Published in final edited form as:
Trop Med Int Health. 2010 September ; 15(9): 1077–1081. doi:10.1111/j.1365-3156.2010.02579.x.

Differentiating *Taenia* eggs found in human stools - Does Ziehl Neelsen staining help?

Juan A. Jimenez[1], **Silvia Rodriguez**[1,2], **Luz M. Moyano**[1], **Yesenia Castillo**[1], and **Héctor H. García**[1,2,3] **for the Cysticercosis Working Group in Peru**

[1]Department of Microbiology, School of Sciences, (JJ, SR, YC, HG) and Center for Global Health - Tumbes (LM, HG), Universidad Peruana Cayetano Heredia, Av. H. Delgado 430, SMP, Lima 31, Lima, Perú

[2]Cysticercosis Unit, Instituto Nacional de Ciencias Neurologicas, Jr. Ancash 1271, Lima 1, Lima, Perú

[3]Instituto Peruano de Parasitología Clínica y Experimental (INPPACE), Lima, Perú

SUMMARY

Introduction—Unlike other tapeworms, *T. solium* infections carry risk for neurocysticercosis. Differential diagnosis of human tapeworm infections relies on morphology of the scolex or proglottids, frequently unavailable. DNA-based assays are poorly available in endemic areas. Ziehl Neelsen staining has been suggested but not tested in controlled designs. We validated whether Ziehl Neelsen staining could differentiate *T. solium* and *T. saginata* eggs.

Methods—Tapeworm proglottids (33 specimens, 23 *T. solium* and 10 *T. saginata*) and eggs (31 specimens, 13 *T. solium* and 10 *T. saginata*) were stained. Four eggs from each sample were measured and average diameters were recorded.

Results—*T. saginata* eggs stained entirely magenta in seven of 13 cases. *T. solium* eggs stained entirely blue/purple in 4/18 cases and entirely magenta in one. Eggs of *T. saginata* were slightly larger and always ovoid, while *T. solium* eggs were smaller and were mostly spheric.

Conclusions—Ziehl Neelsen staining can occasionally distinguish fully mature *T. solium* from *T. saginata* eggs. This distinction is poorly sensitive and not completely specific. Differential staining suggest differences in embryophore components between species, evident along egg maturation. In this small series, egg morphology (shape, maximal diameter) provided appropriate differentiation between *T. solium* and *T. saginata* eggs.

Keywords

Taenia; *Taenia solium*; *Taenia saginata*; Ziehl Neelsen; cestodes; Perú

INTRODUCTION

Three big tapeworms lodge in the human intestine: *Diphyllobothrium* sp, *Taenia solium*, and *Taenia saginata*, with a fourth, *Taenia asiatica*, still in debate on whether it is a new species or a *T. saginata* subspecies.(Flisser *et al.*, 2004; Garcia *et al.*, 2007) From these, only *Taenia solium* can lead to severe disease because of its capacity to infect the human brain with its larval form causing neurocysticercosis, the major cause of acquired epilepsy in most of the

[*]**Corresponding author:** Hector H. Garcia, Department of Microbiology, Universidad Peruana Cayetano Heredia, Avenida H. Delgado 430, SMP, Lima, 31, Perú. Telephone: +511 328 7360 Fax: +51 1 3284038 hgarcia@jhsph.edu.

Europe PMC Funders Author Manuscripts

world.(Garcia & Del Brutto, 2005) The differential diagnosis between these tapeworms is based on morphology of the adult tapeworm scolex or proglottids. In most cases, however, only tapeworm eggs are found in stool samples, and no parasite tissue is available. Although *Diphyllobothrium* eggs are easily distinguishable, eggs from *T. solium* and *T. saginata* can not be differentiated by microscopical examination. Only DNA-based probes have obtained specific identification between these eggs; this type of assays are hardly available in endemic areas.(Flisser *et al.*, 2004; Garcia *et al.*, 2007; Gonzalez *et al.*, 2000; Mayta *et al.*, 2000)

A few decades ago, Capron, A. and Rose, F. (1962) described the use of the acid-fast (Ziehl Neelsen) staining to distinguish *T. solium* from *T. saginata*.(Capron & Brygoo, 1959; Capron & Rose, 1962) While their work is occasionally quoted, it has neither been replicated nor refuted. We examined fresh and preserved material from both parasite species to validate whether this method can differentiate between these two tapeworm species.

