Fibropapillomatosis in Marine Turtles: Insights into Chelonid Alphaherpesvirus 5 (ChAHV5) and its detection in Marine Ecosystems through Environmental DNA (eDNA)

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ABSTRACT

Fibropapillomatosis (FP) is a tumor-forming disease that poses a significant threat to global marine turtle populations. This review explores the multifaceted nature of FP, examining its clinical manifestations, management strategies, and current understanding of its etiology, epidemiology, prevalence, and distribution. Central to FP pathology is Chelonid alphaherpesvirus 5 (ChAHV5), a virus strongly linked to tumor development in infected turtles. The review

provides insights into ChAHV5, elucidating its viral structure, classification, known variants, and modes of transmission, alongside a critical assessment of established and emerging detection methods. Given the solitary nature and conservation status of marine turtles, the use of non-invasive monitoring is highly preferred, and the use of environmental DNA (eDNA) presents a promising approach for viral detection in marine ecosystems. With this, the review discusses the persistence and abundance of eDNA in aquatic environments, alongside its methodological advantages and inherent limitations. By integrating current knowledge on FP and ChAHV5 with the emerging potential of eDNA, this paper aims to provide a holistic perspective on the disease, highlight critical knowledge gaps, and propose future research avenues and conservation strategies to mitigate the impact of fibropapillomatosis on vulnerable marine turtle species.

Keywords: marine turtles, fibropapillomatosis, ChAHV5, eDNA

INTRODUCTION

Marine or sea turtles are air-breathing reptiles in the superfamily Chelonioidea (Arulmoorthy & Srinivasan, 2019). There are seven extant species—the green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*), flatback (*Natator depressus*), olive ridley (*Lepidochelys olivacea*), Kemp's ridley (*Lepidochelys kempii*), and leatherback (*Dermochelys coriacea*; Avise & Hamrick, 1997; Paladino & Morreale, 2001)—which inhabit all oceans except the Arctic and Antarctic (Bennet, 2018). Five species are found in Philippine waters: the green, hawksbill, leatherback, loggerhead, and olive ridley (IUCN Red List, 2022).

Their life is almost completely spent in the water (Robinson & Paladino, 2013), but is inevitably tied to land at certain stages, such as during nesting (Paladino & Morreale, 2001). As adults, sea turtles are capable of travelling long distances, from hundreds to thousands of kilometers, between foraging grounds and nesting sites (Luschi, 2013). As keystone species, their extensive migratory behavior plays a significant role in the facilitation and preservation of marine biodiversity as they transport other organisms and maintain food web balance (Cáceres-Farías et al., 2022; González-Paredes, 2011; Lutz et al., 2002).

Unfortunately, their populations are in decline due to various natural and anthropogenic threats. The majority of mortality occurs in the early stages of their life, mainly through predation or habitat destruction. Upon maturity, they are at less risk due to their enlarged size, however they remain susceptible to anthropogenic factors like poaching, bycatch, pollution, habitat loss, and climate change (Mazaris et al., 2017; Stanford et al., 2020). They may also be at risk of diseases from various pathogens (Innis, 2014).

Fibropapillomatosis (FP), a panzootic disease in marine turtles, has been reported in many regions–Americas, Australia, and Southeast Asia–over the years. Recent cases of increased prevalence in FP infection of green sea turtles were reported in Mabul Island, Sabah, Malaysia, wherein a 7.8% (n = 115) prevalence during the

2015-2016 survey reached 42.9% (n = 63) in a 3-year span. Infection in hawksbill and olive ridley turtles were also reported (Loganathan et al., 2021; Robben et al., 2023). The increased FP prevelance in Malaysia increases the risk of exposure of marine turtle species to neighboring countries, such as the Philippines, given the potential interoceanic transmission of ChAHV5 facilitated by turtle migration (Talib et al., 2004, Pilcher et al., 2019).

