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MORPHOLOGICAL CHARACTERIZATION OF THE ROSTELLAR HOOKS OF *TAENIA SOLIUM* LINNAEUS, 1758 (CESTODA: CYCLOPHYLLIDEA) METACESTODES EXTRACTED FROM A NATURALLY INFECTED PIG OF GUATEMALA

CARACTERIZACIÓN MORFOLÓGICA DE LOS GANCHOS ROSTELARES DE METACESTODOS DE *TAENIA SOLIUM* LINNAEUS, 1758 (CESTODA: CYCLOPHYLLIDEA) EXTRAÍDOS DE UN CERDO NATURALMENTE INFECTADO DE GUATEMALA

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ABSTRACT

Taenia solium Linnaeus, 1758 the etiological agent of the taeniasis/cysticercosis complex causes taeniasis in humans (definitive host), and swine cysticercosis in pigs (intermediate host). Major complications occur when humans accidentally ingest *T. solium* eggs and acquire neurocysticercosis, the leading cause of epilepsy in developing countries. Swine cysticercosis greatly affects rural farmers due to the economic losses from confiscation or loss of the infected meat. Although the morphological features of *T. solium* are well established, limited information is available on the complete morphology of the rostellar hooks of metacestodes. The objective of this study was to characterize morphologically the rostellar hooks of metacestodes of *T. solium* using morphometric features that have not been previously applied for this species. In addition, a massive infection of swine cysticercosis was described. As part of a pilot study, a male pig from the department of Zacapa was examined and diagnosed with swine cysticercosis. Lingual palpation and necropsy were performed and an exceptionally heavy infection was found. The estimated number of metacestodes was 40,500, of which, 340 were placed in bile solution to estimate the percentage of viability. Others were used for morphometric analysis of the rostellar hooks. About 97.6% were found evaginated and with contractile movements. The hooks we found were similar in length and width to other studies of *T. solium*. Future studies should compare the morphometric parameters of rostellar hooks to reject or determine intraspecific morphological variation of *T. solium*. Morphological features of rostellar hooks should be considered as a rapid identification guide during necropsies of swine, particularly when cysts of *Taenia* sp. are found in the viscera. Our findings suggest the presence of high endemic rural areas of *T. solium* in Guatemala.

Keywords: Guatemala – metacestode – morphology – neglected tropical disease – swine cysticercosis – *Taenia solium*

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RESUMEN

Taenia solium Linnaeus, 1758 es el agente etiológico del complejo teniasis/cisticercosis, causante de teniasis en el humano (hospedador definitivo) y cisticercosis porcina en los cerdos (hospedador intermediario). La mayor complicación ocurre cuando los humanos accidentalmente ingieren huevos de *T. solium* y adquieren la neurocisticercosis; la causa principal de epilepsia en los países subdesarrollados. La cisticercosis porcina afecta inmensamente a los productores rurales debido a las pérdidas económicas por decomisos o pérdida de la carne infectada. Aunque las características morfológicas de *T. solium* están bien establecidas, existe limitada información sobre la morfología completa de los ganchos rostelares de los metacestodos. El objetivo de este estudio fue caracterizar morfológicamente los ganchos rostelares de metacestodos, utilizando características morfométricas aún no aplicadas en *T. solium*. Además se describe una infección masiva por cisticercosis porcina. Como parte de un estudio piloto, un cerdo macho del departamento de Zacapa fue examinado y diagnosticado con cisticercosis porcina. Se realizó la palpación lingual y necropsia, y se encontró una alta carga parasitaria, el número estimado de metacestodos fue de 40.500. De los cuales 340 fueron colocados en una solución de bilis para estimar el porcentaje de viabilidad. El 97,6% fue encontrado evaginado y con movimientos contráctiles. En el análisis morfológico, los ganchos que se encontraron fueron similares en largo y ancho a los reportados en otros estudios de *T. solium*. Futuros estudios deben ser comparados usando los parámetros morfométricos de los ganchos rostelares para descartar o determinar variación morfológica intraespecífica de *T. solium*. Las características morfológicas de los ganchos rostelares deberían considerarse como una guía rápida de identificación durante las necropsias de cerdos de traspatio, principalmente cuando se encuentran quistes de *Taenia* sp. en las vísceras. Nuestros hallazgos sugieren la presencia de áreas rurales altamente endémicas de *T. solium* en Guatemala.

