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First molecular detection of *Spirometra* spp. in Cuba

Primera detección molecular de *Spirometra* spp. en Cuba

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Human sparganosis is a zoonosis caused by the plerocercoid larvae (Sparganum) genus *Spirometra* (Cestoda: Diphyllobothriidae). More than 2000 cases have been reported in the world, 80% occur in China, followed by Korea, Thailand and Japan. Few cases are described in Africa, Europe and America. The disease has been recorded in individuals who travel to endemic regions or who migrate from endemic to non-endemic countries.[1,2]

The life cycle of the parasite involves three intermediate hosts. The first are the copepods (Cyclopidea) that carry the procercoid larvae. These are ingested by amphibians, reptiles, chickens, pigs and wild animals (second hosts) where the

development to plerocercoid occurs. The most common definitive hosts are canids and felines.[3]

Humans are mainly infected through the consumption of raw or undercooked meat from second intermediate hosts, the ingestion of untreated water, or the use of raw meat in traditional poultices. Sparganum have been found in the brain, eyes, breasts, spinal cord, and subcutaneous tissue, producing local tissue damage, paralysis, blindness, and death.[1,2,3]

Recently, at the "Pedro Kourí" Institute of Tropical Medicine (IPK), our group detected for the first time *Spirometra* spp. in a case of cerebral Sparganosis using molecular methods. The case was a 48-year-old male patient, rural origin from the province of Villa Clara, habitual consumer of insufficiently cooked snake meat and bullfrog, who also used to ingest untreated water from river in his place of residence. He began to manifest confusion, seizure, headache, convulsions and progressive weakness. He is surgically intervened for diagnostic studies.

A flat ivory-white worm was obtained from the right parietal lobe. The parasite was examined under a stereoscopic microscope. The plerocercoid showed immature scolex at the anterior end and has a cleft-like invagination. In addition, the body wall was irregular producing pseudosegmented appearance.

Histopathological findings included body wall with variable thickness and composed by a layer of microvilli, tegument (10 µm), two layers of smooth muscle and row tegumental cells. The parenchyma contains irregularly scattered bundles of longitudinal muscle fibers, branching excretory channels, mesenchymal fibers and calcareous corpuscles in a loose stroma. Reproductive organs were not found. This description was compatible with plerocercoid larvae of *Spirometra* spp.

In the present study, morphological observations were conducted and two mitochondrial genes of the *Spirometra* species were analyzed. The primers (spcox1f: 5′-GTA TTG AAG GAA TTA GTT AGG TTA-3′ and spcox1r: 5′-CAA CCC AAT TAA ATT AAG TTC CAC-3′) and nad3 region (spnad3f: 5′-GTG TGT TTT TGC ACT GTG-3′ and spnad3r: 5′-ATT GAC AAT AGA TTA TTA GCA-3′) were used to amplify the cytochrome C oxidase subunit 1 (*cox1*) and subunit 3 of NADH dehydrogenase (*nad3*) sequence, respectively. PCR was performed in 50 µl reaction mixture with 0.01 µg/µl genomic DNA, 10× PCR

buffer (20 mM Mg+), a 10 mM dNTP mixture, 10 pmols of each primer, and 2.5 U/µl Taq DNA polymerase (High Fidelity PCR system, Roche, Mannheim, Germany). PCRs were performed in a 3Primer Thermal Cycler (DOT Scientific Inc., EE.UU) as follows: 1 cycle of initial denaturation at 94 ˚C for 3 min, 30 cycles at 94 ˚C for 1 min, 50 ˚C for 1 min, 72 ˚C for 2 min, and extension at 72 ˚C for 10 min. This resulted in *cox1* (1,566 bp) and *nad3* (346 bp) DNA fragments were isolated on a 1.0% agarose gel. This result allowed the optimization of polymerase chain reaction conditions for *cox*1 and *nad*3.[4]

The first diagnosed case of Sparganum in humans was identified in Xiamen, Fujian province, China in 1882.[1] In Cuba, Kourí et al. carried out the initial studies on *Spirometra* spp in 1947. These authors show that 20% to 30% of Cuban banana frogs (*Osteopilus septentrionalis*) were infected by the parasite.[5] Others isolated cases with the disease were described by conventional histological methods, which do not allow confirming the *Spirometra* species.[6,7,8]

At present, distribution, prevalence and species of the parasite in Cuba are unknown. For this reason, it is essential to have a laboratory that has a standardized technique to reach these proposals. Studies aimed at solving the problem of the disease and the *Spirometra* species in Cuba are under development at the IPK.

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Conflict of interests

The authors declare that does not exist an interest conflict.