Green Turtle Fibropapillomatosis: Challenges to Assessing the Role of Environmental Cofactors

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Green turtle fibropapillomatosis (GTFP) is a growing threat to the survival of green turtle (Chelonia mydas) populations worldwide. Recent transmission studies point to an infectious etiology. Several field studies suggest that high GTFP prevalence is associated with marine habitats that have been impacted by agricultural, industrial, or urban development. Environmental contaminants could be involved in GTFP through several plausible mechanisms including cocarcinogenesis and contaminant-induced immune suppression. However, an association of contaminants with GTFP has not been established. A broader perspective is needed when studying infectious diseases such as GTFP in complex ecosystems. Alternative explanations for high GTFP prevalence in some near-shore habitats include the following: a) these habitats provide an optimum physical environment for survival and transmission of the infectious agent; b) these habitats attract a high density of susceptible turtles or harbor a higher density of potential vectors, facilitating transmission of the pathogen in a density-dependent fashion; and c) these habitats may contain other stressors that render turtles more susceptible to GTFP. Application of scientifically rigorous criteria in the epizootiology of GTFP in free-ranging populations remains a formidable challenge. — Environ Health Perspect 103(Suppl 4):27–30 (1995)

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Emergence and Impact of Green Turtle Fibropapillomatosis

All seven existing species of marine turtles have suffered various severe population declines from overharvesting for their eggs, meat, leather, and shells, from entrapment by fishing lines and nets, from collisions with boats, from dredging operations, and from destruction of nesting beaches and foraging habitat; these species are currently either threatened or endangered (1). A serious new threat to the survival of endangered green turtles (Chelonia mydas) has emerged in the form of an epizootic disease, green turtle fibropapillomatosis (GTFP) (2–4).

Fibropapillomatosis is characterized by single to multiple histologically benign fibroepithelial tumors (ranging from 0.1 cm to greater than 30 cm in diameter) that are found commonly on areas of soft skin (flippers, neck, chin, inguinal and axillary regions, and tail base) and conjunctivae (Figure 1). Green turtles with multiple cutaneous and ocular fibropapillomas may become severely debilitated. Visceral tumors may also be present, and their expansive growth disrupts normal organ functions which leads to death. GTFP primarily affects age groups of high reproductive value, large juveniles and, to a lesser extent, adult green turtles (1,4). As a result, GTFP poses a significant threat to the long-term survival of this endangered species. Exact mortality is unknown but is probably high based on disease severity (4).

Fibropapillomatosis was first reported over 50 years ago in green turtles from Florida (5). A survey of the Key West turtle fishery at that time revealed a prevalence of 1.5%, indicating that the disease was sporadic. Additional early reports showed that the prevalence of GTFP in several populations around the world was probably very low (6,7).

In the last decade, GTFP has emerged as a significant worldwide epizootic in green turtle populations, with documentation of the disease at new localities and prevalences as high as 92% in some population samples (2,4). In addition, lesions similar to GTFP have been observed in other marine turtle species, including olive ridleys (Lepidochelys olivacea), flatbacks (Natator depressus), and loggerheads (Caretta caretta) (4), raising concerns about disease impacts on these species as well.

Etiology and Prevalence of GTFP

Evidence from controlled transmission experiments now implicates a filterable infectious agent, most probably a virus, as the primary cause of GTFP (8). Similar epithelial and fibro-epithelial tumors in other vertebrate species have also been shown to have viral etiologies (4,9).

GTFP prevalence varies considerably among geographic locations, ranging from 0 to 92%, and substantial differences that
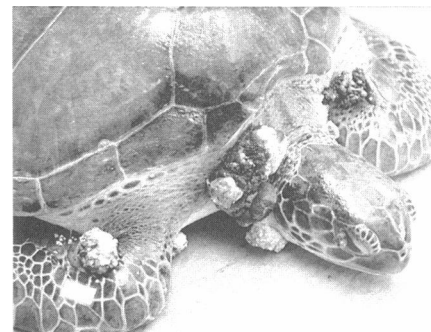

Figure 1. Green turtle (Chelonia mydas) with cutaneous fibropapillomatosis.

