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EPIZOOTIOLOGY OF SPIRORCHIID INFECTION IN GREEN TURTLES (*CHELONIA MYDAS*) IN HAWAII

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ABSTRACT: We describe the epizootiology of spirorchiid trematode infections in Hawaiian green turtles (*Chelonia mydas*) by quantifying tissue egg burdens in turtles submitted for necropsy and by assessing antibody response to crude adult worm and egg antigens among a variety of age groups. *Hapalotrema* sp. and *Laeredius* sp. predominated in turtles infected with spirorchiids. Tissue egg burdens decreased with increasing size and increased with deteriorating body condition of turtles. No relationship was found between tissue egg burdens and sex or fibropapillomatosis status. Tissue egg burdens increased in turtles from southeast to northwest in the main Hawaiian Islands (Hawaii to Kauai). Hatchling and captive-reared turtles had significantly lower levels of antibodies against crude worm and egg antigens. Based on tissue egg burdens and antibody status, we hypothesize that immature turtles become infected with spirorchiids shortly after recruiting into coastal foraging pastures from the pelagic environment, that infection levels decrease with age, and that spirorchiids detrimentally affect the body condition of sea turtles independent of tumor burden. The low intensity of infection in turtles with the endemic trematode *Carettacola hawaiiensis* suggests either that turtles are less susceptible to infection with this parasite or that the parasite is outcompeted by species of *Hapalotrema* and *Laeredius*. Given that the 2 latter species are found in the Pacific and other oceans, they are not likely endemic and were probably introduced into Hawaii through an undetermined route.

The major health issue affecting green turtles (*Chelonia mydas*) in Hawaii is fibropapillomatosis (FP), a neoplastic disease that is a significant cause of strandings (Murakawa et al., 1999; Work et al., 2004) and that presents complications such as immunosuppression (Work and Balazs, 1999; Work et al., 2001) and bacteremia (Work et al., 2003). A high percentage of turtles with FP in Hawaii (Dailey , 1992; Aguirre et al., 1998) and the Pacific (Gordon et al., 1998) are also infected with blood flukes. Blood flukes (spirorchiids) could play an etiologic role in FP (Dailey and Morris, 1995), but more recent evidence implicates an FP-associated turtle herpesvirus (FPTHV) as the most probable cause of the disease in Hawaii (Quackenbush et al., 1998) and Florida (Lackovich et al., 1999). However, given that most turtles that strand with FP are infected with spirorchiids and that these parasites can cause significant pathology (Aguirre et al., 1998; Work et al., 2004), understanding their epizootiology may shed light on their role in the health of sea turtles, thereby aiding conservation efforts to recover the species.

Four species of spirorchiids in 3 genera (*Laeredius*, *Hapalotrema*, and *Carettacola*) have been described from Hawaiian green turtles (Dailey et al., 1992, 1993). Adult worms infect the vessels of various organs, where they copulate and oviposit. Eggs migrate and lodge in tissues, where they elicit a granulomatous response in multiple organs. Adult worms can cause severe vasculitis (Aguirre et al., 1998; Work et al., 2004). Detection of infection with spirorchiids in turtles usually is done at necropsy, when adult worms or eggs are observed either grossly or at microscopy. Antemortem detection of infection is more difficult and currently limited to serology. Graczyk et al. (1995) used enzyme-linked immunosorbent assays (ELISAs) to detect antibodies to adult worm antigens in Hawaiian green turtles and found 71% to be seropositive in Kaneohe, Oahu (where FP is enzootic), and 100% to be seropositive on the west coast (Kona) of the island of Hawaii, where FP rarely occurs. Those authors concluded that no association existed between seropositivity to spirorchiids and FP status. Herbst et al. (1998) used ELISA to examine green turtles from Florida for exposure to spirorchiids and immunohistochemistry to measure antibodies against FPTHV; they also found no association between seropositivity to spirorchiids and FP.

Serology is useful to detect exposure of sea turtles to spirorchiids, but it does have limitations. Serology may not reflect the intensity of infection with spirorchiids, nor will it indicate the relative role of individual species of flukes infecting turtles (Herbst et al., 1998). Existing studies (Graczyk et al., 1995; Herbst et al., 1998) have examined serology against adult worms in immature turtles within foraging habitats. However, a more complete picture of the epizootiology of infection with spirorchiids in turtles might be gained by examining serology against both worms and eggs in other turtle life stages (hatchling and adult). Finally, serological responses to spirorchiids can be confounded if animals have concomitant infections with other helminths (Alarcon de Noya et al., 1996). Our objectives in the present study were, first, to evaluate the serological response of various age and health categories of sea turtles against spirorchiid worm and egg antigen and, second, to examine the relationship between the intensity of infection with spirorchiids in stranded turtles and serology, body condition, and FP burden.

