J. Parasitol., 90(3), 2004, pp. 645–647

Learedius learedi Infection in Black Turtles (*Chelonia mydas agassizii*), Baja California Sur, Mexico

A. Cordero-Tapia A., S. C. Gardner*, J. Arellano-Blanco, and R. B. Inohuye-Rivera, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo No. 195, Playa Palo de Santa Rita, A.P. 128, La Paz, Baja California Sur 23090, Mexico; *To whom correspondence should be addressed. *e-mail: sgardner@cibnor.mx*

ABSTRACT: Black turtle (*Chelonia mydas agassizii*) carcasses, recovered as a result of incidental capture in Magdalena Bay, Mexico, revealed invasion by spirorchiid trematode eggs in liver, kidney, intestines, muscle, heart, pancreas, and duodenum. Seventy-five adult *Learedius learedi* Price, 1934, were recovered from the heart of 1 turtle. Most of the organs showed a mild or absent inflammatory response in histological sections, with the exception of a pancreatic–duodenal section that revealed severe lymphocyte and phagocyte infiltration associated with an infestation of more than 200 eggs. A linear formation of 35 eggs from the pancreas toward the intestinal lumen is described as resembling migration. This is among the first reports of a parasitic infection of *L. learedi* Price 1934, in *C. m. agassizii* in Mexico.

Spirorchiid trematode infections occur frequently in sea turtles (Lauckner, 1985; Dyer et al., 1991), with reports of up to 85% of examined turtles infected by eggs or adult trematodes (Dailey et al., 1993). The life cycle of marine spirorchiids is not well known; adult forms are frequently located in heart chambers, whereas the eggs (with similar form and size for different genera) can be located in the blood vessels of any organ (Lauckner, 1985). Infection in herbivorous sea turtles has been associated with incidental ingestion of invertebrates, such as small gastropods, which potentially serve as intermediate hosts for the parasite (Pérez Ponce de Leon et al., 1996; Raidal et al., 1998).

Infection by trematodes has been associated with several diseases and is suspected to be the cause of death in stranded sea turtles (Rand and Wiles, 1985; Gordon et al., 1998). Infected turtles revealed tissue consolidation and degeneration, nodules, aneurysms, papillary proliferation in blood vessels, endocarditis, periarterial and multifocal edema, thrombosis, and infarcts caused by the eggs (Glazebrook et al., 1981; Aguirre et al., 1998; Raidal et al., 1998). Microscopic lesions observed include hemosiderosis with intracellular load of hemosiderin in hepatic cells, pulmonary alveolar cells, epithelial cells, and red pulp cells in the spleen (Rand and Wiles, 1985); vacuolization and endothelial edema in the affected organs (Glazebrook et al., 1989); and a granulomatous response showing parasite eggs surrounded by gigantic cells, lymphocytes, eosinophils, plasma cells, and fibroblasts (Dyer et al., 1991). Inflammatory response has been associated with the number of eggs found per microscopic field, ranging from mild to severe (Glazebrook et al., 1981; Rand and Wiles, 1985).

Black turtles (*Chelonia mydas agassizii*, also known as Eastern Pacific green turtles, a subspecies of *C. mydas*) occur along the western coast of North and South America. In Mexico, this turtle has experienced a drastic decline during the past 30 yr because of human-related causes such as habitat modification, and incidental and direct fisheries capture of eggs, juveniles, and adults (Cliffton et al., 1982; Gardner and Nichols, 2001). As part of a larger project evaluating the health status of black turtles along the Baja California Peninsula, a systematic study was conducted to assess the presence of infective agents in this region. The objective of the present study was to describe the prevalence of parasitic infection in black turtles and the associated tissue lesions.

Tissues were collected from black turtles killed as a result of incidental fisheries capture in Magadalena Bay, Baja California Sur. Magdalena Bay is located (25°43′N–24°20′N) on the west coast of the Baja California Peninsula, Mexico. The fishing activity in this region, together with the permanent occurrence of black turtles, results in a low, but constant, mortality of turtles by incidental capture (Gardner and Nichols, 2001). Samples of liver, kidney, intestines, and muscle were collected from 11 turtles according to the procedures described by Work (2000). In a subset of these turtles, heart (4), pancreas (5), and duodenum (8) were also removed at necropsy. The samples were processed by conventional histological techniques (Prophet, 1992) and stained with hematoxylin–eosin before optical microscopy with image processing (Image-pro plus, 4.1). Complete hearts were collected from an additional 4 turtles and dissected using a stereomicroscope to identify and quantify adult parasites. The recovered trematodes were fixed in 70% alcohol and stained with Gomori trichrome. Identification was made based on the structural characteristics of *Learedius* species reported by Caballero et al. (1950).

