# Spirorchiidiosis (Digenea: Spirorchiidae) and lesions associated with parasites in Caribbean green turtles (*Chelonia mydas*)

M. SANTORO, J. A. MORALES, B. RODRÍGUEZ-ORTÍZ

Forty-seven nesting green turtles (*Chelonia mydas*) were examined for parasitic pathogens. Four species of cardiovascular flukes (Digenea: Spirorchiidae), *Learedius learedi, Hapalotrema postorchis, Monticellius indicum* and *Amphiorchis solus* were collected from 39 of 40 of the turtles, and *Neospirorchis* species were identified in seven of the 47 by histological examination. The pathological changes associated with the spirorchiids and their eggs included aneurysms, arteritis of great vessels with dark nodular thickenings, endocarditis, haemorrhagic lesions, thrombi, vasculitis, and granulomatous reactions in all the turtles. *Ozobranchus branchiatus* (Hirudinea: Ozobranchidae) leeches were found on the skin of 27 of the 47 turtles and were associated with traumatic purulent and ulcerative dermatitis. Oesophagitis associated with *Rameshwarotrema uterocrescens* (Digenea: Pronocephalidae) was recorded in 11 of 43 of the turtles. Petechial haemorrhages and unspecific crater-like ulcerated lesions on the gastric mucosa associated with *Charaxicephaloides* species and *Charaxicephalus robustus* (Digenea: Pronocephalidae) were observed in four of 40 of the turtles. Cholycystitis and ectasia of mucosal glands associated with *Rhytidodoides similis* (Digenea: Rhytidodidae) were observed in one of the 47 turtles. Fibropapillomas were observed on the skin of one turtle and fibromas on the skin of two of them.

THE green turtle (Chelonia mydas) is a herbivorous reptile, distributed in the tropical and subtropical waters of the Atlantic, Pacific and Indian oceans (Plotkin 2003). During the past 30 years there has been an increase in the prevalence of epidemic diseases among several sea turtle populations. Infections by cardiovascular flukes (Digenea: Spirorchiidae) and fibropapillomatosis, a neoplastic disease associated with a herpesvirus, have been reported as the most common causes of the stranding of green turtles worldwide (Rand and Wiles 1985, Jacobson and others 1989, Williams and others 1994, Aguirre and others 1998, 2002, Gordon and others 1998, Raidal and others 1998, Work and others 2004, 2005). However, there is little information about the pathological findings in freeranging Caribbean green turtles, and, to the authors' knowledge, there has been only one report of an epizootic of fibropapillomas in stranded turtles (Williams and others 1994).

Tortuguero National Park on the northern Caribbean coast of Costa Rica is the most important nesting ground for green turtles of the Atlantic Ocean (Troëng and Rankin 2005). This paper describes the prevalence and morphology of the lesions caused by spirorchiid trematodes and other parasites in nesting green turtles at the park.

### **MATERIALS AND METHODS**

Between June and October of 2003 and 2004, 47 nesting green turtles were found dead on the beach and examined postmortem by the methods described by Wolke and George (1981). The turtles' curved carapace length (CCL) was measured and tissue samples were collected from the heart, great vessels, lungs, oesophagus, stomach, intestine, spleen, liver, gall bladder, kidneys, urinary bladder, oviduct, skin and skeletal muscle; the oesophagus and stomach could not be collected from four of them. The tissue samples were fixed in 10 per cent neutral buffered formalin, embedded in paraffin and sectioned at 6  $\mu$ m. The sections were stained with haematoxylin and eosin, and Gram's, Ziehl-Neelsen and periodic acid-Schiff stains were applied to selected tissue samples to detect bacteria or fungi. The eggs of cardiovascular flukes from the tissue samples were recorded as type 1 (eggs with bipolar processes)

or type 3 (ellipsoid eggs without polar processes) (Wolke and others 1982, Work and others 2005). Tumours were classified as fibropapillomas or fibromas by using the criteria of Jacobson and others (1989) and Aguirre and others (2002).

