



Pathology of Spirorchiidae (Digenea: Schistosomatoidea) infection in green turtles (*Chelonia mydas*) on Campos Basin, Rio de Janeiro¹

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ABSTRACT.- Cartagena R.I.A., Ikeda J.M.P., Neto I.M., Pereira G.O., Pereira A.H.B., Simões R.O., Luque J.L. & Ubiali D.G. 2023. **Pathology of Spirorchiidae (Digenea: Schistosomatoidea) infection in green turtles (*Chelonia mydas*) on Campos Basin, Rio de Janeiro.** *Pesquisa Veterinária Brasileira* 43:e07235, 2023. Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: danielubiali@ufrj.br

Spirorchidiasis is one of the most common diseases in green turtles. Eggs and the adult form of trematodes are mainly found in the heart, liver, lung, spleen, and gastrointestinal tract. The present study aimed to describe the pathological aspects of Spirorchiidae trematode infection in green turtles (*Chelonia mydas*) in the Campos Basin, Rio de Janeiro. The current study was based on the necropsy of 99 green turtles (*Chelonia mydas*, Chelonidae) from April to December 2021. Epidemiological data were obtained from the Aquatic Biota Monitoring Information System (SIMBA). A histopathological examination was performed at the “Setor de Anatomia Patológica” (SAP) from “Universidade Federal Rural do Rio de Janeiro” (UFRuralRJ). Tissue fragments (buccal papilla, esophagus, trachea, lung, heart, kidney, bladder, stomach, liver, spleen, intestines, gonads, adrenal, thyroid, pancreas, salt glands, muscle, and skin) were sampled for histological examination. The green turtles had lesions compatible with parasitic infection in 89% (88/99) cases. Out of 88 turtles, 87 were juveniles (99%), 75% (66/88) were females, and 25% (22/88) were males. Adult parasites and their eggs were found to cause granulomatous tissue reactions in 14% (12/88) of turtles. Egg-associated granulomatous inflammation was found in 86% (76/88) of the turtles. The organs with the highest number of egg-associated granulomas in order of occurrence were: the spleen, small intestine, adrenal gland, pancreas, and lungs. The adult trematodes were observed in histological lesions in order of occurrence, mainly in the esophagus, stomach, and small intestine. Out of the total number of turtles parasitized, 33% (29/88) of deaths occurred due to multiple causes such as fractures, interaction with fishing, predation, and fibropapillomas. The other 67% (59/88) presented multisystemic granulomatous disease caused by Spirorchiidae infection as a cause of death. Evidence is presented that spirorchidiasis is highly prevalent in young green turtles, is responsible for extensive chronic lesions, and responsible for significant debilitation and mortality.

INDEX TERMS: Marine biology, veterinary pathology, Spirorchiidae, Testudines, green turtle, *Chelonia mydas*, blood flukes, trematodes, histopathology.

