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Bacteraemia in free-ranging Hawaiian green turtles *Chelonia mydas* with fibropapillomatosis

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ABSTRACT: Past studies of free-ranging green turtles *Chelonia mydas* with fibropapillomatosis (FP) in Hawaii have shown that animals become immunosuppressed with increasing severity of this disease. Additionally, preliminary clinical examination of moribund turtles with FP revealed that some animals were also bacteraemic. We tested the hypothesis that bacteraemia in sea turtles is associated with the severity of FP. We captured free-ranging green turtles from areas in Hawaii where FP is absent, and areas where FP has been endemic since the late 1950s. Each turtle was given an FP severity score ranging from 0 (no tumours) to 3 (severely affected). A fifth category included turtles that were stranded ashore and moribund with FP. We found that the percentage of turtles with bacteraemia increased with the severity of FP, and that the majority of bacteria cultured were *Vibrio* spp. Turtles with severe FP were more susceptible to bactaeremia, probably in part due to immunosuppression. The pattern of bacteraemia in relation to severity of disease strengthens the hypothesis that immunosuppression is a sequel to FP.

KEY WORDS: Bacteria · Bacteraemia · *Chelonia mydas* · Green turtle · Haematology · *Vibrio*

INTRODUCTION

Fibropapillomatosis (FP) is a globally distributed neoplastic disease of marine turtles. The disease causes external and internal tumours and debilitation that mainly affects immature animals (Herbst 1994). Although factors such as contaminants (Aguirre et al. 1994a), parasites (Dailey & Morris 1995, Aguirre et al. 1998), and marine toxins (Landsberg et al. 1999) have been suspected of contributing to FP, herpesviruses appear to be a common denominator (Herbst et al. 1998, Quackenbush et al. 1998, Lackovich et al. 1999, Lu et al. 2000). However, attempts to culture this virus in the laboratory have been unsuccessful, and Koch's postulates have not been fulfilled.

In Hawaii, prevalence of FP in immature green turtles *Chelonia mydas* fluctuates between 40 and 60% depending on where and how animals are sampled. The disease is present in turtles from all the main Hawaiian islands except the Kona/Kohala (west) coast of the island of Hawaii, where FP has historically been rare (Balazs 1991, Murakawa et al. 1999, Balazs et al. 2000).

The pathophysiology of FP in green turtles remains elusive. Debilitation from tumour loads involving the eyes and mouth (Balazs et al. 1997) certainly explains why animals with severe FP strand or become emaciated. However, severe debilitation also occurs in turtles with few external or internal tumours. Aguirre et al. (1995) concluded that turtles with severe FP were stressed and immunosuppressed based on haematology and blood cortisol levels. Work & Balazs (1999) and Work et al. (2001) showed, through immune function tests and haematology, that immunosuppression in

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free-ranging turtles was most likely a sequel and not a pre-requisite to FP. Turtles that strand with FP can also be severely infested with vascular flukes (Dailey & Morris 1995, Aguirre et al. 1998). The role of herpesviral infection in debilitation is unclear.

Preliminary clinical studies of moribund stranded turtles with FP revealed that some animals were bacteraemic. We wanted to see whether bacteraemia in free-ranging green turtles from Hawaii was associated with severity of FP.

MATERIALS AND METHODS

Non-stranded turtles were sampled from Oahu (Kaneohe and Kailua Bay) and the west coast of Hawaii (Kona/Kohala). Stranded turtles moribund with FP were salvaged from Oahu, Maui, and east Hawaii (Hilo) where FP is endemic (Fig. 1). Turtles in west Hawaii were sampled to represent an area historically free of FP. West Hawaii has an open coastline, and differs from the other sampling sites that consisted of sheltered embayments.

Non-stranded free-swimming turtles were captured by hand using a combination of scoop nets from the water surface, and snorkeling or SCUBA diving. For each turtle, we recorded weight (kg) and straight carapace length. Turtles were evaluated for gross evidence of tumours and classified as to severity of disease by assigning a tumour score (Work & Balazs 1999). Briefly, size of tumours on animals was estimated (cm) and placed into 4 categories (<1, 1 to 4, >4 to 10, and >10 cm diameter). Based on the number and size of tumours, animals were then assigned a score ranging from 0 (no tumours) to 3 (heavily affected). Moribund turtles with FP found stranded on the shore were salvaged and classified as stranded.

