



Research note

Infection status of the estuarine turtles *Kinosternon integrum* and *Trachemys scripta* with *Gnathostoma binucleatum* in Sinaloa, Mexico

Estado de la infección con *Gnathostoma binucleatum* de las tortugas estuarinas *Kinosternon integrum* y *Trachemys scripta* en Sinaloa, México

Sylvia Páz Díaz-Camacho^{1*}, María del Carmen de la Cruz-Otero¹, Edith Hilario Torres-Montoya², Sergio Sánchez-Gonzales², Francisco Delgado-Vargas¹ and Yukifumi Nawa³

¹Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Ciudad Universitaria 80010, Culiacán Rosales, Sinaloa, México.

²Escuela de Biología, Universidad Autónoma de Sinaloa, Ciudad Universitaria 80010, Culiacán Rosales, Sinaloa, México.

³Professor-Emeritus, University of Miyazaki, Miyazaki, Japan.

*Correspondent: spdiacz@uas.uasnet.mx

Abstract. Human gnathostomosis, a serious public health issue in Mexico, is endemic to Sinaloa. The disease is mainly caused by consumption of the raw meat of freshwater or estuarine fishes infected with the advanced third stage larvae (AL3) of *Gnathostoma binucleatum*. In the present study, we examined estuarine turtles with a sample consisting of 23 *Trachemys scripta* and 5 *Kinosternon integrum* from Sinaloa, Mexico for the presence of *Gnathostoma* larvae; such examination was made by the pressing method of skeletal muscles between 2 glass plates. The results showed that both turtles harbored *G. binucleatum* AL3; identification was achieved by morphology and also by PCR/sequencing of the ITS2 region of ribosomal DNA of the larvae. Infection prevalence was higher for *K. integrum* (80%) than for *T. scripta* (69.6%), but heavy infection (> 10 AL3/turtle) was observed in the larger sized individuals of *T. scripta*. Consumption of the raw meat of these turtles represents a risk to acquire the disease.

Key words: estuarine turtles, *Kinosternon integrum*, *Trachemys scripta*, paratenic host, *Gnathostoma binucleatum*.

Resumen. La gnathostomosis humana, un serio problema de salud pública en México, es endémica de Sinaloa. La enfermedad es principalmente ocasionada por el consumo de carne cruda de pescado de agua dulce o salobre infectado con larvas del tercer estadio avanzado (AL3) de *Gnathostoma binucleatum*. En la presente investigación, se examinaron tortugas estuarinas, 23 *Trachemys scripta* y 5 *Kinosternon integrum*, de Sinaloa, México para identificar la presencia de larvas de *Gnathostoma*; para ello se utilizó el método de compresión del tejido muscular entre 2 placas de vidrio. Los resultados mostraron que ambas especies de tortugas son hospederas de larvas AL3 de *G. binucleatum*; la identificación específica se basó en la morfología y composición molecular (por PCR/secuenciación de la región ribosomal ITS2) del DNA de las larvas. La prevalencia de la infección fue mayor en *K. integrum* (80%) que en *T. scripta* (69.6%), pero la intensidad fue más alta (> 10 AL3/tortuga) en las tortugas de mayor tamaño, *T. scripta*. El consumo de carne cruda de estas tortugas representa un riesgo para adquirir la enfermedad.

Palabras clave: tortugas estuarinas, *Kinosternon integrum*, *Trachemys scripta*, hospedero paraténico, *Gnathostoma binucleatum*.

Gnathostomosis is a serious public health issue in Mexico, especially in the States of Nayarit and Sinaloa (Lamothe-Argumedo, 1997). In Mexico, 3 species of *Gnathostoma* have been identified: *G. turgidum*, *G. binucleatum*, and *G. lamothei* (Pérez-Álvarez et al., 2008). To date, the advanced third stage larva (AL3) of *Gnathostoma binucleatum* is the only entity associated with

human infection in Mexico (Almeyda-Artigas et al., 2000). These larvae are prevalent among estuarine fishes (Díaz-Camacho et al., 2002, 2008a; Álvarez-Guerrero and Alba-Hurtado, 2007) as well as in several other animal species and some of them have been characterized as paratenic/intermediate hosts (Lamothe-Argumedo, 1997; León-Règagnon et al., 2005). In particular, the estuarine turtles, *Kinosternon integrum* and *Trachemys scripta* have been identified as important paratenic hosts of *G. binucleatum*

in Nayarit, Mexico (Álvarez-Guerrero and Alba-Hurtado, 2007); however, infection status of estuarine turtles has not been studied in detail in Sinaloa, Mexico, which is an endemic area of human gnathostomosis (Díaz-Camacho et al., 2003).

