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Author: K. Jones, E. Ariel, G. Burgess, M. Read

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Review

A review of fibropapillomatosis in Green turtles (*Chelonia mydas*)

K. Jones [a,*], E. Ariel [a], G. Burgess [a], M. Read [b]

[a] *College of Public Health, Medical and Veterinary Sciences, James Cook University, 4811 Townsville, Queensland, Australia*
[b] *Reef Recovery Group, Great Barrier Reef Marine Park Authority, 2-68 Flinders Street, Townsville 4810, Queensland, Australia*

* Corresponding author. Tel.: +61 7 47816915.
E-mail address: karina.jones@my.jcu.edu.au (K. Jones).

Highlights

- Fibropapillomatosis (FP), an emerging disease in green turtles, is reviewed
- Chelonid herpesvirus 5 is the likely aetiological agent of FP
- The route of transmission and conditions facilitating lesion development are uncertain
- High prevalence of FP is observed in areas of reduced water quality
- A multi-factorial interplay between a range of factors is likely to be occurring

Abstract

Despite being identified in 1938, many aspects of the pathogenesis and epidemiology of fibropapillomatosis (FP) in marine turtles are yet to be fully uncovered. Current knowledge suggests that FP is an emerging infectious disease, with the prevalence varying both spatially and temporally, even between localities in close proximity to each other. A high prevalence of FP in marine turtles has been correlated with residency in areas of reduced water quality, indicating that there is an environmental influence on disease presentation.

Chelonid herpesvirus 5 (ChHV5) has been identified as the likely aetiological agent of FP. The current taxonomic position of ChHV5 is in the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Scutavirus*. Molecular differentiation of strains has revealed that a viral variant is typically present at specific locations, even within sympatric species of marine turtles, indicating that the disease FP originates regionally. There is uncertainty surrounding the exact path of transmission and the conditions that facilitate lesion development, although recent research has identified atypical genes within the genome of ChHV5 that may play a role in pathogenesis. This review discusses emerging areas where researchers might focus and theories behind the emergence of FP globally since the 1980s, which appear to be a multi-factorial interplay between the virus, the host and environmental factors influencing disease expression.

Keywords: Fibropapillomatosis; Marine turtle; Herpesvirus; Chelonid herpesvirus 5; Green turtle

Introduction

The Green turtle (*Chelonia mydas*) is one of seven species of marine turtle and is internationally recognised as endangered by the International Union for the Conservation of Nature (Seminoff, 2004). Eleven discrete population segments of Green turtles have been identified, each of which is considered biologically and ecologically significant (NMFS and USFWS, 2014). Green turtles also hold great cultural significance for many indigenous peoples and are of economic interest, playing a significant role in ecotourism (Dobbs, 2001; Gulko, 2004). The species has a global distribution and a complex life history, occupying a range of habitats. Hatchling turtles have a pelagic existence and recruit into benthic inshore waters at the age of 3-5 years (Reich et al., 2007). With the exception of migration for breeding, turtles typically remain in these inshore environments, which are commonly associated with seagrass meadows or coral reefs, for the remainder of their life (Musick and Limpus, 1997) (Fig. 1).

Green turtles are exposed to a number of threats including ingestion of marine debris, degradation, urbanisation and pollution of nesting habitats and foraging areas, nest and hatchling depredation by wild, feral and domestic animals, boat strike, traditional hunting and egg harvest, the impacts of climate change on the marine and terrestrial environment, and entanglement in fishing nets and lines (Bjorndal, 1995; Herbst and Klein, 1995a; Lutz, 2002; Van Houtan et al., 2010). Conservation efforts which aim to abate many of these threats have assisted in the recovery of some of the major Green turtle populations (Chaloupka et al., 2008a). However, outbreaks of disease are also contributing to morbidity and mortality in this already vulnerable species (Foley et al., 2005; Chaloupka et al., 2008; Flint et al., 2010c).

Fibropapillomatosis (FP) is a disease that has now been reported in every species of marine turtle; Green (Smith and Coates, 1938), Loggerhead (*Caretta caretta*) (Harshbarger,

1991), Kemp's Ridley (*Lepidochelys kempii*) (Barragan and Sarti, 1994), Hawksbill (*Eretmochelys imbricata*) (D'Amato and Moraes-Neto, 2000), Olive Ridley (*Lepidochelys olivacea*) (Aguirre et al., 1999), Flatback (*Natator depressus*) (Limpus et al., 1993), and Leatherback (*Dermochelys coriacea*) (Huerta et al., 2002) turtles. FP is of greatest concern in Green turtles as it has only reached a panzootic status in this species (Williams et al., 1994).

