



## RESEARCH NOTE/ NOTA CIENTÍFICA

### DIAGNOSIS OF EXPERIMENTAL INFECTION BY *ECHINOSTOMA PARAENSEI* LIE AND BASCH, 1967 (TREMATODA, ECHINOSTOMATIDAE)

### DIAGNÓSTICO DE LA INFECCIÓN EXPERIMENTAL POR *ECHINOSTOMA PARAENSEI* LIE Y BASCH, 1967 (TREMATODA, ECHINOSTOMATIDAE)

Vinicius M. Tunholi-Alves<sup>1</sup>, Victor M. A. Tunholi<sup>1</sup>, Sócrates Costa-Neto<sup>1</sup>, Ludmila S Amaral<sup>2</sup>, Arnaldo Maldonado Jr<sup>3</sup>, Jairo Pinheiro<sup>4</sup> & Maria de Lurdes de A. Rodrigues<sup>5</sup>

<sup>1</sup>Programa de Pós-Graduação em Ciências Veterinárias, Deptº Parasitologia Animal, Instituto de Veterinária, UFRuralRJ;

<sup>2</sup>Programa de Pós-Graduação em Saúde Pública, Departamento de Saneamento e Saúde Ambiental, Escola Nacional de Saúde Pública, Fiocruz, RJ; <sup>3</sup>Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Instituto Oswaldo Cruz, Fiocruz, RJ; <sup>4</sup>Área de Biofísica, Departamento de Ciências Fisiológicas, Instituto de Biologia, UFRuralRJ; <sup>5</sup>Deptº de Parasitologia Animal, Inst. Veterinária, UFRuralRJ.- BR 465 Seropédica, RJ, 23890-000, Brazil.

mlazrod@uol.com.br

Neotropical Helminthology, 2015, 9(1), jan-jun: 171-176.

## ABSTRACT

---

The diagnosis of infection by trematodes is essentially based on the microscopic observation of eggs of parasites in feces, through qualitative and quantitative techniques. In this study, we investigated the efficiency and sensitivity of the techniques of Kato-Katz and Hoffman used to diagnose *Echinostoma paraensei* in infected and uninfected hamsters under experimental conditions. The method of Hoffman showed the highest sensitivity and efficiency when compared with the Kato-Katz method for the diagnosis of *E. paraensei*.

---

**Keywords:** *Echinostoma paraensei* - efficiency - Hoffman - Kato-Katz - sensitivity.

## RESUMO

---

El diagnóstico de la infección por tremátodos se basa esencialmente en la observación microscópica de los huevos de parásitos en las heces, a través de técnicas cualitativas y cuantitativas. En este estudio, se investigó la eficacia y la sensibilidad de las técnicas de Kato-Katz y Hoffman que se utilizan para diagnosticar *Echinostoma paraensei* en hámsters infectados y no infectados en condiciones experimentales. El método de Hoffman mostró mayor sensibilidad y eficacia en comparación con el método de Kato-Katz para el diagnóstico de *E. paraensei*.

---

**Palabras clave:** *Echinostoma paraensei* - eficiencia - Hoffman - Kato -Katz - sensibilidad.

## INTRODUCTION

---

Trematodes diagnosis is essentially based on the microscopic observation of eggs of parasites in faeces, through qualitative and quantitative techniques. The Kato-Katz technique receives special attention, and is mainly used in schistosomiasis control programs, in addition to acting as an auxiliary tool in the quantitative and qualitative diagnosis of intestinal helminth infections (Kato & Miura, 1954; Katz *et al.*, 1972; Feldmeier & Poggensee, 1993). The efficiency and sensitivity of parasitological techniques have been the focus of some studies (Táparo *et al.*, 2006). The Kato-Katz, widely used as a standard for the diagnosis of *Schistosoma mansoni* Sambon, 1907, has shown over time, the results of effectiveness (Santos *et al.*, 2005) when compared with the sedimentation techniques (Martin & Beaver, 1968; Kongs *et al.*, 2001). Coproparasitologic techniques results allow estimating the intensity of the infection as main parameter for the evaluation of the pathology, and prognosis response to chemotherapy (Feldmeier & Poggensee, 1993). Adults of *Echinostoma* infect the small intestine definitive host, in which we highlight the waterfowl, mammals, including humans, reptiles and fish (Lie & Basch, 1967; Fried & Graczyk, 2000; Kanev *et al.*, 2000; Maldonado *et al.*, 2001a, b;). Serological techniques and molecular biology has been developed for the diagnosis of different helminths (Barbosa Campos *et al.*, 1988; Graczyk & Fried, 1994), but function as a complementary tool for research studies, restricting its use in routine laboratory because of the high cost of implementation. For the first time, a study was conducted comparing the techniques of Kato-Katz and Hoffman in the diagnosis of *Echinostoma paraensei* Lie & Basch, 1967 in hamsters experimentally infected in order to confirm the establishment of the fluke in experimental and clinical studies, assisting epidemiological studies focused on the

characterization of the dynamics of this helminthiasis.