In this study, a total of 28 turtles, 23 *T. scripta* and 5 *K. integrum*, were obtained at Tanque Vía and Ojo de Agua, Tecualilla, Sinaloa, Mexico (22°45'27.97" N 105°40'25.41" W) during October 2001 and March 2008. Turtles were dissected, skeletal muscle collected, every piece of meat compressed between 2 glass plates and visually examined for the presence of larvae. The larvae were recovered and routine identification was carried out by light microscopy and occasionally by electron microscopy with previously described methods (Díaz-Camacho et al., 2002). Species identification was confirmed further by sequence alignment of the amplified DNA sequence of the Internal Transcribed Spacer (ITS2) region with the primers and PCR conditions previously described (León-Règanon et al., 2003).

We found *Gnathostoma* larvae in the turtles *T. scripta* and *K. integrum* with a prevalence of 69.6% and 80.0%, respectively (Table 1). The size of the turtles and the intensity of infection showed a positive correlation, with the larger ones having more larvae (Table 2). The larvae found in the estuarine turtles were identified exclusively as the advanced third stage larvae (AL3) of *G. binucleatum*. Our results were similar to those reported in Nayarit but we found a higher mean intensity of infection for *T. scripta* (15.6 vs. 1.3) (Álvarez-Guerrero and Alba-Hurtado, 2007). Comparing to their results, the size of turtles in our study, especially *T. scripta* captured in 2008, were larger than theirs, even comparing with *K. integrum* in this study; and

as expected for paratenic hosts, the larger ones showed the higher intensity of infection (Table 2); in fact, the size of turtles and the intensity of infection showed a positive correlation for *T. scripta* ($r = 0.65$) and for *K. integrum* ($r = 0.75$).

Interestingly, in the studied geographic area, not only *G. binucleatum* but also *Gnathostoma turgidum* is highly prevalent (Díaz-Camacho et al., 2002, 2008a, 2008b, 2009). Recently, Mosqueda et al. (2009) reported the presence of *G. turgidum* AL3 in *K. integrum* from Tres Palos Lagoon, Mexico. Advanced third stage larvae of *G. binucleatum* and *G. turgidum* can be easily distinguished from each other by the shape of the hooklets on the head bulb. Nevertheless, during 7 years of research, we have not yet found turtles, or even fish or birds infected with *G. turgidum* (Díaz-Camacho et al. 2002, 2008a, 2009). The knowledge of *G. binucleatum* life cycle is important to decrease the risk of human infection. In particular, rural people in the studied area have a custom of eating turtle meat with the belief of curative effects on some diseases (Álvarez -Guerrero and Alba Hurtado 2007). As a result, people who consume raw meat of turtle are at risk of infection with *G. binucleatum*, and estuarine turtles might be an important source of infection in humans.

This work was supported by PROFAPI-UAS No. PI-PROFAPI-06070 and CONACYT-México No. P48161-M. Authors wish to thank Magda Luz Zazueta Ramos, Ángel Bojórquez Contreras, Josefina Sicarios Félix and Roberto Guzmán Loreto for their technical assistance. Molecular identification of *Gnathostoma binucleatum* was done by Dra. Virginia León Règanon, Instituto de Biología, UNAM.

Table 1. Epidemiological data of *G. binucleatum* infection in estuarine turtles

Turtles	Prevalence positive/ examined (%)	No. of AL3 recovered	Mean Intensity	Range of intensity
<i>Trachemys scripta</i>	16/23 (69.6)	249	15.6	1-64
<i>Kinosternon integrum</i>	4/5 (80)	9	2.3	1-4

Table 2. Relationship between the size of turtles and the prevalence and the intensity of infection

Turtles*	Length** (cm)	Prevalence positive/examined (%)	Intensity mean (range)
<i>Trachemys scripta</i>	18.5-23	3/4 (75)	1.3 (1-2)
	25-33	4/4 (100)	32.8 (5-48)
<i>Kinosternon integrum</i>	9.5-11	1/2 (50)	0.5 (1)
	14-16	3/3 (100)	2.7 (1-4)

* Samples were limited to those collected during 2008.