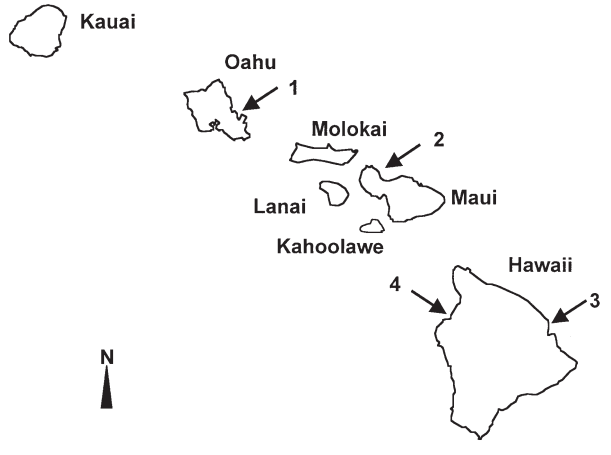

Fig. 1. Main Hawaiian islands and sampling sites for bacteriology study on green turtles *Chelonia mydas*: (1) Kailua/Kaneohe Bay; (2) Kahului, Maui; (3) Hilo; (4) Kona/Kohala

Prior to taking a blood sample, the skin overlying the dorsal neck was cleaned with soap and water, scrubbed for 10 minutes with betadine scrub solution, rinsed with 70% ethanol, and allowed to dry. The scrubbed skin was swabbed using a sterile Dacron swab that was immediately placed in sterile Columbia broth (BBL Septi-Check, Becton Dickinson). A blood sample was then taken using a sterile 12 cc syringe and 20 gauge 3.8 cm needle (Owen & Ruiz 1980). A new needle was placed on the syringe prior to dispensing 8 ml of whole blood into 70 ml of sterile Columbia broth. The swab and blood broth mixture were cultured on sheep blood agar (Becton Dickinson) with and without marine salts (National Wildlife Health Center) at 37°C for approximately 24 h. Colonies were isolated and identified using Biolog GN2 MicroPlates (Biolog) or the appropriate API or Vitek system (bioMerieux). *Vibrio* spp. were further identified using 16S rDNA sequencing via the MicroSeq™ system (Accugenix). Separate aliquots of blood were placed in heparinized tubes and analyzed for routine haematology including total white blood cell (WBC) count, differential, packed cell volume (%) and estimated total solids (Work et al. 1998).

The following data were summarized (means, median, and SD) for animals in each of 5 categories (tumour score 0, 1, 2, 3, and stranded): Percentage of animals bacteraemic, heterophil:lymphocyte ratio, monocyte:eosinophil ratio, packed cell volume, estimated total solids, and straight carapace length. Analytes were compared using ANOVA, and non-parametric Kruskal-Wallis ANOVA was used for data that violated assumptions of normality or equal variance. Alpha was adjusted for number of analytes (7) (Rice 1989), and, for significant differences in ANOVA, post-hoc pairwise comparisons were done using Student-Newman-Keuls test. Chi-square (Daniel 1987) was used to test for the association between positive-status skin bacteria and positive-status blood culture. Logistic regression (Hosmer & Lemeshow 1989) was used to test the relationship between tumour score and blood culture status.

RESULTS

We sampled 85 turtles (63 from Oahu, 19 from west Hawaii, 2 from east Hawaii, and 1 from Maui). The percentage of animals that were bacteraemic generally increased with severity of disease (Table 1), and there was a significant relationship between blood cul-

Table 1. Number of bacteraemic turtles (Blood+) partitioned by fibropapillomatosis (FP) status. TS: tumour score (0, no tumours, to 3, severely affected); Total: number of turtles at each tumour score; %: percentage of turtles affected

Status	Blood+	Total	%	No. bacteria species per turtle
TS 0	0	33	0	0
TS 1	0	15	0	0
TS 2	7	14	50	1
TS 3	4	9	44	1
Stranded	12	14	86	1–4

ture status and tumour score (likelihood ratio = 47.9, $p < 0.001$). For each unit-increase in tumour score, turtles were 4.6 (95% CI = 2.4 to 8.5) times more likely to be blood culture positive. Significantly more species of bacteria ($t = 2.7$, $df = 11$, $p < 0.01$) were cultured from the blood of stranded turtles with FP than from free-ranging captured turtles with a tumour score of 2 or 3 (Table 1).