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RESUMO.- [Patologia da infecção por Spirorchiidae (Digenea: Schistosomatoidea) em tartarugas verdes (*Chelonia mydas*) na Bacia de Campos, Rio de Janeiro.] A espiroquidíase é uma das doenças mais comuns em tartarugas verdes. Os ovos e a forma adulta dos trematódeos são encontrados principalmente no coração, fígado, pulmão, baço e trato gastrointestinal. O objetivo do presente estudo foi descrever os aspectos patológicos da infecção por trematódeos Spirorchiidae em tartarugas verdes (*Chelonia mydas*) na Bacia de Campos, Rio de Janeiro. O presente trabalho foi baseado na necropsia de 99 tartarugas verdes (*Chelonia mydas*, Cheloniidae) no período de abril a dezembro de 2021. Os dados epidemiológicos foram obtidos do Sistema de Informação de Monitoramento da Biota Aquática (SIMBA). Realizou-se exame histopatológico no Setor de anatomia Patológica (SAP) da Universidade Federal Rural do Rio de Janeiro (UFRuralRJ). Os fragmentos de tecidos (papila bucal, esôfago, traqueia, pulmão, coração, rim, bexiga, estômago, fígado, baço, intestinos, gônadas, adrenal, tireoides, pâncreas, glândulas de sal, músculo e pele) foram coletados e fixados em formalina a 10% tamponada, processados rotineiramente para exame histológico. Em 89% (88/99) dos casos, as tartarugas-verdes apresentaram lesões compatíveis com infecção parasitária. Das 88 tartarugas, 87 eram jovens (99%), 75% (66/88) correspondiam a fêmeas, 25% (22/88) a machos. Em 14% (12/88) das tartarugas foram encontrados parasitas adultos e seus ovos causando inflamação granulomatosa. Em 86,4% (76/88) das tartarugas foram encontradas lesões granulomatosas associadas aos ovos dos trematódeos. Os órgãos com maior quantidade de granulomas associados a ovos foram: baço, intestino delgado, adrenal, pâncreas e pulmões. Trematódeos adultos foram principalmente observados nas lesões histológicas, em ordem de ocorrência, no esôfago, estômago e intestino delgado. Do total de tartarugas parasitadas, 33% (29/88) das mortes ocorreram em consequência de múltiplas causas como fraturas, interação com pesca, predação e fibropapilomas. As outras 67% (59/88) apresentaram como causa de morte a doença granulomatosa multissistêmica causada pela infecção por trematódeo da família Spirorchiidae. São apresentadas evidências de que a espiroquidíase é altamente prevalente em tartarugas verdes jovens no Rio de Janeiro, responsável por extensas lesões crônicas, quadro clínico de debilidade e mortalidade significativas.

TERMOS DE INDEXAÇÃO: Biologia marinha, patologia veterinária, Spirorchiidae, Testudines, tartaruga verde, *Chelonia mydas*, parasitas intravasculares, trematódeos, histopatologia.

INTRODUCTION

Five sea turtles species occur in Brazil, out of seven that inhabit the world. They are green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), loggerhead turtle (*Caretta caretta*), olive turtle (*Lepidochelys olivacea*), and leather turtle (*Dermochelys coriacea*) (Cremer et al. 2020). In Brazil, all sea turtle species are included in the Red Book of the Brazilian Fauna Threatened by Extinction (Brasil 2018). According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, *C. mydas* is endangered (Seminoff 2004). *Chelonia mydas* has a wide distribution, encompassing tropical and occasionally subtropical regions in the Atlantic, Pacific, Indian Oceans and the Mediterranean

and Red Seas (Pritchard & Mortimer 1999). In the Brazilian coastal areas, the green turtle can be found in Rio Grande do Norte, Pernambuco, Espírito Santo, and Rio de Janeiro states (Marcovaldi & Marcovaldi 1999, Awabdi et al. 2013).

Sea turtles play an essential role in the cycle of energy and nutrients in different environments, controlling the population of species on which they feed and, when young, as a source of food for crustaceans, birds, fish, and mammals (Bjorndal 1996). Young green turtles are carnivorous; when they are adults, they become herbivores and feed on algae. After migratory movements of adult *C. mydas*, hundreds to thousands of kilometers are crossed into international waters (Cubas & Baptistotte 2006). The feeding habits influence the composition and richness of parasite communities in different geographic areas (Santoro et al. 2006). Various parasitic agents, mainly nematodes, digenetic trematodes, and ectoparasites, affect sea turtles (George 1997). In Rio de Janeiro, endoparasitic infections are one of the leading natural causes of green turtle stranding (Tagliolatto et al. 2019). Spirorchidiasis cases have been reported in different world regions, particularly the Northwest and Central Atlantic Ocean (Werneck et al. 2008, Chapman et al. 2017), Indian Ocean, and Northwest Pacific Ocean (Glazebrook et al. 1989, Santoro et al. 2007) and Mediterranean Sea (Marchiori et al. 2017).

