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Research Note

Helminths of hawksbill turtle (*Eretmochelys imbricata*) from the Pacific coast of Costa Rica

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Summary

Parasitological examination of a stranded hawksbill turtle (*Eretmochelys imbricata*) from Pacific coast of Costa Rica revealed the presence of a rich digenean fauna including *Carettacola stunkardi* (Spirorchiidae), *Enodiotrema reductum* (Plagiorchiidae), *Cricocephalus albus*, *Adenogaster serialis*, *Epibathra crassa*, *Pleurogonius lobatus*, *P. trigonocephalus*, *P. linearis*, and *Pyelosomum posterorchis* (Pronocephalidae). All helminths except *C. albus* and *P. lobatus* represent new geographical records for Costa Rica. *Carettacola stunkardi* is reported for first time in an Eastern Pacific hawksbill turtle and its pathological changes are here described. Histologically, nodular lesions on the serosal surface of intestine revealed a mixed infiltrate of heterophils, lymphocytes, and histiocytes within necrotic debris. Granulomas with spirorchiid eggs were observed in the mucosa, sub-mucosa and muscular layers of stomach and intestine, gallbladder and liver.
Keywords: hawksbill turtle; *Eretmochelys imbricata*; helminths; digeneans; spirorchiid eggs; Costa Rica

Introduction

The hawksbill turtle (*Eretmochelys imbricata*) is distributed in the tropical waters throughout much of Atlantic, Pacific and Indian Oceans, but it is uncommon from both Atlantic and Pacific Costa Rican coasts (Sasa *et al*., 2009). In the Pacific, it uses neritic habitats at >30 cm curved carapace length in close association with hard substrate communities such as coral reefs, where feed primarily on sponges (Jones & Seminoff, 2013).

The knowledge about the helminth fauna of hawksbill turtles includes at least 50 species of digeneans and a single larval cestode, most of which are reported from the Caribbean sea and the coasts of Atlantic and Asiatic regions (Oguro, 1936; Chattopadhyaya, 1972; Fischthal & Acholonu, 1976; Dyer *et al*., 1995; Werneck *et al*., 2008, 2014; Greiner, 2013). In contrast, few data exist from hawksbill turtle of the Eastern Pacific; to our knowledge only two helminth species including *Orchidasma amphiorchis* and *Pleurogonius linearis* have been reported from the Pacific coast of Mexico (Caballero & Zerecero, 1950). The present paper reports the rich digenean fauna from a juvenile hawksbill turtle stranded on the north Pacific coast of Costa Rica and the first record of *Carettacola stunkardi* (Spirorchiidae) in a Eastern Pacific hawksbill turtle. Pathology associated with *C. stunkardi* is also described in an hawksbill turtle for first time. This represents the first report on helminths of hawksbill turtle from Costa Rica.

Materials and Methods

On February 2013, an emaciated juvenile male of hawksbill turtle (curved carapace length of 46 cm) was found stranded by a local fisherman on San Juanillo beach, on the Nicoya peninsula (northern Pacific coast of Costa Rica). During post-mortem examination, the heart, trachea, lungs, kidneys, urinary bladder, spleen, liver, gallbladder, and the whole digestive tract including oesophagus, stomach and intestine were examined for helminths following the methods by Greiner *et al*. (1980). Digeneans were washed in saline solution, fixed in 70 % ethanol, stained with Mayer's acid carmine, and mounted in Canada balsam for identification using light microscopy. Tissue samples from all examined organs were fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 6 µm, and stained with haematoxylin and eosin. Finally, since most of helminths of sea turtles are transmitted through the trophic chain (Santoro & Mattiucci, 2009; Greiner, 2013), stomach contents were classified by stereomicroscope observation (Casale *et al*., 2008).

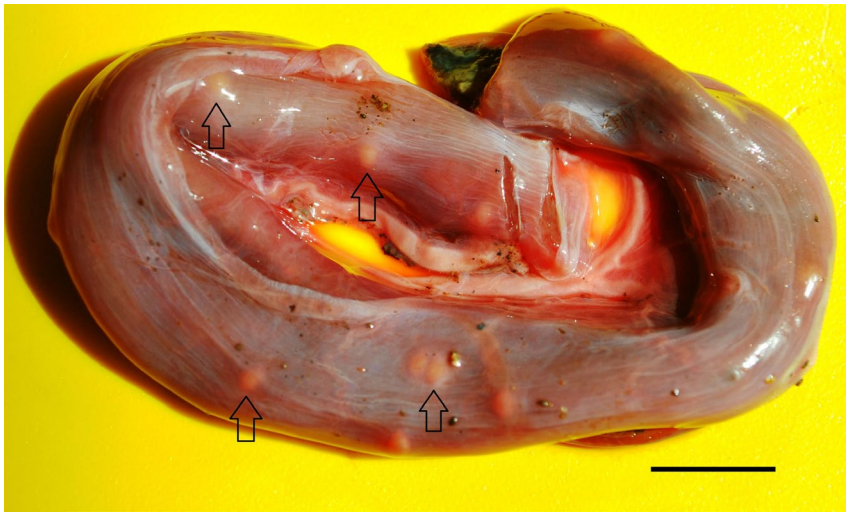

Fig. 1. Diffuse nodular lesions (black arrows) on the serosa of the intestine in the hawksbill turtle (*Eretmochelys imbricata*). Bar = 0.5 mm.