MATERIALS AND METHODS

Free-ranging turtles were captured on a nesting beach or in coastal waters using tangle nets, SCUBA, or snorkeling. Turtles were bled from the cervical sinus (Owens and Ruiz, 1980) using 10-ml syringes and 20-gauge, 2.57-cm needles. Blood was collected in heparin (7 IU/ml), centrifuged at 300 *g* for 10 min, plasma harvested, and stored frozen (−70 C). Immature turtles were sampled from Kaneohe Bay, Oahu, (an FP enzootic area) and the west (Kona-Kohala) coast of the island of Hawaii (an area where FP is rarely observed). Hatchlings and nesting adults were sampled from French Frigate Shoals (FFS) in the northwestern Hawaiian Islands (Balazs and Chaloupka, 2004). Captive-bred and-reared, immature green turtles of Hawaiian origin were sampled from Sea Life Park (SLP), Oahu. Some of these turtles were then translocated within 2 mo of hatching to coastal holding ponds at the Mauna Lani Bay Resort (MLBR) on the Kona-Kohala coast of the island of Hawaii.

Stranded turtles that were moribund and judged to have poor prog-

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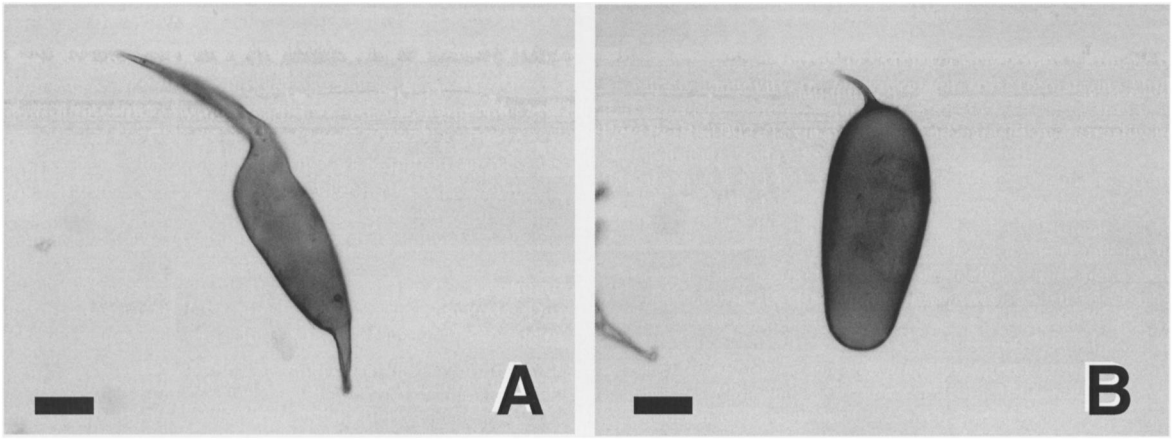

FIGURE 1. Type I (**A**) and type II (**B**) spirorchiid eggs recovered from spleens of green turtles from Hawaii. Bar = 30 μm.

nosis for survival based on severity of lesions were humanely killed by intracardiac administration of barbiturate solution (Beuthanasia D Special, Scherling Plough Animal Health, Kenilworth, New Jersey) after a blood sample was taken. Turtles from KB and stranded turtles with FP were assigned a tumor score (TS) that quantified the severity of FP (Work and Balazs, 1999). Briefly, this score involved classifying each turtle into 4 categories, depending on whether FP was absent (TS-0) or severe (TS-3), based on the size and location of tumors. For all turtles, straight carapace length (SCL; 0.1 cm) was recorded with calipers.

At necropsy, turtles were weighed (0.5 kg) with a spring scale. A body condition index (BCI) was calculated using weight and SCL (weight/SCL^3) to assess body condition (Bjorndal et al., 2000). All turtles underwent a systematic external and internal examination. Based on gross necropsy, turtles were categorized as to cause of stranding, including FP (those with external or internal tumors) or causes other than FP (trauma or undetermined). For turtles with FP, each external tumor was measured at its 2 widest cross-sectional dimensions with a ruler. Multilobulated external tumors on a single stalk were measured as a single unit. We estimated cross-sectional area (cm^2) of each tumor using the formula for circle (πr^2) or ellipse ($\pi \cdot 0.5a \cdot 0.5b$ for the major [a] and minor [b] axis of an ellipse) depending on whether the cross-section approximated a circle or an ellipse, respectively (Work et al., 2004).

At necropsy, adult trematodes were collected directly from the vasculature, bladder, or ventriculus and then washed extensively in phosphate-buffered saline (PBS), placed in cryovials, and stored frozen (−70 C). Spirorchiid eggs were isolated from the spleen as described previously (Dailey and Morris, 1995) with the following modifications. Preweighed (g) portions of spleen were allowed to sit at 27 C for 24 hr, diced, mixed with small amounts of PBS, and homogenized in a blender. Homogenates were then digested in 2% (w/v) pepsin dissolved in 1% NaCl solution and 0.03% HCl in a warm (37 C) water bath on a shaker for 24 hr. Eggs were recovered with a Flukefinder (Visual Difference, Moscow, Idaho), a sieve with coarse- and fine-mesh filters, washed extensively with PBS, and quantified as type I (eggs with bipolar spines) or type II (eggs with a unipolar spine) (Wolke et al., 1982) (Fig. 1). Total splenic egg burden for each turtle was expressed as eggs per gram spleen (eggs/GOS).