There was no association between positive-status skin bacteria and positive-status blood bacteria ($\chi^2 = 0.61$, $df = 1$, $p = 0.44$). In bacteraemic turtles, *Vibrio* was the most prevalent genus of bacteria with *V. harveyi* most commonly encountered. *Bacillus* sp. was the most frequently cultured bacteria from scrubbed skin (Table 2). In no turtle did we see the same species of bacterium cultured from scrubbed skin as was found in the blood. Estimated total solids and packed cell volume were significantly lower ($p < 0.001$) for animals with tumour scores ≥2 and for stranded turtles. Stranded turtles, all with FP, had a significantly lower WBC count ($p < 0.002$) and a significantly higher heterophil:lymphocyte ratio and monocyte: eosinophil ratio ($p < 0.001$) than the other 4 groups. Straight carapace length was significantly greater for tumour score 3 and stranded turtles ($p < 0.001$) than other groups (Table 3).

Table 2. Species of bacteria cultured from green turtle (*Chelonia mydas*) blood and scrubbed skin and number (n) of turtles with positive cultures

Blood	n	Scrubbed skin	n
Vibrio harveyi	13	*Bacillus* sp.	12
Vibrio alginolyticus	3	*Acinetobacter* sp.	2
Vibrio campbellii	2	Gram-negative rod	2
Vibrio tubiashii	2	*Aeromonas hydrophila*	1
Aeromonas hydrophila	2	*Comomonas acidovorans*	1
Aeromonas sobria	1	*Enterobacter cloacae*	1
Burkholderia capacia	1	*Flavobacterium* sp.	1
Gram-negative rod	1	*Pseudomonas aeruginosa*	1
Shewanella putrefasciens	1	*Pseudomonas* sp.	1
		Shewanella putrefasciens	1
Aeromonas caviae	1	*Staphylococcus* sp.	1
Aeromonas popoffii	1	*Xanthomonas maltophila*	1
Pseudomonas sp.	1		
Staphylococcus sciuri	1		
Vibrio aestuarianus	1		

DISCUSSION

Free-ranging green turtles have an increasing likelihood of being bacteraemic as severity of FP increases. The increasing number of different species of bacteria cultured from stranded turtles with FP also indicates that turtles with FP are most vulnerable to infection with bacteria when they are clinically most severely affected. Work et al. (2001) showed that immunosuppression is most likely a sequel to FP, with suppression of cellular and humoral immune status being most severe in turtles with tumour scores 2 and 3. The pattern of bacteraemia in free-ranging turtles closely follows that of immunosuppression (Work et al. 2001), suggesting that defenses against foreign agents collapse with severe FP.

Bacteraemia in turtles with severe FP would also explain debilitation. In reptiles, bacteraemia can cause anorexia and lethargy (Novak & Seigel 1986), both clinical signs that are seen in stranded turtles with

Table 3. Mean (±SD) values for packed cell volume (PCV in %), estimated total solids (ETS in g dl^{-1}), heterophil:lymphocyte (H:L) ratio, monocyte:eosinophil (M:E) ratio, total white blood cell (WBC) count ($\times 10^3$ ul^{-1}), and straight carapace length (SCL in cm) partitioned by tumour score for Hawaiian green turtles. TS: tumour score (0, no tumours, to 3, severely affected); sample size (n) for TS1, TS3 and stranded were 15, 9, and 9, respectively. Sample sizes (n) for TS0 and TS2 are included; *: values significantly different ($p < 0.007$) across a row

	TS0	n	TS1	TS2	n	TS3	Stranded
PCV	32 (5)	33	31 (5)	26 (7)*	14	20 (7)*	17 (7)*
ETS	4.7 (1)	33	4.6 (0.9)	2.9 (1.1)*	14	2.7 (.8)*	2.3 (1.1)*
H:L	0.12 (0.06)	31	0.15 (0.07)	0.12 (0.08)	13	0.27 (0.15)	0.94 (0.51)*
M:E	0.41 (0.36)	31	0.42 (0.38)	0.5 (0.43)	13	1.37 (1.34)	4.73 (6.98)*
WBC	25 (9)	31	24 (9)	24 (10)	13	18 (5)	13 (7)*
SCL	54 (7)	33	54 (7)	58 (10)	14	65 (12)*	61 (8)*

severe FP. Bacteraemia in animals also results in metabolic changes such as altered electrolyte balance, hypoglycaemia, altered clotting profiles, and toxaemia (Dow 1995). Indeed, in the more severely affected turtles in our previous study, heterophils appeared toxaemic with basophilic vacuolated cytoplasm and clumped granules (Campbell 1996). Likewise, the haematological changes observed in severely affected animals (heterophilia, monocytosis, leukopenia, anaemia, hypoproteinaemia) are compatible with bacteraemia.