MATERIAL AND METHODS

Species diagnosis of tapeworm material

Expelled parasite material from human origin was used for this study. Cases were defined as *T. solium* or *T. saginata* on the basis of carmine staining and counting of main uterine branches (Flisser *et al.*, 2004; Garcia *et al.*, 2007; Mayta *et al.*, 2000) as well as PCR in tapeworm material.(Mayta *et al.*, 2000) There were no discordances between results with either method.

Tapeworm proglottids

Pre-existing archive parasite material was used for this part of the study, comprising mature and gravid proglottids obtained from 33 patients (23 *T. solium* carriers, 10 *T. saginata* carriers) after anti-parasitic treatment.(Jeri *et al.*, 2004) In some cases multiple gravid and pre-gravid proglottids were available (14 and 9 proglottids from two *T. solium* tapeworms, and 7 and 4 proglottids from two *T. saginata* tapeworms) and were processed to assess changes in staining according to proglottid maturation. As per our standard routine, proglottids were washed with distilled water, fixed in 10% formalin-phosphate buffered saline (PBS) and stored at room temperature to be later processed by histology.

Defined fresh and archive stool samples

Eggs from 8 pre- or post- treatment stool samples (2 *T. saginata* and 6 *T. solium*) were examined and stained in fresh, before any fixation. Also archive stool samples from 23 patients, preserved in 5% formalin-PBS, were used for staining (11 *T. saginata*, 12 *T. solium*). Stool samples were concentrated by tube sedimentation. Sediments were placed in microscopy slides with polilysine and left to dry for staining. Four eggs from each sample were measured and the average maximal and transversal diameters were recorded (Table 1).

Histology

Proglottids were washed to eliminate the excess of formalin and then passed through increasing ethanol concentrations (70°, 80°, 90°, and 100°) and then three times in xilol. Proglottid samples were then placed in paraffin blocks, sliced in 6 um sections, de-paraffined, and placed on microscopy slides with polilysine for staining (Luna, 1968).

Ziehl-Neelsen staining

Samples were stained with carbol-fuchsin 3% for 15′, washed with tap water, and then decolored with 70% ethanol 1% HCl for 2′. After a second washing the slide was contrasted

with 3% methylene blue for 5′, washed again, and left to dry at room temperature.(Chapin & Lauderdale, 2007; Clavel *et al.*, 1999).

RESULTS

Staining of *Taenia* eggs in proglottid material

Staining of more proximal and more distal gravid *T. solium* and *T. saginata* proglottids showed that the oncospheres always stain blue in both species, with magenta hooks. As the eggs mature, a blue oncospheral membrane is clearly defined, around which magenta blocks begin to form the embryophore. A substance apparently secreted from the oncosphere then begins to fill the space between blocks. This substance is initially blue in both species. As the embryophore matures and becomes thicker, coloration gets more intense, departing from blue to gradually acquire some mixed magenta tones in *T. solium*, and a more marked magenta color in material from *T. saginata* (Figure 1).

Staining of *Taenia* eggs from stool samples

There was no difference in staining of eggs from fresh versus preserved stool samples. In *T. saginata* eggs the external cover or embryophore was colored entirely magenta on Ziehl Neelsen in 7/13 cases (Figure 2a), and magenta with dark blue (some close to dark purple) areas in the remaining six. In *T. solium* eggs the embryophore stained usually in a mix of magenta and blue being entirely blue in four of the 18 cases (Figure 2b) and entirely magenta in one case (Table 1).