FP contributes to the increasing mortality rate by causing tumor growths that significantly affect their overall health. The suspected causative agent of this disease is chelonid alphaherpesvirus 5 (ChAHV5) of the genus *Scutavirus*, subfamily *Alphaherpesvirinae*, family *Orthoherpesviridae* (Gatherer et al., 2021; Page-Karjian, 2019). ChAHV5 is consistently linked to fibropapillomatosis across all marine turtle species but remains understudied in terms of its morphology and infection mechanisms. This is due to difficulties in culturing the virus *in vitro* and in obtaining tissue samples from infected turtles in the wild (Lu et al., 1999; Moore et al., 1997). Furthermore, this sampling method is invasive, highlighting the need for alternative approaches.

Environmental DNA (eDNA) methods offer a non-invasive means of species and pathogen detection from environmental samples (Farrell, Whitmore et al., 2021). As such, recent studies have explored its use in the detection of ChAHV5 in marine waters, but only in setups under controlled conditions such as in holding tanks, where the impacts of environmental factors are minimized (Farrell et al., 2022; Farrell, Yetsko, et al., 2021; Yetsko et al., 2020). Thus, further investigation on ChAHV5 detection using eDNA is needed, especially *in situ* marine environments.

Despite numerous reports on fibropapillomatosis, studies on its definitive causative agent and effective detection methods remain limited. As such, a narrative review approach was adopted because the existing literature on chelonid alphaherpesviruses is characterized by substantial heterogeneity and incomplete documentation. At present, only ChAHV5 is formally recognized,

and even its reported variants and inferred evolutionary relationships are shaped by uneven research efforts, limited sequence availability, and geographic biases in sampling. Several proposed variants were named in the absence of complete sequence data, and the broader field lacks standardized nomenclature and consistent methodological reporting. These limitations prevent the rigorous comparison, structured data extraction, and quantitative synthesis required for a systematic review or meta-analysis. Consequently, a narrative format provides the most appropriate means to integrate and contextualize the available evidence while transparently acknowledging current gaps and constraints in the literature.

FIBROPAPILLOMATOSIS

Fibropapillomatosis (FP) is a condition that only affects the seven extant marine turtle species. It is characterized by benign dermal tumors that can grow throughout the turtle's body (Garcês & Pires, 2022). The disease is believed to be caused by a viral agent, but studies also show evidence of the effects of environmental and anthropogenic factors on exacerbating FP growth (dos Santos et al., 2010; Manes et al., 2022; Van Houtan et al., 2010). Moreover, fibropapillomatosis is considered panzootic as it has been reported in many regions around the world, affecting all species of marine turtles (Aguirre & Lutz, 2004; Jones et al., 2016; Williams et al., 1994).

Disease Manifestation and Impact

The primary indication of fibropapillomatosis is the presence of cauliflower-like fibroepithelial tumors that vary in color, size, and frequency (Whitehouse, 2015). Smith and Coates (1938) described these as a combination of the characteristics of papillomata and fibromata in a single neoplasm, manifesting as epidermal and dermal hyperplasia, respectively. Moreover, histological examinations further described fibropapilloma as an extensive proliferation of the epidermis attached to the fibrovascular stromal stalk (Herbst, 1994). These descriptions became the basis for

characterizing and identifying fibropapillomatosis. However, such gross anatomical and histological characteristics are not unique to fibropapillomatosis in marine turtles. Other animal groups may also exhibit diseases with similar manifestations that are caused by different etiologic agents such as bovine papillomavirus causing cutaneous warts in cattle and sarcoids in horses (Ugochukwu et al., 2018), as well as avian papillomavirus in birds (Najihah et al., 2023)

Fibropapillomas can grow externally and internally. These tumors commonly develop near the eyes and mouth and around visceral organs (Blackburn et al., 2021; Garcês & Pires, 2022). Manifestations of fibropapillomatosis differ; some cases are non-life-threatening and may resolve spontaneously (Machado Guimarães et al., 2013; Muñoz Tenería et al., 2022), while others are progressive and debilitating. In severe FP cases, locomotion, vision, and organ function are impaired, significantly decreasing the quality of marine turtles' health (Jones et al., 2016). In Herbst (1994), it was suspected that organ dysfunction, changes in blood chemistry, and other abnormalities in the lungs and kidneys were the primary causes of death of affected individuals.

