

## *Cryptosporidium* sp. Infections in Green Turtles, *Chelonia mydas*, as a Potential Source of Marine Waterborne Oocysts in the Hawaiian Islands

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**For the first time, *Cryptosporidium* sp. oocysts were identified in fecal and intestinal samples from free-ranging marine turtles, *Chelonia mydas*, from the Hawaiian Islands. The oocysts produced positive reactions with commercial test kits recommended for the detection of human-infectious waterborne oocysts of *Cryptosporidium parvum*.**

One of the eight *Cryptosporidium* species (16), *Cryptosporidium parvum*, infects humans and is a global public health threat, having caused massive waterborne epidemics (21). The infectious stage, the oocysts, can be transmitted via water, and marine waters extend their viability (20, 21). *Cryptosporidium* oocysts were found in Hawaii near Waikiki Beach on the island of Oahu (19), but the origin and epidemiological importance of these oocysts remain unknown. The green turtle, *Chelonia mydas*, is a marine species under protection of the U.S. Endangered Species Act whose habitat is the Hawaiian Islands (2, 4). *Cryptosporidium* infections have been reported for turtles (7, 8, 12, 15, 17) but not for *C. mydas*. The purpose of this study was to test *C. mydas* for *Cryptosporidium* infections and to determine the reaction of turtle-recovered oocysts with commercial tests for detection of human-infectious oocysts of *C. parvum*.

Fecal and intestinal samples collected between November 1994 and October 1996 at necropsy originated from 34 free-ranging turtles: 22 from Oahu and 12 from the western shores of Maui. Thirty-one turtles were euthanized due to severe fibropapillostomatosis (1, 5), and 3 animals died as a result of entanglement and drowning in a fisherman's gill net or due to fishhook ingestion. Three turtles were adults; all others were immature. The oocysts extracted from fecal and intestinal samples (13) were measured (14), and oocyst aliquots were subjected to in vitro excystation (9). The excystation rate (9) and the excystation index (18) were determined. In vitro excystation was observed by using a Nomarski interference contrast optic. Aliquots of the oocysts were acid-fast stained or examined in wet preparation in fluorescent light (14), as some algae and coccidia, e.g., *Cyclospora*, produce an autofluorescent "halo." The oocysts were processed with immunofluorescent antibodies of the following commercial test kits: the MERIFLUOR *Cryptosporidium/Giardia* direct immunofluorescence assay (IFA) (Meridian Diagnostics, Inc., Cincinnati, Ohio) and the Hydrofluor-

Combo *Cryptosporidium/Giardia* indirect IFA (Ensyss, Inc., Research Triangle Park, N.C.). Also included was the commercial enzyme immunoassay (EIA) ProSpect *Cryptosporidium* Rapid Assay (Alexon, Inc., Sunnyvale, Calif.). The tests are used for detection of *C. parvum* in environmental, fecal, and water samples. The specificity and sensitivity of these tests to *Cryptosporidium* were higher than 97% (15), and they produced negative reactions with more than 40 various enteric agents, including coccidia other than *Cryptosporidium* (15).

The 34 turtles had a straight carapace length of  $59.9 \pm 12.8$  cm (mean  $\pm$  standard deviation) and a weight of  $28.1 \pm 22.0$  kg. The turtles from Maui were significantly heavier ( $P < 0.05$ ;  $t$  test) than those from Oahu, indicating that Oahu turtles were more emaciated from fibropapillostomatosis. Six of 34 (18%) fecal and intestinal samples from immature turtles contained *Cryptosporidium* sp. oocysts; five turtles originated from Oahu (23% prevalence) and one originated from Maui (8% prevalence). Of the positive turtles from Oahu, two came from Kaneohe Bay and one each came from Waiialua, Punaluu, and Laie. Two of six positive turtles (both from Oahu) were not affected by fibropapillostomatosis. The positive turtle from Maui originated from Maliko. All collection sites represent waters used to some extent for recreational purposes. The oocyst size varied from 4.9 to 7.0  $\mu\text{m}$  ( $\bar{x}$ ,  $5.9 \pm 0.3 \mu\text{m}$ ) (Table 1), and the differences in size among oocyst isolates were not significant ( $F = 1.95$ ,  $P > 0.07$ ; analysis of variance). The concentration of fecal oocysts was  $3.7 \times 10^4$  to  $3.1 \times 10^5$  per ml of sedimented feces ( $\bar{x}$ ,  $1.3 \times 10^5$  oocysts/ml) (Table 1); differences among the turtles were significant ( $G = 0.47$ ,  $P < 0.05$ ;  $G$  test). The total number of feces-recovered oocysts,  $8.4 \times 10^6$ , was significantly higher ( $G = 0.57$ ,  $P < 0.05$ ;  $G$  test) than the total number of oocysts ( $4.3 \times 10^6$ ) recovered from intestinal samples (range,  $3.2 \times 10^5$  to  $9.4 \times 10^5$ ;  $\bar{x}$ ,  $5.9 \times 10^5$ ). The mean excystation rate for the pool prepared from six oocyst isolates was 40%, and the excystation index varied from 0.61 to 0.73. None of *Cryptosporidium* sp. oocyst isolates produced an autofluorescent "halo." All six *Cryptosporidium* sp. oocyst isolates produced positive direct- and indirect-IFA reactions. None of the isolates produced a positive EIA reaction (ProSpect test), indicating that the oocysts did not represent *C. parvum* (15). Turtle-recovered oocysts were bigger than *C.*