All the black turtles examined were juveniles with an average weight of 30 (±10) kg. Trematode eggs, observed as brownish-green keratin structures with a central cellular aggregation inside, were seen in histological sections of every tissue type examined. Fusiform eggs contained 2 polar prolongations, one with a hooked end and the other spherical with a suckerlike appearance (Fig. 1). Egg density in the affected tissues reached more than 50 eggs per microscopic field at ×50. The highest intensity was observed in a pancreatic–duodenal sample (up to 190 eggs counted in 1 section using a stereomicroscope) (Fig. 2a).

Adult parasites were found only in the heart chambers. In the 4 hearts in which a complete dissection was conducted, the average parasite density was 31 (0, 18, 31, and 75) adults per heart, with some possessing eggs occasionally. The parasite structural characteristics were consistent with descriptions of *Learedius learedi* Price, 1934, i.e., a lanceolate and flat body; length, 3.4–5.1 mm; oral and ventral sucker present; pharynx absent; esophagus at least one-third as long as body; intestinal ceca long and not fused; testes numerous (20 or more), filling

TABLE I. Immunological response observed in black sea turtle (*Chelonia mydas agassizii*) tissues infected with spirorchiid trematode eggs. Values represent the percentage of tissues examined that demonstrated the particular response (n = 11 for liver, kidney, intestines, and muscle; n = 4 for heart; n = 5 for pancreas; and n = 8 for duodenum).

Response	Liver	Kidney	Muscle	Intestine	Heart	Pancreas	Duodenum
Giant cells	18.2	36.4	27.3	9.1	0	20	38
Hemosiderin	54.5	0	0	0	0	0	0
Lymphocyte infiltration	0	0	27.3	63.6	25	20	38
Polymorphonuclear leukocyte infiltration	0	0	9.1	9.1	0	0	0
Plasma cell infiltration	0	9.1	0	0	0	0	0
Autholises	18.2	9.1	9.1	18.2	25	0	0
Thrombis	0	9.1	0	9.1	0	0	0

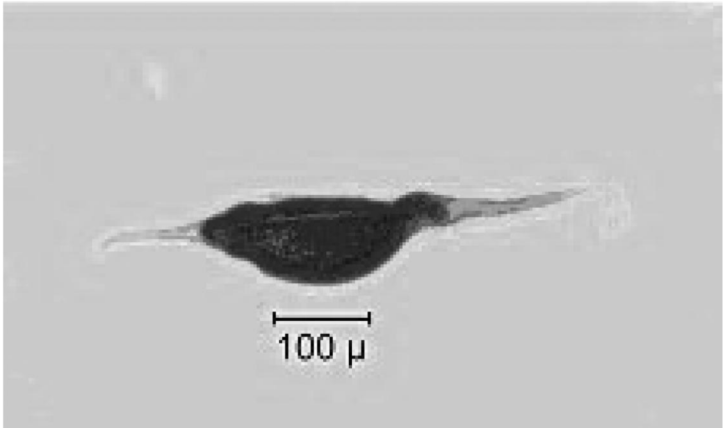

FIGURE 1. Spirorchiid trematode egg observed in tissues of black sea turtles (*Chelonia mydas agassizii*) collected from Baja California Sur, Mexico (×150).

intercecal area; ovary posterior to testes; and a short uterus with few eggs (see Inohuye-Rivera et al., 2004, for complete description).

The most common histological findings in the affected organs included the presence of giant cells and hemosiderin and inflammatory infiltration by lymphocytes, polymorphonuclear leukocytes, and plasma cells (Table I). Only 1 sample, i.e., pancreatic–duodenal, presented severe lymphocyte and phagocyte infiltration. In this same sample, 35 eggs were associated in a linear formation united by polar prolongations (Fig. 2b), giving the appearance that the eggs were migrating or being transported passively from the pancreas into the intestinal lumen (Fig. 2c).