Some apparent morphological differences could also be observed. The eggs of *T. saginata* were slightly larger, with a maximal diameter of 35.58 +/− 0.91 μm compared with 32.08 +/− 1.45 μm for *T. solium* eggs (n=13 for *T. saginata*, n=18 for *T. solium*; mean +/− SD; $p<0.001$, Mann Whitney test). *T. saginata* eggs were always ovoid (ratio between larger diameter and its transverse diameter was 1.14+/− 0.07), while most *T. solium* eggs were spheric (ratio was 1.03+/− 0.03; $p<0.001$ compared to *T. saginata*, Mann Whitney test). In 3 out of 18 cases, however, *T. solium* eggs looked ovoid in shape (ratios 1.11, 1.08, and 1.07) (Table 1).

In direct comparison of sensitivity and specificity with *T. saginata* and *T. solium* eggs, egg size and form were better predictors for species differentiation than Ziehl Neelsen staining (Table 2).

DISCUSSION

Taenia spp. eggs are covered by a thick embryophore composed by prismatic keratin blocks (which give it its typical radial appearance), kept together by a cement substance. By the time they reach the environment the embryophore is still surrounded by a colloid vitellum layer. It has been previously described that the vitelogen glands in the oncosphere produce a acid-fast resistant substance which takes the spaces between embryophoric blocks, likely responsible by the changes in coloration along egg maturation.(Capron & Brygoo, 1959; Capron & Rose, 1962)

This series showed that Ziehl Neelsen staining can occasionally distinguish fully mature *T. solium* from *T. saginata* eggs, in cases where the cover is entirely magenta (7/13 *T. saginata*), or entirely blue/purple (4/18 *T. solium*). Staining was mixed in 19/31 cases, and equivocal (magenta) in one case of *T. solium.* While this distinction provided a species diagnosis in 35% of cases, it is by no means absolute and seems poorly useful in practice. Its application assumes eggs are fully mature (which is not possible to determine in a proglottid), and carries some degree of subjectivity because color differences may be subtle,

Europe PMC Funders Author Manuscripts

adding to the requirement of highly trained personal. In most centers, the numbers of specimens to be tested for species differentiation will be small, as will be the experience of the operators. Oncospheres stained blue in both species, not helping in differentiation. Morphological criteria (diameter and shape) seemed more consistent in this series. In this small series, an arbitrary cut off of 35 μm had 100% predictive value for differentiation. This had been reported before (Verster, 1969) but not really replicated. Morphological differences are however minor and would need to be replicated with *T. saginata* and *T. solium* material from other parts of the world.

Of interest, our data suggest differences in specific components of the embryophore between these two species, evident along egg maturation, compatible with previous data on different chemical composition of embryophoric blocks.(Morseth, 1966) Given that the ability of the oncosphere of *T. solium* to infect the human host and cause cysticercosis is not shared by *T. saginata,* further understanding and characterization of the enzymes and other active molecules present in one species but absent in the other may provide species-specific diagnostics and potential vaccine targets.

Acknowledgments

FINANCIAL SUPPORT

This work was partially supported by the Fogarty International Center, National Institutes of Health, Bethesda, USA (H.G., training grant number D43 TW001140); and the Bill and Melinda Gates Foundation (H.G., grants number 23981 and 33848).

REFERENCES

Capron A, Brygoo ER. [Constitution of helminth eggs. I. Presence and formation of an acid-alcohol resistant substance in the shell]. Bull Soc Pathol Exot Filiales. 1959; 52:574–577. [PubMed: 13807576]

Capron A, Rose F. [On the composition of helminth eggs. II. Alcohol-acid-resistance in cestodes. Difference of Ziehl stainability of embryophores of *Taenia saginata* and *Taenia solium*]. Bull Soc Pathol Exot Filiales. 1962; 55:765–767. [PubMed: 14018471]