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TABLE 1. Characteristics of *Cryptosporidium* sp. oocysts recovered from free-ranging green turtles, *C. mydas*, in the Hawaiian Islands<sup>a</sup>

Turtle origin	Carapace length (cm)	Date of sample collection (mo/day/yr)	Oocyst size (μm)		Fecal oocysts		Reaction <sup>a</sup>		
			Range	Mean ± SD	No. (10 <sup>6</sup> )	Concn (10 <sup>3</sup> /ml)	IFA		EIA
							Direct	Indirect	
Oahu									
Laie <sup>b</sup>	43.5	5/24/95	5.0–7.0	5.6 ± 0.6	2.48	310	+	+	–
Punaluu <sup>b</sup>	57.6	5/3/95	4.9–7.0	6.0 ± 0.6	2.25	250	+	+	–
Waialua	77.5	4/4/95	4.9–7.0	6.1 ± 0.6	0.74	49	+	+	–
Kahaluu	54.2	6/29/95	4.9–7.0	6.0 ± 0.7	0.67	37	+	+	–
Mokapu	64.8	5/25/95	4.9–7.0	6.0 ± 0.7	1.68	84	+	+	–
Maui (Maliko)	67.5	2/22/96	5.0–7.0	5.9 ± 0.6	0.57	57	+	+	–

<sup>a</sup> The oocyst isolates were tested with commercial IFAs and an EIA for detection of *C. parvum* oocysts as follows: direct IFA, MERIFLUOR *Cryptosporidium/Giardia*; indirect IFA, Hydrofluor-Combo *Cryptosporidium/Giardia*; and EIA, ProSpect *Cryptosporidium* Rapid Assay.

<sup>b</sup> Nondiseased and with no clinical signs of fibropapillomas.

*parvum* oocysts, 3.5 to 5.0 μm (16), and their excystation rate and excystation index were lower than *C. parvum* values, 65% (9) and 1.00 (18), respectively.

The present study constitutes the first report of *Cryptosporidium* sp. infection in the green turtle and in marine turtle species. The range of oocyst concentrations in green turtle feces was similar to the oocyst concentrations reported for snakes (13, 14). However, it is difficult to evaluate the 18% level of *Cryptosporidium* sp. prevalence in green turtles due to the lack of a report(s) of similar values. Screening of 528 wild and captive reptiles (mainly snakes) for *Cryptosporidium* demonstrated a 3% prevalence (22). Intestinal fragments were utilized in the present study to enhance the numbers of recovered oocysts, and it should not be inferred that cryptosporidial infection originates in the intestine.

Raw-sewage disposal into marine waters, a common practice in many coastal countries (11), has enhanced the risk of pathogen transmission to recreational swimmers (11, 19). Accidental sewage spills in Hawaii's nearshore waters have been reported (6). The state of Hawaii has announced waterborne *Cryptosporidium* contamination of Hawaiian beaches (19); the pathogen also has been found in drinking water in Hawaii. Positive reactions of the turtle-recovered oocysts with commercial tests for *C. parvum* are of epidemiological importance to the state of Hawaii's Department of Health. If those or similar test kits are used, positive reaction with turtle-derived oocysts can be misleading and can needlessly exacerbate public health concerns. The present study indicates that green turtles may also be a source of waterborne oocysts contaminating recreational beaches. In 1989, large numbers of green turtle fecal pellets (approximately 5,500) washed ashore on Oahu at Kualoa Beach, Kaneohe Bay, forcing the Department of Health to close the beach as an epidemiological precaution (6). The 18% *Cryptosporidium* prevalence in the present study suggests that approximately 1,000 fecal specimens could carry the oocysts. Interestingly, two of six positive fecal specimens in the present study originated from Kaneohe Bay. Fecal pellets of green turtles have occasionally appeared on certain Hawaiian beaches since 1976 (3). Thus, if a beach or adjacent waters are found to be contaminated with *Cryptosporidium*, we recommend that any turtle feces from that site be tested. Although the tests used in the present study were designed to identify *C. parvum*, they have provided positive reactions with other *Cryptosporidium* organisms noninfectious to humans (15). The molecular similarities of the turtle-recovered oocysts to *C. parvum* or to *Cryptosporidium serpentis* and their infectivity for immu-

nocompromised or immunosuppressed people are unknown. *C. serpentis* isolates were found to be noninfectious when tested in the neonatal BALB/c mouse model (10).

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