## MATERIAL AND METHODS

---

### Samples

The cycle of *E. paraensei* is maintained in experimental Laboratory of Parasitology of Wild Mammals Reservoirs (LABPMR) of the Oswaldo Cruz Institute (IOC), FIOCRUZ, Rio de Janeiro, RJ, Brazil and faeces samples of 15 hamsters infected were collected with the aid of plastic bags previously identified and kept under refrigeration for twenty-four hours until processing.

### Parasitological Techniques

The experiment was conducted in LABPMR, IOC, FIOCRUZ, Rio de Janeiro, RJ. The fecal samples were homogenized, weighed and processed in duplicate by the techniques of Hoffman (Lutz, sedimentation) and Kato-Katz (García *et al.*, 1985).

### Lutz (natural sedimentation)

Approximately one gram of faeces was homogenized in water and the solution filtered through a gaze to a bent sedimentation cup of 100 ml, and the volume completed with tap water at room temperature. This suspension allowed to stand for 30 min and then examined under a light microscope using a 20x objective. This is the routine technique used in LABPMR.

### Kato-Katz

One gram of feces was placed on absorbent paper to remove excess moisture. Shortly thereafter, a nylon fabric was compressed with the aid of a spatula causing part of the stool pass through its meshes. These were collected with a spatula and pressed into the hole of a perforated plate situated on a slide, until the latter is full. The excess was removed with the feces side of the spatula. Subsequently, the perforated plate was removed, tilting initially

one end so as to remain on the glass slide of a cylinder fecal sample. About this cylinder was placed a cellophane coverslip, previously soaked in solution Diafix component of commercial Kit Kato-Katz. The slide was then inverted on a flat surface and pressed so as to spread the material evenly between the slide and coverslip cellophane preventing the leakage of feces. Waiting for 30 min, to clarify the fecal smear for t analysis in a light microscope.

#### Statistical analyses

To evaluate the sensitivity, efficiency, number of eggs recovered and to verify the normality of the data the Kolmogorov Smirnov analyses were conducted. The results were expressed as mean  $\pm$  standard error and submitted to the Tukey test for comparison of means ( $\alpha = 5\%$ ).

## RESULTS

The results indicated significant variations in their sensitivity, efficiency, and number of eggs recovered between the two methods applied in the diagnosis of fecal *E. paraensei* (Table 1). The method of Hoffman showed the highest sensitivity when compared to the method of Kato Katz, indicating positive in all samples. Regarding the number of eggs retrieved, the method of Kato Katz showed lower efficacy ( $14.35 \pm 62.05$ ) when compared to Hoffman method ( $165.60 \pm 11.22$ ), resulting in approximately a result 62.53% lower in the total number of eggs observed (Table 2).

**Table 1.** Samples and results of fecal eggs counting in hamsters by Kato-Katz and Hoffman techniques.

Hamsters number	Parasitological techniques			
	Kato-Katz (duplicate)		Hoffman (duplicate)	
1	30	47	95	90
2	12	29	85	82
3	13	45	105	67
4	130	75	124	92
5	31	89	85	77
6	12	24	69	105
7	28	-	94	83
8	66	21	91	98
9	09	37	86	79
10	54	30	94	88
11	43	77	95	75
12	17	-	70	97
13	-	-	69	57
14	-	-	98	68
15	-	72	78	96

**Table 2.** Comparison of Techniques Kato-Katz and Hoffman, percentage of positivity and mean number of eggs and standard error of *Echinostoma paraensei* in hamsters.  $\bar{X} \pm \text{SEM}$  Mean  $\pm$  standard error of the mean.

Techniques	N	Positivity (%)	$\bar{X} \pm \text{SEM}$
Kato-Katz	15	86.66 (13)	62.05 $\pm$ 14.35 <sup>a</sup>
Hoffman	15	100 (15)	165.60 $\pm$ 11.23 <sup>b</sup>

Different letters indicate mean that differ at the 5% level of significance.

