

SHORT COMMUNICATION

Isozyme Analysis of *Taenia solium* Isolates from Mexico and Colombia

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Mexican and Colombian Taenia solium cysticerci and some species of Taenia adults were assayed using cellulose acetate electrophoresis to distinguish between isolates. Isozyme patterns for ARK, GOT, G3PD, GPI, and MPI were identical in all cysticerci suggesting homozygotic profiles. G6PD and MDH showed different patterns between Mexican and Colombian cysticerci, suggesting regional differences. ME activity was mainly detected in the adult stage suggesting that this enzyme is active in anaerobic environment, while MDH, detected in cysticerci, could be related to an environment that contains oxygen. Finally, the species of taeniid adults analyzed showed different patterns among them.

Key words: *Taenia solium* - isozymes - cysticerci - Mexico - Colombia

Taeniosis and cysticercosis are two diseases caused by the cestode *Taenia solium*. Thompson and Lymbery (1995) argue that knowledge of genetic structure of cestodes can be applied to the epidemiology and the control of these parasites, because genetic variation within and between populations, determines future evolutionary changes, genetic differentiation, and speciation. In order to distinguish within isolates from *T. solium*, isozyme electrophoresis analysis was performed in parasites recovered from Mexico and Colombia, and in adult tapeworms.

Ten *T. solium* cysticerci were excised from each of seven naturally infected pigs from central states of Mexico and one pig from Colombia, parasites were washed with 0.01 M phosphate-buffered 0.15 M saline, pH 7.2 (PBS), dried over filter paper, frozen (-70°C) and individually analyzed for isozymes. Mexican immature proglottids of *T. solium* and *T. saginata* adult worms from human hosts recovered prior to cestocidal treatment were frozen and similarly analyzed. Immature proglottids from *Taenia taeniaeformis* recovered from experimental cat infections were also used. Cellulose acetate (CA) electrophoresis of enzymes was conducted according to the methods of Hebert and Beaton (1993) and Johnston and Fernando (1997). Eight isoenzymes were tested: ARK, EC. 2.7.3.3; G6PD, EC. 1.1.1.49; GPI, EC. 1.2.1.12; G3PD, EC. 1.2.1.12;

GOT, EC. 2.6.1.1; MDH, EC, 1.1.1.37; ME, EC. 1.1.1.40; MPI, EC. 5.3.1.8. Individual parasites were homogenized in an equal weight-volume ratio of distilled water and centrifuged in at 14,000 rpm for 15-30 s. Supernatants were chilled on ice and loaded onto Titan III 76x76 mm CA plates (Helena Laboratories, Beaumont, TX). Electrode buffers and electrophoresis conditions are outlined in the Table. One "hit" (~ 3 µl) per plate for each enzyme was delivered employing the Super Z-12 applicator kit (Helena Laboratories, Beaumont, TX). Soluble meat extracts from a healthy pig were used as control.

The electrophoretic mobility of G6PD, GPI, GOT, ARK, and G3PD from all cysticerci recovered from the isolates showed only one band of activity, MDH and ME (only for *T. solium* adult) showed 2 bands and MPI presented 3 bands making a total of 10 putative alleles. Enzyme mobility was the same for Mexican and Colombian cysticerci, except for GPI and MDH that were slightly faster with Colombian than Mexican cysticerci (Fig. 1A). Each *Taenia* species tapeworm tested showed its own enzyme pattern, being clearly distinct for GPI, ME (data not shown), and MPI (Fig. 1B). Activity of ME was observed in adult tapeworms but not in cysticerci, suggesting to be stage-specific. To confirm this observation, two other methods were used: isoelectrofocusing and spectrophotometry as described by Ponce-Macotela et al. (1993). The former confirmed this result, while the latter measured 4 times less activity in cysticerci than in adult parasites (0.122 vs 0.512 absorbance values at 570 nm using 20 mg/ml total protein in each extract), indicating that although there is some activity in cysticerci it is higher in adult worms. Isozyme profiles from cysticerci were different from those seen in pig meat (Fig. 1, lane 1) which confirms lack of meat contamination.

The trimeric pattern of GPI from *T. solium*, *T. saginata*, and *T. taeniaeformis* was according to profiles published by Le Riche and Sewell (1977) as well as Okamoto and

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TABLE
Electrophoresis conditions

Enzyme	Tank buffer	Time (min)	Volts
G6PD, MDH, G3PD	CAAPM ^a	120	38
GOT, GPI	TM ^b	120	38
ARK, ME, MPI	TM	20	170

^a: 0.22 M citric acid, 5% (v/v) 4-(3-aminopropyl) morpholine, pH 8.2; ^b: 0.25M TRIS, 0.05 M malic acid, pH 7.8

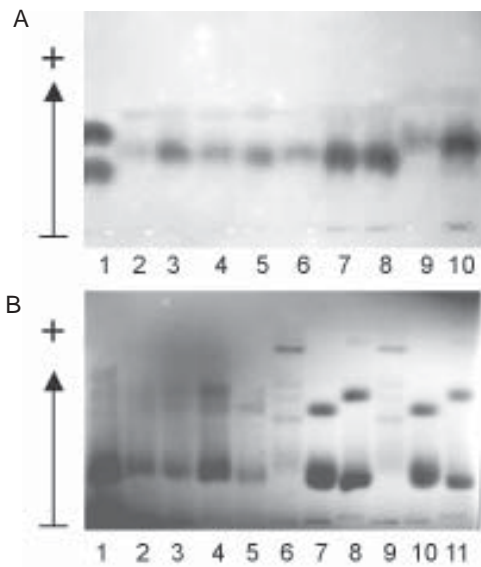


Fig. 1: isoenzyme patterns - A: MDH from Mexican cysticerici (lanes 2-8), and Colombian cysticerici (lanes 9-10); B: MPI from Mexican cysticerici (lanes 2-5) adult *Taenia taeniaeformis* (lanes 6 and 9), adult *T. solium* (lanes 7 and 10), and adult *T. saginata* (lanes 8 and 11). Swine muscle extract was analyzed in lanes 1.

colleagues (1995). Housekeeping enzymes, such as GPI, therefore, confer a phenotypic identity to each parasite, and could be used for differential diagnosis with modern molecular methods. Various enzymes have been tested in order to learn about evolution ecology and population genetics of cestodes (Okamoto et al. 1995, Siles-Lucas et

al. 1996, Turèková et al. 2003). In the present study no differences in the migration of 5 isoenzyme profiles (ARK, GOT, G3PD, GPI, and MPI) were found suggesting homozygotic profiles. On the other hand, the mobility of G6PD and MDH was slightly faster in Colombian than in Mexican cysticerici suggesting regional differences that have to be confirmed with a larger sample of cysticerici from Colombia or from other countries. The presence of detectable ME activity in the adult stage can be explained because this enzyme has high activity in anaerobic environment (like the intestinal lumen), while MDH, that was only detected in cysticerici, has better activity in an environment that contains oxygen (such as skeletal muscle, where the *T. solium* cysticerici develop); this feature could be exploited in studies focused for the treatment and control of this parasite.

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