Management and Treatment

There is currently no cure for fibropapillomatosis, but several treatments can remove lesions on the skin. These include conventional surgery, radio scalpel surgery, cryosurgery, electrocautery, electrochemotherapy, and CO_2-laser-mediated tumor removal (Brunner et al., 2014; Morris & Balazs, 1994). The most widely used method is conventional surgery, where visible tumors are excised under anesthesia (Page-Karjian, 2019). Alternatively, CO_2-laser-mediated tumor removal is now often used as it controls hemorrhaging during surgery and lessens the need for suturing (Wyneken et al, 2006). Other treatment options are also being considered such as vaccination (Castro et al., 2024). Documented treatment procedures were performed mostly on rescued and captured marine turtles for rehabilitation, which were initiated

through collaborative efforts of government agencies as well as marine turtle conservation and rehabilitation organizations (Brunner et al., 2014; Castro et al., 2024; Futema et al., 2020; Glazkova, 2015; Narita et al., 2021; Whilde et al., 2024). These procedures have shown promising results in treating fibropapillomatosis (Florida Fish and Wildlife Conservation Commission, n.d.; Glazkova, 2015), although some cases of surgical removal still pose a risk of regrowth (Blackburn et al., 2021; Page-Karjian et al., 2019).

Etiology

Since its first diagnosis, fibropapillomatosis has been the subject of studies investigating its nature and cause. Unfortunately, there has been no conclusive report determining its true causative agent. Nonetheless, decades of research have shown that fibropapillomatosis is consistently and strongly associated with the viral agent chelonid alphaherpesvirus 5 (Domiciano et al., 2019; James et al., 2021; Page-Karjian, 2019). Molecular analysis of skin lesions often detects the presence of its genetic material (Lu, Wang et al., 2000; Quackenbush et al., 1998). Moreover, electron microscopy showed signs of viral particles, indicating that infection may be caused by a virus (Jacobson et al., 1991). However, scientists believe that FP infection is multifactorial, suggesting that tumor formation is not solely because of ChAHV5. Environmental and anthropogenic factors are also hypothesized to promote the growth and transmission of the disease (dos Santos et al., 2010; Van Houtan et al., 2010).

Epidemiology

Numerous studies have reported the incidence of fibropapillomatosis in all species of marine turtles (Aguirre et al., 1999; D'Amato & Moraes-Neto, 2000; Herbst, 1994; Huerta et al., 2002; Rossi et al., 2015). However, FP was found to be most prevalent in green sea turtles (Manes et al., 2022). Studies inferred that the disease was not constrained to a certain life stage, although it was commonly observed in juveniles (Ene et al., 2005; Herbst, 1994). Girard et al. (2010) contradicted this claim when they found that

larger marine turtles (CCL size > 60 cm) had a higher FP prevalence. Since clinical signs of FP usually manifest later (Herbst, 1994), a clear linkage of FP infection to a particular life stage cannot be determined. It is possible that the older individuals were infected as juveniles, but the tumors became more visible only when they matured.

The prevalence of FP in juveniles was also attributed to their environmental conditions. Studies have shown that coastal waters with reduced water quality contribute to the development of tumors (dos Santos et al., 2010; Van Houtan et al., 2010). Moreover, anthropogenic activities, such as discharging of waste, affect the nutrient levels and abundance of biotoxin-producing macrophytes in the water. These environmental changes, along with changes in sea surface temperature (Manes et al., 2022), can compromise the immune systems of marine turtles, promoting tumor growth (Norton & Walsh, 2012). Furthermore, mechanical vectors, such as marine leeches (*Ozobranchus* spp.), which are known parasites of green sea turtles, may also contribute to the development of fibropapillomatosis as these species may carry high viral DNA loads of the viral etiologic agent allowing its transmission and infection to marine turtles (Greenblatt et al., 2004). A more recent study revealed the strong association of marine leeches with fibropapillomatosis, clustering on FP tumors to feed on its high vascularization (Rittenburg et al., 2021). Such association indicates that the former are likely vectors of ChAHV5 (Santoro & Mattiucci, 2009; Truong & McGowin, 2011). The contribution of potential vectors, combined with the high transmissibility and infectivity of ChAHV5, increases the risk of infection and development of fibropapillomatosis (Jones et al., 2016). The high prevalence of FP-infected juveniles further increases the risk of transmission due to the interactions between cohabiting healthy and infected individuals.