## DISCUSSION

The data obtained revealed that the Kato-Katz method showed lower sensitivity (positive) and specificity when compared to Hoffman method for the diagnosis of *E. paraensei*, which is in agreement with Zaman & Cheong (1967) to *Schistosoma*, strengthen and even for eggs *Ascaris* and *Trichuris*. The calculation of eggs per g by the Kato-Katz method is based on the assumption that the density of a stool sample is equal to 1.0, and several factors such as consistency, preservation solution, parasitic load and physiology of the host can influence in the diagnosis of trematodíases (Scott, 1938) leading to numerous adaptations technique Kato-Katz (Engels *et al.*, 1993). In this study, the distribution of eggs of *E. paraensei*, followed the pattern of abnormality demonstrated by dispersion of the values around the average and large standard error, reflecting, thus, a limitation of this technique, which can be justified by work of Teesdale *et al.* (1985). These authors report that a dry stool sample can provide counts of eggs *S. mansoni* seven times greater than a moist sample obtained from the same host. Watery feces or very dry and thick fibrous could compromise the semi-quantitative diagnostic technique (Feldmeier & Poggensee, 1993). Moreover, in this study the technique of Lutz showed the highest sensitivity and efficacy for the diagnosis of eggs *E. paraensei* even the parasite load is low. Similar results were observed by Santos *et al.* (2005), in a comparative study of parasitological methods for diagnosis of intestinal helminthes

identifying the sedimentation technique spontaneously with sensitivity to the Kato-Katz. It is noteworthy that studies have shown higher efficiency of sedimentation techniques, especially the method of Lutz, for the diagnosis of trematodes (Palumbo *et al.*, 1976; Leal, 2003), which was also observed by Silva & Abboud (2001) in diagnosing *Platynosomum* sp. In this study 100% of samples were positive method of Hoffman *et al.* (1934), agreeing with the results of Holland (1993) with 85% of positive results for *S. mansoni* in relation to the method of Ritchie (68%), which leads us to consider the technique of Lutz as the most sensitive in the recovery of eggs *E. paraensei* compared with Kato Katz. Therefore it is recommended technique for diagnosis in Lutz experimental infection and epidemiological monitoring technique, depending on countries considered endemic, the cultural habit of eating from fish, crustaceans, amphibians and freshwater aquatic mollusks raw or undercooked containing metacercariae and is associated with low socio-economic conditions, increases the risk of transmission of human echinostomiasis, as well as other food-borne diseases.

## ACKNOWLEDGEMENTS

This study was supported by Conselho Nacional para o Desenvolvimento Científico e Tecnológico (CNPq); Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and PPGCV-UFRRJ.

