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Source: Journal of Wildlife Diseases, 18(1) : 41-46

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-18.1.41>

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## GASTRIC ULCERATION ASSOCIATED WITH LARVAL NEMATODES (*Anisakis* SP. TYPE I) IN PEN REARED GREEN TURTLES (*Chelonia mydas*) FROM TORRES STRAIT

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**Abstract:** During turtle farming operations in Torres Strait, green turtles (*Chelonia mydas*) penned on Murray Island became infected with a larval nematode (*Anisakis* sp. Type I). The larvae were found associated with haemorrhagic ulcers in the pyloro-duodenal junction of the alimentary tract. The apparent source of infection was a locally abundant sardine (*Harengula ovalis*), on which the Murray Island turtles were fed. Turtles held on other islands in the region were not fed sardines and remained uninfected. Recommendations were made to prevent further infection.

### INTRODUCTION

Turtle farming under governmental sponsorship by selected Torres Strait Islanders began in the early 1970's. The intent was to interest the native population in turtle conservation as well as to reduce local hunting pressure on wild turtle stocks. By 1973, hatchlings of both the green turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*) were being held on 11 islands in the Torres Strait region (10°S, 143°E). Turtle farming methods employed on Torres Strait islands have been previously described by Carr and Main.<sup>1</sup> Briefly, the methods were: Hatchlings were procured by removal of a percentage of eggs from clutches laid by wild turtles. Turtles were penned on the islands in pools and trays of varying sizes and fed on locally available fish.

In October, 1973, a taxidermist, while preparing young turtles for sale as curios, noticed a heavy nematode infection in green turtles from Murray Island.<sup>1</sup> Subsequently, a number of turtles were examined by the Queensland Fisheries Service to determine the source of infection and to assess its impact on the future of the turtle farming project.

### MATERIALS AND METHODS

Twelve 18-month-old turtles were collected under the supervision of officers of the Queensland Department of Aboriginal and Islanders Advancement. These turtles were transported alive to Brisbane for examination by research staff of the Queensland Fisheries Service. The sample included 3 green turtles from Murray Island, 3 green turtles from Yorke Island, 3 hawksbill turtles from Yam Island and 3 hawksbill turtles from Cocoanut Island. Specimens were chosen from four different islands to establish the range of infection. Sample size was limited to only twelve turtles because of difficulties in transporting specimens 1,800 km from Torres Strait to Brisbane. These difficulties also prevented subsequent samplings.

Turtles penned on Yorke, Yam and Cocoanut Islands were fed on a variety of fish excluding sardines. Only green turtles were penned on Murray Island and these were fed entirely on locally caught sardines (*Harengula ovalis*). A sample of 15 sardines was obtained and examined for nematode parasites. No other food species was examined.

Tissue samples were taken from infected turtles, fixed in 10% buffered for-

malin, paraffin embedded, sectioned at 6  $\mu$ m and stained with haematoxylin and eosin. Nematode larvae from both turtles and sardines were cleared in 70% ethanol plus 5% glycerol, and mounted in neutral glycerin jelly, and examined at 200 magnification.

## RESULTS

Of the twelve turtles examined, only the three green turtles from Murray Island were infected by nematode larvae ( $\bar{x}$  = 81, range = 73-96). Nematodes were not found in turtles from the other islands. Most of the larvae (91.8%) were found spirally coiled beneath the serosal surface of the stomach and duodenum (Fig. 1). Larvae were separately encapsulated within a tubular sheath of host connective tissue. In one turtle, 20 larvae (8.2%) were encysted beneath Glisson's capsule of the liver. Larvae were identified as *Anisakis* sp. Type I by comparing specimens to the descriptions of Berland<sup>1</sup> and Cannon.<sup>3</sup>

All of the sardines (15) examined, harboured small numbers ( $\bar{x}$  = 7, range 1-15) of *Anisakis* sp. Type I larvae. These larvae were lying free within the peritoneal cavity.

All nematode specimens recovered from both turtles and sardines were identified as third stage larvae.

Haemorrhagic ulcers were found predominantly in the area of the pyloro-duodenal junction but also occurred in the lower stomach and upper intestine. Ulcers varied from 1-3 mm in diameter and were associated with larvae which had perforated the serosal surface, projecting into the peritoneal cavity.

Histologic sections of the pyloro-duodenal junction of the stomach and intestine revealed nematodes within the submucosa and under the serosa. Larvae observed within the serosal region were encapsulated by a proliferation of fibroblasts (Fig. 2). Larvae within the sub-

mucosa were delineated by a thin, hyaline layer of degenerate host tissue. Lying outside this layer were aggregates of lymphocytes and eosinophils (Fig. 3).

## DISCUSSION

The source of infection in the green turtles penned on Murray Island appeared to be raw sardines. Turtles held on other islands, and fed on fish other than sardines, remained uninfected.

The life cycle of *Anisakis* has been described.<sup>2</sup> The definitive hosts for the adult nematode are piscivorous cetaceans and pinnipeds. Of the two intermediate hosts in the life cycle, the last is commonly a member of the family Clupidae e.g. *Harengula ovalis*. The body temperature of the definitive host is the major factor influencing the final moult into adult form.<sup>5</sup> No fourth stage larvae were observed. Hence, the poikilothermic green turtle would represent a terminal host for the ingested larvae. Consequently, the encysted larvae could not mature within the turtle, nor would they represent any risk of transmission to uninfected turtles.