Organs from 40 of the turtles were carefully examined for helminths by the methods described by Greiner and others (1980). Flukes were collected alive, placed in tap water and refrigerated over night, fixed in alcohol-formalin-acetic acid solution or 70 per cent ethanol, stained with Mayer's acid carmine and mounted in Canada balsam for identification by comparison with available references and voucher specimens. The parasites were archived in the Colección Helmintológica de Costa Rica (accession numbers CHCR 168/283), Universidad de Costa Rica, and the Harold W. Manter Laboratory of Parasitology (accession numbers P-2005-797 and P-2005-800), Nebraska State Museum, Lincoln, USA. Whole mounts and histological sections were studied by light microscopy. The prevalence and mean intensity of the infections were determined according to the definitions of Bush and others (1997). The intensity of infection and the dimensions of eggs and flukes are reported as the mean (sd) and range.

#### **RESULTS**

All 47 green turtles were freshly dead gravid females with a CCL ranging from 92 to 111 cm. There was a wound on the dorsal surface of the neck of all the turtles that died from severe blood loss and/or fractures of the cervical vertebrae, a wound attributed to attacks by jaguars (*Panthera onca*) during the night while the turtles were coming ashore to nest. Jaguar tracks were always observed close to the turtles. The body condition of all the turtles was good, on the basis of an external physical examination and the presence of abundant adipose reserves in the body cavity.

#### **Spirorchild infection**

**Macroscopic findings** A total of 1192 spirorchiids were found in 39 of the 40 turtles examined; there were 1024 *Learedius learedi*, 159 *Hapalotrema postorchis*, eight *Monticellius indicum*, and one *Amphiorchis solus*. *L learedi* was found in all 39 of the turtles, with a mean intensity of 26·2 (21·5) (range

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M. Santoro, DVM, PhD,

J. A. Morales, DVM, PhD,

Departamento de
Patología, Escuela de
Medicina Veterinaria,
Universidad Nacional,
PO Box 86, Heredia 3000,
Costa Rica
B. Rodríguez-Ortíz, PhD,
Laboratorio de
Helminthología, Facultad
de Microbiología,
Universidad de Costa
Rica, San José, Costa Rica

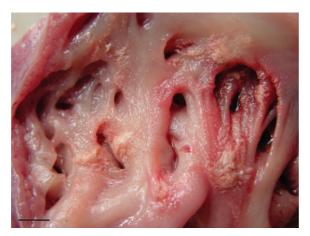


FIG 1: Mural endocarditis in the atrial endocardium of a green turtle (*Chelonia mydas*) affected by spirorchiid infection.

Bar=5 mm

three to 78). The flukes were attached to the inner surface or free within the cardiac chambers of 39 of the 40 turtles and in the great vessels of four of them (Fig 1). Three specimens of *L learedi* were also recorded from the intestinal lumen and a single fluke each from the lumen of the oesophagus, the liver and the gall bladder. *H postorchis* was found in eight of the turtles, with a mean intensity of 19-9 (45-4) (range one to 132); they were attached strongly to the lumen of the cardiac chambers of two of the turtles and in the great vessels of all eight. *M indicum* was found exclusively in the cardiac chambers of five of the turtles, with a mean intensity of 1-6 (0-6) (range one to two). A single specimen of *A solus* was collected from the intestine.

L learedi was associated with mural necrotising endocarditis in 36 turtles (Fig 2), which was most evident on the atrial endocardium and consisted of irregular creamto brown-coloured foci, approximately 2 cm in diameter. H postorchis was associated with aneurysms in two turtles, with arteritis of the great vessels with dark nodular thickenings, thrombi and haemorrhagic lesions in eight, and with mural and valvular endocarditis in one. Aneurysms and thickening of arterial walls with the formation of yellowcream to brown hard mineralised thrombi were associated with mechanical damage due to Hapalotrema acetabulum deeply embedded in the vessel walls. In two turtles large thrombi almost entirely occluded the vascular lumina (Fig 3); Hapalotrema species eggs were frequently observed close to these lesions. Similar lesions were also recorded in the heart and great vessels of three other turtles from which flukes were not collected. Multiple, circular green-black raised spots up to 1 mm in diameter containing necrotic material were observed on the mucosal surface of the lower

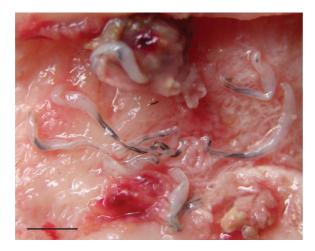


FIG 2: Specimens of *Learedius learedi* associated with mural endocarditis in the ventricular endocardium of a green turtle (*Chelonia mydas*). Bar=3 mm

intestine of eight of the 47 turtles (Fig 4). These lesions were not associated with the presence of adult parasites, but contained parasite eggs.