Spirorchids are trematodes that cause parasitosis and affect primarily the heart or visceral blood vessels (Glazebrook et al. 1989, Aguirre et al. 1998). The reported lesions appear similar in the four sea turtles species parasited by spirorchids (green, loggerhead, hawksbill, and olive) for which pathological data were described. The aorta and heart are common organs for adult parasites (Stacy et al. 2010, Chen et al. 2012, Chapman et al. 2017). Herein, we describe the pathological findings of infection by trematodes Spirorchiidae in a stranded sampling of green turtles submitted to necropsy and histopathology.

MATERIALS AND METHODS

The present work was based on the necropsy of 99 green turtles *Chelonia mydas*, Cheloniidae (Linnaeus, 1758) from April to December 2021. The clinical-epidemiological history, sex, age, and origin were obtained from the “Sistema de Informação de Monitoramento da Biota Aquática” (Aquatic Biota Monitoring Information System - SIMBA) and the responsible veterinarians. The green turtles were found stranded on the beaches of the municipalities of São Francisco de Itabapoana (N=22), Arraial do Cabo (N=20), Armação dos Búzios (N=18), Cabo Frio (N=16), Rio das Ostras (N=6), Macaé (N=4), Araruama (N=1) and Casimiro de Abreu (N=1) (Fig.1).

A histopathological examination was performed at the “Setor de Anatomia Patológica” (SAP) from “Universidade Federal Rural do Rio de Janeiro” (UFRuralRJ). The tissue fragments (buccal papilla, esophagus, trachea, lung, heart, kidney, bladder, stomach, liver, spleen, intestines, gonads, adrenal, thyroid, pancreas, salt glands, muscle, and skin) from the necropsies were fixed in 10% buffered formalin, processed routinely for histological examination, cut into 4µm sections and the histological slides were stained with hematoxylin and eosin (HE). Tissue sections with specific lesions were subjected to Masson Trichrome staining. Slides were examined by light microscopy.

The diagnosis of parasitic infection by Spirorchiidae trematodes in this population of green turtles (*Chelonia mydas*) from the Brazilian Southeast coast was based on the histopathological aspects of lesions containing adult parasites and eggs. All cases with lesions

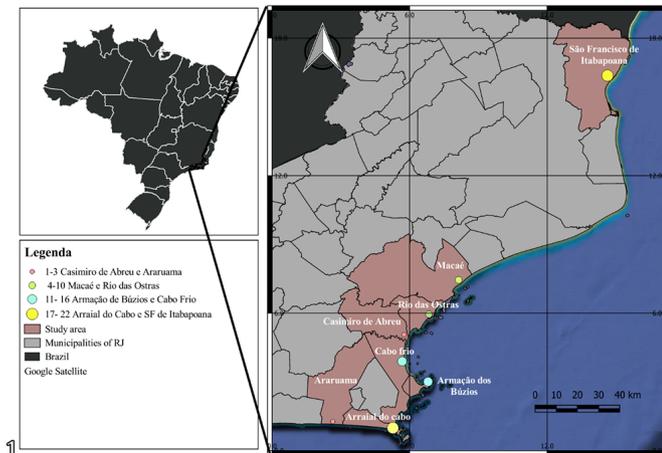


Fig.1. Geographic sampling distribution from 88 stranded green turtles (*Chelonia mydas*) with spirorchidiasis in the Campos Basin, Rio de Janeiro, Brazil.

compatible with parasitic infection by trematodes were selected, and the lesions were described. To establish the Spirorchiidae infection were considered the eggs and adult trematodes morphology (Werneck et al. 2008, Werneck & Silva 2015, Flint et al. 2010). Based on the lesion morphology, the evolution acute were designed in cases with heterophils predominance surrounding eggs, subacute were designed in cases with lymphocyte predominance, and chronic lesions were the cases with almost one multinucleated giant cell (Innis et al. 2009).