FP is a neoplastic condition which may lead to the growth of lesions on the skin, oral cavity, shell, eyes and internal organs of the affected turtle, which in severe cases reduces the probability of survival (Flint et al., 2010a; Herbst, 1995; Work et al., 2004). The disease was first identified in a Green turtle with multiple wart-like lesions on display at the New York Aquarium, although originally from Key West, Florida (Smith and Coates, 1938). Despite being described in 1938 (Lucke, 1938; Smith and Coates, 1938), FP did not reach epizootic proportions until the 1980s (Herbst et al., 1994, 2004) and has now been reported from every major ocean basin that Green turtles inhabit (Herbst, 1994).

This review covers the epidemiology and proposed aetiology of FP in Green turtles, with considerable emphasis on the primary candidate for the aetiological agent, chelonid herpesvirus 5 (ChHV5).

Disease presentation

FP can be identified in marine turtles by the presence of single or multiple benign fibroepithelial lesions. The characteristic lesions are easily noticed and are pathognomonic for FP, often limiting or obstructing the vision, feeding and locomotive ability of the affected turtle (Herbst, 1994, 1995; Work et al., 2004; Flint et al., 2010a). Cutaneous lesions are typically present on the external soft tissue of the turtle, but may grow on the carapace, plastron (Smith and Coates, 1938; Jacobson et al., 1989; Balazs and Pooley, 1991; Brooks et al., 1994; Herbst,

1994) and cornea of affected turtles (Brooks et al., 1994; Flint et al., 2010a). The lesions can be observed on all visceral organs (Herbst 1994; Work et al., 2004; Foley et al. 2005) and are thought to develop during later stages of the disease (Herbst et al. 1999; Wyneken et al. 2006). However, as most visceral lesions are observed during post mortem investigations, the data available on the prevalence of this type of lesion are skewed. Individual lesions can range from 0.1 to 30 cm in diameter and can be sessile or pedunculated. The appearance of these lesions can vary from smooth to verrucous and the colour is dependent on the pigment at the site of origin (Herbst, 1994) (Fig. 2).

Myxofibromas, fibrosarcomas, papillomas, fibromas and fibropapillomas have all been found to be associated with FP (Norton et al., 1990; Work et al., 2004). Three of these lesions are thought to be linked with different stages of lesion development (Herbst, 1994; Kang et al., 2008). The early development phase is associated with papilloma lesions, proliferation of epidermal cells, with little or no involvement of the dermal layer. The chronic phase of lesion development is marked by the presence of fibromas, with proliferation of the dermal layer, while the epidermal layer remains normal. Fibropapillomas represent the intermediate phase of lesion development and consist of characteristics of both the papillomas and fibromas (Herbst, 1994; Kang et al., 2008).

Histological studies on FP lesions have observed orthokeratotic hyperkeratosis and varying degrees of epidermal hyperplasia. Key features observed in FP lesions include cytoplasmic vacuolation and ballooning degeneration of superficial epidermal cells (Jacobson et al., 1989, 1991; Herbst, 1994; Adnyana et al., 1997).

Haematological and biochemical signs of immunosuppression, chronic stress, and chronic inflammation such as anaemia, lymphocytopenia, neutrophilia, monocytosis, hypoproteinaemia and hyperglobulinaemia have been observed in turtles with clinical signs of FP (Aguirre et al., 1995; Work et al., 2001; dos Santos et al., 2010; Page-Karjian et al. 2014). Although it is still unclear whether the immunosuppression occurs as a result of or as a precursor to FP development, it has been suggested that immunosuppression occurs as a result of FP (Work et al., 2001). While further study is essential to confirm the relationship between immunosuppression and FP infection, it is clear that immunosuppression leaves turtles with FP lesions susceptible to secondary infections and opportunistic pathogens (Work et al., 2001, 2003; Stacey et al., 2008; dos Santos et al., 2010). Impacts of such secondary infections, combined with FP in marine turtles, are a major cause for concern in an already vulnerable species.

Epidemiology of fibropapillomatosis in marine turtles

FP typically occurs in marine turtles inhabiting neritic tropical and sub-tropical areas (Herbst, 1994; Adnyana et al., 1997; Work et al., 2004; Ene et al., 2005). The disease is most frequently observed in juvenile turtles; FP has also been reported in sub-adults and less commonly in adults (Herbst, 1994; Herbst and Klein, 1995b; Adnyana et al., 1997; Work et al., 2004; Ene et al., 2005; Patrício et al., 2012; Page-Karjian et al., 2014). This apparent age differentiation in certain locations may indicate that affected juveniles perish from the population altogether or recover with acquired immunity that protects them as adults (Van Houtan et al., 2010). Alternatively, it is possible that these adults were never exposed to this disease.