Microscopic findings Acute and chronic inflammatory reactions were recorded in all the turtles and in all the tissues in which cardiovascular flukes and their eggs were found. Fluke egg granulomas and cardiovascular lesions were the most common findings. Egg granulomas containing from one to approximately 100 eggs were observed in all the tissues examined, except for the skeletal muscle, of all 47 turtles. The majority of the granulomas consisted of five to 10 eggs and they were characterised by syncytia of giant cells and a lymphocytic infiltrate surrounded by reactive fibrosis. Microscopically, two types of egg were distinguished in the fixed tissues. The type 1 eggs resembled those from Hapalotrema and Learedius species (Fig 5), were yellow-brown, fusiform with large bipolar processes; 24 of them measured 208 (30·9) (range 143 to 250)  $\mu$ m × 24 (4·8) (range 19 to 32) µm. The type 3 eggs resembled those from Neospirorchis species, were smaller, brown and round to oval without polar processes (Fig 6); 40 of them measured 38 (6.4) (range 26 to 52) μm x 32 (5·8) (range 22 to 45) μm. Both types were found most frequently in the spleen. The lungs and lower intestine followed in frequency for the type 1 eggs, and the kidney and heart for the type 3 (Table 1).



FIG 4: Black raised foci (arrows) on a congested mucosal surface of the lower intestine, associated with packets of spirorchiid eggs, in a green turtle (*Chelonia mydas*). Bar=10 mm

FIG 3: Specimens of Hapalotrema postorchis associated with a mineralised thrombus in the aorta of a green turtle (Chelonia mydas). Bar=5 mm

TABLE 1: Distribution of two types of spirorchiid eggs in the tissues of green turtles (*Chelonia mydas*) from Tortuguero National Park, Costa Rica

Tissue	Number examined		er of turtles inf Egg type 3 <sup>†</sup>	fected (%) Total positive
Heart	47	13 (28)	28 (60)	35 (74.5)
Great vessels	47	4 (8.5)	2 (4)	5 (11)
Lung	47	33 (70)	24 (51)	46 (98)
Spleen	47	47 (100)	44 (94)	47 (100)
Oesophagus	43	2 (5)	2 (5)	4 (9)
Stomach	43	13 (30)	16 (37)	24 (56)
Higher intestine	47	17 (36)	19 (40)	25 (53)
Lower intestine	47	19 (40)	21 (45)	27 (57.5)
Liver	47	10 (21)	17 (36)	22 (47)
Gall bladder	47	7 (15)	10 (21)	14 (30)
Kidney	47	13 (28)	35 (74.5)	35 (74.5)
Urinary bladder	47	6 (13)	8 (17)	11 (23.5)
Oviduct	47	4 (8.5)	9 (19)	10 (21)
Skeletal muscle	47	0	0	0
Skin	47	1 (2)	3 (6)	4 (8.5)
Fibroma	3	1 (33)	0	1 (33)
Fibropapilloma	1	0	0	0

<sup>\*</sup> Resembling *Hapalotrema* and *Learedius* species eggs † Resembling *Neospirorchis* species eggs

Proliferative multifocal endoarteritis of the small peripheral vessels of the lungs, entire gastrointestinal tract, liver, gall bladder, kidneys and urinary bladder was characterised by the myointimal and perivascular proliferation of leucocytes and fibrous connective tissue (Fig 7). In many cases the lumen of smaller vessels was totally obliterated by the inflammatory response. Necrosis of the endothelial cells was also recorded. Small raised foci on the mucosal surface of the lower intestine contained a core of necrotic debris with fluke eggshells surrounded by granulomatous inflammation. Several sections of adult flukes compatible with Neospirorchis species were found in the heart of seven of the 47 turtles (Fig 8), and in the small vessels of the oesophagus of one, of the stomach of two, of the intestine of three and of the urinary bladder of one. The flukes were always associated with a predominantly granulocytic vasculitis. Cross-sections of 16 flukes from the heart measured 210 (65.8) (range 130 to 325)  $\mu$ m × 70 (23.1) (range 32 to 117) μm. Mixed bacterial colonies were frequently recorded from the cardiovascular, gastrointestinal and pulmonary lesions in association with the flukes and their eggs.