RESULTS

Necropsy and histopathological examination of 99 green turtles resulted in 88 cases of parasitic fluke infection, of which 83% (73/88) were found dead, and 17% (15/88) were found alive and died during rehabilitation. Regarding age, 99% were young (87/88), and an adult 1% (1/88). Regarding sex, 75% (66/88) corresponded to females, and 25% (22/88) to males. The clinical picture of these 15 turtles was characterized by a skin load of epibionts and algae, weakness, low responsiveness to stimuli, emaciation, and pale mucous membranes.

Gross pathological changes varied from no apparent lesions in turtles slightly affected to severe cachexia. Nonspecific findings were characterized by changes meaning cachexia and anemia (excess of clear pericardial or peritoneal fluid, diffuse visceral pallor, absent fat overlying the viscera, or reduced jelly fat). The specific necropsy hallmarks of spirorchiid fluke infection were the thickening and hardening of arterial walls and thrombosis. Multiple, 0.2-2cm diameter yellow, irregularly demarcated, and sometimes gritty granulomas were observed scattered in the spleen, adrenal, lungs, and heart. Multiple 0.2-0.5cm diameter black nodules were observed at the intestinal serosa in 63% (55/88) turtles.

In histology, were observed acute 1.1% (1/88), subacute 8% (7/88), and chronic 91% (80/88) inflammatory reactions from multiple tissues (Table 1). In the 88 turtles, trematode eggs were morphologically characterized by oval or elliptical structures, the average size of 20x60µm, refracting yellow wall and often fragmented, with an average of 2-3µm in thickness, containing round and basophilic round aggregates (miracids). Adult trematodes presented morphology containing oral sucker, eosinophilic external integument, somatic musculature,

Table 1. Age, sex, and inflammatory reactions in green turtles diagnosed with spirorchidiasis in the "Setor de Anatomia Patológica" (SAP) of UFRuralRJ

Age	Sex	Inflammatory reactions
99% (87/88) Young	75% (66/88) Females	1% (1/88) Acute
1% (1/88) Adult	25% (22/88) Males	8% (7/88) Sub-acute
		91% (80/88) Chronic

parenchymal matrix, vitellaria, and, sometimes, a uterus eggfully compatible with trematodes of the Spirorchiidae family.

Adult parasites and their eggs were found in 14% (12/88) of the turtles. Tissue with granulomatous reaction eggs-associated was observed in 86% (76/88) of *Chelonia mydas* studied. Trematode egg granulomas (Fig.2-3) and lesions associated with adult trematodes (Fig.4-5) were the most common findings. Spirorchiidae parasite caused egg-associated severe granulomatous lesions in the buccal papilla, esophagus, trachea, lung, heart, kidney, bladder, stomach, liver, spleen, intestines, adrenal gland, thyroid, and pancreas. The eggs were located in the lumen of veins and arteries or the interstitium, often within multinucleated giant cells, surrounded by lymphohistiocytic infiltrate, fibrin, and necrosis. Most granulomas comprised five to 15 eggs, but lesions with over 120 eggs were observed in only one multinucleated giant cell in a histological section.

The organs most affected by granulomatous lesions egg-associated were: the spleen 81% (71/88), the small intestine 72% (63/88), the adrenal gland 70% (62/88), and the pancreas 68% (60/88). The adult trematodes were observed mainly in histological lesions, in order of occurrence in the esophagus, stomach, and small intestine (Table 2). The lesions associated with adult parasites were necrotizing granulomatous vasculitis with lymphohistiocytic inflammatory infiltrate, few neutrophils and eosinophils, and moderate to severe multinucleated giant cells, and large amounts of fibrin. Masson Trichrome staining revealed granulomas had a fine collagenous connective tissue capsule. We observed that 67% (59/88) presented granulomatous lesions only associated with trematode from the family Spirorchiidae. The other 33% (29/88) parasitized turtles showed additional lesions: 15% (13/88) cases of fractures, 9% (8/88) cases of interaction with fishing, 7% (6/88) lesions compatible with predation, and 2% (2/88) cutaneous fibropapillomas.