The histological evaluation of organs and tissues from *C. m. agassizii* revealed a similar distribution of the eggs and adult trematodes as reported previously for other sea turtle species from different geographic areas, i.e., Caballero et al. (1955) in Panama; Rand and Wiles (1985) in Bermuda; Dyer et al. (1991, 1995) in Puerto Rico; Perez Ponce de Leon et al. (1996) in Oaxaca, Mexico; and Gordon et al. (1998) in Australia. In other studies adult parasites were found in the heart, whereas eggs invaded various organs including brain, heart, liver, lung, spleen, kidney, skin, stomach, intestines, and major vessels (Rand and Wiles, 1985; Glazebrook et al., 1989; Aguirre et al., 1998). Similarly, in the black turtles that we examined, adult spirorchiids occurred at high density in the heart (up to 75 parasites per heart), whereas trematode eggs were found in every tissue examined, e.g., liver, kidney, muscle, intestines, and pancreas.

Previous reports of egg granuloma response against spirorchiids in sea turtles indicate that the magnitude of the response was related to the degree of infestation, ranging from light (in organs with 2–3 eggs) to severe (with 20–50 eggs) (Rand and Wiles, 1985; Glazebrook et al., 1989). In the black turtle tissues that we examined, only mild or absent inflammatory response was observed, regardless of the level of infestation; an exception occurred in the pancreatic–duodenal tissues of a single turtle in which the largest number of eggs (200) were observed. Gordon et al. (1998) reported a similar response in green turtles in which inflammation was observed only with the highest parasite densities (200 eggs). The reason for this low inflammatory response in these turtles is not known. However, exterior factors such as molecular mimesis of the parasite (Kretschmer et al., 2000), climate and diet (Badillo, 1996; Aznar et al., 1998), and associated viral or bacterial pathogens (Gordon et al., 1998; Raidal et al., 1998) have been implicated by studies of other species.

In Hawaii, reports that 94% of the sea turtles with clinical manifestation of fibropapillomatosis contained trematode eggs raised the question of whether there might be an association between spirorchiid infection and this disease (Aguirre et al., 1998). However, the high prevalence of *L. learedi* infection in Mexican turtles, and the absence of any clinical evidence of fibropapillomatosis in the black turtle population in Mexico, argues against the hypothesis that such an association exists. This finding agrees with that of others (Graczyk et al., 1995),

