'Cebiche'– a potential source of human anisakiasis in Mexico?

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Abstract

Five fish species used for preparation of a popular dish (cebiche) made with raw fish flesh in Mexico were obtained from five localities of the coast of Yucatan. *Lutjanus synagris*, *Gerres cinereus*, *Sphyraena barracuda*, *Epinephelus morio* and *Haemulon plumieri* were examined for the presence of larvae of anisakid nematodes, causative agents of human anisakiasis. The nematode *Pseudoterranova* sp. was found in *E. morio* and *S. barracuda* with a total prevalence of 83% and 6.5 ± 6.2 worms per fish for *E. morio*, and a prevalence of 33% and 10.2 ± 30.0 worms per fish for *S. barracuda. Contracaecum* sp. was found to infect *G. cinereus* with a prevalence of 57% and 7.6 ± 11.4 worms per fish. The relatively high prevalence of *Pseudoterranova* sp. indicates that this parasite is a potential causal agent of anisakiasis on the coast of Yucatan. Although all larvae were found only in the mesentery of the fish host, their importance as a potential source of human infection cannot be excluded as larval migration to the muscles in dead fish is possible.

Introduction

Anisakid nematode larvae such as *Anisakis simplex* (Rudolphi, 1809) and *Pseudoterranova decipiens* (Krabbe, 1878) have been reported as the most frequent cause of the zoonotic infection in humans termed anisakiasis (Ishikura *et al*., 1993). The final hosts of both species are marine mammals, from which partially embryonated eggs are released into the water via host faeces. Upon entering sea water, the eggs hatch out into free swimming larvae, which are then ingested by primary invertebrate hosts such as copepods and shrimps. Marine fish are paratenic, or carrier hosts that ingest these invertebrates and become infected with third-stage larvae (Mehlhorn, 1988). Human beings acquire anisakid larvae by eating raw or under-cooked fish, such as is used in preparing the dish called 'cebiche' made with raw fish flesh marinated in lemon juice for 30 min to 12 h and seasoned with spices. The presence of the larvae (L_3–L_4) in the gastrointestinal tract causes epigastric pain, diarrhoea, nausea, vomiting, and may result in death if not treated adequately (Petithory *et al*., 1990; Bouree *et al*., 1995). Anisakiasis is most widely known in countries like Japan, where 12,586 cases have been documented up to 1993 (Ishikura *et al*., 1993). Likewise, numerous cases have been reported in Canada, USA, Chile and Brazil (Ishikura *et al*., 1993; Smith, 1999). Interestingly, there is not an up-to-date epidemiological report for anisakiasis in Mexico; however, the larvae of *A. typica* (Diesing, 1860) and *P. decipiens* (Krabbe, 1878) have been identified in the mesentery of the red grouper (*Epinephelus morio* (Valenciennes)) from the Yucatan coast (Moravec *et al.*, 1995, 1997). Given the frequent presence of anisakid larvae in commercially important fish, and the use of raw fish in the preparation of cebiche in Yucatan, there is the potential for these larvae to infect humans, especially in coastal communities. Therefore, the main aim of this paper was to determine the prevalence (proportion of fish infected with a single nematode species) and abundance (mean number of a single nematode species per examined fish) of anisakid nematodes in fish used for cebiche preparation.

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https://doi.org/10.1017/S0022149X00700824 Published online by Cambridge University Press

Material and methods

The present study was undertaken in the following localities of the state of Yucatan, Mexico: Celestun (20°52′N and 90°24′W); Sisal (21°10′N and 90°02′W); Progreso (21°17′N and 89°40′W), Telchac (21°20′N and 89°16′W); and San Felipe (21°35′N and 88°09′W). Visits were made to these localities from October 1996 to January 1997, during which restaurant managers and fishermen at each locality were asked to answer two different questionnaires. The questionnaires, one for restaurant managers and the other for fisherman, were designed to collect information on which fish species are most commonly used for cebiche preparation. The viscera and muscle from each fish of the most frequently named fish species were examined. The viscera were examined for anisakids under a dissection microscope. To look for anisakid nematodes in muscle, the flesh of each fish was mechanically disintegrated with a domestic food processor (Brattey, 1988), and then placed in a beaker containing digesting solution (see Deardorff & Throm, 1988). To corroborate internal features of the larvae, transverse histological sections were prepared and stained with haematoxylin and eosin. Larval identification was based on the characteristics proposed by Yamaguti (1961), Hartwich (1975) and Moravec (1998).

Nematodes were identified only to the genus level as experimental infections were not possible for the recovery of adult stages, which would have permitted identification to species level. Measurements were based on 15 specimens for each genus, and are given in millimetres. The infection level parameters (prevalence and abundance) were used as proposed by Margolis *et al*. (1982).