are stable through time may be found over relatively short distances (4,10). Data from several field studies indicate that GTFP is more prevalent in near-shore ecosystems such as lagoons and bays (4). For example, the prevalence of fibropapillomatosis among juvenile green turtles in the Indian River lagoon has averaged about 50% since 1982, whereas a nearby (< 1 km away) demographically matched population from the ocean side of the barrier island system has had 0% disease prevalence (10). The maintenance of large differences in GTFP prevalence through time over relatively small geographic distances suggests that environmental cofactors may be important for the full expression of this disease. Alternatively, the availability of susceptible (naive) hosts and the presence or absence of the infectious agent in various locations may be sufficient to explain the variation in prevalence among sites. Anecdotal reports suggest that GTFP is most prevalent in those near-shore habitats that have been impacted by human activities, including agricultural, urban, and industrial development within the catchment areas (reviewed in 4,7,10,11).

Do Environmental Contaminants Play a Role in GTFP?

Identification of putative environmental cofactors and analysis of their specific roles in GTFP development is a major challenge. Development of hypotheses for specific cofactors in GTFP pathogenesis must be met with suitable criteria for accepting or rejecting them. Koch's postulates are one set of criteria that are appropriate for primary infectious disease, but they are probably inappropriate for complex, multifactorial diseases (12). Hill's criteria (12) are more appropriate for epidemiologic analysis and conclusions based on statistical inference and should be used when deciding between alternate explanations for the observed GTFP prevalence patterns .

The perceived association of high GTFP prevalence with near-shore habitats in proximity to human activities has led to speculation that environmental contaminants may play a role in GTFP pathogenesis. Associations between contaminant levels and neoplastic diseases have been made in several aquatic species (13–18). Biologically plausible mechanisms (hypotheses) for environmental chemical contaminant effects in GTFP currently include cocarcinogenesis, including induction of latent virus infections, and

contaminant-induced immune suppression, with subsequent failure of turtles to recognize or eliminate the relevant pathogen or fibropapilloma tumor cells that facilitate development or persistence of this disease. Contaminants could also suppress the immune system of turtles or act as cocarcinogens indirectly by disrupting neuroendocrine functions (19). Cocarcinogenic effects have been documented for a wide range of xenobiotics (20,21). Similarly, a wide variety of chemical contaminants are known to disrupt immune system functions (22–24).

Criteria relating to the strength of the association between GTFP and environmental contaminants, as well as the consistency and specificity of this association, are certainly not met by the available data. The perceived association between GTFP and pollution is based upon subjective assessment of human impacts in certain catchment areas (reviewed in 4). Objective documentation of contaminant exposure in high and low GTFP prevalence sites is needed.

Problems arise in how to document the contaminant exposure of marine turtles. Few data are available for comparing contaminant residue levels in water, sediment, or benthic organisms from high GTFP prevalence areas with those from areas where GTFP is rare. Similarly, data on contaminant levels in green turtle tissues are scant and difficult to obtain because of the endangered status of this species. The few studies that have been published are difficult to interpret in the context of GTFP. For example, whereas one study in 1983 found significant amounts of hydrocarbons in two green turtles that stranded after a major oil spill (25), most surveys of organochlorine and polychlorinated biphenyl residues in green turtle tissues including eggs have yielded relatively low levels, often below the limits of detection of the methods (26–29).

Where data exist, there are problems with relating contaminant levels to the prevalence of this infectious disease. First, the biologic effect (toxicity) of any particular residue level in green turtles is unknown. Second, surveys of residue levels are usually limited to those chemicals that persist in the environment or bioaccumulate, although important toxic effects such as genetic damage (in a multistage carcinogenesis model) can result from transient exposures to compounds that do not bioaccumulate. In addition, exposure to a potent chemical carcinogen or immunotoxin may occur transiently in a completely different

habitat from that being monitored. For example, before entering near-shore feeding habitats, marine turtles spend several years in the open ocean where they become associated with convergence zones in which the potential for exposure to concentrated marine debris and pollutants is high. Third, toxic effects may not be direct as in some experimental models but may involve complex interactions with other abiotic and biotic factors. Thus, fulfilling the criteria for implicating specific chemical contaminants as important cofactors for GTFP expression could be extremely difficult (12,30). Finally, the same biological effects may be caused by any of a number of different classes of compounds acting through several different mechanisms. Decisions about which contaminant residues to measure should be made with specific a priori mechanistic hypotheses in mind and in light of a documented history of exposure to specific compounds.

