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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 euthanatized 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 MER*IFLUOR 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 ± 12.8 cm (mean ± standard deviation) and a weight of 28.1 ± 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 Waialua, 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 μm ($\bar{x}$, 5.9 ± 0.3 μm) (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 3.7×10^4 to 3.1×10^5 per ml of sedimented feces ($\bar{x}$, 1.3×10^5 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, 8.4×10^6, was significantly higher ($G = 0.57$, $P < 0.05$; *G* test) than the total number of oocysts (4.3×10^6) recovered from intestinal samples (range, 3.2×10^5 to 9.4×10^5; $\bar{x}$, 5.9×10^5). 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[a]

Turtle origin	Carapace length (cm)	Date of sample collection (mo/day/yr)	Oocyst size (μm)		Fecal oocysts		Reaction[a]		
			Range	Mean ± SD	No. (10^6)	Concn (10^3/ml)	IFA		EIA
							Direct	Indirect	
Oahu									
Laie[b]	43.5	5/24/95	5.0–7.0	5.6 ± 0.6	2.48	310	+	+	−
Punaluu[b]	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	+	+	−

[a] The oocyst isolates were tested with commercial IFAs and an EIA for detection of *C. parvum* oocysts as follows: direct IFA, MER*IFLUOR Cryptosporidium*/*Giardia*; indirect IFA, Hydrofluor-Combo *Cryptosporidium*/*Giardia*; and EIA, ProSpect *Cryptosporidium* Rapid Assay.
[b] 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 immunocompromised 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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REFERENCES

1. **Aguirre, A. A., G. H. Balazs, B. Zimmerman, and T. R. Spraker.** 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. J. Wildl. Dis. **30:**8–15.
2. **Balazs, G. H.** 1976. Green turtle migrations in the Hawaiian Archipelago. Biol. Conserv. **9:**125–140.
3. **Balazs, G. H.** 1980. Field methods for sampling the dietary components of green turtles, *Chelonia mydas*. Herpetol. Rev. **11:**5–6.
4. **Balazs, G. H.** 1980. Synopsis of biological data on the green turtle in the Hawaiian Islands, p. 141. NOAA technical memo NMFS-SWFC-7. U.S. Department of Commerce, Washington, D.C.
5. **Balazs, G. H., and S. G. Pooley.** 1991. Research plan for marine turtle fibropapillomas, p. 113. NOAA technical memo NMFS-SWFSC-156. U.S. Department of Commerce, Washington, D.C.
6. **Balazs, G. H., R. Fujioka, and C. Fujioka.** 1993. Marine turtle faeces on Hawaiian beaches. Marine Pollut. Bull. **26:**392–394.
7. **Barnard, S. M., and S. J. Upton.** 1994. A veterinary guide to the parasites of reptiles, vol. 1. Protozoa, p. 240. Krieger Publishing Co., Malabar, Fla.
8. **Bourdeau, P.** 1988. Diseases of turtles: disease of the skin and digestive tract. Point Vet. **20:**871–884.
9. **Campbell, A. T., L. J. Robertson, and H. V. Smith.** 1992. Viability of *Cryptosporidium parvum* oocysts: correlation of in vitro excystation with inclusion or exclusion of fluorogenic vital dyes. Appl. Environ. Microbiol. **58:**3488–3493.
10. **Fayer, R., T. K. Graczyk, and M. R. Cranfield.** 1995. Multiple heterogenous isolates of *Cryptosporidium serpentis* from captive snakes are not transmissible to neonatal BALB/c mice (*Mus musculus*). J. Parasitol. **80:**482–484.
11. **Fleisher, J. M., D. Kay, R. L. Salmon, F. Jones, M. D. Wyer, and A. F. Godfree.** 1996. Marine waters contaminated with domestic sewage: nonenteric illness associated with bather exposure in the United Kingdom. Am. J. Public Health **86:**1228–1234.
12. **Funk, R. S.** 1987. Implications of cryptosporidiosis in emerald tree boas, *Corallus caninus*, p. 139–141. *In* M. J. Rosenberg (ed.), Proceedings of the 10th and 11th International Herpetological Symposium on Captive Propagation and Husbandry. Chicago, Ill. Zoological Consortium, Inc., Thurmont, Md.
13. **Graczyk, T. K., and M. R. Cranfield.** 1996. Assessment of the conventional detection of fecal *Cryptosporidium serpentis* oocysts of subclinically infected captive snakes. Vet. Res. **27:**185–192.
14. **Graczyk, T. K., M. R. Cranfield, and R. Fayer.** 1995. A comparative assessment of direct fluorescence antibody, modified acid-fast-stain, and sucrose flotation techniques for detection of *Cryptosporidium serpentis* in snake fecal specimens. J. Zoo Wildl. Med. **26:**396–402.
15. **Graczyk, T. K., M. R. Cranfield, and R. Fayer.** 1996. Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescence antibody (IFA)

test kits for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. **54:**274–279.
16. **Graczyk, T. K., R. Fayer, and M. R. Cranfield.** Zoonotic transmission of *Cryptosporidium parvum*: implications for waterborne cryptosporidiosis. Parasitol. Today, in press.
17. **Heuschele, W. P., J. Oosterhuis, D. Janssen, P. T. Robinson, P. K. Ensley, J. E. Meier, T. Olson, M. P. Anderson, and K. Benirschke.** 1986. Cryptosporidial infections in captive wild animals. J. Wildl. Dis. **22:**493–496.
18. **Holton, J., P. Nye, and V. McDonald.** 1994. Efficacy of selected disinfectants against *Mycobacteria* and *Cryptosporidia*. J. Hosp. Infect. **27:**105–115.
19. **Johnson, D. C., K. A. Reeynolds, C. P. Gerra, L. Pepper, and J. B. Rose.** 1995. Detection of *Giardia* and *Cryptosporidium* in marine waters. Water Sci. Technol. **31:**439–442.
20. **Robertson, L. J., A. T. Campbell, and H. V. Smith.** 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Appl. Environ. Microbiol. **58:**3494–3500.
21. **Rose, J. B., J. T. Lisle, and M. LeChevallier.** 1997. Waterborne cryptosporidiosis: incidence, outbreaks and treatment strategies, p. 93–109. *In* R. Fayer (ed.), *Cryptosporidium* and cryptosporidiosis. CRC Press, Inc., Boca Raton, Fla.
22. **Upton, S. J., C. T. McAllister, P. S. Freed, and S. M. Barnard.** 1989. *Cryptosporidium* spp. in wild and captive reptiles. J. Wildl. Dis. **25:**20–30.