Species of the genus *Vibrio* were the most commonly isolated bacteria, with *V. harveyi* predominated in blood from FP affected turtles (tumour scores 2 and 3). *V. harveyi* has been documented as a significant pathogen of crustaceans (Jiravanichpaisal et al. 1994, Diggles et al. 2000), seahorses (Alcaide et al. 2001), and finfish (Zhang & Austin 2000). However, this is the first documentation of infection with this bacterium in sea turtles. Other *Vibrio* spp. (*V. alginolyticus*, *V. tubiashii*, *V. campbellii*) cultured from turtles in this study are known pathogens of molluscs (Hada et al. 1984, Zheng et al. 1991), crustaceans (Lee et al. 1996, Sahul Hameed et al. 1996), and fish (Austin et al. 1993). *Vibrio* spp. have been documented to cause endocarditis in a free-ranging leatherback turtle *Dermochelys coriacea* (Obendorf et al. 1987), skin disease in captive loggerhead turtles *Caretta caretta* (Wiles & Rand 1987) and green turtles (Glazebrook & Campbell 1990a,b), and rhinitis, pneumonia, stomatitis and septicaemia in captive green turtles (Glazebrook & Campbell 1990a,b). However, *Vibrio* spp. are also apparently common commensals in cloacal flora of green turtles from Hawaii with and without FP (Aguirre et al. 1994b). The role of *Vibrio* spp. in debilitation of free-ranging green turtles remains to be shown, and the toxicity of *Vibrio* spp. involved in bacteraemia is not well understood.

A variety of other bacteria were infrequently cultured from the blood of green turtles in this study. *Aeromonas hydrophila* and *Pseudomonas* sp. have been documented to cause dermatitis, stomatitis, rhinitis, pneumonia, osteomyelitis, and septicaemia in captive green turtles (Glazebrook & Campbell 1990a,b) while *Aeromonas* sp. has caused skin disease in captive loggerhead turtles (Sinderman 1977) and captive Kemp's ridley turtles *Lepidochelys kempi* (Clary & Leong 1984).

Except for 4 species of bacteria (*Aeromonas hydrophila*, *Shewanella putrefasciens*, *Pseudomonas* sp., and an unidentifiable gram-negative rod), bacteria from cultured scrubbed skin were different from those cultured from blood. The lack of significant association between positive-status blood bacteria and positive-status skin bacteria, and the difference in bacteria species cultured, would argue against collection technique being responsible for the results seen here.

The source of bacterial infection in these turtles is unknown. However, we suspect dermal, cloacal or oral routes as potential avenues of bacterial entry into the blood stream of sea turtles. Some turtles with FP have necrosis of the epithelium covering the fibropapillomas, and this may provide a ready portal of infection. In fact, bacteraemia in fresh-water turtles often begins with skin infections (Jacobson 1992). Alternatively, many of the bacteria cultured from the blood are ubiquitous in marine environments. Possible routes of infection could be oral, either through abrasion of the oral cavity during grazing, or through depressed gastrointestinal immune response, or could be through heavy exposure to faecal coliforms (Balazs et al. 1993) from turtle faeces that may serve as a source of bacterial skin infections. Indeed, *Vibrio* spp. were cultured from the mucosal surface of the oral cavity in 18 out of 19 apparently healthy turtles from west Hawaii (R.Morris unpubl. data), suggesting that exposure of turtles to this bacterium is common.

This is the first documentation of bacteraemia associated with FP in free-ranging green turtles from Hawaii. Bacteraemia (*Citrobacter* sp., *Salmonella* sp., *Moraxella* sp. and *Escherichia coli*) in free-ranging green turtles from Western Australia has been documented in association with vascular flukes (Raidal et al. 1998). Given that many Hawaiian green turtles with FP (including those stranded and necropsied in this study) have concomitant vascular-fluke infestations (Dailey & Morris 1995, Aguirre et al. 1998), it is entirely possible that infestation with flukes may provide a portal of infection with bacteria. Glazebrook & Campbell (1990b) documented septicaemia in 2 out of 22 wild green turtles but did not indicate the species of bacteria involved. The similar pattern of bacteraemia with increasing tumour score in this study to that of immunosuppression (Work et al. 2001) would suggest that bacteraemia may be a good indicator of immunosuppression in green turtles. Future studies might focus on the pathophysiological changes associated with bacteraemia.