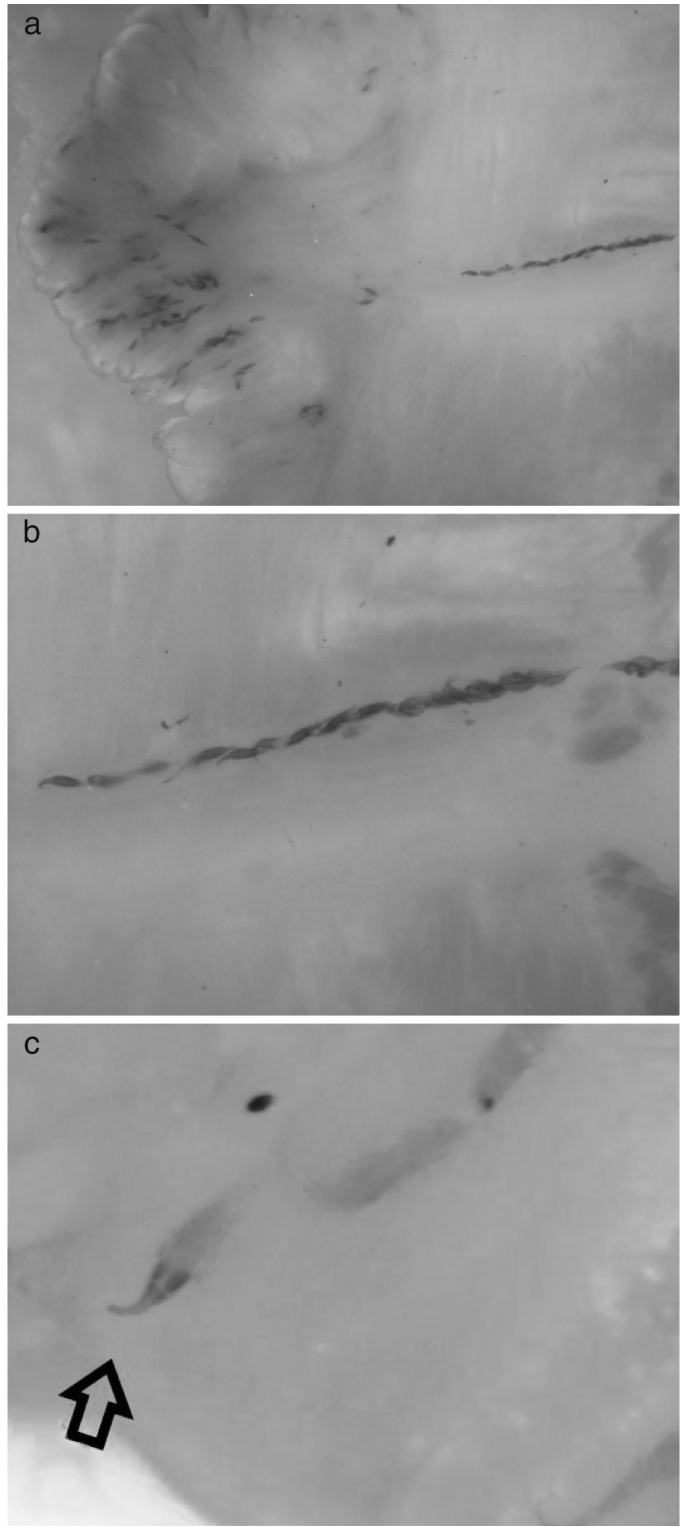

FIGURE 2. **a.** Spirorchiid trematode egg infestation throughout the pancreatic and duodenal tissues to the intestinal lumen (×6). **b.** Eggs associated in a linear formation united by polar prolongations (×20). **c.** Eggs appearing to emerge across the intestinal epithelium.

who reported that 85% of the Hawaiian turtles with fibropapilloma were negative for spirorchiids.

These results are among the first reports of parasitic infection by adult forms of *L. learedi* in *C. m. agassizii* in Mexico or the Pacific coast of North America. The orientation of the eggs observed in the duodenal–pancreatic sample, which appeared to spin on their axes in a formation resembling egg migration or passive transport from the organs through the connective and parenchyma tissues to the intestinal lumen (Fig. 2b, c), has not been reported previously. Although it is reasonable to assume that the eggs observed belonged to the same species as the adults identified, invasion of eggs from 1, or more, spirorchiid trematode species is frequently observed in sea turtles even in the assumed absence of adults of that species (Lauckner, 1985; Dyer et al., 1991). This precludes us from definitively concluding that the eggs observed in our samples belong to *L. learedi*. Confirmation of the species of the eggs in these turtles is currently being undertaken in our laboratory by using molecular methods.

Funding for this project was provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT, G35437-B), the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), and Sistema de Investigación de Mar de Cortéz (SIMAC, 990197010). The authors wish to express their appreciation to Wallace J. Nichols (Wildcoast) for his assistance in the establishment of this project and C. Rodriguez for her assistance in the CIBNOR histology laboratory. We thank T. Zenteno-Savin, G. McConnell, and the editorial staff at CIBNOR, whose comments resulted in a much improved manuscript. This research was conducted in accordance with Mexican laws and regulations, under permits provided by the Secretaria de Medio Ambiente y Recursos Naturales (SGPA/DGVS/002-2895).

LITERATURE CITED

Aguirre, A. A., T. Spraker, G. Balazs, and B. Zimmerman. 1998. Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian islands. Journal of Wildlife Diseases **34:** 91–98.

Aznar, F. J., F. J. Badillo, and J. A. Raga. 1998. Gastrointestinal helminths of loggerhead turtles (*Caretta caretta*) from the western Mediterranean: constraints on community structure. Journal of Parasitology **84:** 474–479.

Badillo, F. J. 1996. Estudio taxonomico y ecológico de los Helmintos de la tortuga boba (*Caretta caretta*) (Linnaeus, 1758), en el Mediterráneo occidental. B.D. Thesis. Universidad de Valencia, Valencia, Spain, 74 p.

Caballero, E., D. Zerecero, and R. Grocott. 1950. Trematodos de las tortugas de México. Revista de Medicina Veterinaria y Parasitología **9:** 123–132.

———, ———, and ———. 1955. Helmintos de la Republica de Panamá, trematodos de *Chelonia mydas* (L.) tortuga marina comestible del Océano Pacifico del Norte, 2a parte. Anales del Instituto de Biología México XXVI **1:** 149–191.