There are no reports of FP in pelagic post hatchlings or new recruits that have recently taken up residence in inshore foraging habitats (Herbst, 1994). Sex is not thought to be a

contributing factor, as no significant difference has been observed in prevalence between males and females (Work et al., 2004).

Disease prevalence and impact

Smith and Coates (1938) reported a prevalence of 1.5% in the Florida Keys region. The disease was not documented in the area again until the 1980s, where the prevalence was then reported to range between 20-60% throughout the subsequent decade. The early to mid-1990s saw FP emerge in the Eastern Pacific, Hawaiian Islands, Indonesia and Australia. As the disease reached epizootic status in several locations globally, it is now considered a panzootic (Williams et al., 1994). Due to the conspicuous presentation of FP, any prior presence would have been noticed in a region where it currently occurs. The incidence of turtles with FP lesions as a percentage of total turtles captured is reported in the Appendix (Supplementary Table 1). Although age class is a risk factor, not all reports of FP prevalence have been corrected by demographic proportions and future reports would benefit from making this distinction.

The prevalence of FP varies both spatially and temporally - see Appendix (Supplementary Table 1). The sporadic reports of the disease over time, in combination with a lack of oral history prior to the 1980s, indicate that FP is globally emerging (Greenblatt et al., 2005b; Duarte et al., 2012). In several cases, a significantly different prevalence of the disease in nearby regions has been observed. In Florida, a prevalence of approximately 50% was observed in Green turtle aggregations in the Indian River region. However, less than 1 km away at the Sabellariid worm reef, FP was not observed at all (Herbst, 1994). At Pala'au, Molokai, FP was not observed at all until 1985, with the prevalence increasing from 1% in 1987 to 60.7% in 1995 - see Appendix (Supplementary Table 1).

A shift in FP prevalence at two closely monitored sites in Puerto Rico has been observed in recent years; FP prevalence began decreasing Puerto Manglar and increasing at Tortuga Bay in 2009 (Patrício et al., 2011). In Australia, FP has been reported in a number of locations since it was first observed in Queensland in the early 1970s (C. Limpus, personal communication).

The contribution of this disease to morbidity and mortality in affected turtles has also been widely discussed (Herbst, 1994; Ene et al., 2005; Foley et al., 2005; Chaloupka et al., 2008, 2009; Flint et al., 2010c). A study on Green turtles at Palaau, Hawaii found that this population was already recovering from previous overharvesting at the time of the FP outbreak in this region. The FP prevalence in this region has also been in decline since the mid-1990s (Chaloupka et al., 2009).

Studies on regions in Australia (Flint et al., 2010c), Puerto Rico (Patrício et al., 2011) and Florida (Hirama and Ehrhart, 2007) have all concluded that FP is not a significant factor in mortality of turtles. Conversely, a study conducted on data accumulated over 21 years from Hawaii implicated FP as the primary cause of strandings (Chaloupka et al., 2008).

Despite some conflicting conclusions, the overwhelming consensus is that FP does not significantly impact the survival of turtle populations. However, Hamann et al. (2010) highlights that understanding and managing this disease is a priority research area for sea turtle conservation. Without a more complete understanding of the fundamental elements of this disease, FP cannot be discounted as a threat to the survival of this species.

Aetiology of fibropapillomatosis in marine turtles

Research to date suggests that FP is associated with a herpesvirus infection (Herbst et al., 1995; Quackenbush et al., 1998, 2001; Lackovich et al., 1999). Despite ongoing research, this virus cannot be cultured in vitro and therefore Koch's postulates have not been fulfilled (Herbst, 1994, 1995; Moore et al., 1997; Lu et al., 1999; Work et al., 2009). Molecular techniques (Quackenbush et al. 1998, 2001; Lackovich et al. 1999) have proven a strong association between FP and a herpesvirus and, according to the criteria established by Hill (1965), the relationship seems to be that of cause and effect. Chelonid herpesvirus 5 (ChHV5) is now the primary focus of research in this area and belongs to the subfamily *Alphaherpesvirinae*, genus *Scutavirus* (Davison and McGeoch, 2010). However, there are still some uncertainties surrounding the transmission of the virus, the circumstances that lead to lesion development and the role of environmental factors in the development of this disease.

Infectious nature of fibropapillomatosis

The epizootic nature of FP and the significant variation in the prevalence of FP between different populations of marine turtles, even between nearby localities, led to speculation that FP was primarily caused by an infectious agent.