Palabras clave: cisticercosis porcina – enfermedad tropical desatendida – Guatemala – metacestodos – morfología – *Taenia solium*

INTRODUCTION

Swine cysticercosis caused by the larval stage of *Taenia solium* Linnaeus, 1758 is a parasitic infection of pigs causing great economic losses with public health implications and concerns, primarily in endemic countries of Latin America, Asia and Africa (Shonyela *et al*., 2017). Pigs act as intermediate hosts of *T. solium* and rarely present associated symptoms of swine cysticercosis. Infection of pigs occurs through ingestion of fecal material contaminated with eggs of the *T. solium* tapeworm from a human carrier, the definitive host. Consequently, free-roaming pigs from endemic rural areas with access to human feces are commonly infected (García *et al*., 2003a). The taeniasis/cysticercosis complex is associated with poverty, lack of sanitation and lack of education. It is well known that in developing countries the trade of pigs is usually informal, and the slaughter of animals is carried out without veterinary inspection (García *et al*., 2003b). Thus, the economic losses of swine cysticercosis are due to the decrease in the economic value of pigs, or as a result of pork meat confiscation or total loss of the carcasses (Adesokan & Adeoye, 2019). The diagnosis and prevalence of swine cysticercosis are very important considering that this problem demonstrates an active transmission of the *T. solium* life cycle. Unfortunately, in many developing countries the only available diagnostic method is the tongue examination, which is highly specific but with low sensitivity (Sciutto *et al*., 1998; Dorny *et al*., 2004). In Guatemala, few studies have been carried out regarding swine cysticercosis, and the actual prevalence is unknown. Nevertheless, any finding of an infected pig with *T. solium* metacestodes is important in order to determine new foci of the infection, particularly in remote rural areas, where *T. solium* represent a serious threat to the public health.

Although the morphology of the metacestode of *T. solium* is well known, there is limited information of the detailed morphology of rostellar hooks, considering that most of the studies carried out have been focused only on the total length and width of the small and large hooks (Verster, 1969; Boa *et al*., 1995). Therefore, the objective of this

study was to characterize the morphology of the rostellar hooks of *T. solium* within Guatemala, based on the complete morphometric analysis. Moreover, the intensity of infection with metacestodes of *T. solium* in a naturally infected pig is reported here.

MATERIAL AND METHODS

Necropsy and extraction of metacestodes

A male pig of 10 months of age (63 kg) from the village San Juan (14°55'06"N/89°36'45"O), municipality of San Jorge, department of Zacapa, Guatemala, was examined through lingual palpation due to the report of lingual cysts by the owner. The mouth was opened with a wooden stick and the tongue pulled gently with a cotton cloth to confirm the presence of metacestodes. Afterwards, the pig was bought and anesthetized. The necropsy was performed as described elsewhere (Chembensofu *et al*., 2017), and the striated muscle tissue of the carcass, heart, brain, eyes and viscera were inspected for the extraction of metacestodes. To determine the intensity of infection by metacestodes in the carcass, 1 kg composed of different muscles including the tongue, masseters, fore- and hind limbs was weighed, and subsequently, the metacestodes contained in that kilogram of pork meat were counted using a manual counter (Assana *et al*., 2010). After veterinary inspection, the whole carcass and organs were incinerated for proper disposal of the biological material. To estimate the viability of metacestodes, a number of them were extracted and washed in 0,9% saline, then placed in Petri dishes containing a 1:1 mixture of pig bile and 0,9% saline, and incubated at 37°C for 12 h (Gonzalez *et al*., 1990). Metacestodes were considered viable when the protoscolices were evaginated and displayed contractile movements under a stereoscopic binocular microscope (Olympus SZ61). The number of evaginated metacestodes was counted and the percentage of viability was determined.