Soluble worm antigen (SWA) was prepared as described previously (McLaren et al., 1978) with modifications. From 2 turtles, 198 *Laeredius* sp., 36 *Hapalotrema* sp., and 24 *Carettacola hawaiiaensis* were recovered and homogenized in 3 ml of PBS for 30 min using a Ten-Broeck grinder and then centrifuged at 20,000 *g* at 4 C for 1 hr, and the supernatant was harvested, aliquoted, and frozen (−70 C). Bladder (*Pyelosomum* sp.) and ventricular (an unknown proportion of *Polyangium* sp. and *Angiodyctyum* sp.) trematode antigens were prepared similarly. Eggs for soluble egg antigen (SEA) were harvested from the lung and spleen of 5 turtles as described above. Eggs were cleaned of tissue debris by overlaying eggs in 3 ml of PBS on 10 ml of Ficoll 1119 and then centrifuging at 300 *g* for 10 min. Eggs were subsequently washed in PBS, and the Ficoll centrifugation and PBS washes were repeated twice. The eggs were then stored frozen (−70 C). For preparation of SEA, 900 μl of eggs were homogenized in 3 ml of 8 M urea buffer (Tsang, 1981) for 30 min and then centrifuged at 20,000 *g* at 4 C for 1 hr, and the supernatant was harvested and stored as described for SWA. Protein content (mg/ml) of all antigen preparations was assessed using bicinchoninic acid quantification according to manufacturer instructions (Pierce, Rockford, Illinois).

Antibody response to SWA and SEA was measured by ELISA as described previously (Herbst et al., 1998; Work et al., 2000). Briefly, plates were coated with 1 μg/well of either SWA or SEA, and duplicate wells were incubated with 50 μl of turtle plasma diluted 1:25 in blocking buffer. Following primary staining with turtle sera, the plates were stained with rabbit antisera to green sea turtle immunoglobulins (rabbit anti-turtle 7S IgY heavy chain [RAT]) and counterstained with goat anti-rabbit IgG directly conjugated to horseradish peroxidase (GHRP; Alpha Diagnostics, San Antonio, Texas). Staining was detected by reaction of horseradish peroxidase with 5-aminosalicylic acid (5-AS), and optical density (OD) was measured at 450 nm on a standard ELISA plate reader. The following negative controls in SWA- or SEA-coated wells were used: Substrate, substrate + GHRP, or substrate + 5-AS + GHRP + RAT. The OD readings of test samples were corrected for background by subtracting the highest negative-control OD.

To correct for plate-to-plate variation, 3 wells on each plate were coated with 5 μg/well of egg white lysozyme (EWL) and overlaid with a 1:200 dilution of plasma collected from a green turtle that had been inoculated with EWL 10 wk previously and had a strong antibody response to EWL (Work et al., 2000). Test results for each sample were expressed as units of EWL-positive turtle plasma by dividing the mean OD for each test sample by the mean OD of EWL-positive plasma for each plate.

To ensure that the SEA and SWA ELISAs were not detecting antibodies that cross-reacted against bladder or ventricular trematodes, we evaluated the SEA and SWA ELISAs against crude antigens from these species. Plasma from turtles that were infected with spirorchiids and bladder trematodes only or with spirorchiids and stomach trematodes only were preincubated for 1 hr with SEA, SWA, soluble antigen from bladder trematodes, or soluble antigen from stomach trematodes at concentrations ranging from 0.02 to 200 μg/ml. The plasma was then assayed by indirect ELISA for SWA or SEA as described above, with the exception that results were expressed as OD.

Data for splenic egg burden were log transformed to meet assumptions of normality and equal variance. Multiple linear regression was used to relate splenic egg counts in stranded turtles to units of activity against SWA and SEA, BCI, total surface area of tumors per turtle, and SCL. Student's *t*-test was used to compare splenic egg burdens between stranded turtles with and without FP and between males and females. For comparison of antibody titers, turtles were categorized into 10 groups as follows: Hatchlings from FFS (FFS-hatchlings), adults from FFS (FFS-adults), Kona-Kohala, stranded; Kaneohe Bay (KB) tumor score 0 (KB0), 1 (KB1), 2 (KB2), and 3 (KB3), SLP, and MLBR. Analysis of variance was used to compare antibody titers against SWA