**BIBLIOGRAPHIC REFERENCES**

- Barbosa Campos, DM, Oliveira, OS, Barbosa, W, Campos, LL, Rosa, ZS & Souza, OC. 1988. *Antígeno de Strongyloides cebus (Darling, 1911) no diagnóstico da estrongiloidíase humana*. Revista de Patologia Tropical, vol. 17, pp. 17-23.
- Engels, D, Ndoricimpa, S & Gryseels, B. 1993. *Schistosoma mansoni in Burundi: Progress in its control since 1985*. Bulletin of the World Health Organization, vol. 71, pp. 207-214.
- Feldmeier, H & Poggensee, G. 1993. *Diagnostic techniques in schistosomiasis control*. Acta Tropica, vol. 52, pp. 205-220.
- Fried, B & Graczyk, TK. 2000. *Echinostomes as experimental models for biological research*. Kluwer, Dordrecht. pp. 245-266.
- García, JA, Martín, AM, Pérez, MJ. 1985. *Valoración de los métodos utilizados em el diagnóstico de parasitosis intestinales*. Laboratório, vol.79, pp.473.
- Hoffman, VA, Pons, JS & Janer, JL. 1934. *Sedimentation concentration method in Schistosomiasis mansoni*. Puerto Rico Journal Public Health Tropical, vol. 9, pp. 283-298.
- Holland, CMCX. 1993. *Estudo comparativo entre as técnicas de sedimentação espontânea e a de centrifugo-sedimentação no diagnóstico coproparasitológico*. Revista Brasileira de Análises Clínicas, vol. 25, pp. 29-32.
- Kanev, I, Sterner, M, Radev, V & Fried, B. 2000. *An overview of the biology of echinostomes*. In: *Echinostomes as Experimental Models for Biological Research*. Eds. Bernard Fried, Taddeus Graczyk. Kluwer Academic Publ. Boston, London: 1-29.
- Kato, K & Miura, M. 1954. *Comparative examinations*. Japanese Journal of Parasitology, vol. 3, pp. 35.
- Katz, N, Chaves, A & Pellegrino, J. 1972. *A simple device for quantitative stool thick smear technique in Schistosomiasis mansoni*. Revista do Instituto de Medicina Tropical de São Paulo. vol. 14, pp. 397-400.
- Kongs, A, Marks, G, Verleâ, P & Stuyft, PVD. 2001. *The unreliability of the Kato-Katz technique limits its usefulness for evaluating Schistosoma mansoni infections*. Tropical Medicine and International Health, vol. 6, pp. 163-169.
- Leal, PDS. 2003. *Diagnóstico da infecção por Platynosomum fastosum (Braun, 1901) Kossack, 1910 (Trematoda: Dicrocoelidae) em gatos domésticos (Felis catus L.)*. 2003. Dissertação (Mestrado em Parasitologia) – Faculdade de Medicina Veterinária, Universidade Federal Rural do Rio de Janeiro.
- Lie, KJ & Basch, PF. 1967. *The life history of Echinostoma paraensei (Trematoda: Echinostomatidae)*. Journal of Parasitology, vol. 53, pp. 1192-1199.
- Maldonado, JrA, Coura, R, Garcia, JS, Lanfredi, RM & Rey, L. 2001. *Changes on Schistosoma mansoni (Digenea: Schistosomatidae) worm load in Nectomys squamipes (Rodentia: Sigmodontinae) concurrently infected Echinostoma paraensei (Digenea: Echinostomatidae)*. Memórias do Instituto Oswaldo Cruz, vol. 96, pp. 193-198.
- Maldonado, JrA, Locker, ES, Morgan, JÁ, Rey, L & Lanfredi, R. 2001. *Description of a new Brazilian isolate of Echinostoma paraensei (Platyhelminthes: Digenea) from its natural vertebrate host Nectomys squamipes by light and scanning electron microscopy molecular analysis*. Parasitology Research, vol. 87, pp. 840-848.
- Martin, LK & Beaver, PC. 1968. *Evaluation of Kato tick-smear technique for*

- quantitative diagnosis of helminth infections*. American Journal of Medical and Tropical Hygiene, vol. 17, pp. 382-391.
- Palumbo, NE, Taylor, DO & Perri, SF. 1976. *Evaluation of fecal technics for the diagnosis cat liver fluke infection laboratory*. Animal Science vol. 26, pp. 490-493.
- Santos, FLN, Cerqueira, EJM & Soares, NM. 2005. *Comparação das técnicas de sedimentação espontânea e Kato-Katz para diagnóstico das helmintoses intestinais*. Revista da Sociedade Brasileira Medicina Tropical, vol. 38, pp. 196-198.
- Scott, JA. 1938. *The regularity of egg output of helminth infestations, with special reference to Schistosoma mansoni*. American Journal of Hygiene. vol. 27, pp.155-175.
- Silva, LL & Abboud, LCS. *Estudo comparativo da Incidência de endoparasitoses entre felinos jovens e adultos*. In: *Anais do II Congresso Internacional de Medicina Felina – CIMFEL*. Rio de Janeiro. 2001. p. 30.
- Graczyk, TK & Fried, B. 1994. *Elisa method for detecting anti-echinostoma caproni (Trematoda; Echinostomatidea) immunoglobulins in experimentally infected mice*. Journal of Parasitology, vol. 80, pp. 544-549.
- Táparo, CV, Perri, SHV, Serrano, ACM, Ishizaki, MN, Costa, TP, Amarante, AFT & Bresciani, KDS. 2006. *Comparação entre técnicas coproparasitológicas no diagnóstico de ovos de helmintos e oocisto de protozoários em cães*. Revista Brasileira de Parasitologia Veterinária, vol. 15, pp. 1-5.
- Teesdale, CH, Fahringer, K & Chitsulo, L. 1985. *Egg count variability and sensitivity of a thin smear technique for the diagnosis of Schistosoma mansoni*. Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 79, pp. 369-373.
- Zaman, V & Cheong, CH. 1967. *A comparison of Kato thick smear technique with zinc sulfate flotation method for the detection of helminth ova in faeces*. Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 61, pp. 751.

Received January 8, 2015.

Accepted February 9, 2015.