Rostellar hooks morphology

Morphometric analysis was conducted using 50 viable metacestodes. They were placed between two slides with 70% alcohol for 24 h, and destained in 5% acid alcohol; then were placed in Petri dishes with 70% alcohol again, for a few minutes. Then the cysts were dehydrated in 90% alcohol for 1 h, then placed in xylene and mounted in Canada balsam (Chawhan *et al*., 2014). Nine measurements were included for morphometric analysis of the small and large rostellar hooks, according to Haukisalmi *et al.* (2011), Fig. 3. The specimens were observed under a light microscope, 40X and 10X magnifications (Leica DM500), and when the rostellum was observed invaginated not allowing proper observation of the hooks, the sample was discarded. Drawings of the hooks were made with the aid of a Lucida camera attached to the compound microscope. For each metacestode, the measurements were obtained from a single small and large hook of each crown. In addition, the mean, standard deviation (SD) and range of the morphometric measurements of the hooks were obtained.

Ethical Aspects: The pig's necropsy was performed according to the consent of the Animal Welfare and Bioethics Committee, Facultad de Medicina Veterinaria y Zootecnia, Universidad de San Carlos de Guatemala (No.EEP.15.2019).

RESULTS

Necropsy findings

Metacestodes of *T. solium* were detected first, by tongue examination and after necropsy, by dissection of the whole carcass. The intensity of infection found was extremely high and the estimated number of metacestodes was 40,500, which were distributed throughout the striated muscle tissue, tongue, heart, eyes, and brain (Fig. 1). The metacestodes were small (0.5 mm) and had intact walls with semitransparent membranes and transparent vesicular fluid. Three hundred forty were extracted to determine the percentage of viability. Out of these, 332 (97.6%) evaginated after 12 h of incubation. The majority was observed with movements and with two rows of well-developed rostellar hooks (Fig. 2A). Few metacestodes were found with only small hooks and only one without hooks.

Morphometric analysis

Out of 50 fixed and mounted metacestodes, 25 were used according to an adequate observation of

the rostellar hooks. The rostella had 20-26 hooks, rearranged in two rows (mean=23, n=25). The small (mean=123 µm, n=25) and large hooks (mean=163 µm, n=25) were characterized by having a curved blade. Large hooks had a thickened handle distally and the guard was rounded and slightly thinner distally. Meanwhile, the small hooks had a thickened handle in the medial part and the guard was slightly thinner distally (Fig. 2B, 3B). All hook measurements are shown in Table 1.

Figure 1. Metacestodes of *T. solium* were found in the whole carcass of the pig. A) Cysts with visible protoscolices were found during lingual palpation, and during necropsy in B) eyes, C) striated muscle and D) the brain.

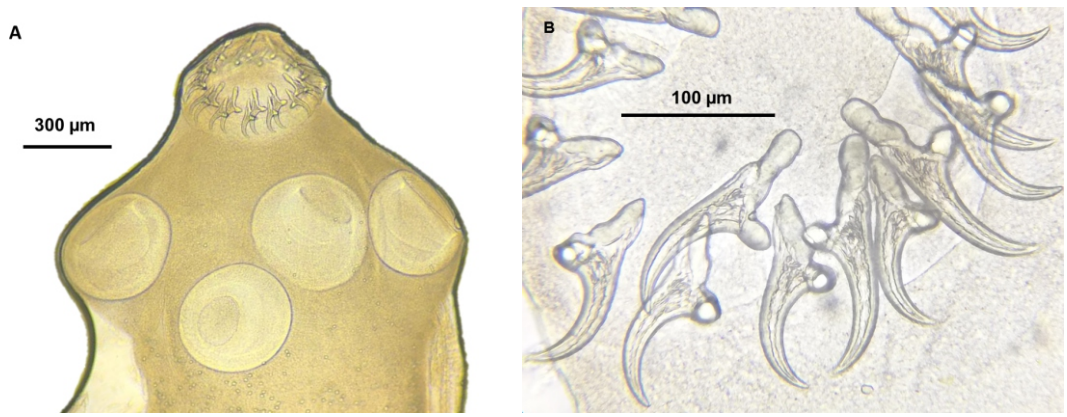

Figure 2. A) Evaginated metacestodes were observed with a double row of hooks. B) Small and large rostellar hooks of a *T. solium* metacestode.