Chapin, K.; Lauderdale, TL. Reagents, stains, and media: bacteriology. In: Murray, PR.; Baron, EJ.; Jorgensen, JH.; Landr, ML.; Pfaller, MA., editors. Manual of Clinical Microbiology. 9th ed. American Society for Microbiology; Washington, D.C: 2007. p. 344

Clavel A, Varea M, Doiz O, et al. Visualization of hydatid elements: comparison of several techniques. Journal of Clinical Microbiology. 1999; 37:1561–1563. [PubMed: 10203521]

Flisser A, Viniegra AE, Aguilar-Vega L, Garza-Rodriguez A, Maravilla P, Avila G. Portrait of human tapeworms. Journal of Parasitology. 2004; 90:914–916. [PubMed: 15357104]

Garcia HH, Del Brutto OH. Neurocysticercosis: updated concepts about an old disease. Lancet Neurology. 2005; 4:653–661. [PubMed: 16168934]

Garcia, HH.; Jimenez, JA.; Escalante, H. Cestodes. In: Murray, PR.; Baron, EJ., editors. Manual of Clinical Microbiology. ASM Press; Washington DC: 2007. p. 2166-2174.

Gonzalez LM, Montero E, Harrison LJ, Parkhouse RM, Garate T. Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. Journal of Clinical Microbiology. 2000; 38:737–744. [PubMed: 10655377]

Jeri C, Gilman RH, Lescano AG, et al. Species identification after treatment for human taeniasis. Lancet. 2004; 363:949–950. [PubMed: 15043964]

Luna, LG., editor. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Ed. McGraw-Hill; New York: 1968. p. 28

Mayta H, Talley A, Gilman RH, et al. Differentiating *Taenia solium* and *Taenia saginata* infections by simple hematoxylin-eosin staining and PCR-restriction enzyme analysis. Journal of Clinical Microbiology. 2000; 38:133–137. [PubMed: 10618076]

Morseth DJ. Chemical composition of embryophoric blocks of *Taenia hydatigena, Taenia ovis*, and *Taenia pisiformis* eggs. Experimental Parasitology. 1966; 18:347–354. [PubMed: 5949306]

Verster A. A taxonomic revision of the genus *Taenia* Linnaeus, 1758 *s. str*. Onderstepoort Journal of Veterinary Research. 1969; 36:3–58. [PubMed: 5407584]

Europe PMC Funders Author Manuscripts

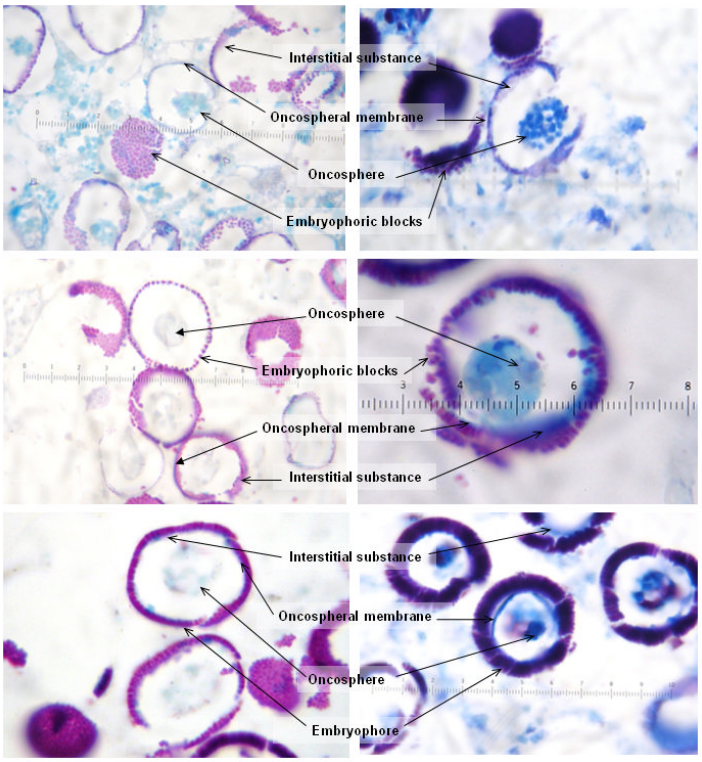

Figure 1.
Histological sections showing stages of maturation of eggs in proglottids of *Taenia saginata* (left) and *Taenia solium* (right) showing oncospheres surrounded by an oncospheral membrane, small, magenta embryophoric blocks, and interstitial substance.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