Prevalence and Distribution

The first case of fibropapillomatosis was reported in the 1930s when warty growths were observed on the bodies of three green sea turtles in Florida, USA. However, the study was limited

to histological examination and the tumors were initially hypothesized to be caused by prolonged exposure to the sun. The study also suggested the possible viral etiology of the diseases (Smith & Coates, 1938).

Over the years, cases of fibropapillomatosis have been reported in many regions worldwide, especially along the tropics and subtropics. Recent data suggests that FP infection has increased by at least 35% worldwide from 2020-2022 (Ip et al., 2022). Moreover, numerous studies report that the prevalence of fibropapillomatosis varies between 0% and 65% (Girard et al., 2010; Kelley et al., 2022; Jones et al., 2022; Loganathan et al., 2021; Muñoz Tenería et al., 2022; Robben et al., 2023; Roost et al., 2022; Russet Rodríguez et al., 2021; Shaver et al., 2019). Most of these studies provide the first comprehensive investigation and analysis of FP prevalence across various countries. In Borneo, the first evidence of ChAHV5-linked FP infection in green sea turtles was reported in 2021, having a prevalence rate of approximately 7.8% (*n = 115;* Loganathan et al., 2021). A more recent study on the same study site revealed that the prevalence reached 42.9% (*n = 63*) in just a span of 3 years (Robben et al., 2023). Additionally, the study reported the first evidence of ChAHV5 infection in hawksbills and olive ridleys, with a prevalence of 60% (*n = 5*) and 100% (*n = 1*), respectively.

In the Philippines, a case of fibropapillomatosis was reported by Lucero et. al (2012). An olive ridley turtle and a hawksbill turtle reared in captivity at the Southern Philippines Agri-Business and Marine and Aquatic School of Technology Marine Research Station, Davao del Sur were observed to have pinkish lesions around the eye, indicating possible fibropapillomatosis. However, the investigation was limited to physical examination and no confirmatory tests were performed to ascertain that the lesion was indeed FP. Apart from this, there have been no other official reports in the country. This shows that fibropapillomatosis is underreported and understudied in the Philippines, despite being a marine turtle hotspot.

With this, the viral disease may be more widespread and its prevalence may potentially be higher than expected. This may pose a great risk to the marine turtle populations in the country as there has been no concrete management plan and actions undertaken to detect the virus and prevent its transmission across turtle populations and foraging grounds within and outside the Philippines. Therefore, it may be necessary to undertake more comprehensive studies on the prevalence of fibropapillomatosis in marine turtles in the Philippines, as well as to establish an efficient method for viral detection.

Overall, the data providing evidence on the presence and prevalence of fibropapillomatosis in marine turtles has been documented from various regions around the world (Whitehouse, 2015), however these studies are outdated, and the data may not represent the accurate prevalence values of infection. Nonetheless, the increase in global reporting has led to FP being classified as panzootic.