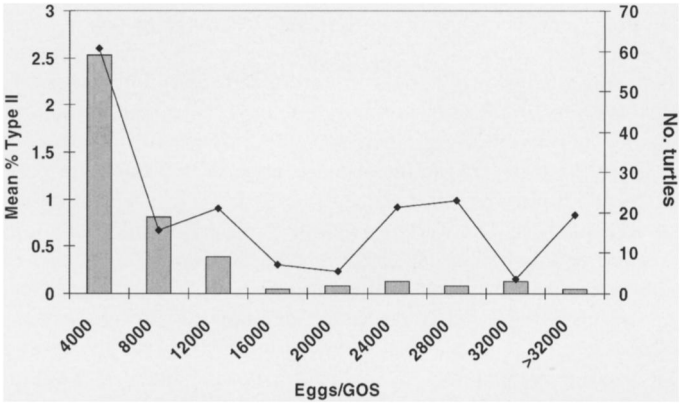

FIGURE 2. Frequency distribution of numbers of turtles with different numbers of eggs/g of spleen (GOS) (bars, right axis) and mean percent of eggs in each category that were type II (diamonds and lines, left axis).

and SEA between the 10 groups of turtles, splenic egg burdens between TS categories of stranded turtles, and splenic egg burdens between the various Hawaiian islands (Kauai, Oahu, Maui-Molokai-Lanai, and Hawaii). The α value for all comparisons was 0.05 (Daniel, 1987).

RESULTS

We digested the spleen from 99 turtles, of which 91 were stranded with FP and the remainder were stranded from non-FP causes (trauma or undetermined). Splenic egg burden could be described by a negative binomial distribution and ranged from 10 to 239,865 eggs/GOS. The majority of eggs were bipolar spine type I, and the percentage of unipolar spine type II eggs (range 0–67%) decreased rapidly to 1% or less with increasing total splenic egg burden (Fig. 2). No significant difference was found in splenic egg burden between turtles with (n = 91) and without (n = 8) FP, between males (n = 49) and females (n = 50), or between TS-1 (n = 3), TS-2 (n = 38), or TS-3 (n = 50) categories. Splenic egg burden decreased significantly with increasing SCL ($P < 0.001$) and increasing BCI ($P < 0.05$) (Fig. 3). No significant relationship was found between total tumor area per turtle and splenic egg burden, between BCI and SCL, or between BCI and total tumor area per turtle (data not shown). We did observe a trend of increasing mean number of eggs per GOS from southeast to northwest in the main Hawaiian islands (Table I). Of 2,170 blood flukes recovered from dead turtles, 53%, 34%, and 13% were *Laeredius* sp., *Hapalotrema* spp., and *C. hawaiiensis*, respectively.

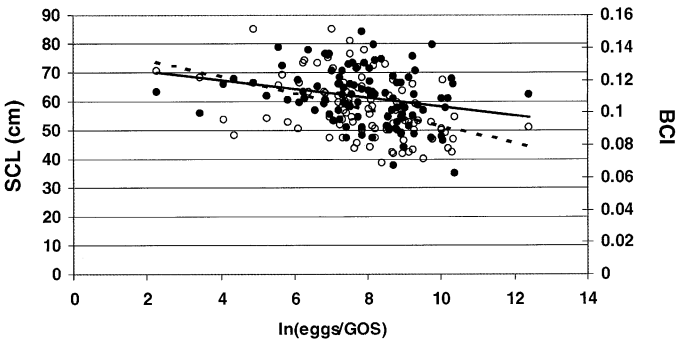

FIGURE 3. Relationship between eggs per gram spleen and body condition index (black dots, solid line; right axis) and straight carapace length (SCL; white dots, dashed line; left axis).

TABLE I. Natural log of eggs/spleen by island.

Group name	No. of turtles	Mean	SD
Kauai	2	8.557	0.986
Oahu	64	8.008	1.392
Maui-Molokai-Lanai	23	7.815	1.572
Hawaii	3	6.773	2.922

A total of 191 plasma samples were tested for SWA and SEA. These comprised 15 hatchlings and 20 adults from FFS, 26 turtles from SLP, 10 turtles from MLBR, 20 turtles stranded with FP from various locations, 20 turtles from Kona, and 20 turtles each from KB in TS categories 0, 1, 2, and 3. Hatchlings from FFS, SLP, and MLBR turtles had significantly lower titers ($F = 22.16$, $P < 0.001$) against SWA compared to juvenile turtles from KB, Kona, stranded, or adults from FFS (Fig. 4A).