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LITERATURE CITED

Aguirre AA, Balazs GH, Zimmerman B, Galey FD (1994a) Organic contaminants and trace metals in the tissues of

Hawaiian green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian islands. Mar Pollut Bull 28:109–114

Aguirre AA, Balazs GH, Zimmerman B, Spraker TR (1994b) Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomatosis. J Wildl Dis 30:8–15

Aguirre AA, Balazs GH, Spraker TR, Gross TS (1995) Adrenal and haematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. Physiol Zool 68:831–854

Aguirre AA, Spraker TR, Balazs GH, Zimmerman B (1998) Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. J Wildl Dis 34:91–98

Alcaide E, Gil-Sanz C, Sanjuan E, Esteve D, Amaro C, Silveira L (2001) *Vibrio harveyi* causes disease in seahorse, *Hippocampus* sp. J Fish Dis 24:311–313

Austin B, Stobie M, Robertson PAW, Glass HG, Stark JR, Mudarris M (1993) *Vibrio alginolyticus*: the cause of gill disease leading to progressive low-level mortalities among juvenile turbot, *Scophthalmus maximus* L, in a Scottish aquarium. J Fish Dis 16:277–280

Balazs GH (1991) Current status of fibropapillomatosis in the Hawaiian green turtle, *Chelonia mydas.* In: Balazs GH, Pooley S (eds) Research plan for marine turtle fibropapillomas. US Department of Commerce (NOAA-TM-NMFS-SWFC-156), Washington, DC, p 47–57

Balazs GH, Fujioka R, Fujioka C (1993) Marine turtle faeces on Hawaiian beaches. Mar Pollut Bull 26:392–394

Balazs GH, Aguirre AA, Murakawa SKK (1997) Occurrence of oral fibropapillomas in the Hawaiian green turtle: differential disease expression. Mar Turtle News 76:1–2

Balazs GH, Rice M, Murakawa SKK, Watson G (2000) Growth rates and residency of immature green turtles at Kiholo Bay, Hawaii. US Department of Commerce (NOAA-TM-NMFS-SEFSC-436), Washington, DC, p 283–285

Campbell TW (1996) Clinical pathology. In: Mader DR (ed) Reptile medicine and surgery. WB Saunders, Philadelphia, p 248–257

Clary JC III, Leong JK (1984) Disease studies aid Kemp's ridley sea turtle headstart research. Herp Rev 15:69–70

Dailey MD, Morris R (1995) Relationship of parasites (Trematoda: Spirorchidae) and their eggs to the occurrence of fibropapillomatosis in the green turtle (*Chelonia mydas*). Can J Fish Aquat Sci 52:84–89

Daniel WW (1987) Biostatistics: a foundation for analysis in the health sciences. John Wiley & Sons, New York

Diggles BK, Moss GA, Carson J, Anderson CD (2000) Luminous vibriosis in rock lobster *Jasus verreauxi* (Decapoda: Palinuridae) phyllosoma larvae associated with infection by *Vibrio harveyi*. Dis Aquat Org 43:127–37

Dow SW (1995) Diagnosis of bacteraemia in critically ill dogs and cats. In: Bonagura JD, Kirk RW (eds) Current veterinary therapy XII. WB Sanders, Philadelphia, p 137–139

Glazebrook JS, Campbell RSF (1990a) A survey of the diseases of marine turtles in northern Australia. I. Farmed turtles. Dis Aquat Org 9:83–95

Glazebrook JS, Campbell RSF (1990b) A survey of the diseases of marine turtles in northern Australia. II. Oceanarium-reared and wild turtles. Dis Aquat Org 9:97–104

Hada HS, West PA, Lee JV, Stemmler J, Colwell RR (1984) *Vibrio tubiashii* sp. nov, a pathogen of bivalve molluscs. Int J Syst Bacteriol 34:1–4

Herbst LH (1994) Fibropapillomatosis of marine turtles. Annu Rev Fish Dis 4:389-425

