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Parasitic Ulcerous Caseous Gastroesophagitis Associated with Rameshwarotrema uterocrescens Rao, 1975 (Digenea: Pronocephalidae) in a Juvenile Green Turtle [Chelonia mydas, Linnaeus 1758 (Testudines: Cheloniidae)]: A Case Report

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Abstract:	Here we report a case of ulcerative caseous gastroesophagitis associated with Rameshwarotrema uterocrescens; Rao, 1975 (Digenea: Pronocephalidae) in a juvenile green turtle (Chelonia mydas) from Southern Brazil. Similar pathologies have been reported only in adult green turtles from Costa Rica. This paper presents the second report of parasitic esophagitis due R. uterocrescens and the first occurrence in juvenile green turtles along coastal Brazil.

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ABSTRACT: Here we report a case of ulcerative caseous gastroesophagitis associated with *Rameshwarotrema uterocrescens*; Rao, 1975 (Digenea: Pronocephalidae) in a juvenile green turtle (*Chelonia mydas*) from Southern Brazil. Similar pathologies have been reported only in adult green turtles from Costa Rica. This paper presents the second report of parasitic esophagitis due *R. uterocrescens* and the first occurrence in juvenile green turtles along coastal Brazil.

Esophageal injuries constitute important alterations to the digestive tract of sea turtles. Some injuries are caused by anthropogenic activities, such as perforations due to the ingestion of hooks or injuries caused by monofilament nylon lines (Orós et al., 2005). Other times, esophageal lesions are associated with bacterial agents (Glazebrook and Campbell, 1990; Torrent et al., 2002) or parasites, as Santoro et al. (2007) found in the esophagus of *Chelonia mydas* infected by *Rameshwarotrema uterocrescens*; Rao, 1975. The aim of this case report is to present the first report of macroscopic and microscopic findings of parasitic ulcerous caseous gastroesophagitis in a juvenile green sea turtle found in Southern Brazil.

In December 2015, a female juvenile green sea turtle (*C. mydas*) weighing 3.4 kg with 33 cm of curved carapace length and 31.4 cm of curved carapace width was found stranded on Cascalho beach, located in the municipal district of Penha (-26°46' 53.4"N, -48°36'6.4794"W) in the state of Santa Catarina, Brazil. The animal was housed in the rehabilitation facilities of Projeto Tamar, in the city of Florianópolis, located in the same state. On admission the turtle was weak and lethargic, with a poor body condition and exhibited signs of cachexia, dehydration, anemia (PCV 13%) and positive buoyancy. Initially it was maintained out of water, placed into a dry fiberglass container padded with foam, to prevent drowning. A light coating of vaseline ointment was applied all over the carapace, head and flippers to avoid dehydration and to prevent the skin from drying out. For volemic replacement, a 6% solution of hydroxyethylamide colloid (Istarhes) was administered intravenously (IV) at a dose of 15 ml/kg, through a catheter placed in the occipital sinus. Additionally, a 15% amino acid intravenous infusion (Aminoven®) (10 ml/kg, by continuous solution), ceftazidime (20 mg/kg every 72 hr), dexamethasone (1 mg/kg every 24 hr) and an intravenous lipid infusion (Lipovenos®) (2 ml/kg every 24 hr) were also administered during the treatment. Five days after arrival, the turtle was placed in a 500 L tank, half-filled with sea water, maintained at 26 C. Different food items were offered (e.g., fish, shrimp, and algae – *Hypnea musciformis*) however, the turtle didn't show any interest in food and little clinical improvement was observed.

Natural death occurred 10 days after initial supportive care. A complete necropsy was performed following a standardized protocol (according to Wyneken, 2001), revealing a caseous esophageal mass. Moreover, a massive thrombus in the right atrium and moderate pulmonary edema were found.

Five specimens of *Ruterocrescens* were found in the distal esophagus (in the gastric transition). The parasites were placed in 70% alcohol, stained with carmine and cleared with eugenol. Morphometric data were determined with the aid of a Nikon Eclipse 80i microscope (Kurobane Nikon Co., Ltd., Otawara, Tochigi, Japan) using the NIS Elements BR software program. Analyses of the parasites were authorized by federal licenses for activities with scientific purposes (SISBIO 30600-1 and 9329-1). The helminths were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC 38197) in the state of Rio de Janeiro, Brazil.

Tissue samples of the esophagus were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned (5 µm) and stained with hematoxylin and eosin (HE) for subsequent analysis using an optical microscope. For parasite identification and morphometric comparisons, the key to genera found in Blair (2005) and specific reports by Rao (1975) and Santoro et al. (2007) were used to confirm the findings.