**Other parasitic lesions** Traumatic purulent and ulcerative dermatitis associated with the leech *Ozobranchus branchiatus* (Hirudinea: Ozobranchidae) was observed on the skin of 27 of the 47 turtles. Mixed bacterial infections were associated with the lesions. *O branchiatus* eggs were also recorded on the

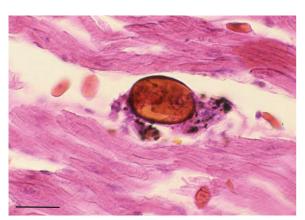


FIG 6: Type 3 spirorchiid egg resembling a *Neospirorchis* species egg, in the heart of a green turtle (*Chelonia mydas*). Haematoxylin and eosin. Bar=20 µm

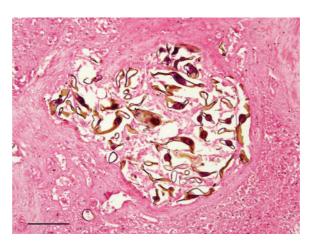


FIG 5: Granulomatous inflammatory reaction caused by type 1 spirorchiid egg, resembling Hapalotrema and Learedius species eggs, in the spleen of a green turtle (Chelonia mydas). Haematoxylin and eosin. Bar=100 µm

external surfaces of 39 of the 47 turtles, in one of them on the surface of a fibroma.

Multifocal green to yellow plaques, ranging from approximately 0·1 to 0·5 cm in diameter, were observed on the mucosal surface of the distal oesophagus of 11 of 43 of the turtles. These lesions were associated with *Rameshwarotrema uterocrescens* (Digenea: Pronocephalidae) (Santoro and others 2007). Petechial haemorrhages and unspecific crater-like ulcerated lesions up to 0·5 cm in diameter were recorded on the gastric mucosal surface of four of 40 of the turtles and were associated with *Charaxicephaloides* species and *Charaxicephalus robustus* (Digenea: Pronocephalidae) infections. Microscopically, these lesions were characterised by hyperplasia of the mucosal surface flanking a necrotic circular area that extended into the submucosa.

Cholycystitis and ectasia of the mucosal glands, with retention of material and small numbers of leucocytes, were recorded in one turtle in association with 14 flukes identified as *Rhytidodoides similis* (Digenea: Rhytidodidae); this species of fluke was recorded in 10 of 40 turtles.

**Tumours** Four cutaneous spherical tumours ( $4\cdot2$  cm or less in diameter) were recorded on the flippers and neck of three of the 47 turtles; three of them were diagnosed as fibromas and one as a fibropapilloma ( $3\cdot8$  cm in diameter). Fusiform eggs, resembling type 1 spirorchiid eggs, were observed in one of the fibromas. No neoplastic lesions were recorded in the internal organs of any of the turtles examined.

#### **DISCUSSION**

Spirorchiids are cosmopolitan flukes that have been found in green, loggerhead turtles (*Caretta caretta*), black turtles

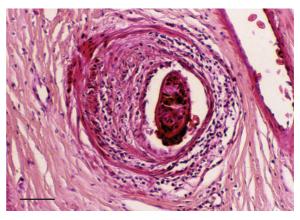


FIG 7: Neospirorchis species fluke in a small vessel in the stomach of a green turtle (Chelonia mydas), associated with proliferative vasculitis. Haematoxylin and eosin. Bar=50 µm

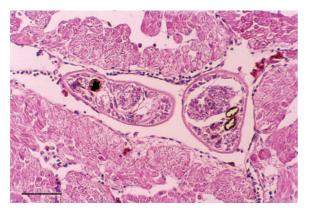


FIG 8: Neospirorchis species sections in the heart of a green turtle (Chelonia mydas). Haematoxylin and eosin. Bar=50 µm