CHELONID ALPHAHERPESVIRUS 5 (ChAHV5)

Chelonid alphaherpesvirus 5 (ChAHV5), with species name recently changed to *Scutavirus chelonidalpha5* (Gatherer et al., 2021; Siddell et al., 2020), was formerly referred to as chelonid herpesvirus 5 (ChHV5; National Center for Biotechnology Information, 2023), however its former virus name is still used due to its greater recognition. ChAHV5 infects all species of marine turtles (Whitehouse, 2015) and is commonly associated with fibropapillomatosis tumor growth, though it has also been detected in healthy skin biopsies (Loganathan et al., 2021; Page-Karjian et al., 2012; Page-Karjian, Norton, Ritchie, et al., 2015). This may be explained by latent infection, allowing the virus to lie dormant and manifest at a later stage of the disease (Alfaro-Núñez et al., 2016). Its reactivation may be triggered by changes in environmental conditions, weakening the immune system and promoting tumor growth (Arthur et al., 2008; Dujon et al., 2021; Manes et al., 2022; Van Houtan et al., 2010). However, the exact viral mechanisms and

external-factor influences on the promotion of tumor growth remains unknown.

Viral structure

The morphology of chelonid alphaherpesvirus 5 (ChAHV5) has not been comprehensively studied due to difficulties in cultivation using conventional monolayer cell cultures (Work et al., 2017). Its viral structure is only often observed through electron microscopy of fibropapilloma tissues from infected individuals or from complex tissue cultures (Jacobson et al., 1991; Work et al., 2017). Being a species in the subfamily *Alphaherpesvirinae*, family *Orthoherpesviridae*, the virus is known to have a spherical virion (150–200 nm in diameter) comprised of a single linear, double-stranded DNA core, an icosahedral capsid, an outer and inner tegument, and a lipid envelope (Aurelian, 1999; Gatherer et al., 2021; The UniProt Consortium, n.d.). Moreover, the genomes of members of the family range from 125–241 kbp containing 70–170 genes (Gatherer et al., 2021). Among these, the capsid protein (UL18), glycoprotein B (UL27), glycoprotein H (UL22), and DNA polymerase subunit pol (UL30) genes are commonly used to detect ChAHV5 through molecular methods such as polymerase chain reaction (PCR) and quantitative PCR (Li et al., 2022; Loganathan et al., 2021). These highly conserved gene regions encode for proteins that are essential to the virus and its structure.

Classification and Variants of Chelonid Alphaherpesvirus 5

There are six known variants of chelonid herpesvirus, ChHV1–4 and ChAHV5–6, which infect certain species of tortoises and turtles (Loganathan et al., 2021; Teifke et al., 2000). Among them, only ChAHV5 is legitimately recognized by the International Committee on Taxonomy of Viruses (ICTV). Although ChAHV6 has been studied, the lack of genetic data prevents its formal classification

Being the only recognized chelonid alphaherpesvirus species, ChAHV5 sequence data has been analyzed to understand its evolutionary history and geographical distribution. However,

without the requisite sequence data (Davison & McGeoch, 2010). It is possible that they may or may not be distinct species since they were only identified through electron microscopy. It is also because of this that it is difficult to determine the evolutionary relationships between the six chelonid herpesvirus variants.

Being the only recognized chelonid alphaherpesvirus species, ChAHV5 sequence data has been analyzed to understand its evolutionary history and geographical distribution. However, much of the available data comes from a small number of regions with active sea turtle research efforts and better access to sequencing resources, while many other geographic populations remain understudied or entirely unsampled. This uneven research effort creates gaps in genomic data and inconsistent reporting. As a result, perceived viral diversity and evolutionary patterns may reflect sampling bias more than true global ChAHV5 dynamics. Given this situation, chelonid alphaherpesvirus 5 was initially identified to have regional variants: Eastern Pacific, Midwest Pacific, Western Atlantic/ Eastern Caribbean, and Atlantic (Herbst et al., 2004; Patrício et al., 2012). Existence of distinct variants in localized geographies was also reported, such as the four ChAHV5 subvariants (A, B, C, and D) in Florida (Ene et al., 2005) and three clusters (North Australian, North Queensland, and Queensland clusters) in Australia (Ariel et al., 2017). These provide evidence of its transmission within foraging grounds. The clustering shows that a variant may be detected from individuals in the same neighboring areas or wider regions (Jones et al., 2016). However, this does not exclude the possibility of transmission to distant regions, as marine turtles travel long distances when migrating. For instance, some studies found no clear phylogeographic distinction among identified ChAHV5 variants (i.e., Mabul Sabah isolates), thus they were not grouped based on their local distribution (Loganathan et al., 2021). Instead, they were clustered according to their phylogenetic placement, where it was found that the said isolates may have originated from multiple regions around the world.