Results

A total of nine digenean species were found including five *Carettacola stunkardi* (Spirorchiidae) from the liver, 10 *Cricocephalus albus* (Pronocephalidae) from the stomach, 26 *Enodiotrema reductum* (Plagiorchiidae), five *Adenogaster serialis* (Pronocephalidae), 16 *Epibathra crassa* (Pronocephalidae), 22 *Pleurogonius lobatus* (Pronocephalidae), three *P. trigonocephalus* (Pronocephalidae), seven *P. linearis* (Pronocephalidae), and three *Pyelosomum posterorchis* (Pronocephalidae) all from the intestine. All digeneans except *C. albus* and *P. lobatus* represent new record for Costa Rica and Eastern Pacific. The only gastrointestinal contents seen were the green alga *Codium isabelae*.
The large intestine had numerous serosal yellow circular nodular lesions of approximately 0.1 mm in diameter with a caseous center throughout its distal portion (Fig. 1). Histopathological examination of intestinal nodules revealed a mixed infiltrate of heterophils, lymphocytes, and histiocytes within necrotic debris (Fig. 2). Granulomas with brown spirorchiid eggs bearing miracidia were observed in the mucosa, sub-mucosa and muscular layers of stomach and intestine (Fig. 2), gallbladder and liver (Fig. 3). Granulomas consisted of one or two eggs and were characterized by central necrosis with syncytia of giant cells and a lymphocytic infiltrate surrounded by reactive fibrosis. Eggs inside the granulomatous lesions were approximately in size 130 x 50 µm resembling those of *Carettacola* spp. (see Wolke *et al*., 1982).

Discussion

Studies on sea turtles suggest that ontogenetic and ecological factors influence strongly their helminth communities (Pérez Ponce de León *et al*., 1996; Santoro *et al*., 2006, 2010). Unfortunately, no information exists on helminth community of hawksbill turtle at both hierarchical levels of organization (the component community and infracommunity), and the known data is fragmented and based on occasional findings of stranded individuals (Oguro, 1936; Chattopadhyaya, 1972; Fischthal & Acholonu, 1976; Dyer *et al*., 1995; Werneck *et al*., 2008; Greiner, 2013).

Fig. 2. A section of a serosal intestinal nodule of fig. 1. The inflammatory infiltrate is composed of heterophils, lymphocytes, and histiocytes with necrosis of muscular layer. Two spirorchiid eggs (black arrows) are also visible. Haematoxylin and eosin; bar = 1 mm.

Fig. 3. Liver of hawksbill turtle (*Eretmochelys imbricata*). Granulomatous inflammatory reaction caused by a *Carettacola* egg. Haematoxylin and eosin. Bar = 50 µm.

Greiner (2013) suggested that the low number and diversity of digeneans found in hawksbill turtle from Florida (only six digeneans and a larval cestode species from four individual turtles with the number of helminth species ranging from 2 to 4) might reflect its specialized feeding habit (mainly sponges) which would limit the potential ingestion of mollusks serving as intermediate hosts for digeneans. The turtle in this study had ingested mainly algae that is unusual for this species and would indicate inability to forage effectively in its environment. It might explain the rich helminth fauna here found since sea turtles with vegetarian diet would ingest accidentally and more easily great amount of mollusk intermediate hosts whose live on marine pasture with respect to turtles with mainly spongivorous or carnivorous habits (Pérez Ponce de León *et al*., 1996; Santoro *et al*., 2006, 2010; Santoro & Mattiucci, 2009).

Infection by spirorchiid flukes represents the main parasitological health problem of sea turtles. Adult flukes live in the blood vessels of host where they copulate and oviposit disseminating eggs throughout internal organs against which turtle mounts a chronic inflammatory response (Work *et al*., 2005; Santoro *et al*., 2007; Santoro & Morales, 2007). When spirorchiids localize in the brain and spinal cord may cause a fatal neurological disorder (Jacobson *et al*., 2006).

A total of six spirorchiid species have been previously reported from sea turtles in Costa Rica including *Learedius learedi, Hapalotrema postorchis, Monticellius indicum, Amphiorchis solus* and *Neospirorchis* sp. In that study from the Caribbean green turtle, multiple tissues contained only two different type of eggs resembling *Hapalotrema* and *Learedius* spp. the first type, fusiform with large bipolar processes; and *Neospirorchis* sp. the second, smaller and round to slightly oval (Santoro *et al*., 2007). Histologically, the tissue most frequently infected by those eggs was the spleen (100 % for the first type, and 94 % for the second type eggs; Santoro *et al*., 2007). In contrast we found here, *Carettacola* eggs just from liver, gallbladder, stomach and intestine. Egg histopathological features here observed were overlapping with those observed in green turtles infected with spirorchiid flukes (Santoro *et al*., 2007) with the exception for distribution of their eggs. *Carettacola* eggs may be distinguished by the first two types above cited because those are ovoid (approximately bigger more than two times than those of *Neospirorchis* spp.) with a sharp short terminal process (see Wolke *et al*., 1982). *Carettacola stunkardi* represents the only blood fluke species known to data from the Pacific coast of Costa Rica. Other records of this latter fluke include green turtle from Baja California and Pacific coast of Panama (Martin & Bamberger, 1952; Caballero *et al*., 1955), and hawksbill turtle of Atlantic coast of Brazil (Werneck *et al*., 2008).

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