DISCUSSION

The diagnosis of parasitic infection by Spirorchiidae trematodes in this stranded sampling of green turtles (*Chelonia mydas*) from the Brazilian Southeast coast was based on the histological aspects of lesions containing adult parasites and eggs (Wolke et al. 1982, Gordon et al. 1998, Cordero-Tapia et al. 2004, Werneck et al. 2008, Flint et al. 2010, Werneck & Silva 2015). Similar to our pathological findings, Jerdy et al. (2023) found serious egg granulomas by fatal spirorchidiasis in 16 wild green turtles found stranded in South and Southeast Brazil.

We noticed a predominance of diagnoses of spirorchiids causing lesions in females (75%) and young (99%) *C. mydas*. This was expected since, in the juvenile phase, green turtles tend to remain in a neritic environment, extending from the coast to 200 meters from the marine region (Reis & Goldberg

2017). The same occurred for the number of females; since males, mainly adults, do not usually stay close to the coast due to reproductive behavior (Brasil 2018). The Campos Basin is located from the north coast of Rio de Janeiro to the south of Espírito Santo. Of 40 green sea turtles (*Chelonia mydas*) investigated with parasitic infections at Tortuguero National Park, Costa Rica, 100% were adult females (Santoro et al. 2006).

According to Esch et al. (1990), the average density of parasites is higher in the first years of the life of turtles and tends to decrease in the adult stage. This age group is related to the change in feeding habits, where juveniles are omnivorous, and adults are especially herbivores. All cases evaluated in this study presented parasites with marked granulomatous inflammatory reactions in the parasitized organs. For Greiner

& Mader (2006), knowledge of turtle parasites is scarce, and often, it is not possible to know if the parasites are the causative agents of diseases (pathogenic agents) or if they live peacefully in the host without underlying disease. Werneck & Silva (2015) reported that geographic distribution, ocean currents, and possibly phylogenetic history could influence the sea parasite community.

In the present study, 67% of the turtles had only injuries caused by the trematodes, while 33% had other injuries that contributed to the death. Flint et al. (2010) described other causes of death in green turtles, such as gastrointestinal impactions (11.8%), human actions such as asphyxiation by gillnets in fishing nets (7.2%), trauma (5.2%), fungal and bacterial infections (5.2%) and coccidial enteritis by *Caryospora cheloniae* (1.2%). To investigate the pathogenicity of spirorchids,