The macroscopic lesion was formed by multifocal areas of friable, yellow-brownish ulcerated plaques measuring approximately 0.5 cm and 3 caseous masses measuring up to 1.5 cm associated with *R. uterocrescens*. Histologically, there was ulceration of the esophageal mucosa associated with a proliferative reaction of fibrous connective tissue (sclerosis) covered by abundant caseous material in which 4 specimens of *R. uterocrescens* replete with eggs were immersed (Fig. 1A). The caseous mass was strongly eosinophilic and formed by an intense response of heterophils, fibrin, cell debris and bacterial coccus colonies with different sizes. Additionally, 2 parasites were in the internal circular muscle layer and one was between the internal muscle layer and the external longitudinal muscle layer. These parasites were ruptured, with macrophages, lymphocytes, plasma cells, heterophils

inflammatory infiltrate (Fig. 1B) and multinucleated foreign body giant cells surrounding eggs (Fig. 1C).

The morphometric findings of the 5 parasites found in the esophagus are expressed in µm, with mean and ± standard deviation values (minimum and maximum values)]: total length: 1,288 ± 184 (1,095-1,583) µm; total width: 523 ± 60 (448-610) µm; length of oral sucker: 98 ± 14 (81-111) µm; width of oral sucker: 85 ± 24 (52-104) µm; ovary length: 69 ± 11 (56-81) µm; ovary width: 73 ± 13 (59-90) µm; right vitelline fields: 203 ± 32 (158-232) µm by 94 ± 14 (81-112) µm; left vitelline fields: 185 ± 36 (145-232) µm by 89 ± 20 (74-113) µm; length of right testicle: 165 ± 24 (129-194) µm; width of right testicle: 143 ± 19 (127-173) µm; length of left testicle: 178 ± 38 (147-235) µm; width of left testicle: 161 ± 15 (147-182) µm; length of eggs: 28 ± 3 (23-34) µm; and width of eggs: 14 ± 2 (10-19) µm.

Similar lesions are described by Santoro et al. (2007), who found multifocal green to yellow plaques on the mucosal surface of the distal esophagus that ranged in diameter from approximately 0.1 to 0.5 cm. Through the plaques, the lesions extended into the submucosa as cystic spaces, which contained yellow caseous material and were lined by a distinct wall of connective tissue. Histologically, Santoro et al. (2007) found that the cystic spaces grossly corresponded to ectatic, inflamed esophageal glands and trematodes consistent with species of *Rameshwarotrema* were found within the lumen of the glands. In all cases, these trematodes were associated with an inflammatory infiltrate composed of heterophils, lymphocytes, macrophages and a few plasma cells. In the present report, some parasites were found immersed in a caseous mass and others in muscle layer of the esophagus showing that *R. uterocrescens* get access in deep wall layers of esophagus, different from that reported by Santoro et al. (2007). The inflammatory infiltrate consisted of heterophils,

macrophages and giant cells were only found surrounding ruptured parasites with the exposure of eggs.

The caseous material, ulceration, sclerosis, fibrin, cell debris and bacterial colonies were indicative of a chronic process. Likewise, Santoro et al. (2007) described chronic lesions characterized by the accumulation of necrotic material, degenerated heterophils and cell debris, which dilated the glands and were often colonized by Gram-positive cocci. The bacterial colonies in this case were considered a secondary infection to the lesion associated with the parasites, as the bacteria were only found superficially, which is in agreement with data reported by Santoro et al. (2007).

Rao (1975) erected the genus *Rameswarotrema* (type species *R. uterocrescens*; Rao, 1975) found in the intestine of *C. mydas* from the Gulf of Manar, in India. Only one other species, denominated *R. chelonii*; Rao, 1975, belongs to the genus and was found in the intestine of the same host in the same location. Dyer et al. (1995) subsequently analyzed 2 individuals of *Eretmochelys imbricata* from Puerto Rico and identified 9 specimens of *R. uterocrescens* in the intestinal mucosa. This was the second report of the parasite and the first in this host. Analyzing 47 adult *C. mydas* females from Costa Rica, Santoro et al. (2006) found *R. uterocrescens* in 10%, with a mean intensity of 5.7 ± 1.9 (range: 3 to 7) in the esophagus.

According to Rao (1975), *R. uterocrescens* differs from *R. chelonii* by having a less evident cephalic collar and a less spiraled shape of the external seminal vesicle. Unfortunately, the author did not indicate the institution where the type species was deposited, which hinders a better taxonomic analysis. The specimens described herein most closely resemble *R. uteroscrescens* because of the less evident cephalic collar and all morphomertic data collected in this study conform to Rao (1975) except for a

longer and wider body.

This paper reports esophageal lesions stemming from parasitism by *R. uterocresccens* in a juvenile green sea turtle found on the southern coast of Brazil and contributes to a better understanding of parasitosis in sea turtles in the region as well as the impact on these hosts.

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Figure 1. (**A**) *R. uterocrescens* immersed in caseous formed by degenerate heterophils, fibrin and cell debris. (**B**) Ruptured parasite (arrow) associated with caseous (*) and inflammatory infiltrate composed of macrophages, heterophils, lymphocytes and plasma cells. (**C**) Multinucleated foreign body giant cells (arrows) invading parasite and surrounding eggs.

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Figure

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