Cliffton, K., D. O. Cornejo, and R. S. Felger. 1982. Sea turtles of the Pacific coast of México. *In* Biology and conservation of sea turtles, K. A. Bjorndal (ed.). Smithsonian Institution Press, Washington, D.C., p. 199–109.

Dailey, M., M. Fast, and G. Balazs. 1993. *Hapalotrema dorsopora* sp. n. (Trematoda: Spirorchidae) from the heart of the green turtle (*Chelonia mydas*) with a redescription of *Hapalotrema postorchis*. Journal of the Helminthological Society of Washington **6:** 5–9.

Dyer, W., E. Williams, and L. Bunkley-Williams. 1991. Some digeneans (Trematoda) of green turtle, *Chelonia mydas* (Testudines: Cheloniidae) from Puerto Rico. Journal of the Helminthological Society of Washington **58:** 176–180.

———, ———, and ———. 1995. *Angiodictyum mooreae* n. sp. (Digenea: Microscaphidiidae) and other digeneans from an Atlantic hawksbill turtle *Eretmochelys imbricata* from Puerto Rico. Journal of Aquatic Animal Health **7:** 38–41.

Gardner, S., and W. Nichols. 2001. Assessment of sea turtles mortality rates in the Bahía Magdalena region, Baja California Sur, México. Chelonian Conservation and Biology **4:** 197–199.

Glazebrook, J., R. Campbell, and D. Blair. 1981. Pathological changes associated with cardiovascular trematodes (Digenea: Spirorchiidae) in a green sea turtle *Chelonia mydas* (L). Journal of Comparative Pathology **9:** 361–368.

———, ———, and ———. 1989. Studies on cardiovascular fluke (Digenea: Spirorchiidae) infections in sea turtles from the Great Barrier Reef, Queensland, Australia. Journal of Comparative Pathology **101:** 231–250.

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. Balazs. 1995. Detection by ELISA of circulating anti-blood fluke (*Carettacola*, *Hapalotrema*, and *Learedius*) immunoglobulins in Hawaiian green turtles (*Chelonia mydas*). Journal of Parasitology **81:** 416–421.

Inohuye-Rivera, R. B., A. Cordero-Tapia, J. Arellano-Blanco, and S. C. Gardner. 2004. *Learedius learedi* Price, 1934 (Trematoda: Spirorchiidae) in black turtle (*Chelonia mydas agassizii*) hearts from Magdalena Bay, Baja California Sur, Mexico. Journal of Comparative Parasitology **71**: 37–41.

Kretschmer, R., G. Rico, and J. Giménez. 2000. Isolation and structural studies of the monocyte locomotion inhibitory factor (MLIF) produced by *Entamoeba histolytica*. Archives of Medical Research **31:** 76–77.

Lauckner, G. 1985. Diseases of reptilia. *In* Diseases of marine animals, O. Kinne (ed.). Biologische Anstalt Helgoland, Hamburg, Germany, p. 553–626.

Perez Ponce de León, G., L. García, and V. Leon. 1996. Gastrointestinal digenetic trematodes of Olive Ridley's turtle (*Lepidochelys olivacea*) from Oaxaca, México. Taxonomy and infracomunity structure. Journal of the Helminthological Society of Washington **63:** 76–82.

Prophet, E. 1992. American registry of pathology. Laboratory methods in histotechnology. Armed Forces Institute of Pathology, Washington, D.C., 275 p.

Raidal, S., M. Ohara, R. Hobbs, and R. Prince. 1998. Gram-negative bacterial infections and cardiovascular parasitism in green sea turtles (*Chelonia mydas*). Australian Veterinary Journal **76:** 415–417.

Rand, T. G., and M. Wiles. 1985. Histopathology of infections by *Learedius learedi* Price, 1934 and *Neospirorchis schistosomatoides* Price, 1934 (Digenea: Spirorchiidae) in wild green turtles, *Chelonia mydas* L., from Bermuda. Journal of Wildlife Diseases **21:** 461–463.

Work, T. 2000. Manual de necropsia de tortugas marinas para biólogos en refugios o áreas remotas. U.S. Geological Survey, National Wildlife Health Center, Hawaii Field Station, Honolulu, Hawaii, 25 p.