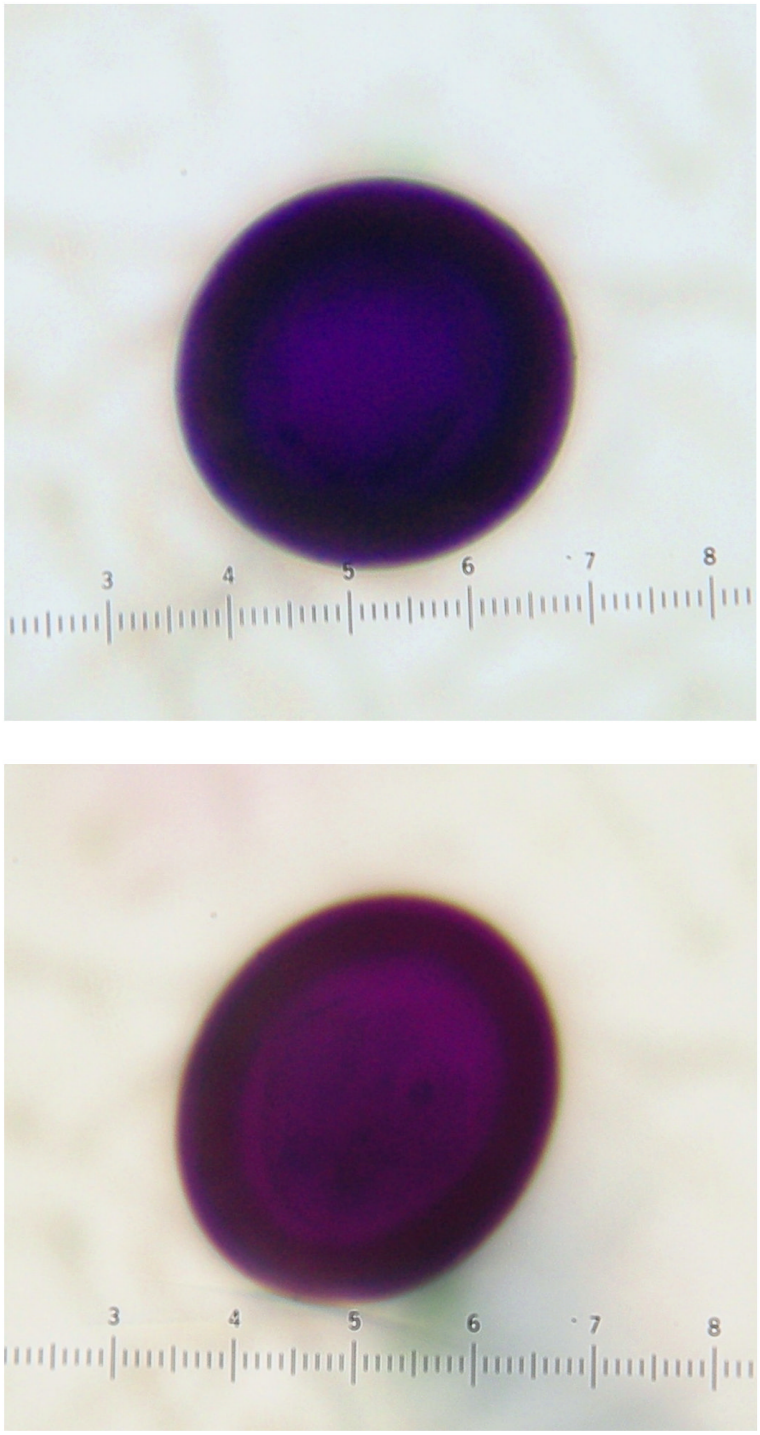

Figure 2.
Mature eggs of *Taenia solium* (left) and *Taenia saginata* (right) as seen in stool samples, showing differences in staining tonalities.