Mode of Transmission

ChAHV5 most likely spreads through horizontal transmission (Jones et al., 2020). Although there have been studies investigating the possibility of vertical transmission—from parent to offspring (Farrell, Yetsko et al., 2021)—they have not found any compelling evidence to support this (Jones et al., 2020). As of now, transmission is known to happen via direct contact, indirect interactions, mechanical vectors, or viral sheddings (Loganathan et al., 2021; Page-Karjian et al., 2021).

Mechanical vectors such as marine leeches, cleaner fish, saddleback wrasse, and spirorchid trematodes are considered to be contributing factors in the transmission of ChAHV5 as these organisms are found in the same ecosystem as marine turtles, have the same parasites as marine turtles, create entry sites for the virus through open wounds, or may be found in the FP tumors themselves (Jones et al., 2016). While some have hypothesized that saddleback wrasse, cleaner fish, and trematodes are potential vectors of ChAHV5, there is still a lack of evidence to corroborate this (Smith and Coates, 1938; Williams et al. 1994; Losey et al., 1994; Lu et al., 2000; Jones et al., 2016). Among the possible mechanical vectors, only marine leeches (*Ozobranchus* spp.) showed strong association with fibropapillomatosis as a study showed that they could carry a substantial load of ChAHV5 (Greenblatt et al., 2004; Rittenburg et al., 2021).

ChAHV5 is also thought to be dispersed into the environment through fluid secretion and tumor shedding (Farrell, Yetsko et al., 2021; Yetsko et al., 2020) where it can survive and eventually infect healthy marine turtles. There have been studies indicating the presence of ChAHV5 in blood and urine, as well as nasal, ocular, and cloacal discharges, using molecular diagnostic techniques such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) targeting viral DNA (Alfaro-Núñez et al., 2016; Chaves et al., 2017; Page-Karjian, Norton, Ritchie, et al., 2015). These methods amplify viral genetic material from swabs, tissue samples, or bodily

fluids, allowing researchers to confirm the presence of viral DNA even at low concentrations. Moreover, an environmental DNA approach revealed that viral shedding is strongly correlated with the tumor burden of infected individuals (Yetsko et al., 2020). This detection may have been possible due to the fact that most viruses can survive for a long period of time in the environment. Although no published study confirms the ability of ChAHV5 to persist in coastal waters, a study of ChAHV6 showed that it can survive up to 120 hours in seawater at ambient temperature (Curry et al., 2000).

Detection Method

To date, the most widely used approach to detect chelonid alphaherpesvirus 5 is through molecular analysis of FP-infected tissue samples such as the skin, visceral organs, and bodily fluids (Blackburn et al., 2021; Chaves et al., 2017; Loganathan et al., 2021; Page-Karjian, Norton, Ritchie, et al., 2015). However, this method is invasive and negatively affects the behavior and survival of the living individual. As such, a non-invasive approach, such as using environmental DNA (eDNA), is preferable. Recent studies have implemented this method to detect ChAHV5 in oceanic water and sand tracks (Farrell et al., 2022; Farrell, Yetsko et al., 2021; Yetsko et al., 2020); both approaches apply polymerase chain reaction (PCR) and its variations (Farrell et al., 2022; Lawrance et al., 2018; Mashkour et al., 2021).

ENVIRONMENTAL DNA (eDNA)

Environmental DNA refers to the mixture of genetic material (e.g., nuclear, mitochondrial, and/or chloroplast) expelled by organisms into their surroundings (Herder et al., 2014). Sources of eDNA encompass biological matter (e.g., feces, mucus, hair, urine, skin, and gametes) which can be retrieved from varying sources (e.g., water, sand, soil, sediment, mud, ice, and air) without the need to isolate the specific target organism (U.S. Geological Survey, 2018). The composition of samples can exist as either intracellular or extracellular DNA, which is analyzed using modern molecular methods. This approach allows species identification, even from

deceased organisms, irrespective of sex and life stage (Herder et al., 2014).