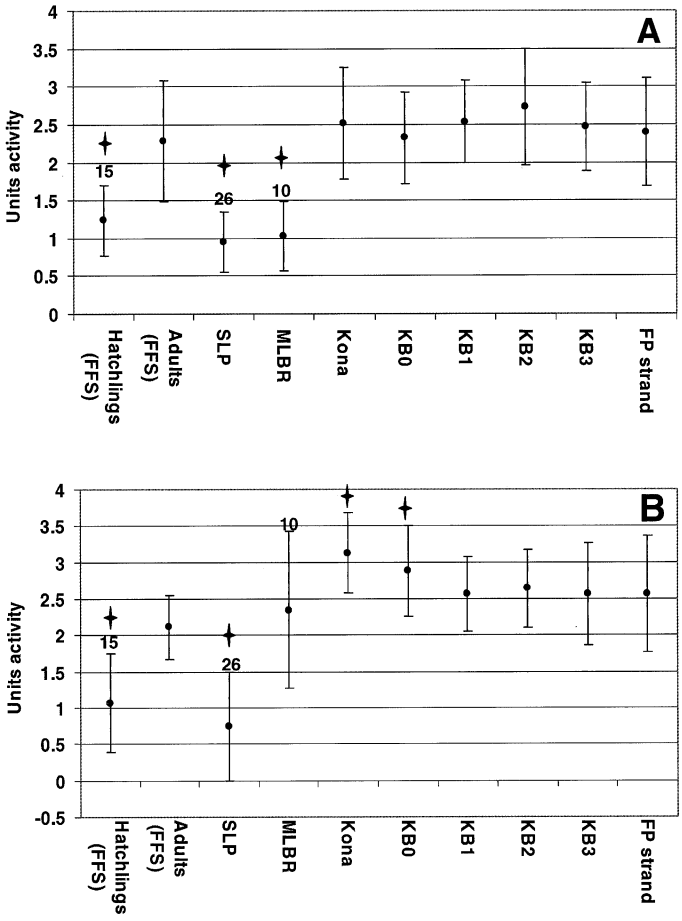

FIGURE 4. Mean units (SD) of activity against soluble spirorchiid worm (**A**) and egg (**B**) antigen for 10 groups of turtles. The number for each group is 20 unless otherwise indicated above the data point. Turtles originated from French Frigate Shoals (FFS), Sea Life Park (SLP), Mauna Lani Bay Resort (MLBR), Kaneohe Bay (KB), Kona, or were found stranded (FP Strand). Numbers after KB refer to tumor score categories. Data points with a star were significantly different ($P <$ 0.05).

Hatchlings from FFS and SLP turtles had significantly lower titers, and Kona and KB0 turtles had significantly ($F = 28.36$, $P < 0.001$) higher titers against SEA than adults from FFS, KB1, KB2, KB3, and stranded turtles (Fig. 4B). No significant relationship was found between units of activity of antibodies against SWA or SEA and splenic egg burden. No cross-reactivity existed in antibodies to SWA or SEA with bladder or ventricular trematodes, but cross-reactivity did exist between antibodies against SEA and SWA (data not shown).

DISCUSSION

The criteria used by Dailey and Morris (1995) to differentiate eggs of *Hapalotrema* spp. versus those of *Laeredius* sp. recovered from turtle tissues were not explicit. We opted to be more conservative and categorize eggs recovered from the spleen as type I or type II (Wolke et al., 1982). Based on morphology, type I eggs with bipolar spines came from either *Laeredius* sp. (Dailey, 1992) or *Hapalotrema* spp. (Dailey, 1993), and type II eggs originated from *C. hawaiiensis* (Dailey et al., 1991). To assess severity of infection with spirorchiids, we opted to quantify eggs in the spleen to ensure we were dealing only with eggs from spirorchiids. Tissue egg burdens probably are more reflective of chronic accumulation and infection and are not necessarily correlated with adult worm burdens. However, in the absence of methods to quantify adult worm burdens in turtles both reliably and completely, splenic egg burden is a useful surrogate.

From splenic egg counts, *Laeredius* sp. and *Hapalotrema* spp. were the most commonly encountered spirorchiids in stranded turtles regardless of FP status. Dailey and Morris (1995) also found these 2 species of spirorchiids to be dominant based on evaluation of tissue egg burdens in green turtles from Hawaii. In addition, predominance of these 2 species was reflected in our recovery of adult worms. Mean percentage of eggs from *C. hawaiiaensis* was low and averaged a maximum of 3%, rapidly decreasing to 1% or less as total egg burden in the spleen increased. Either green turtles are more susceptible to infection with *Hapalotrema* spp. and *Laeredius* sp. or these parasites outcompete *C. hawaiiensis* in either the definitive (turtle) or the intermediate (unknown) host. To our knowledge, *C. hawaiiensis* has been described only from green turtles in Hawaii and is probably an endemic parasite (Dailey et al., 1991). In contrast, *Laeredius* sp. has been described from green turtles in the western Atlantic (Greiner et al., 1980) and *Hapalotrema* spp. from the western Atlantic (Greiner et al., 1980) and Australia (Cribb and Gordon, 1998). This suggests that *Hapalotrema* spp. and *Laeredius* sp. are not endemic to Hawaii but were introduced into the islands through an unknown route. Phylogenetic analyses for evaluating which of these trematode species are truly endemic versus cryptogenic (Chapman and Carlton, 1991) may shed light on this issue.