Europe PMC Funders Author Manuscripts

Table 1

Ziehl Neelsen staining and morphological characteristics of *T. solium* and *T. saginata* eggs in fresh and preserved stool samples.

	Fresh /		Ziehl Neelsen	Maximal	Transversal
487	Fresh	*T. saginata*	Magenta	36.5	34
492	Fresh	*T. saginata*	Magenta	36	32.5
268	Preserved	*T. saginata*	Magenta	35	28
274	Preserved	*T. saginata*	Magenta/blue	35	28
279	Preserved	*T. saginata*	Magenta	38	31
301	Preserved	*T. saginata*	Magenta/blue	36	32
421	Preserved	*T. saginata*	Magenta	36	30
453	Preserved	*T. saginata*	Magenta	35	32.5
492	Preserved	*T. saginata*	Magenta	35	32.5
500	Preserved	*T. saginata*	Magenta/blue	35	30
508	Preserved	*T. saginata*	Magenta/blue	35	32.5
536	Preserved	*T. saginata*	Magenta/blue	35	32.5
543	Preserved	*T. saginata*	Magenta/blue	35	32.5
402	Fresh	*T. solium*	Magenta/blue	32.5	32.5
411	Fresh	*T. solium*	Magenta/blue	32.5	32.5
459	Fresh	*T. solium*	Magenta/blue	32.5	32.5
461	Fresh	*T. solium*	Magenta/blue	32	30
472	Fresh	*T. solium*	Magenta/blue	31	30
489	Fresh	*T. solium*	Magenta/blue	33.5	32.5
403	Preserved	*T. solium*	Magenta	32	32
405	Preserved	*T. solium*	Blue	29	29
408	Preserved	*T. solium*	Magenta/blue	32.5	32
409	Preserved	*T. solium*	Magenta/blue	33	32.5
410	Preserved	*T. solium*	Blue	32	32
411	Preserved	*T. solium*	Magenta/blue	33.7	32
413	Preserved	*T. solium*	Magenta/blue	32.5	32.5
428	Preserved	*T. solium*	Magenta/blue	32.5	31.5

	Fresh /		Ziehl Neelsen	Maximal	Transversal
457	Preserved	*T. solium*	Magenta/blue	32.5	30
458	Preserved	*T. solium*	Blue	33.3	30
472	Preserved	*T. solium*	Blue	32.5	31.5
474	Preserved	*T. solium*	Magenta/blue	28	28

* Values correspond to the assessment of all examined egs (staining) or to the mean value obtained from measuring 4 eggs from the same sample (diameters).

Europe PMC Funders Author Manuscripts

Table 2

Sensitivity and specificity of Ziehl Neelsen staining and egg morphological to differentiate *T. solium* and *T. saginata* eggs.

Criteria	Species	Sensitivity	Specificity	Youden's J
Entirely magenta	*T. saginata*	54% (7/13)	96% (17/18)	0.50
Entirely blue/purple	*T. solium*	50% (4/8)	100% (13/13)	0.50
Ovoid	*T. saginata*	100% (13/13)	83% (15/18)	0.83
Spheric	*T. solium*	83% (15/18)	100% (13/13)	0.83
> 35 μm	*T. saginata*	100% (13/13)	100% (18/18)	1.00
< 35 μm	*T. solium*	100% (18/18)	100% (13/13)	1.00

*Values for spheric shape or maximal diameter < 35 μm as criteria to define *T. solium* are inverse to those of ovoid shape or larger eggs for *T. saginata*, due to direct group comparison