** The length of the back shell.

Literature Cited

- Almeyda-Artigas, R. J., M. D. Bargues and S. Mas-Coma. 2000. ITS-2 rDNA sequencing of *Gnathostoma* species (Nematoda) and elucidation of the species causing human gnathostomiasis in the Americas. *Journal of Parasitology* 86:537-544.
- Álvarez-Guerrero, C. and F. Alba-Hurtado. 2007. Estuarine fish and turtles as intermediate and paratenic hosts of *Gnathostoma binucleatum* in Nayarit, Mexico. *Parasitology Research* 102:117-122.
- Díaz-Camacho, S. P., M. C. de-la-Cruz-Otero, M. L. Zazueta-Ramos, A. Bojórquez-Contreras, J. Sicaños-Félix, S. Campista-León, R. Guzmán-Loreto, F. Delgado-Vargas, V. León-Règagnon and Y. Nawa. 2008a. Identification of estuarine fish *Dormitator latifrons* as an intermediate host and *Eleotris picta* as a paratenic host for *Gnathostoma binucleatum* in Sinaloa, Mexico. *Parasitology Research* 103:1421-1425.
- Díaz-Camacho, S. P., K. Willms, M. C. de-la-Cruz-Otero, M. L. Zazueta-Ramos, S. Bayliss-Gaxiola, R. Castro-Velázquez, I. Osuna-Ramírez, A. Bojórquez-Contreras, E. H. Torres-Montoya and S. Sánchez-Gonzales. 2003. Acute outbreak of gnathostomiasis in a fishing community in Sinaloa, Mexico. *Parasitology International* 52:133-140.
- Díaz-Camacho, S. P., K. Willms, M. L. Zazueta-Ramos, M. C. de-la-Cruz-Otero, Y. Nawa and H. Akahane. 2002. Morphology of *Gnathostoma* spp. isolated from natural hosts in Sinaloa, Mexico. *Parasitology Research* 88:639-645.
- Díaz-Camacho, S. P., K. Willms, J. G. Rendón-Maldonado, M. C. de-la-Cruz-Otero, F. Delgado-Vargas, L. Robert-Guerrero, S. Antuna-Bizarro, V. León-Règagnon and Y. Nawa. 2009. Discovery of an endemic area of *Gnathostoma turgidum* infection among opossums, *Didelphis virginiana*, in Mexico. *Journal of Parasitology* 95:617-622.
- Lamothe-Argumedo, R. 1997. Hospederos definitivos, intermediarios y paraténicos de *Gnathostoma* en Veracruz y Oaxaca, México. *Cuadernos Mexicanos de Zoología* 3:22-28.
- León-Règagnon, V., D. Osorio-Sarabia, L. García-Prieto, R. Lamothe-Argumedo, F. Bertoni-Ruiz and A. Ocegüera-Figueroa. 2005. New host records of the nematode *Gnathostoma* sp. in Mexico. *Parasitology International* 54:51-53.
- León-Règagnon, V., L. García-Prieto, D. Osorio-Sarabia, E. Martínez-Salazar, A. Ocegüera-Figueroa, R. Lamothe-Argumedo, S. P. Díaz-Camacho, S. Nuamtanong, J. Waikagul, H. Akahane and R. Lazo. 2003. Molecular systematics of *Gnathostoma* spp. The Bulletin of Central Research Institute Fukuoka University. Series E: Interdisciplinary Sciences 1:237-247.
- Mosqueda-Cabrera, M. A., E. Sánchez-Miranda, L. Carranza-Calderón and H. E. Ortiz-Najera. 2009. Finding advanced third-stage larvae of *Gnathostoma turgidum* Stossich, 1902 in Mexico from natural and experimental host and contributions to the lifecycle description. *Parasitology Research* 104:1219-1225.
- Pérez-Álvarez, Y., L. García-Prieto, D. Osorio-Sarabia, R. Lamothe-Argumedo and V. León-Règagnon. 2008. Present distribution of the genus *Gnathostoma* (Nematoda: Gnathostomatidae) in Mexico. *Zootaxa* 1930:39-55.