Egg burden in stranded turtles decreased with increasing SCL and increasing BCI. As turtles age (increased SCL), density of eggs in tissues decreases either through fewer opportunities for new infection, increased immunity against parasites, or decreased fecundity of parasites. Cheever et al. (1977) found that tissue egg burdens in humans infected with *Schistosoma mansoni* and *Schistosoma haematobium* also decreased with increasing age of the host, suggesting that this phenomenon may occur in a wide range of vertebrates. Decrease in splenic egg burdens with increasing BCI indicated either that worms in turtles with a better body condition produced fewer eggs, that turtles with a better body condition have stronger immunity against worms and eggs in tissue, that worms in nutritionally compromised turtles produced more eggs, or that turtles in poor body condition are more susceptible to having larger numbers of adult worms. The role of nutrition in vascular fluke infection is unclear. Lawrence (1973) found that *Schistosoma matthei* in calves at a higher plane of nutrition produced fewer eggs than worms in calves at a lower plane of nutrition. Conversely, *S. mansoni* infecting mice on a low-protein diet had lower egg production compared with mice on a normal diet (de Meillon and Patterson, 1958). The significant association between poor body condition and splenic egg burdens and the lack of a relationship between total tumor area per turtle and splenic egg burden suggests that spirorchiids are playing a detrimental role in turtles independent of FP status.

We did not see a relationship between splenic egg burden and levels of antibodies against adult worms or eggs. In humans, antibody response to crude adult worm or egg antigens showed a relatively low correlation to fecal egg counts, but partially purified antigens showed better correlation (Mott and Dixon, 1982). Using SEA from a single species of *Schistosoma*, Mott and Dixon (1982) found a significant relationship between antibody response and fecal egg counts in humans. In the present study, we used crude antigens composed of at least 3 species of adult worms. This almost certainly lowered the specificity of our assay and may explain the lack of correlation between splenic egg burden and antibody response in turtles. The sera we used to evaluate the relationship of tissue egg burden to antibody response came from stranded turtles that were severely debilitated, some of which may have been anergic. Future investigations should focus on detecting parasite antigen in the circulation to better assess the degree of infection. For example, the circulating microsomal antigen for *S. mansoni* was highly specific, sensitive, and indicative of parasite burden in humans (Tsang and Wilkins, 1997). Obtaining sufficient quantities of such soluble antigen required using a large number of parasitic infections in guinea pigs (Tsang et al., 1983). Currently, however, this is unrealistic for spirorchiids from green turtles unless an experimental host for rearing large numbers of turtle spirorchiids is developed.

Although the serology did not reflect worm burdens, this test may be useful in evaluating exposure on a temporal basis and in determining relationships between groups of turtles. Graczyk et al. (1995) used 2 standard deviations (SD) above negative controls to determine seropositivity against worm antigens, and Herbst et al. (1998) used 3 SD. Because of the uncertain specificity and sensitivity of this serological assay, we opted not to assign arbitrary cutoffs to assess whether turtles were seropositive. Instead, we compared units of activity between groups (FP positive and negative) and arrived at similar conclusions regarding exposure to spirorchiids and FP status.

The generally higher antibody activity against SEA versus that against SWA was similar to findings in humans, in whom crude SEA is reputed to be a more sensitive antigen (Mott and Dixon, 1982). As expected, captive turtles that are housed in tanks containing filtered seawater at SLP, presumably free of cercariae, had the lowest levels of antibodies to SWA. Antibody

levels in hatchlings on the nesting beaches at FFS were slightly higher, and this may be caused by transfer of maternal antibodies through egg yolk from chronically infected adults. Free-ranging adults and free-ranging immature turtles appeared equally exposed to adult worms. Turtles that were transferred from captivity into oceanside seawater ponds at the MLBR had higher levels of antibodies against SEA. We suspect that these turtles were undergoing recent infection with spirorchiids and that antibodies against cercariae may be cross-reactive to SEA. Confirming this would require monitoring serological response of turtles over time and a reliable way to assess tissue worm or egg burden in live turtles.

The relatively low level of antibodies to both SEA and SWA in hatchlings suggests that green turtles become infected with spirorchiids shortly after entering foraging pastures from the pelagic environment. Tissue egg burdens indicate that the intensity of infection wanes with age. The low antibody levels in hatchlings probably result from maternal transfer of antibody through yolk, because it is unlikely that hatchlings would be exposed to trematode cercariae before leaving the nesting beach for the open sea. Supporting this argument is the observation that 100% of necropsied nearshore turtles that died from various causes, including FP, were positive for spirorchiids at microscopic examination of tissues (Work et al., 2004), whereas several species of turtles, including greens, caught in pelagic longline fisheries had little or no evidence of infection with spirorchiids (Work and Balazs, 2002). In another geographic area (Costa Rica), we have also failed to detect evidence of spirorchiid infection in hatchling leatherback and olive ridley turtles on microscopic examination of tissues (T. Work, unpubl. obs.). We suspect that infection with spirorchiids does not occur in hatchling Hawaiian green turtles.

The finding of increasing tissue egg burdens in turtles along the main Hawaiian Islands from the southeast to northwest was provocative. The island of Hawaii is the youngest island of the archipelago, and islands become geologically older from southeast to northwest. Knowing the identity and range of the intermediate hosts for spirorchiids and, hence, opportunities for infection of turtles may help to explain higher tissue egg burdens in turtles from the more northwestern islands. Factors associated with the age of the islands, such as latitudinal or erosional gradients, also may play a role, along with the time available for intermediate hosts to colonize these islands.