Herbst et al. (1995) successfully transferred FP between animals by using cell-free lesion extracts from turtles with lesions to inoculate young captive-reared turtles that were theoretically naive to FP. All turtles in 3/4 experimental groups developed FP lesions. Control animals, which were housed in the same facility and conditions as the experimental turtles, did not develop FP during the same study period. The lesion extracts used in this experiment were filtered through a 0.45 µm syringe tip filter to prevent most pathogens, other than viruses, from being transferred. These findings support the case for the role of a viral agent in FP transmission in marine turtles.

Although in their initial description of FP, Smith and Coates (1938) did not identify any viral elements in histological examination of FP lesions, modern theories have focused on viruses as the primary aetiological agent of FP. A range of viruses are capable of producing neoplasms such as those seen in Green turtle FP. As a result, papillomavirus (Herbst, 1994), papova-like virus (Lu et al., 2000a), retrovirus (Casey et al., 1997) and herpesviruses (Jacobson et al., 1991; Quackenbush et al., 1998; Herbst et al., 1994, 2004) have all been proposed as potential candidates for the aetiological agents of FP in marine turtles.

Current research suggests that FP is associated with ChHV5 infection. Early molecular studies tested a range of tissues from turtles both with and without FP lesions and all concluded that while ChHV5 could be detected in lesion biopsies from turtles with FP, the virus was rarely detected in normal skin samples from the same turtles (Quackenbush et al., 1998; Lackovich et al., 1999). Samples from turtles without FP lesions did not react in any of the PCR assays conducted in these early studies (Quackenbush et al., 1998; Lackovich et al., 1999; Lu et al., 2000b). These results support a strong association between the presence of ChHV5 and the presence of FP lesions.

Quackenbush et al. (2001) first successfully amplified ChHV5 from skin samples collected from turtles without FP lesions. Although only a subset of samples from turtles without FP lesions reacted in the assay, the results showed that the virus may be present in turtles despite a lack of clinical signs of disease. More recently, ChHV5 sequences have been amplified from skin samples of turtles without FP lesions with greater success (Page-Karjian et al., 2012; Alfaro-Núñez et al., 2014). These results indicate that early or latent infection with ChHV5 is more common than previously thought. The prevalence of turtles with FP lesions may be small relative to the number of turtles infected with ChHV5. Therefore, an absence of FP lesions does

not imply absence of ChHV5 infection. As latency is a typical feature of herpesviruses (Fields et al., 2013), such results are to be expected. The improved sensitivity and specificity of the assays used in these studies have revealed a feature of the disease that was undetectable using earlier assays.

If disease presentation is not dependent on viral infection alone, other factors contributing to lesion development must be considered. An interaction between host, pathogen and the environment (García-Sastre and Sansonetti, 2010) which tips the balance in favour of lesion development may be at play. Differences in host immunity may be preventing certain turtles from mounting a response to the virus (Griffin et al., 2010). Studies on other viral infections have shown that variants of a virus can have different levels of virulence and as such, disease presentation and severity may differ with each variant (Laegreid et al., 1993; Kaashoek et al., 1996; Berumen et al., 2001; Zhang et al., 2001; Yunis et al., 2004).

It is possible that the development of FP lesions is dependent on which viral variant a turtle is infected with. It is also possible that turtles infected with the virus only develop lesions when the viral load surpasses a certain threshold. While the relationship between viral titre and lesion development has not been resolved for ChHV5, this relationship has been described in other viral infections (Brodie et al., 1992; Liu et al., 2000; Zhang et al., 2000; Rosell et al., 2000; Quintana et al., 2001; Ladekjær-Mikkelsen et al., 2002; Rovira et al., 2002; Olvera et al., 2004; Islam et al., 2006; Ravazzolo et al., 2006; Nsubuga et al., 2008; Haralambus et al., 2010). The consistent association of high viral load and lesion development provides support for the theory that this may be the case for ChHV5.

Chelonid herpesvirus 5

Nomenclature and taxonomy

There are currently six herpesviruses documented in chelonids, named chelonid herpesvirus 1 to 6 (ChHV1-6). Chelonid herpesvirus 1, 5 and 6 are described in marine turtles whilst the others have been reported in freshwater turtles (Tidona and Darai, 2011). In the absence of sequence data, ChHV1, ChHV2, ChHV3 and ChHV4 remain unrecognised by the International Committee on Taxonomy of Viruses (ICTV) and their taxonomic place is unclear (Davison and McGeoch, 2010). With respect to the marine turtle herpesviruses, ChHV1 is described in association with grey patch disease (Haines et al., 1974; Rebell et al., 1975), ChHV5 is associated with FP and ChHV6 is known to be associated with lung-eye-trachea disease (Jacobson et al., 1986; Curry et al., 2000; Coberley et al., 2001a, 2002).