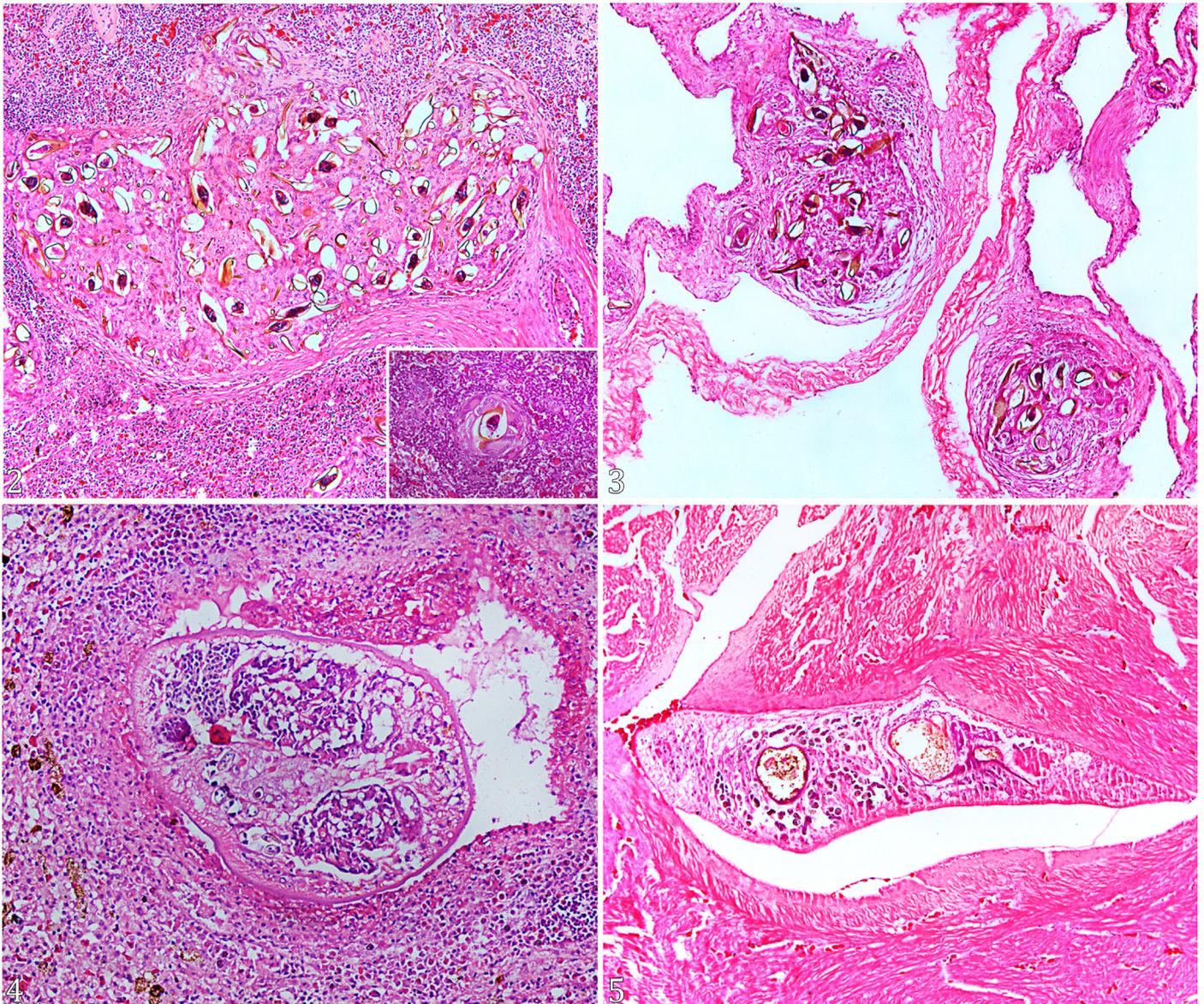


Fig.2-5. Spirorchidae infection in green turtle (*Chelonia mydas*) (2) Eggs of trematodes Spirorchidae are characterized as oval structures with a refracting yellow wall and often fragmented, containing basophilic, round punctate aggregates (miracidia) in the spleen of the green turtle. Insertion demonstrates eggs within giant cells. HE, obj.20x. (3) Trematode eggs in the lung of *C. mydas*. HE, obj.20x. (4) Adult trematode dissecting sinusoids in *C. mydas* liver. HE, obj.20x. (5) Adult trematode dissecting myocardial fibers in the heart of *C. mydas*. HE, obj.20x.

appropriate necropsy, bacteriological, parasitological, and histological examinations should be considered in sea turtles diagnoses (Chapman 2019). Santoro et al. (2007) concluded that the injuries caused by spirorchids had little impact on the health of the adult turtles, and such injuries could only affect the juvenile turtle community, contributing to their death, as young turtles do not have good acquired immunity. Innis et al. (2009) described the severity of lesions diagnosed in 28 tortoises (*Lepidochelys kempii*) from Massachusetts. They pointed out that mild lesions caused by parasites alone were insufficient to cause the turtles' death, suggesting additional factors, such as metabolic injuries, hypothermia, and drowning. On the one hand, the inference of the trematode's clinical importance was based on the pronounced multisystemic lesions and emaciation in this study. On the other hand, because the free-ranging turtles were naturally infected, it was not possible to determine the clinical evolution or make inferences regarding the causes of death. This study provides insight into the variety of pathologic findings that may be found in stranded green turtles. The results provide relevant data that may contribute to rehabilitation wildlife clinics.