Turtles with severe FP are immunosuppressed (Work et al., 2001). Although we saw no significant association between severity of FP and splenic egg burdens, all stranded turtles affected with FP are infected with blood flukes (Aguirre et al., 1998; Work et al., 2004). A herpesvirus has been strongly associated with the presence of tumors (Quackenbush et al., 1998), and recent evidence suggests that leeches, but not spirorchiids, could serve as potential mechanical vectors for this virus (Greenblatt et al., 2004). In humans, schistosomiasis can cause immunosuppression and decrease cell-mediated immune response against certain viruses (Marshall et al., 2001). Investigating immune mediation of green turtles by spirorchiids in light of FPTHV infection and identifying the potential intermediate hosts of these parasites are steps that would help to clarify the role of the environment in disease of sea turtles, thereby aiding management and recovery of the species.

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LITERATURE CITED

AGUIRRE, A. A., T. R. SPRAKER, G. H. BALAZS, AND B. ZIMMERMAN. 1998. Spirorchiidiasis and fibropapillomatosis in green turtles from the Hawaiian islands. Journal of Wildlife Diseases **34:** 91–98.

ALARCON DE NOYA, B., C. COLMENARES, S. LOSADA, Z. FERMIN, G. MASROUA, L. RUIZ, L. SOTO, AND O. NOYA. 1996. Do intestinal parasites interfere with the seroepidemiologic surveillance of *Schistosoma mansoni* infection? Epidemiology and Infection **116:** 323–329.

BALAZS, G. H., AND M. CHALOUPKA. 2004. Thirty-year recovery trend in the once depleted Hawaiian green turtle stock. Biological Conservation **117:** 491–498.

BJORNDAL, K. A., A. B. BOLTEN, AND M. Y. CHALOUPKA. 2000. Green turtle somatic growth model: Evidence for density dependence. Ecological Applications **10:** 269–282.

CHAPMAN, J. W., AND J. T. CARLTON. 1991. A test of criteria for introduced species: the global invasion by the isopod *Synidotea laevidorsalis* (Miers, 1881). Journal of Crustacean Biology **11:** 386–400.

CHEEVER, A. W., I. S. KAMEL, A. M. ELWI, J. E. MOSIMANN, AND R. DANNER. 1977. *Schistosoma mansoni* and *S. haematobium* infections in Egypt. II. Quantitative parasitological findings at necropsy. American Journal of Tropical Medicine and Hygiene **26:** 702–716.

CRIBB, T. H., AND A. N. GORDON. 1998. *Hapalotrema* (Diginea: Spirorchiidae) in the green turtle (*Chelonia mydas*) in Australia. Journal of Parasitology **84:** 375–378.

DAILEY, M. D. 1992. A survey of the trematoda (Platyhelminthes: Diginea) parasitic in green turtles, *Chelonia mydas* (L.) from Hawaii. Bulletin of the Southern California Academy of Sciences **91:** 84–91.

———. 1993. *Hapalotrema dorsospora* sp. n. (Trematoda: Spirorchiidae) from the heart of the green turtle (*Chelonia mydas*) with a redescription of *Hapalotrema postorchis*. Journal of the Helminthological Society of Washington **60:** 5–9.

———, M. L. FAST, AND G. H. BALAZS. 1991. *Carettacola hawaiiensis* n. sp. (Trematoda: Spirorchiidae) from the green turtle, *Chelonia mydas*, in Hawaii. Journal of Parasitology **77:** 906–909.

———, AND R. MORRIS. 1995. Relationship of parasites (Trematoda: Spirorchiidae) and their eggs to the occurrence of fibropapillomas in the green turtle (*Chelonia mydas*). Canadian Journal of Fisheries and Aquatic Science **52**(Suppl. 1): 84–89.

DANIEL, W. W. 1987. Biostatistics: A foundation for analysis in the health sciences. 4th ed. John Wiley & Sons, New York, New York, 734 p.

DE MEILLON, B., AND S. PATERSON. 1958. Experimental bilharziasis in animals. VII. Effect of a low-protein diet on bilharziasis in white mice. South African Medical Journal **32:** 1086–1088.

GORDON A. N., W. R. KELLY, AND T. H. CRIBB. 1998. Lesions caused by cardiovascular flukes (Digenea: Spirorchidae) in stranded green turtles (*Chelonia mydas*). Veterinary Pathology **35:** 21–30.

GRACZYK, T. K., A. A. AGUIRRE, AND G. H. BALAZS. 1995. Detection by ELISA of circulating anti-blood flukes (*Carettacola*, *Hapalotrema*, and *Laeradius*) immunoglobulins in Hawaiian green turtles (*Chelonia mydas*). Journal of Parasitology **81:** 416–421.

GREENBLATT, R. J., T. M. WORK, G. H. BALAZS, C. A. SUTTON, R. N. CASEY, AND J. W. CASEY. 2004. The *Ozobranchus* leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (*Chelonia mydas*). Virology **321:** 101–110.

