inhabit the coastal waters of Trinidad and Tobago. Herein we document lesions consistent with fibropapillomatosis found in green turtles that stranded across Trinidad between 2010 and 2016, and discuss the implications.

**Key words** marine turtles, fibropapilloma, tumours, herpesvirus, *Chelonia mydas*, Caribbean

## INTRODUCTION

Green turtles *Chelonia mydas* (Chelonia: Cheloniidae) are distributed throughout the coastal waters of the tropics, and to a lesser extent, the subtropics (Seminoff 2004). They are currently listed as Endangered by the IUCN (Seminoff 2004), and have been designated as an Environmentally Sensitive Species in Trinidad and Tobago. They are considered important indicators of the health of the marine environment, especially since juveniles spend many years resident in nearshore feeding habitats (Aguirre and Lutz 2004). The distribution of green turtles has not been studied in Trinidad, but juveniles have been reported from Saut D'eau, Matelot, Grande Riviere, Toco, Matura, Mayaro, Soldado Rock and Chacachacare (Forestry Division *et al.* 2010), and they have been harvested in nearshore fisheries throughout Trinidad including multiple fishing depots on north, south and east coasts in addition to Carenage in the Gulf of Paria (Fisheries Division data and Chu Cheong 1995). Based on their herbivorous diet and preference for *Thalassia testudinum* in the Caribbean (Bjorndal 1980, Bjorndal 1985), their distribution around Trinidad has generally been assumed to be coincident with seagrass beds (Forestry Division *et al.* 2010), however some green turtles in the Atlantic also feed on algae and sometimes sponges (Mortimer 1981, Sazima and Sazima 1983, Makowski, Seminoff, and Salmon 2006, Nakashima 2008), therefore they may also inhabit coral reef and hard-bottom habitats, as has been found in Tobago (Cazabon-Mannette 2016), and they may be widely distributed around Trinidad.

Fibropapillomatosis (FP) is a disease of marine turtles, most commonly seen in green turtles (*Chelonia mydas*), though it has been reported in low frequencies in all hard-shell turtle species (George 1997, Guillen and Villalobos 1999, D'Amato and Moraes-Neto 2000) and at least once on a leatherback (Huerta *et al.* 2000). First documented by

Smith and Coates (1938) in a green turtle in Florida, outbreaks have been increasingly recorded around the world since the 1980s, including the Caribbean (Williams *et al.* 1994) and most prominently in the Hawaiian Islands and Florida (Jacobson *et al.* 1989, Balazs and Pooley 1991, Balazs, Aguirre, and Murakawa 1997, Murakawa *et al.* 1999, Work *et al.* 2004, Foley *et al.* 2005). FP now has a circumtropical distribution and has been observed in all major oceans (George 1997, Aguirre 1998). In Hawaii, FP prevalence increased rapidly since an outbreak in the late 1980s, peaked during the mid-1990s, and has been in decline since the late 1990s (Chaloupka *et al.* 2009).

FP is characterised by external benign tumours of the skin, flippers, periocular tissues, carapace, and plastron (Aguirre and Lutz 2004), which can grow to more than 30 cm in diameter (George 1997, Aguirre 1998). Fibropapilloma tumours are most common on the axillary region of the flippers, and on the eyes, neck, and tail (Balazs, Aguirre, and Murakawa 1997). Depending on the location, larger tumours can impair vision, locomotion, breathing and the ability to locate, capture, and swallow food, and avoid predators (Herbst and Klein 1995, Balazs, Aguirre, and Murakawa 1997, Aguirre and Lutz 2004), and may ultimately prove fatal (George 1997). Internal tumours may be found in the oral cavity (Balazs, Aguirre and Murakawa 1997), kidneys (Norton, Jacobson and Sundberg 1990), lungs, heart, liver, spleen, gastrointestinal tract and gonads (Campbell 1996, cited by Aguirre and Lutz 2004), and are present in 39% of stranded turtles with external tumours in Hawaii (Work *et al.* 2004). Such internal or visceral tumours can disrupt organ function and lead to death (Herbst and Klein 1995). Concomitant infections with vascular spirorchid trematodes have also been recorded among some fibropapilloma turtles (Aguirre *et*