In sea turtles, the definitive diagnosis of spirorchidiasis in tissues occurs through histopathology (Wolke et al. 1982, Gordon et al. 1998, Jerdy et al. 2023). In this study, 89% of the necropsied turtles presented parasitosis by trematodes morphologically compatible with the Spirorchidae family. Werneck & Silva (2015) found a prevalence of 50.7% in *C. mydas* parasitized by Spirorchidae trematodes in Ubatuba, on the coast of São Paulo. Binoti et al. (2016) reported the occurrence of trematode eggs mainly in the spleen and lungs and sometimes within multinucleated giant cells by histopathological examination. In the occasional sampling of this work (turtles found stranded or dead), it was also possible to observe that the lesions caused by the adult trematodes and their eggs are systemic, granulomatous, containing numerous multinucleated giant cells. Similar findings were observed by Santoro et al. (2007) in a study carried out with 47 adult green turtles in Costa Rica. They found that lesions associated with Spirorchidae parasites contained acute and

chronic inflammation, giant cells, and lymphocytic infiltrate surrounded by fibrosis.

The life cycle of spirorchid species in sea turtles has not been completely elucidated, but it has already been described in freshwater hosts. Adults infect blood vessels, and macroscopically they can be observed in the cardiac chambers and distal aorta (Flint et al. 2010). According to Glazebrook et al. (1989), the lesions develop due to eggs in the blood circulation of several organs, generating a marked inflammatory response, most of the time, granulomatous with multinucleated giant cells, as observed in this study. Granuloma formation responds to infection by various etiologic agents, including bacteria, parasites, and fungi (Montali 1988). In this population sampled by necropsy, granulomas with eggs or adult trematodes were evidenced mainly in the esophagus, stomach, small intestine, spleen, and lung of the studied individuals. Infection with these trematodes can favor the development of secondary bacterial diseases (Wolke et al. 1982, Gordon et al. 1998, Cordero-Tapia et al. 2004). Raidal et al. (1998) isolated *E. coli*, *Salmonella* spp., *Citrobacter freundii*, and *Moraxella* spp. from wild green turtles with clinical signs of spirorchid cardiovascular flukes infection.

In addition, Binoti et al. (2016) observed that entanglements caused by fishing nets are the most significant cause of death in green turtles, followed by parasitosis on the coast of Espírito Santo. The histological lesions associated with these parasites and their eggs include granulomatous mural endocarditis, hepatitis, cystitis, myositis, enteritis, splenitis, nephritis, thrombosis, and infarctions (Flint et al. 2010). The histological lesions of the parasites in turtles in the present study showed that the distribution of eggs between the organs was similar. However, the esophagus, stomach, spleen, and small intestine were the organs that presented the highest amount of adult parasites and their eggs. Studies are needed to understand the importance of spirorchidiasis on the coast of Brazil compared to other causes of turtle mortality.

CONCLUSIONS

We observed a high frequency of spirorchid infections in green turtles (*Chelonia mydas*) on the coast of Rio de Janeiro from May to December 2021.

Granulomatous lesions, mainly in the spleen and lungs, were observed. Spirorchid eggs were found in the lesions of the majority of turtles. The frequency of finding adult parasites and their eggs was six times lesser than the finding of only eggs.

Injuries caused only by the trematodes occurred in two-thirds of the cases of this necropsy sampling, and one-third had other injuries that contributed to the death. Further studies are needed on the biological cycle and the pathogenesis of trematodes from the Spirorchidae family in sea turtles.

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Conflict of interest statement.- The authors declare no conflicts of interest.

Table 2. Anatomical distribution of adult parasites and eggs in *Chelonia mydas* inflammatory lesions

Organ	Eggs %	Adults %
Spleen	81	1.3
Small intestine	63	3.2
Adrenal	62	-
Pancreas	60	-
Lung	59	1.3
Stomach	55	8
kidney	53	1.4
Bladder	53	-
Heart	47	1.2
Large intestine	43	-
Thyroid	42	-
Buccal papilla	35	-
Liver	34	1.3
Esophagus	24	12
Trachea	5	-

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