Results and Discussion

On the questionnaires, 22 fish species were cumulatively listed by the restaurant managers and fishermen as being used to prepare cebiche (table 1). Of these, five were identified as suitable for helminthological examination based on their availability (table 2): *Lutjanus synagris* (Linnaeus), lane snapper, from Celestun, *Gerres cinereus* (Walbaum), yellowfin mojarra, from Progreso, *Epinephelus morio* (Valenciennes), red grouper, from Sisal, *Haemulon plumieri* (Lacépède), white grunt, from Telchac, and *Sphyraena barracuda* (Walbaum), great barracuda, from San Felipe. Only the mesentery, and not the muscle of three of these species was found to be infected with third-stage anisakid nematode larvae; 230 *Contracaecum* sp. larvae were recovered from 30 *G. cinereus*, 39 *Pseudoterranova* sp. larvae from six *E. morio*, and 59 *Pseudoterranova* sp. from nine *S. barracuda* (table 3).

The following are descriptions of larval morphology of these two nematodes, together with relevant comments on the two genera.

Contracaecum sp. third-stage larva

Description. Body elongate, 4.80 ± 0.63 (3.80–6.30) long by 0.16 ± 0.01 (0.15–0.18) wide. Cuticle with conspicuous annulations. Boring tooth at the anterior extremity, projecting anteroventrally from the base. Mouth with three lip primordia, one dorsal and two ventrolateral. Excretory pore situated ventrally to tooth, at the base of the lip primordia. Oesophagus muscular, 0.57 ± 0.14 (0.41–0.96) long. Ventriculus small and round, 0.05 ± 0.01 (0.03–0.07) long. Ventricular appendix extends posteriorly and is 0.38 ± 0.05 (0.26–0.48) long and 0.05 ± 0.01 (0.03–0.06) wide. Intestinal caecum extends anteriorly,

Table 1. Fish species used in 'cebiche' preparation on the coast of Yucatan, as recorded using questionnaires.

Scientific name and common name in English (Spanish) of fish	Localities				
	Celestun	Sisal	Progreso	Telchac	San Felipe
Acanthocybium solanderi, Wahoo (Peto)			●		●
Bagre marinus, Gafftopsail catfish (Bagre)		●	●		
Balistes vetula, Queen triggerfish (Cochinita)				●	
Centropomus undecimalis, Snook (Robalo)	●				
Cynoscion nebulosus, Spotted seatrout (Corvina)	●	●		●	●
Epinephelus morio, Red grouper (Mero)	●	●	●	●	●
Gerres cinereus, Yellowfin mojarra (Mojarra blanca)	●		●		
Haemulon bonariense, Black grunt (Ronco)	●	●		●	
H. plumieri, White grunt (Chac-chí)	●	●	●		●
Lachnolaimus maximus, Hogfish (Boquinete)			●	●	●
Lutjanus campechanus, Red snapper (Huachinango)	●	●		●	
L. griseus, Grey snapper (Pargo)			●		
L. synagris, Lane snapper (Rubia)			●		
Mugil cephalus, Striped mullet (Lisa)			●	●	
M. curema, White mullet (Liseta)			●		
Ocyurus chrysurus, Yellowtail snapper (Canané)	●				
Ortopristis chrysoptera, Pigfish (Armado)	●				
Scomberomorus cavalla, King mackerel (Carito)			●	●	
S. maculatus, Spanish mackerel (Sierra)	●	●	●	●	●
Seriola dumerili, Greater amberjack (Coronado)			●		●
Sphyraena barracuda, Great barracuda (Picuda)	●				●
Umbrina coroides, Sand drum (Tambor)			●	●	

https://doi.org/10.1017/S0022149X00700824 Published online by Cambridge University Press

Table 2. Fish species most frequently used for the preparation of cebiche on the coast of Yucatan.

	Fishermen			Restaurant managers		
Locality	Questionnaires answered	Fish species mentioned	%	Questionnaires answered	Fish species mentioned	%
Celestún	35	*Lutjanus griseus*	68	4	*Epinephelus morio*	75
		L. synagris	66		*L. synagris*	75
Progreso	12	*Gerres cinereus*	100	18	*G. cinereus*	78
		E. morio	58		*E. morio*	89
San Felipe	24	*Sphyraena barracuda*	100	1	*E. morio*	100
		E. morio	79			
Sisal	41	*Haemulon plumieri*	95	7	*H. plumieri*	57
		E. morio	80		*E. morio*	43
Telchac	30	*E. morio*	100	10	*E. morio*	100
		H. plumieri	77		*H. plumieri*	60

0.28 ± 0.05 (0.23–0.44) long and 0.05 ± 0.01 (0.04–0.06) wide. Nerve ring surrounding the oesophagus at 0.20 ± 0.01 (0.18–0.23) from the anterior extremity. Tail 0.09 ± 0.01 (0.06–0.11) long, with round tip. The excretory canal is situated at the basal region and the lateral chords are butterfly-shaped. There are 13–15 cells in the intestinal caecum and basal nuclei; 13–20 large cells in the intestine, and 40–50 muscle cells per quadrant. The ventricular appendix possesses glandular tissue.

Host. *Gerres cinereus* (infected/examined = 17/30; abundance = 7.6 ± 11.4).

Habitat. Mesentery.

Locality. Progreso, Yucatan.