*Anisakis* larvae have been reported to cause ulcers in the stomach wall of porpoises.<sup>6</sup> Acute gastric ulceration observed in infected turtles was considered to lead to appetite depression, thereby aggravating the effect of their already narrow dietary spectrum. Carr and Main<sup>4</sup> observed stunting of growth in turtles older than one year and suggested a possible link with the nematode infection. Infiltration of eosinophils and lymphocytes into the site of inflammation indicated an immunological response within the turtles which could lead to chronic ulceration and subsequent appetite depression.

Because of the labour intensive methods employed on the turtle farms,<sup>1</sup> any stunting of growth or mortalities resulting from nematode infestation in the turtles would be a serious financial



FIGURE 1. Stomach of a green turtle with encapsulated *Anisakis* larvae on the serosal surface.



FIGURE 2. Chronic inflammation of the gastric serosa illustrating proliferation of fibroblasts (F) around an *Anisakis* larva (A). H & E  $\times 400$ .

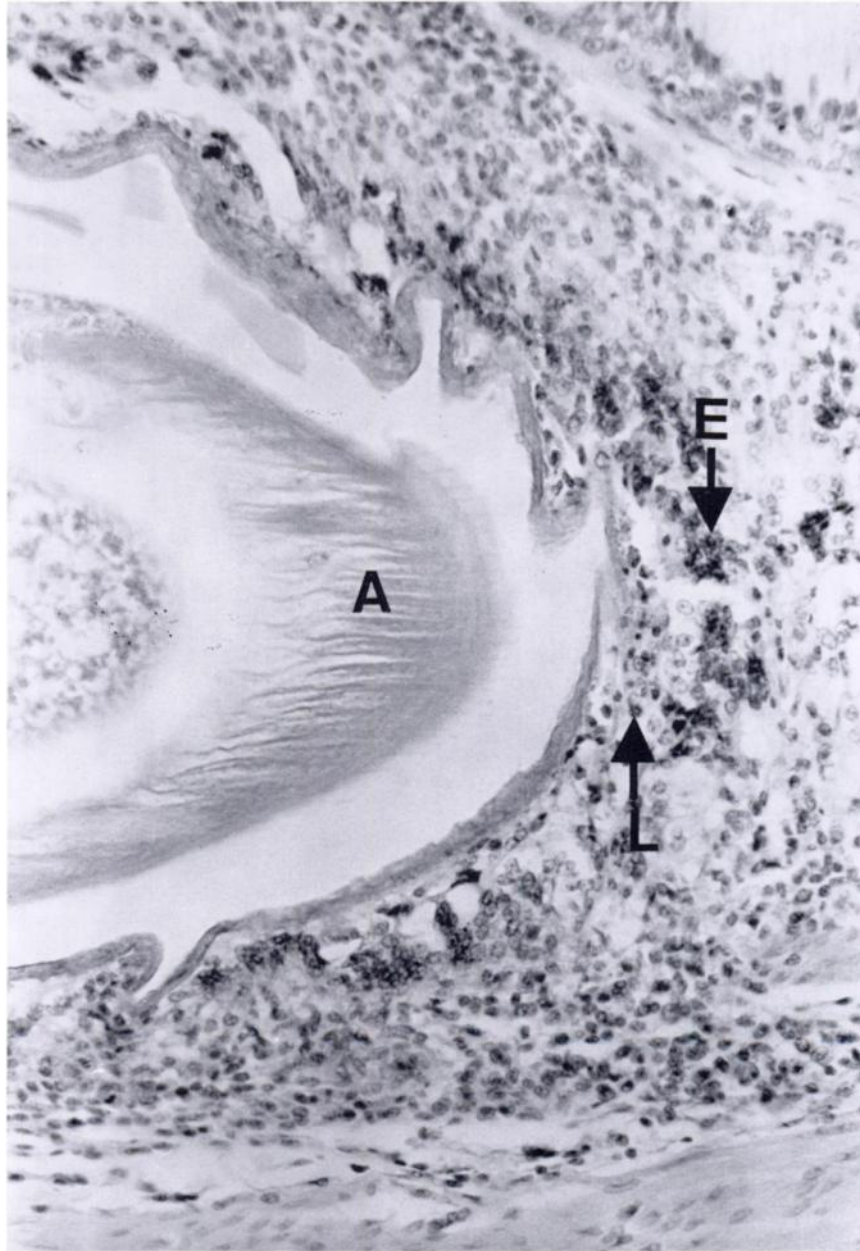


FIGURE 3. Acute inflammation of the gastric submucosa depicting infiltration of eosinophils (E) and lymphocytes (L) following penetration by an *Anisakis* larva (A). H & E  $\times 200$ .

setback to the turtle farmers. Since an alternative food source was not readily available, recommendations were made that all sardines be either cooked or frozen at -20 C for 24 h, to ensure the death of *Anisakis* larvae.<sup>4</sup>

#### Acknowledgements

We wish to acknowledge Prof. J.F.A. Sprent, Parasitology Department, University of Queensland who identified the larval nematodes; Mr. P.J. Killoran, Director of the Queensland Department of Aboriginal and Islanders Advancement, who arranged the supply of turtle specimens; Dr. N.C. Gillespie for his advice and criticism in the preparation of this manuscript.

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*Received for publication 8 December 1980*