Table 1. Measurements (µm) of rostellar hooks of *T. solium* metacestodes, extracted from a backyard pig (Zacapa, Guatemala). The morphometric parameters were based on the system used by Haukisalmi *et al.* (2011).

Small hooks	n=25		
	Mean	Range	SD
Total length (TL)	125.9	107-135	6.8
Total width (TW)	45.3	36-55	4.4
Basal length (BL)	66.5	55-72.5	5.0
Apical length (AL)	68.2	62.5-77	3.7
Blade curvature length (BCL)	56.7	50-62.5	3.3
Blade curvature (BC)	16.7	15-17.5	1.1
Guard width (GW)	19.1	17.5-25	1.8
Guard length (GL)	20.4	17.5-22.5	1.8
Handle width (HW)	21.7	17.5-25	2.6
Large hooks	n=25		
	Mean	Range	SD
Total length (TL)	163.3	150-175	5.9
Total width (TW)	47.5	37.5-57	5.5
Basal length (BL)	81.0	67.5-9	5.5
Apical length (AL)	87.9	85-92.5	5.1
Blade curvature length (BCL)	76.6	60-82.5	4.4
Blade curvature (BC)	15.2	10-17.5	1.91
Guard width (GW)	19.0	15-22.5	2.8
Guard length (GL)	24.1	22-25	1.2
Handle width (HW)	19.3	17.5-25	2.3

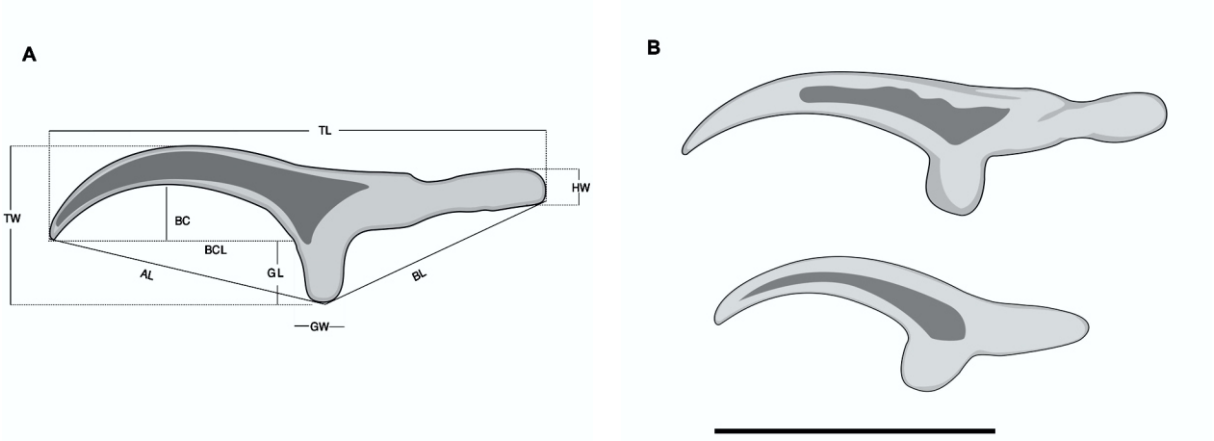

Figure 3. A) Morphometric parameters used for morphological analysis and B) examples of the shapes of the small and large rostellar hooks of *T. solium* metacestodes (scale bar 100 µm).

DISCUSSION

Although we could only examine one backyard pig for the diagnosis of swine cysticercosis, this research sample was very important in order to start new epidemiological surveys and discover new foci of the taeniasis/cysticercosis complex in rural communities from Zacapa, Guatemala. The infection intensity of metacestodes of *T. solium* was excessively high, considering that exceptionally heavy infections are classified from 10,000 metacestodes (Lightowlers *et al*., 2015).