Chelonid fibropapilloma-associated herpesvirus (CFPHV) or ChHV5 (Davison and McGeoch, 2010) is now the more commonly used name for this virus. However, it should be noted that previous studies have used a range of names for this virus – see Appendix (Supplementary Table 2). This review refers to the virus as ChHV5.

Histological investigations of FP lesions showed indications of herpesvirus infection and subsequent studies using electron microscopy concluded that the virus-like particles that were observed were likely to belong to the family Herpesviridae based on location, size and morphology (Jacobson et al., 1989, 1991; Herbst et al., 1995).

More recent studies using a range of molecular techniques have confirmed herpesviral elements are present in FP lesions (Quackenbush et al., 1998, 2001; Lackovich et al., 1999; Lu et al., 2000a, b, 2003; Yu et al., 2000, 2001; Nigro et al., 2004a, b). Phylogenetic analysis of the ChHV5 genes DNA polymerase and DNA binding protein sequences revealed that ChHV5

clusters closely with, but separate to, other members of the *Alphaherpesvirinae* subfamily (Greenblatt et al., 2005b; McGeoch and Gatherer, 2005). Davison and McGeoch (2010) targeted the single-stranded DNA-binding protein, glycoprotein B, the major capsid protein, DNA polymerase and two subunits of the DNA packaging terminase (genes UL29, UL27, UL19, UL30, UL15 and UL28, respectively). The resulting Bayesian phylogenetic tree shows that ChHV5 exists as an out-group, clearly separate from the current genera. A Minimum Evolution phylogenetic tree of *Alphaherpesvirinae* based on full length DNA polymerase sequence further supports this result (Fig. 3). Consequently, it has been proposed that ChHV5 be placed in its own genus. The proposed genus, *Scutavirus*, sits within the *Alphaherpesvirinae* subfamily of *Herpesviridae*.

Variants of chelonid herpesvirus 5

Based on nucleotide sequence diversity, four viral variants of ChHV5 have been recorded in waters around Florida. At present, they are known as A, B, C and D (Herbst et al., 2004; Ene et al., 2005). Variant A is the most prevalent in the region, yet there is variation in relative prevalence of variants at each site. Co-infection with variants A and B was also found in one Green turtle (Ene et al., 2005). Perhaps even more significantly, different species of marine turtle shared the same variant if they were present in the same locality (Herbst et al., 2004; Ene et al., 2005). This indicates a strong geographic role in the transmission of the virus.

In a recent study, ChHV5 was examined using samples from a variety of locations in order to create a global phylogeography of the virus. Four phylogeographical groups of ChHV5 were identified: eastern Pacific, western Atlantic/eastern Caribbean, mid-west Pacific and Atlantic (Patrício et al., 2012). The results of the study showed that the viral variant is similar between nearby foraging grounds while distant regions are considerably divergent. The study by

Patrício et al. (2012) also found that sympatric species of marine turtle were infected with the same viral variant, further supporting the results of Herbst et al. (2004) and Ene et al. (2005). These findings indicate that individual turtles are likely to be infected with the virus through horizontal transmission in neritic bays (Patrício et al., 2012).

Co-evolution of virus and host

Herbst et al. (2004) suggested that the virus diverged prior to the separation of avian and mammalian alphaherpesviruses. This would mean that ChHV5 became specific to marine turtles approximately 300 million years ago (mya). In addition, it was estimated that the two most divergent clades were separated approximately 1.6-4.0 mya. These results led to speculation that the rise of the Isthmus of Panama (3.1-3.5 mya) was responsible for the divergence as it prevented genetic exchange between these clades. Patrício et al. (2012) found that the most recent common ancestor of the currently known variants of this virus existed 193-430 years ago. This estimate is considerably more recent than the work of Herbst et al. (2004) but both studies demonstrate that ChHV5 has evolved with marine turtles and, in either case, it is likely ChHV5 has undergone region specific co-evolution with its host.

While further research is needed to resolve the time of divergence, there is one clear conclusion; it is not a new virus, or even recent mutations in an old virus, that is causing lesions to develop. This evidence further supports the theory that the recent emergence of FP is linked to modern day extrinsic environmental factors promoting lesion development.