The Broader Perspective

Although the hypothesis that contaminants may be involved in GTFP epidemiology should be considered, a broader perspective is needed when studying disease in complex ecosystems. A comprehensive analysis must begin by outlining all of the abiotic and biotic factors that may explain differences in GTFP susceptibility, transmissibility, and severity between different habitats.

The strongest association of GTFP prevalence is with habitat type (near-shore embayments). These marine environments may provide favorable physical conditions for either infectious or noninfectious disease agents. For example, certain sediment types may accumulate chemical contaminants and, combined with low flushing rates, could increase the level of exposure to chemical carcinogens or immunotoxins. However, these same sediment properties and hydrodynamic conditions may also favor the accumulation and maintenance of high concentrations of infectious agents. More variable water temperatures in shallow embayments could affect the rate of xenobiotic metabolism, tumor cell proliferation, immune system function, and pathogen replication. For example, thermal stress has been shown to exacerbate virus infection in hatchling green turtles (31). Variable salinity in near-shore habitats may have similar stress effects.

Certain marine habitat types may also provide an optimum biotic environment for survival and transmission of an infectious etiologic agent. Disease transmission could be enhanced by high population densities of

vectors or intermediate host species. Feeding grounds may attract a high density of susceptible turtles that would facilitate the transmission of pathogens in a density-dependent fashion, as has been shown for horizontally transmitted damselfish neurofibromatosis (*32*) and the herpesvirus of Lucké's renal adenocarcinoma (*33*). Recruitment of susceptible turtles from many different breeding stocks into common foraging grounds may allow the exchange of many diseases, including GTFP, from exposed to naive individuals. Habitat differences in levels of other stressors such as concurrent infectious disease (parasites) and disturbance by human activity (fishing, boating, dredging) may render turtles more susceptible to or less able to recover from GTFP.

The Challenges

It is clear that the primary etiologic factor in GTFP is an infectious agent (*8*), and the major goal at present is to identify this agent and fulfill Koch's postulates. Successful isolation and characterization of the infectious GTFP agent must be followed by the development of appropriate diagnostic tools to enable us to study the epidemiology of this disease. Individuals and populations could then be monitored for exposure to the GTFP agent

allowing the natural routes of transmission, potential vectors and reservoirs, and the effects of environmental cofactors on susceptibility to be identified.

Assessment of the role of environmental cofactors in GTFP will require objective documentation of all potential interacting factors and human impacts in selected study areas, including monitoring of contaminant levels and monitoring of turtles for evidence of sublethal biologic damage (biomarkers). Testing hypotheses concerning a role for environmental contamination will first require objective documentation of the association of GTFP-affected turtles with sources of contaminants. This could be done by screening for residue levels but will require *a priori* selection of residues to monitor based on a known history of exposure to specific contaminants. Alternatively, hypotheses linking specific contaminants with GTFP could be developed using data from biomarker studies that point to the disruption of key physiological systems in turtles residing in contaminated habitats. For example, hypotheses that contaminants influence GTFP prevalence or pathogenicity through endocrine disruptive effects or immune suppression will require demonstration of these perturbations in turtles from near-shore areas. Controlled laboratory and field experiments, as have been carried

out in some other aquatic species (*34,35*), would provide the best data linking contaminants and susceptibility to GTFP. All of these studies will be difficult to conduct in free-ranging populations of this endangered species.

In conclusion, although there may be an association of high GTFP prevalence with certain habitat types and with human impacts including contaminants, it is premature to conclude that environmental contaminants are involved in the etiology of this infectious disease. Whereas plausible hypotheses about contaminant effects on GTFP susceptibility or GTFP expression can be developed, it is important to rule out alternative hypotheses and to remember that virulent pathogenic agents (in contrast to opportunistic pathogens) need not depend on prior host debilitation (e.g., immune suppression) to cause disease. Diseases caused by virulent pathogens are an ever present risk to animals in both captive and wild populations and are capable of causing significant population declines (*36,37*). It is clear that further understanding of any interactions between the infectious agent and environmental contaminants in producing GTFP epizootics will require thorough sampling in many different populations and locations, consideration of alternative hypotheses, and controlled experimentation.

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