GREINER, E. C., D. J. FORRESTER, AND E. R. JACOBSON. 1980. Helminths of mariculture-reared green turtles (*Chelonia mydas*) from Grand

Cayman, British West Indies. Proceedings of the Helminthological Society of Washington **47:** 142–144.

HERBST, L. H., E. C. GREINER, L. M. EHRHART, D. A. BAGLEY, AND P. A. KLEIN. 1998. Serological association between spirorchiidiasis, herpesvirus infection, and fibropapillomatosis in green turtles from Florida. Journal of Wildlife Diseases **34:** 496–507.

LACKOVICH, J. K., D. R. BROWN, B. L. HOMER, R. L. GARBER, D. R. MADER, R. H. MORETTI, A. D. PATTERSON, L. H. HERBST, J. OROS, E. R. JACOBSON, S. S. CURRY, AND P. A. KLEIN. 1999. Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. Diseases of Aquatic Organisms **37:** 89–97.

LAWRENCE, J. A. 1973. *Schistosoma matthei* in cattle: variation in parasite egg production. Research in Veterinary Science **14:** 402–404.

MARSHALL, M. A., D. JANKOVIC, V. E. MAHER, A. SHER, AND J. A. BERZOFSKY. 2001. Mice infected with *Schistosoma mansoni* develop a novel non-T-lymphocyte suppressor population which inhibits virus-specific CTL induction via a soluble factor. Microbes and Infection **3:** 1051–61.

MCLAREN, M., C. C. DRAPER, J. M. ROBERTS, E. MINTER-GOEDBLOED, G. S. LIGTHART, C. H. TEESDALE, M. A. AMIN, A. H. S. OMER, A. BARTLETT, AND A. VOLLER. 1978. Studies on the enzyme-linked immunosorbent assay (ELISA) test for *Schistosoma mansoni* infections. Annals of Tropical Medicine and Parasitology **72:** 243–253.

MOTT, K. E., AND H. DIXON. 1982. Collaborative study on antigens for immunodiagnosis of schistosomiasis. Bulletin of the World Health Organization **60:** 729–753.

MURAKAWA, S. K. K., G. H. BALAZS, D. M. ELLIS, S. HAU, AND S. M. EAMES. 1999. Trends in fibropapillomatosis among green turtles stranded in the Hawaiian islands, 1982–98. *In* Proceedings of the 19th Annual Symposium on Sea Turtle Biology and Conservation, March 2–5, 1999, South Padre Island, Texas, p. 239–241.

OWENS, D. W., AND G. J. RUIZ. 1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica **36:** 17–20.

QUACKENBUSH, S. L., T. M. WORK, G. H. BALAZS, R. N. CASEY, J. ROVNAK, A. CHAVES, L. DUTOIT, J. D. BAINES, C. R. PARRISH, P. R. BOWSER, AND J. W. CASEY. 1998. Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. Virology **246:** 392–399.

TSANG, V. C. W. 1981. Systematic fractionation of *Schistosoma mansoni* urea-soluble egg antigens and their evaluation by the single-tube, kinetic-dependent, enzyme-linked, immunosorbent assay (K-Elisa). Journal of Parasitology **67:** 340–350.

———, K. HANCOCK, M. A. KELLEY, B. C. WILSON, AND S. E. MADDISON. 1983. *Schistosoma mansoni* adult microsomal antigens, a serologic reagent. Journal of Immunology **130:** 1366–1370.

———, AND P. P. WILKINS. 1997. Immunodiagnosis of schistosomiasis. Immunological Investigations **26:** 175–188.

WOLKE, R. E., D. R. BROOKS, AND A. GEORGE. 1982. Spirorchiidiasis in loggerhead sea turtles (*Caretta caretta*): Pathology. Journal of Wildlife Diseases **18:** 175–185.

WORK, T. M., AND G. H. BALAZS. 1999. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. Journal of Wildlife Diseases **35:** 804–807.

———, AND ———. 2002. Necropsy findings in sea turtles taken as by-catch in the north Pacific longline fishery. Fishery Bulletin **100:** 876–880.

———, ———, R. A. RAMEYER, S. P. CHANG, AND J. BERESTECKY. 2000. Assessing humoral and cell-mediated immune response in Hawaiian green turtles, *Chelonia mydas*. Veterinary Immunology and Immunopathology **74:** 179–194.

———, ———, R. A. RAMEYER, AND R. M. MORRIS. 2004. Retrospective pathology survey of green turtles (*Chelonia mydas*) with fibropapillomatosis from the Hawaiian Islands, 1993–2003. Diseases of Aquatic Organisms **62:** 163–176.

———, R. A. RAMEYER, G. H. BALAZS, C. CRAY, AND S. P. CHANG. 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. Journal of Wildlife Diseases **37:** 574–581.

———, ———, M. WOLCOTT, AND R. M. MORRIS. 2003. Bacteremia in Hawaiian green turtles, *Chelonia mydas*, with fibropapillomatosis. Diseases of Aquatic Organisms **53:** 41–46.