al. 1998). The most severely affected turtles often exhibit physiological stress characterised by immunosuppression, with serum biochemical abnormalities which may include, anaemia, leukopaenia, heterophilia, uraemia, hypoproteinaemia, hypoalbuminaemia, electrolyte imbalance, lowered levels of cholesterol and triglycerides and wound infection which may progress to bacteraemia (Norton, Jacobson, and Sundberg 1990, Work and Balazs 1999, Aguirre and Balazs 2000, Work *et al.* 2003, Work *et al.* 2004).

An alphaherpesvirus, referred to as chelonid fibropapilloma-associated herpesvirus (CFPHV) or chelonid herpesvirus-5 (ChHV5) has been consistently detected in tumour tissue and is recognised as the most likely aetiological agent responsible for fibropapillomatosis (Quackenbush *et al.* 1998, Lackovich *et al.* 1999). Phylogenetic analyses have detected multiple separate lineages of CFPHVs with a shared mutation responsible for FP acquired prior to the emergence of the current panzootic, suggesting environmental or host factors underlie the recent disease expression, rather than recent virulence mutations (Herbst *et al.* 2004, Greenblatt *et al.* 2005, Patricio *et al.* 2012). This genetic evidence is consistent with field observations that a high prevalence of FP is associated with anthropogenically altered environments such as heavily polluted coastal areas (Aguirre and Lutz 2004, Van Houtan, Hargrove, and Balazs 2010, Santos *et al.* 2010). Furthermore, there is molecular evidence that FP is acquired after the recruitment of juveniles into coastal areas (Ene *et al.* 2005), consistent with field observations which find that fibropapilloma tumours are most common in juveniles of intermediate size (Borrowman 2000, Work *et al.* 2004, Foley *et al.* 2005, Tagliolatto *et al.* 2016).

As far as could be ascertained, the earliest published report of fibropapillomatosis in Trinidad is by Jacobson (1991); though no details were provided. The Sea Turtle Recovery Action Plan for Trinidad and Tobago (Forestry Division *et al.* 2010) cites a number of unconfirmed reports of green turtles with fibropapillomas in Chaguaramas, Paria Bay, Matelot and nesting at Grande Riviere, with the earliest report from 1983 in Chaguaramas. Cooper and Seebarsingh (2008) described a case of fibropapillomatosis (confirmed by cytological and histopathological techniques) in a green turtle (*Chelonia mydas*) found in the sea at Chaguaramas, in February 2008 and transported by the Trinidad Wildlife Section to the University of the West Indies School of Veterinary Medicine (UWI-SVM) for post-mortem examination. During a survey of fisherfolk in Tobago, fishers were shown images of turtles with fibropapilloma tumours and 13 of 215 fishers indicated they had encountered turtles with similar growths (Cazabon-Mannette, unpublished data), however

there are, to date, no confirmed reports of this disease in Tobago (personal communication, Giancarlo Lalsingh, Programme Manager, SOS Tobago).

## CASE SUMMARIES

The UWI-SVM has been a repository for reports of fibropapilloma cases around Trinidad since 2008. Following the 2008 case report by Cooper and Seebarsingh, four green turtle cases have since been submitted to the Aquatic Animal Health (AAH) Unit of the UWI-SVM for evaluation. Two specimens bore no lesions consistent with fibropapillomatosis; one was a live juvenile found in a tidal pool in North Manzanilla in 2015, and the other was an adult measuring ~110cm carapace length that died after being struck by a boat in the offshore Soldado Fields, in 2016. The two other specimens, however, bore lesions consistent with fibropapillomatosis. Below we present a summary of the findings from these two cases, as well as the findings on one additional specimen that was found dead on a beach in southern Trinidad. Figure 1 shows a map of Trinidad with the location of each case.