Although fairly novel, eDNA techniques have shown great importance in biodiversity monitoring and conservation. Applications include the detection of endangered or cryptic species, investigation of climate change impacts, and evaluation of overall ecosystem health. Recently, they have also been employed in pathogen surveillance and monitoring—a stage that is crucial in the development of suitable disease mitigation and management strategies (Farrell, Whitmore et al., 2021; Pfleger et al. 2016).

Persistence and Abundance of eDNA in Environments

Species detection using eDNA is influenced by deterioration, which occurs as soon as the biological material is released. Hence, to accurately determine the current presence or absence of a particular species, an understanding of its genetic material's decay is crucial (Holman et al., 2021). Since organisms vary anatomically and physiologically, they also differ in the types of biological material that they shed into the environment (Zhao et al., 2021). Studies suggest that the differences in the type of eDNA shed by various animal species affect the rates of both shedding and degradation (Allan et al., 2020).

The persistence and abundance of eDNA are strongly influenced by habitat type. Generally, cold and dry environments decelerate degradation and vice versa (Willerslev & Cooper, 2005 as cited in Herder et al., 2014). Studies show that eDNA degrades within hours to weeks in aquatic environments but can persist for hundreds to thousands of years in soils and sediments (Bairoliya et al., 2022; Herder et al., 2014). This is partly due to the dispersion of eDNA away from its source. Stagnant water tends to retain eDNA for longer than flowing or open systems, where eDNA is diluted into less detectable concentrations (Farrell, Whitmore et al., 2021; Rees et al., 2014). In contrast, soil and sediment do not facilitate dissolution, causing minimal dispersion, making higher concentrations likely.

Therefore, aquatic samples are better suited to detect the recent presence of a species compared to soils and sediments which are preferred in the reconstruction of historic biodiversity (Herder et al., 2014; Zhao et al., 2021).

Furthermore, the persistence and abundance of eDNA remains highly variable due to the interactions of environmental factors such as temperature, salinity, organic matter load, redox potential, and microbial community structure (Collins et al., 2018; Qian et al., 2022; Saito & Doi, 2021; Strickler et al., 2015). Other environmental factors that are specific to certain habitats, such as hydrolysis in aquatic ecosystems, may also lead to DNA damage (Herder et al., 2014). Aside from these, other factors that may influence eDNA degradation should be further investigated in order to develop detection protocols that are robust to variations in environmental conditions (Thomsen et al., 2012).

Studies on fibropapillomatosis and chelonid alphaherpesvirus 5 have found that marine turtles also shed viral particles into the water column, mainly from individuals with tumor growths, and even from those without visible signs of infection (Farrell, Yetsko et al., 2021; Yetsko et al., 2020). However, there is a lack of research on the virus's resistance to physical and chemical factors such as temperature and pH (World Organization for Animal Health [WOAH], n.d.). As such, the persistence and abundance of viral eDNA, as of now, remains unpredictable under natural settings. Nonetheless, detection of viral particles in the water column supports the possibility of waterborne transmission in marine environments (Curry et al., 2000; Farrell, Yetsko, et al., 2021; Patricio et al., 2012). Based on this, suitable conditions for detecting alphaherpesvirus via eDNA include sampling in areas where marine turtles commonly aggregate, such as foraging grounds, resting areas, and semi-enclosed coastal habitats. In addition, cooler temperatures, low UV exposure, and minimal water movement can further increase the chances of capturing intact viral eDNA (MacCartin et al., 2022; Saito & Doi, 2021; Strickler et al., 2015; Quian et al., 2022).