Genome organisation

The herpesvirus genome is divided into two unique regions, one composed of a unique long (UL) sequence and the other region is composed of a unique short (US) sequence. These

unique sequences are flanked by repeat sequences. The number, position and direction of these sequences can vary and as a result, there are multiple types of herpesvirus genome structures. Current literature lists between four and six known herpesvirus genome types. Fauquet et al. (2005) recognised four herpesvirus genome types (denoted Type 1-4) while Pellet and Roizmann (2007) described six different genome types (denoted Type A-F).

A recent study has described the entire genome of ChHV5 (Ackermann et al., 2012). The extensive sequence data generated from this study showed a clear division of the genome into UL and US regions. Inverted repeat sequences (IRS) were also found to flank the US sequence. This configuration is consistent with ChHV5 having a type D genome (Ackermann et al., 2012).

Ackermann et al. (2012) also described four genes that are atypical for an alphaherpesvirus genome. Two members of the C-type lectin-like domain superfamily (F-lec1, F-lec2), an orthologue to the mouse cytomegalovirus M04 (F-M04) and a viral sialyltransferase (F-sial) were all found to be present in the ChHV5 genome (Ackermann et al., 2012). While the products of these genes may not be critical for viral replication, each one has a potential role in pathogenesis or immune deviation (Ackermann et al., 2012). Orthologues to these genes have been described in other viral families and host cells (Neilan et al., 1999; Wilcock et al., 1999; Voigt et al., 2001; Markine-Goriaynoff et al., 2004). However, until now, none of these genes has ever been reported in the genome of an alphaherpesvirus. Two of these atypical genes (F-sial and F-M04) were found to be expressed in the FP lesions and it has been suggested that these genes may play a role in FP pathogenesis (Ackermann et al., 2012).

Transmission of chelonid herpesvirus 5

As this disease has not been observed in pelagic juveniles, it is thought that turtles are exposed to ChHV5 upon recruitment to neritic zones, indicating horizontal transmission (Herbst, 1994; Ene et al., 2005; Patrício et al., 2012). These new recruits may be exposed to several stressors associated with migration, adaptation to a new environment, and changes in population density, diet and pathogen exposure, which may all combine to reduce the efficacy of the immune system and make these juveniles more susceptible to infection (Ritchie, 2006) with ChHV5 and development of FP. It is also possible that these stressors combine to enhance transmission or elicit herpesviral recrudescence in latently infected turtles (Ritchie, 2006) leading to the development of FP lesions. Alternatively, direct transmission may be occurring between co-habiting turtles via interactions such as mating and aggression.

Researchers have speculated on means of transmission of FP as an infectious disease and possible vectors. Marine turtles host a range of parasites and correlations have been made between parasite load and individual health. Spirorchid trematodes (Jacobson et al., 1989, 1991; Norton et al., 1990; Aguirre et al., 1994, 1998b; Williams et al., 1994), coral reef cleaner fish (Booth and Peters, 1972; Losey et al., 1994; Lu et al., 2000c), saddleback wrasse (*Thalassoma duperrey*) (Lu et al., 2000c) and marine leeches (*Ozobranchus* spp.) (Greenblatt et al., 2004) have all been proposed as potential vectors of ChHV5. Significantly higher viral loads were detected in marine leeches when compared with the other parasites examined (Greenblatt et al., 2004) and they are currently the leading candidate for a mechanical vector. Although *Ozobranchus* leeches are the most likely candidates for transmission vectors of ChHV5, their exact role has not yet been confirmed. This is partly due to the possible latent state of the virus and involvement of other co-factors in disease expression of FP (Greenblatt et al., 2004).

Other marine turtle epibiota, including bladder parasites (*Pyelosomum longicaecum*), barnacles (*Platylepas* spp.), amphipods of the skin and oral cavity (order *Talitroidea*) and blood flukes of the genera *Carretacola*, *Hapalotrema* and *Laeredius* have been ruled out as potential vectors (Greenblatt et al., 2004).

Environmental factors

Marine turtles are particularly susceptible to changes in their environment as they are long-lived animals with a complex life history (Aguirre and Lutz, 2004). A marine turtle will access a range of habitat types during its lifetime, but exhibits a high degree of site fidelity once recruited into a near shore foraging area. Mature female turtles are known to return to the natal area from which they originated as hatchlings in order to lay their eggs (Limpus, 2008). Due to this site fidelity, marine turtles are likely to persist in, or return to, their chosen localities despite unfavourable changes to the environment. As a result, any damage to or destruction of these sites could have extremely detrimental effects on populations that inhabit them (Hawkes et al., 2009; Poloczanska et al., 2010; GBRMPA, 2014).