(Chelonia mydas agassizii) and hawksbill turtles (Eretmochelys imbricata) (Smith 1997, Inohuye-Rivera and others 2004). In the Caribbean, spirorchiids and their eggs have been recovered from free-ranging Puerto Rican green and hawksbill turtles (Fischthal and Acholonu 1976, Dyer and others 1991, 1995a, b, Williams and others 1994), and from captive green turtles from Grand Cayman, British West Indies (Greiner and others 1980, Jacobson and others 1986). L learedi (Dyer and others 1991, Williams and others 1994) and Learedius orientalis (Dyer and others 1995a) are the only cardiovascular flukes that have been collected from free-ranging green turtles in the Caribbean; L learedi and L orientalis are sometimes considered synonymous (Fischthal and Acholonu 1976, Dyer and others 1995b, Inohuye-Rivera and others 2004).

Most studies of the effects of spirorchiid infection on wild sea turtles have been performed on stranded or sick animals. They recorded a spirorchiid prevalence of 33 per cent in Atlantic loggerheads (Wolke and others 1982), and 90 to 100 per cent (Dailey and others 1992, Work and others 2005) and 95 per cent (Gordon and others 1998) in green turtles from Hawaii and Australia. A study of healthy captive and wild Australian turtles, including 23 green and four hawksbill turtles reported a prevalence of 77.2 per cent (Glazebrook and others 1989). Glazebrook and others (1989) and Gordon and others (1998) found macroscopic flukes in only 40 and 45 per cent of the turtles, respectively. Aguirre and others (1998) recorded a spirorchiid prevalence of 94 per cent in wild Hawaiian green turtles affected by fibropapillomas, but they found adult flukes in only 33 per cent of them. In these three last studies, the turtles from which no adult flukes were recovered were still considered positive for infection if there was histological evidence of fluke eggs. Similarly, Work and others (2005) diagnosed a 100 per cent prevalence of spirorchiid infection in stranded Hawaiian green turtles on the basis of the presence of eggs in the spleen. In the present study the prevalence of L learedi (97.5 per cent) was the highest ever recorded in a nonstranded group of green turtles. The highest intensity of infection with *L learedi* was recorded by Dyer and others (1995a) (94 flukes in a Puerto Rican green turtle).

The sites of infection with flukes have varied with geographical location. Gordon and others (1998) reported spirorchiids (*Hapalotrema mehrai*, *H postorchis* and *Neospirorchis schistosomatoides*) and their presumed lesions more frequently in the left (92 per cent) than in the right (54 per cent) aorta or in the heart (38 per cent). Glazebrook and others (1989) found *Learedius* and *Hapalotrema* species more frequently in the heart. Rand and Wiles (1985) and Dailey and others (1992) recorded *Hapalotrema* species *L learedi* and *N schistosomatoides* only in the heart. Greiner and others (1980) recovered 141 *L learedi* from 10 culture-reared green turtles; 12 were found in the great vessels and 129 in the heart. In the present study, *L learedi* was collected more frequently from the heart chambers than from the great vessels, *H postorchis* was col-

lected more frequently from the great vessels than from the cardiac chambers, and *M indicum* was collected exclusively from the cardiac chambers. The spirorchiids recorded in the gall bladder, intestine, liver and oesophagus were probably a consequence of the rupture of blood vessels in these organs.

The high prevalence and intensity of infection recorded in this study may be the result of sampling an old population. However, Work and others (2005) have suggested that levels of infection decrease with age, either because of increases in immunity against the parasites, or because of a reduction in the fecundity of the parasites. Stress associated with the breeding, migration and nesting season could adversely affect the turtles' immune system making them more susceptible to infection and thus prone to higher parasite intensities.

The spirorchiid lesions were considered severe in only four of the turtles, which were also infected with *Hapalotrema*, *Learedius* and *Neospirorchis* species. The lesions observed were consistent with those described in other geographical regions (Rand and Wiles 1985, Aguirre and others 1998, Gordon and others 1998, Raidal and others 1998). The turtles sampled at Tortuguero were all nesting females that were apparently in good health. Given their good body condition and active breeding status, the parasite-induced lesions seemed to have had little impact on their health. However, in juvenile turtles without acquired immunity, similar lesions might contribute to their stranding and death. The biological cycles of the parasites recorded in this study are unknown. This study is the first survey of parasites in non-stranded free-ranging green turtles of the Caribbean Sea.

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