Herbst LH, Garber R, Lockwood L, Klein PA (1998) Molecular biological evidence for the involvement of a unique herpesvirus in the etiology of green turtle Fibropapillomatosis. US Department of Commerce (NOAA-TM-NMFS-SEFSC-412), Washington, DC, p 67

Hosmer DW, Lemeshow S (1989) Applied logistic regression. John Wiley & Sons, New York

Jacobson ER (1992) Reptile dermatology. In: Bonagura JD, Kirk RW (eds) Current veterinary therapy XI. WB Saunders, Philadelphia, p 1204–1210

Jiravanichpaisal P, Miyazaki T, Limsuwan C (1994) Histopathology, biochemistry, and pathogenicity of *Vibrio harveyi* infecting black tiger prawn *Penaeus monodon.* J Aquat Anim Health 6:27–35

Lackovich JK, Brown DR, Homer BL, Garber RL and 8 others (1999) Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. Dis Aquat Org 37:89–97

Landsberg JH, Balazs GH, Steidinger KA, Baden DG, Work TM, Russell DJ (1999) The potential role of natural tumour promoters in marine turtle fibropapillomatosis. J Aquat Anim Health 11:199–210

Lee K, Yu S, Chen F, Yang T, Liu P (1996) Virulence of *Vibrio alginolyticus* isolated from diseased tiger prawn, *Penaeus monodon*. Curr Microbiol 32:229–231

Lu Y, Wang Y, Yu Q, Aguirre AA, Balazs GH, Nerurkar VR, Yanagihara R (2000) Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. Arch Virol 145:1885–1893

Murakawa SKK, Balazs GH, Ellis DM, Hau S, Eames SM (1999) Trends in fibropapillomatosis among green turtles stranded in the Hawaiian islands, 1982–98. US Department of Commerce (NOAA-TM-NMFS-SEFSC-443), Washington, DC, p 239–241

Novak SS, Seigel RA (1986) Gram-negative bacteremia in American alligators (*Alligator mississippiensis*). J Wildl Dis 22:484–7

Obendorf DL, Carson J, McManus TJ (1987) *Vibrio damsela* infection in a stranded leatherback turtle (*Dermochelys coriacea*). J Wildl Dis 23:666–8

Owen DW, Ruiz GJ (1980) New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetol 36: 17–20

Quackenbush SL, Work TM, Balazs GH, Casey RN and 7 others (1998) Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. Virology 246:392–399

Raidal SR, O'Hara M, Hobbs RP, Prince RIT (1998) Gram-negative bacterial infections and cardiovascular parasitism in green sea turtles (*Chelonia mydas*). Aust Vet J 76:415–417

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Sahul Hameed AS, Rao PV, Farmer JJ, Hickman-Brenner FW, Fanning GR (1996) Characteristics and pathogenicity of a *Vibrio campbellii*-like bacterium affecting hatchery-reared *Penaeus indicus* (Milne Edwards, 1837) larvae. Aquacult Res 27:853–863

Sinderman CJ (1977) *Aeromonas* disease in loggerhead turtles. In: Sinderman CJ (ed) Disease diagnosis and control in North American marine Aquaculture, Developments in Aquaculture and Fisheries Sciences, Vol 6. Elsevier North-Holland, New York, p 292–293

Wiles M, Rand TG (1987) Integumental ulcerative disease in a loggerhead turtle, *Caretta caretta*, at the Bermuda Aquarium: microbiology and histopathology. Dis Aquat Org 3: 85–90

Work TM, Balazs GH (1999) Relating tumour score to haematology in green turtles with fibropapillomatosis in Hawaii. J Wildl Dis 35:804–807

Work TM, Raskin RE, Balazs GH, Whittaker S (1998) Morphologic and cytochemical characteristics of blood cells from the green turtle, *Chelonia mydas*, in the Hawaiian islands. Am J Vet Res 59:1252–1257

Work TM, Rameyer RA, Balazs GH, Cray C, Chang SP (2001) Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. J Wildl Dis 37:574–581

Zhang XH, Austin B (2000) Pathogenicity of *Vibrio harveyi* to salmonids. J Fish Dis 23:93–102

Zheng G, Li H, Huang N, Yu Y, Yang J, Wu Y (1991) Characteristics of *Vibrio alginolyticus* isolated from diseased clam *Meretrix meretrix* and histopathological observations on diseased clam by electron microscope. J Fish China 15: 85–95

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