It has been suggested that environmental factors may play a role in the development of FP (Herbst, 1994; Herbst and Klein, 1995a; Adnyana et al., 1997; Aguirre and Lutz, 2004; Chaloupka et al., 2009; dos Santos et al., 2010; Van Houtan et al., 2014). Moreover, the presence of chemical contaminants may be part of a multifactorial problem that leads to FP (Herbst, 1994). Early proponents of a possible relationship between degraded water quality and the presence of FP proposed that chemical contaminants present in the water acted as immunotoxins or were causing damage at the cellular or genetic level (Herbst, 1994).

Indirect disturbances to the immune system may occur if the chemical contaminants create a disruption of neuroendocrine function (Zeeman and Brindley, 1981; Anderson et al., 1984; Dean et al., 1990; Colborn et al., 1993; Arkoosh et al., 1994; Dunier, 1994). Herbst (1994) demonstrated that a positive correlation exists between the prevalence of FP in Green turtle populations adjacent to regions associated with agriculture, industry and urban development. Subsequent studies have observed the same correlation (Adnyana et al., 1997; Foley et al., 2005; dos Santos et al., 2010; Van Houtan et al., 2010). Although initial reports in Puerto Rico observed the same relationship, this trend was reversed after several years; the prevalence of FP at the more pristine site is now considerably higher than at the site which is subjected to high levels of human activity (Patrício et al., 2011; Page-Karjian et al., 2012). Researchers attempted to quantify this relationship in Hawaii by developing an information-rich index of eutrophication from the analysis of 82 different watersheds. The results showed a strong association between FP rates, nitrogen-footprints and macroalgae consumed by turtles (Van Houtan et al. 2010). Different quantification studies were also undertaken in waters around Brazil and found that Green turtles residing in areas with degraded water quality had a higher prevalence of FP. However, this study based the assessment of water quality on the presence of benthic macrophytes and nutrient levels; pollution and the presence of chemical contaminants were not considered (dos Santos et al., 2010).

Only very low concentrations of persistent organic pollutants (Keller et al., 2014) and selected trace metals and organic pollutants (Aguirre et al., 1994) have been detected in turtles with FP lesions. Although these results suggest that the pollutants examined do not significantly contribute to FP development, it is possible that further investigations will uncover a relationship between this disease and other environmental contaminants (Keller et al., 2014).

441 Water temperature may also be a factor in lesion development and growth rate. It is
442 possible that warmer water temperatures during summer promote lesion growth, resulting in
443 lesions of a debilitating size by autumn (Herbst, 1994; Herbst et al., 1995). This seasonal trend
444 has been observed in Florida, where a higher rate of FP is observed in turtles that strand in winter
445 (Herbst, 1994). However, no seasonal trends have been observed in Hawaii (Murakawa et al.,
446 2000), which may be because there is less seasonal fluctuation in water temperature in this
447 region (Foley et al., 2005).
448
449 Natural biotoxins have also been implicated as a co-factor involved in FP development.
450 Landsberg et al. (1999) identified a correlation between high-risk FP areas in the Hawaiian
451 Islands and prevalence of *Prorocentrum,* a species that produces okadaic acid, a known tumour
452 promoter (Suganuma et al., 1988; Haystead et al., 1989; Cohen et al., 1990; Huynh et al., 1997).
453 Similarly, tissue concentrations of lyngbyatoxin A, produced by *Lyngbya majuscula*, have been
454 correlated with the presence of FP lesions in dead Green turtles (Arthur et al., 2006, 2008).
455 However, this species constituted less than 2% of total dietary intake and subsequently, any
456 biotoxins would be at a low concentration in the turtles (Arthur et al., 2008). If the dietary items
457 containing these biotoxins form a natural component of the diet of Green turtles and the amount
458 being consumed was not altered, these toxins should have no influence on the development of
459 FP.
460
461 An increased concentration of arginine in the diet of Green turtles as a result of invasive
462 macroalgae blooms has also been linked to an increasing prevalence of FP (van Houtan et al.,
463 2010). Arginine is a regulator of immune activity (Peranzoni et al., 2008) and is known to
464 promote herpesviruses and contribute to tumour formation (Mannick et al., 1994). This amino