### *Case 1- 3 May, 2011*

A live, emaciated, poorly responsive juvenile green turtle (37.5cm straight carapace length) was found in Chaguaramas (coordinates not reported) and was submitted to the AAH Unit at the UWI-SVM. Two fibropapillomas, each measuring approximately 1cm × 0.75cm, were present on the lateral canthus of the left eye with corneal attachments. A multilobulated fibropapilloma (1.8cm × 1.9cm) was present on the right eye. Numerous larger fibropapillomas covered the dorsal and lateral aspects of the neck, the left and right pectoral and hind flippers, the tail base and plastron. The animal had minimal body fat,

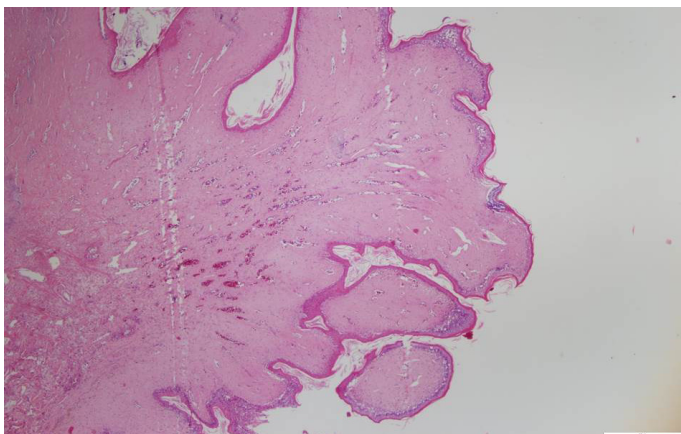


**Fig. 1.** Location of documented strandings of green sea turtles with fibropapilloma tumours at Chaguaramas (Case 1), Matura (Case 2) and Moruga (Case 3).

poor muscling, diffuse softening of the plastron, mild separation of abdominal scute sutures with leakage of haemorrhagic coelomic fluid. Preliminary radiographs showed no evidence of internal masses. Oesophageal tube feeding, additional nutritional supplementation and medical management were initiated to improve the animal's body condition prior to scheduling endoscopic evaluation and surgical excision of external masses. However, the animal did poorly and died on 14 May, 2011. Necropsy findings revealed cachexia, indicating that the animal was debilitated and unable to feed for a considerable period resulting in severe wasting. Histological analysis of the masses revealed characteristic stromal and epidermal hyperplasia, with fibroblast proliferation and zones of cytoplasmic vacuolation of epithelial cells (Fig. 2), consistent with other reports (Herbst 1994, Matushima *et al.* 2001). Samples were collected for confirmatory immunohistochemical and polymerase chain reaction (PCR) analysis. Investigations are ongoing and will be presented in a subsequent report.

#### Case 2- 28 February, 2016

A live, emaciated, weakly responsive juvenile green turtle (38.0cm straight carapace length) was found on Matura beach, Trinidad (coordinates not reported) and was examined at the AAH Unit of the UWI-SVM. The animal was in very poor body condition, with numerous eroded and ulcerated fibropapillomas around the neck and shoulders. There was also evidence of human interaction prior to the animal's rescue, where several other masses were crudely excised, leaving broad-based open wounds that had become infected. The animal was tube fed and antimicrobial therapy initiated, but the animal succumbed to its injuries on March 4<sup>th</sup>, 2016. Necropsy findings also revealed severe inanition and cachexia. Histopathological findings were similar to those presented in figure 2.



**Fig. 2.** External papilloma from a green sea turtle (*Chelonia mydas*) that stranded in Chaguaramas (Case 1). Characteristic stromal and epidermal hyperplasia, with fibroblast proliferation and zones of cytoplasmic vacuolation of epithelial cells are evident. Hematoxylin and Eosin x4.