Advantages and Limitations

Environmental DNA (eDNA) methods are emerging monitoring tools that offer numerous advantages over conventional survey protocols (Herder et al., 2014; Peters et al., 2018). Due to their high sensitivity, they require less effort to detect species—especially for cryptic or low-density taxa—enhancing the probability of detection. Metabarcoding further increases efficiency by enabling the simultaneous identification of multiple species from a single sample, reducing the need for repeated sampling, thus improving cost-effectiveness (Thomsen & Willerslev, 2015; U.S. Geological Survey, 2018). Additionally, these methods are non-invasive, eliminating the need for capture and minimizing harm to both individuals and habitats—an essential consideration when working with threatened species (Ip et al., 2022; Mashkour et al., 2020; Thomsen & Willerslev, 2015). These techniques may serve as an effective tool in the early detection of invasive species, pathogens, and diseases (Farrell, Whitmore et al., 2021; Kawato et al., 2021; U.S. Geological Survey, 2018). Moreover, the use of validated species-specific primers reduces misidentification and improves taxonomic resolution, particularly when morphological differences are subtle (Herder et al., 2014).

The utilization of eDNA-based methods has demonstrated strong potential for rapid, sensitive, and non-invasive biodiversity monitoring. However, as a relatively new and indirect technique, eDNA analysis presents several limitations and potential sources of error that must be addressed (Harrison et al., 2019; Nagarajan et al., 2022). One major limitation is the inability to accurately quantify population densities. Sampling techniques also play a crucial role, with higher eDNA yields typically obtained when samples are collected in close proximity to target organisms. At present, eDNA is primarily reliable for determining species presence or absence. It does not permit the observation of the demographic status of detected species, which is usually obtainable using traditional methods (Herder et al., 2014). Moreover, since environmental conditions vary between each habitat and sampling site, across habitats and sampling

sites, protocols optimized for one system may not be directly transferable to another (Nagarajan et al., 2022). As such, eDNA approaches are susceptible to both false negatives and false positives (Thomsen & Willerslev, 2015; Zhao et al., 2023).

Overall, eDNA techniques offer a number of advantages over traditional methods in biodiversity monitoring. However, it is essential to recognize their limitations and develop strategies to overcome them. The complex relationship between environmental samples and sequence frequencies remains poorly understood, making data interpretation challenging. As such, integrating eDNA metabarcoding with conventional field-based methods is recommended to calibrate and validate results (Yoccoz, 2012). Nevertheless, eDNA approaches can complement traditional biomonitoring, offering novel ecological insights and improving the accuracy and scope of biodiversity assessments (Nagarajan et al., 2022).

CONCLUSION

Despite the significant impact of fibropapillomatosis on marine turtle populations, its probable causative agent, chelonid alphaherpesvirus 5, remains understudied. Hence, further investigation is needed to clarify the link between ChAHV5 infection and the development of FP. Moreover, a comprehensive understanding of the virus's genetic diversity, transmission mechanisms, and interactions with its host is essential for developing effective prevention and control strategies.

Unfortunately, genomic data currently available for ChAHV5 is sparse and not widely distributed, hindering efforts to accurately assess its global distribution and genetic variability. As such, expanding the repository of viral sequences is crucial for a more comprehensive understanding of the virus' distribution and evolution across different regions and populations.

Due to difficulties in studying the virus, especially due to the invasive nature of traditional sampling methods, alternatives

should be explored. The application of environmental DNA holds significant promise for ChAHV5 detection as it can provide early warning signs of virus presence in marine ecosystems, enabling proactive conservation measures. Utilizing eDNA could enhance detection capabilities, allowing for non-invasive monitoring of viral presence in marine ecosystems. However, there is a pressing need to assess and improve current methods to ensure they are both reliable and efficient. Validating and optimizing the protocols used is crucial in effective monitoring and management strategies.

Raising awareness about the significance of ChAHV5 detection and its implications for marine turtle health is critical. Understanding the dynamics of this virus and the effectiveness of detection methods is likewise vital for developing conservation strategies aimed at protecting these vulnerable species. By prioritizing research in these areas, we can contribute to the broader efforts to safeguard marine turtles and mitigate the impact of diseases on their populations.

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