465 acid is also a major component of glycoproteins on the viral envelope of herpesviruses (van
466 Houtan et al. 2010; van Houtan et al. 2014).
467
468 The results of a subsequent study found an association between eutrophication and
469 arginine content of macroalgae, with the intake of arginine in turtles at eutrophied sites being up
470 to 14 times the background level. This increased arginine content may metabolically promote
471 ChHV5, leading to FP lesion development (Van Houtan et al., 2014). Although the conclusions
472 from this study were subsequently challenged (Work et al., 2014), the epidemiological link
473 between the prevalence of disease and feeding ecology found in Van Houtan et al. (2014)
474 provides strong support that environmental factors play a role in the development of this disease.
475 However, the environmental factors leading to the bloom of macroalgae may be causing the
476 development of FP lesions directly, and the algal blooms may not be involved in lesion
477 development at all. If this is the case, it is difficult to link cause and effect.
478
479 Despite there being a strong positive correlation between the prevalence of FP in Green
480 turtle populations and areas with degraded water quality, it is difficult to identify one specific
481 causal contaminant or a combination of such working synergistically to the detriment of the
482 turtles. Studies on toxicity usually focus on chemicals that are persistent in the environment or
483 can bio-accumulate. Genetic damage as a result of a toxin may occur as a consequence of
484 transient exposure and as such, future studies would need to be expanded to include transient
485 chemicals that could have this effect on Green turtles. The practicality of such investigations is
486 daunting considering the vast marine environment and the known and unknown possible causes
487 of FP (Herbst, 1994; Herbst and Klein, 1995a).
488

One way that potential links between FP and anthropogenic contaminants might be identified is to develop a monitoring program that records and compares contaminant residue levels, genetic changes and viral load in blood and/or tissue samples collected from turtles with and without FP lesions over a wide geographic area and across several seasons. Such a program could be integrated into existing turtle monitoring activities. Controlled laboratory studies in a closed experimental system may be needed to conclusively evaluate the roles of various environmental factors in FP development (Herbst and Klein 1995a). Alternatively, results from both field and laboratory based studies may work synergistically to fully resolve this relationship.

Direction of future research

The longevity of marine turtles, coupled with their close association with inshore habitats and seagrass meadows and coral reefs in these habitats, has led to the proposal that they may act as sentinel indicators of marine ecosystem health (Aguirre and Lutz, 2004). Gaining a better understanding of the health and prevalence of diseases in marine turtle populations provides a critical link between ecosystem health and turtle health. Effective management of both the habitat and the species that rely on it is critical for effective species conservation. As FP has been found to be associated with turtles resident in areas exposed to poor water quality (Herbst, 1994; dos Santos et al., 2010; Van Houtan et al., 2010, 2014), FP prevalence may be a vital tool in monitoring inshore marine habitats. Many of these marine environments are also utilised by humans and consequently, research into the epidemiology of this disease could be mutually beneficial for Green turtles, other species in these ecosystems and humans alike (Aguirre and Lutz, 2004; Flint et al., 2010c). Long term monitoring of populations will allow researchers to more accurately establish disease prevalence, corrected by demographic proportions.

Whether the development of FP lesions is a result of a single agent or the interaction between multiple factors is yet to be determined. It is clear that it is an infectious disease with a strong link to ChHV5. In addition, the strong influence of different geographic regions on the prevalence of FP and each of the viral variants indicate that FP is geographically specific (Herbst et al., 2004; Ene et al., 2005; Patrício et al., 2012). The results from molecular studies targeting ChHV5 in samples from turtles show that the virus is present in turtles with and without FP lesions (Quackenbush et al., 2001; Page-Karjian et al., 2012; Alfaro-Núñez et al., 2014). Future molecular studies targeting ChHV5 should consider these results and screen all samples for ChHV5, not only those from turtles with FP lesions. Biosecurity and potential zoonosis should always be considered by those handling marine turtles in both field and captive situations. However, future research should prioritise understanding the triggers for lesion development.

Conclusions

There are many aspects of FP in marine turtles that are yet to be resolved and future research needs to target those gaps which will ultimately aid in managing the disease. Understanding how ChHV5 is transmitted between turtles and between regions is a key priority. Molecular epidemiology is a useful tool for revealing genetic differences in this virus between regions; possible relationships between host lineage and viral strain and the genes responsible for pathogenesis and viral replication. Molecular investigations on ChHV5 from different regions are essential to improve our understanding of the epidemiology and pathogenesis of this virus which will in turn inform the management and conservation of a vulnerable species, the Green turtle.

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Appendix: Supplementary material

Supplementary data associated with this article can be found in the online version at…..setters please insert doi number

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig. 1. The complex life history of Green turtles. Adapted from Lanyon et al. (1989).

Fig. 2. The plastron and hind flippers of a Green turtle severely affected by fibropapillomatosis highlighting the diverse range of lesion appearance.

Fig. 3. A Minimum Evolution phylogenetic tree of *Alphaherpesvirinae* based on full length DNA polymerase sequence retrieved from GenBank (Accession numbers provided in tree). Bootstrap values for each node are provided (1000 replicates). The analysis involved 27 nucleotide sequences resulting in a total of 2593 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013)