#### Case 3- 3 December, 2016

A dead juvenile green turtle (approximately 35cm carapace length), was discovered stranded on shore at L'Anse Mitan, Moruga, between the Moriquite and Moruga Rivers (1115397 N, 0689324 W). The turtle was covered in a heavy burden of external tumours consistent with fibropapillomas (Fig. 3), located on its neck, the axillary and inguinal regions bilaterally, the eyes and the plastron. The largest tumour was approximately 15cm in length and located in the gular region. This is the first record of fibropapilloma for south east Trinidad. A fisherman from the area indicated that they often encounter live turtles with similar tumours within the bay.

### DISCUSSION

Fibropapillomatosis is a severely disfiguring and debilitating disease of sea turtles. A common finding among the cases reported to the AAH Unit of the UWI-SVM was that of severe wasting (cachexia). If animals were found or were admitted for treatment earlier, the prognoses may have improved. Early treatment allows these animals to be rehabilitated and returned to a state of health that would allow for successful surgical excision of the tumours, thus significantly improving the overall survival rate of affected green turtles. Timely detection and intervention is therefore key for cases presenting with external lesions only. If visceral fibropapillomas are detected, however, the prognosis is considered grave.

Green turtles are a species of global and local conservation concern, which are also postulated to play important roles in seagrass and coral reef health through their highly specific diets (Thayer, Engel, and Bjorndal 1982, Thayer *et al.* 1984, Wabnitz *et al.* 2010, Goatley, Hoey, and Bellwood 2012). Therefore threats to individuals' health and more importantly population viability are of immediate conservation concern. The geographic spread of the documented cases around the coast of Trinidad certainly is concerning, however the presence of FP within a green turtle population does not necessarily imply a threat to population viability. While continued passive documentation of FP among turtles encountered by members of the public gives valuable insight into the local distribution of FP, further applied research through in-water studies is also necessary, to allow for a meaningful estimate of FP prevalence and the survivorship of turtles, and to therefore determine the impact of FP on local population viability (Rees *et al.* 2016).

The precise role of environmental factors in the development of FP is not well understood, but FP has generally been observed to be associated with heavily polluted coastal areas (Aguirre and Lutz 2004, Foley *et al.* 2005, Van Houtan, Hargrove, and Balazs 2010). Environmental



**Fig. 3.** (a) (i) and (ii) Juvenile green sea turtle (*Chelonia mydas*) that stranded in Chaguaramas (Case 1). (b) Juvenile green sea turtle (*Chelonia mydas*) that stranded at Moruga (Case 3), (i) dorsal view with GPS unit for scale (ii) view of ventral surface (iii) dorsal view of head, neck and fore flippers.

factors that have been associated with high prevalence of FP include heavy metals in Brazil (da Silva *et al.* 2016), land-based nutrients in Hawaii (Van Houtan, Hargrove, and Balazs 2010), and biotoxins (Landsberg *et al.* 1999, Arthur *et al.* 2008). Therefore the presence of FP in Trinidad may reflect poor water quality due to some form of anthropogenic pollution, and may be a symptom of a larger environmental issue that deserves attention.

Since 2008, we have documented three cases of green turtles stranding with lesions consistent with FP around the coast of Trinidad. This is a relatively small number of cases, and suggests that the disease may be relatively rare locally (at least as a cause of stranding). However, the wide geographic distribution of the cases around Trinidad is cause for concern and it is possible that the disease is more prevalent than these documented strandings indicate, since many cases may go unreported. Members of the public, naturalists, fisherman, yachtsmen and others are urged to report to the SVM if they observe any turtle, alive or dead that may have this disease. It may be helpful to have a national awareness campaign to encourage persons to make such reports. The AAH Unit of the UWI-SVM will continue to conduct systematic necropsies, with histological analysis and confirmatory immunohistochemical and PCR analysis on any stranded turtles with suspected FP.

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