Since the genus *Taenia* was erected by Linnaeus (1758), the most significant morphological

features have been the size, number and measurements of the rostellar hooks (Loos-Frank, 2000). Although the morphology of *T. solium* is specifically known, new morphometric parameters have been proposed and used for the description of rostellar hooks in various species of the genus *Taenia* (Haukisalmi *et al*., 2011). To enhance the knowledge about the morphology of the rostellar hooks of *T. solium* we have used nine morphometric measurements. The results of our study are similar to those reported by Verster (1969) (Brazil and Poland), Boa *et al.* (1995) (Tanzania) and Chawhan *et al.* (2014) (India) concerning the total length of the small and large hooks of the larval stage of *T. solium*; mean: 125-171 µm (small/large); 120-169 µm; 118-159 µm, respectively. Schmidt *et al.* (2015) reported three parameters: total length (128-185 µm), width (47.5-70 µm) and apical length (62.5-97.5µm). Interestingly, all these measurements were similar to the results we obtained from small hooks. Whereas the large hooks we examined were smaller for the three parameters. The blade curvature length was described by Chawhan *et al.* (2014) (mean: 65.6-89.3 µm), and our results differ significantly; we found shorter blade curvatures of small and large hooks. Nevertheless, in that study the point that origin the curvature, between the guard (distal part) and the blade, was not considered as a reference point, and the origin was taken into account from the medial part of the guard. Thus, the differences are due to the use of this inaccurate point of origin. The rest of the morphometric parameters have not been considered in any other study of *T. solium*. However, it would be important to compare the complete morphology of rostellar hooks from specimens of different geographical regions, continents and different genotypes to determine if intraspecific morphological variation exists in metacestodes and adult *T. solium*, as it has been observed in *T. hydatigena*, another species that parasitize domestic pigs (Filip *et al.,* 2019).

The overlap of *T. solium* and *T. hydatigena* is more common than it was thought, and various studies have confirmed the presence of both species in domestic pigs (Conlan *et al*., 2012; Chembensofu *et al*., 2017; Chaisiri *et al*., 2018). Other studies have reported *T. solium* metacestodes in the viscera of pigs (Ostertag, 1913; Chembensofu *et al.*, 2017). Therefore, when sufficient metacestodes are available and are suspected to be of *T. solium* and *T. hydatigena*, morphological differentiation should be considered, specifically when *T. hydatigena* metacestodes are immature and small cysts are found in the liver, lungs and mesentery of pigs. Although the confirmation of *Taenia* spp. must be performed by molecular techniques and sequencing, morphological analysis can provide a quick identification guide for the genus *Taenia* when necropsies are performed in backyard/free-roaming pigs from rural communities. Histological examination for identification of hooks in small cysts with light microscopy is also recommended (L'Ollivier *et al*., 2012). There is limited literature with morphological descriptions of *T. hydatigena* rostellar hooks from swine. However, Filip *et al.* (2019) reported a complete morphometric analysis of metacestodes extracted from the livers of wild boars (*Sus scrofa* Linnaeus, 1758). The main morphological differences concerning the hooks of *T. solium* found are the following: The shape of the blade of *T. hydatigena* hooks is more curved; the basal, apical and guard lengths are significantly larger (77.7-136.3 µm); (85.2-103.1 µm); (30.7-36.4 µm) and were thicker according to width measurements (74.3-74.6 µm). *Taenia hydatigena* hooks are larger for all morphometric parameters. Nevertheless, more studies of metacestodes extracted from pigs of both species, are needed to accurately determine the morphometric differences of the rostellar hooks.

This study provides a better morphological analysis of *T. solium* metacestodes and can be used as a quick guide to identify morphological differences of rostellar hooks, particularly when unknown cysts of similar size are found in different organs, primarily those suspected to be of *T. hydatigena*. Additionally, a massive infection of *T. solium* metacestodes was reported. These results indicate the probable endemicity and active transmission of *T. solium* in rural areas of Zacapa, Guatemala.

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