Comments. Only three authors have reported *Contracaecum* sp. nematodes as anisakiasis causative agents (Schaum & Muller, 1967; Shiraki, 1974; Reichenbach-Klinke, 1975). However, the actual health risk to humans needs to be clarified as Vidal-Martínez *et al*. (1994) demonstrated experimentally the possibility of a bird species of *C. multipapillatum* maturing in mammals (cats).

Pseudoterranova *sp. third-stage larva*

Description, Body elongate, 5.10 ± 0.98 (3.7–7.9) long and 0.13 ± 0.03 (0.08–0.19) wide. Mouth with a small, 0.01 long tooth, and three lip primordia, one dorsal and two ventrolateral. Cuticle with slight annulations. Excretory pore situated ventrally to the tooth, at the base of the lip primordia. Oesophagus muscular, 0.68 ± 0.13 (0.51–1.09) long. Ventricular appendix absent. Ventriculus 0.28 ± 0.05 (0.18–0.40) long and 0.07 ± 0.02 (0.04–0.10) wide. Intestinal caecum extends anteriorly, 0.51 ± 0.13 (0.25–0.97) long and 0.05 ± 0.02 (0.03–0.10) wide. Nerve ring surrounding the oesophagus, 0.20 ± 0.03 (0.01–0.3) from the anterior extremity. Slightly curved tail, with round tip, 0.12 (0.10–0.19) long. The excretory canal is bean-shaped and lies ventrally to the alimentary canal. The lateral nerve chords possess two thick divergent branches. The intestinal dorsal caecum extends to the middle level of the oesophagus. There are 60–70 muscle cells per quadrant and 15–25 cells in the intestine with nuclei situated near the cell base.

Hosts. *Sphyraena barracuda* (infected/examined = 3/9; abundance = 10.2 ± 30.0) *Epinephelus morio* (5/6; 6.5 ± 6.2).

Habitat. Mesentery.

Localities. San Felipe (*S. barracuda*), and Sisal (*E. morio*), Yucatan.

Table 3. Number of fish species examined (n) and infection parameters by locality.

Locality	Fish species	n	Fish length (cm) ± S.D.	*Pseudoterranova* sp.	*Contracaecum* sp.
Celestun	*Lutjanus synagris*	24	14.4 ± 1.8	–	–
Sisal	*Epinephelus morio*	11†	27.5 ± 1.8	–	–
		6	35.2 ± 6.6	83 6.5 ± 6.2	–
Progreso	*Gerres cinereus*	30	15.9 ± 1.1	–	57 7.6 ± 11.4
Telchac	*Haemulon plumieri*	30	19.6 ± 2.6	–	–
San Felipe	*Sphyraena barracuda*	9	48.2 ± 19.0	33 10.2 ± 30.0	–

† Eviscerated.
Upper value, prevalence; lower value, abundance ± S.D.

https://doi.org/10.1017/S0022149X00700824 Published online by Cambridge University Press

Comments. *Pseudoterranova* sp. larvae are known to be causative agents of anisakiasis (Mehlhorn, 1988; Ishikura, *et al*., 1993). Moravec *et al*. (1995, 1997) found *P. decipiens* larvae in *E. morio* collected in Celestun (41% prevalence, intensity 1–2 [mean 7]), Chiquila (5%, 2 [2]), Chelem (20%, 1–18 [6]), Progreso (34%, 1–14 [5]), and Sisal (4%, 2 [2]). In the study by Moravec *et al*. (1997) larvae were only isolated from the mesentery.

In the present study, anisakid nematode larvae were only found in the mesentery, and can be interpreted as harmless to humans. However, post mortem larvae migration from the mesentery to fish flesh has been experimentally demonstrated (Smith & Wotten, 1975; McClelland, 1994). This migration may be due to storage conditions (e.g. icing or freezing) as, during fish examination, liberation from the capsule and active motility of anisakid larvae were observed after 16 h of cooling in the viscera of *S. barracuda* at 4°C (Laffon-Leal, personal observation). Such potential for migration and the fact that the three fish species reported as infected in this study are commercially important (Secretaría de Pesca, 1994), suggests that the presence of anisakid larvae in fish from Yucatan cannot be ignored. Further research should include large samples from other fish species, as well as experimental studies on larval migration of anisakid nematodes. These are critical points to study if the role of commercially important fish species as a potential source of human anisakid nematode infection in Mexico is to be determined.

Acknowledgements

This study was financially supported by grants no. 4689-N9406 'Helmintofauna del mero en la costa de la Penísula de Yucatán' and 26396-B 'Nemátodos anisáquidos que parasitan a peces en la costa de Yucatán' from the Consejo Nacional de Ciencia y Tecnología (CONACyT, México). We are grateful to CONACyT, Mexico for awarding a studentship to S.L.L. This paper is based on the postgraduate thesis of S.L.L. Special thanks are given to L. Aguirre Macedo, R. Rodríguez Canul, T. Scholz, E. Núñez, A. Oropeza and P. Meelu for their comments on the manuscript.

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(Accepted 28 July 1999)

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https://doi.org/10.1017/S0022149X00700824 Published online by Cambridge University Press