

Comparison of the Kato-Katz and Helmintex methods for the diagnosis of schistosomiasis in a low-intensity transmission focus in Bandeirantes, Paraná, southern Brazil

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The diagnosis of schistosomiasis is problematic in low-intensity transmission areas because parasitological methods lack sensitivity and molecular methods are neither widely available nor extensively validated. Helmintex is a method for isolating eggs from large faecal samples. We report preliminary results of a comparative evaluation of the Helmintex and Kato-Katz (KK) methods for the diagnosis of schistosomiasis in a low-intensity transmission area in Bandeirantes, Paraná, southern Brazil. Eggs were detected by both methods in seven patients, whereas only Helmintex yielded positive results in four individuals. The results confirm the previously demonstrated higher sensitivity of the Helmintex method compared with the KK method.

Key words: schistosomiasis - low endemicity - parasitological diagnosis

Improvements in living conditions and the successful implementation of measures to control schistosomiasis have led to reductions in its transmission intensity, prevalence and parasitic burden in several areas (Crompton et al. 2003). These reductions have been detected in recently established foci, such as Esteio, in the southernmost Brazilian state of Rio Grande do Sul. In these areas, classical coproparasitological methods are insufficiently sensitive to reliably detect small numbers of eggs in faeces (Doenhoff et al. 2004). In Esteio, most infected individuals exhibit estimated egg concentrations of < 1 egg per gram (epg) of faeces (Graeff-Teixeira et al. 2004), which is far below the reliable detection capabilities of most methods. Helmintex is a new method for detecting *Schistosoma mansoni* eggs in stool samples that has recently been described and evaluated in seeding experiments (Teixeira et al. 2007). It allows the detection of lower egg concentrations than what is possible with conventional methods because it enables the processing of larger stool samples (30 g).

The objective of this work was to evaluate and compare the performance of the Helmintex and Kato-Katz (KK) methods for the first time in a population study in the schistosomiasis endemic area of Bandeirantes, Paraná (PR), southern Brazil. Eosinophil counts in peripheral blood samples were also evaluated as indicators of infection.

Bandeirantes is a municipality in northern PR (23°06'36"S 50°22'03"W) that covers approximately 446 km² and has approximately 34,000 inhabitants. The study area, Ouro Verde, was chosen based on the suggestion of the local public health service considering the observation of the active transmission of schistosomiasis, the lack of surveys in the past two years and an expected schistosomiasis prevalence of 15% in the area. The total population of Ouro Verde was 280 inhabitants at the time of this study.

The houses included in the study area were located along a street east of the PR-436 road, which runs to the east and turns 90° to the south to reconnect with the PR-436 road. Grassy and swampy fields lie at the northeastern and southeastern margins of the area. In the northeast, many snails were observed next to a street corner. A stream formed a small natural pool where children participated in recreational activities. Some individuals provided sporadic and spontaneous reports of fishing and washing (clothes and utensils) in the contaminated stream, undertaken mainly by individuals from the nearby houses. Most of the houses had recently been improved and many had been rebuilt in locations away from the stream. Although the local school is well organised and schistosomiasis is the focus of regular health education projects, many students were observed conducting leisure activities in contaminated streams, which illustrates the complexity of controlling schistosomiasis.

We aimed to obtain blood and faecal samples from 100 individuals in Ouro Verde, excluding children less than one year old. Sampling proceeded from the northern to southern branch of the street because the northern branch was closest to the transmission focus.

After informed consent was obtained from each participant, venous blood was collected and a thin distention was immediately prepared, air dried and fixed with

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methanol. Later, in the laboratory, the blood distension was stained with Giemsa to obtain eosinophil counts. Serum was separated by centrifugation and stored at 10°C in the field; it was subsequently stored at -20°C in the laboratory for future analysis. At least one total evacuation from each individual was collected in a 1-L plastic vessel with a lid. Blood and faecal samples were obtained from 77 individuals.

In the field laboratory, each faecal sample was weighed and a sample was separated for the KK thick smear. A measured amount of aqueous 4% formalin was added to completely cover the faecal material. The mass per millilitre was estimated by dividing the faeces mass by the volume of the formalin solution.

Two thick smears were prepared from each faecal sample according to the instructions of the manufacturer of the Helm Test (Brazil) and original description of the KK method (Katz et al. 1972). After 24 h, the slides were subjected to complete smear screening by two examiners, at least one of whom was skilled. For the Helminex method, a volume of the faecal homogenised suspension corresponding to 30 g of faeces was processed as described by Teixeira et al. (2007).

Eggs were detected by both methods in seven individuals (12, 12, 30, 96, 108, 174 and 192 epg), whereas only Helminex detected eggs in four individuals (Table I); egg counting was not performed in sediments produced *via* Helminex because part of the material was lost. The kappa estimation (0.75) indicates substantial agreement between the KK and Helminex methods (Landis & Koch 1977). Considering the results of both analyses, the estimated prevalence of schistosomiasis was 12.3% (11/77 individuals). Eosinophilia of > 4% was detected in 10/11 infected individuals, but was also present in 40/66 non-infected individuals, resulting in a 90.9% sensitivity, 39.4% specificity, positive predictive value of 20% and negative predictive value of 96.3% (Table II). The present data on blood eosinophilia do not support its controversial proposal as an indicator of *S. mansoni* infection, especially considering its poor specificity (Dawson-Hahn et al. 2010). The absence of eosinophilia may, however, indicate a lower probability of infection, considering its very high negative predictive value (96.3%) (Table II).

The difficulty of diagnosing schistosomiasis in areas of low-intensity transmission, such as Esteio, has prompted several efforts to improve the sensitivity of egg detection in stool samples, such as using the Percoll and saline density gradient methods (Eberl et al. 2002, Coelho et al. 2009) or polymerase chain reaction analyses of stool samples (Gomes et al. 2010, Oliveira et al. 2010), in addition to the examination of slides using the KK method or combinations of different methods (Siqueira et al. 2011).

The 714-fold higher stool sample volume examined by the Helminex method (30 g) compared with the 42 mg/slide by the KK method explains the higher probability of finding small numbers of eggs with the former method and its much higher expected sensitivity. Increasing the amount of stool examined to improve the sensitivity for schistosomiasis diagnosis was ini-

TABLE I
Comparison of Helminex and Kato-Katz (KK) methods for the diagnosis of schistosomiasis in a low-intensity transmission area (Ouro Verde, municipality of Bandeirantes, Paraná, Brazil) in 2006

KK	Helminex		Total
	Positive	Negative	
Positive	7	0	7
Negative	4	66	70
Total	11	66	77

TABLE II
Evaluation of blood eosinophilia (%) as an indicator for schistosomiasis in comparison with the detection of eggs in faeces

Blood eosinophilia	Eggs in faeces		Total
	Positive	Negative	
> 4%	10	40	50
≤ 4%	1	26	27
Total	11	66	77

tially proposed many decades ago (Faust & Meleney 1924). The Helminex method is based on a sequence of concentration steps that ends with the isolation of eggs through their interaction with paramagnetic beads in a magnetic field. The present evaluation confirms the superior performance of the Helminex method, which was demonstrated previously in seeding experiments (Teixeira et al. 2007). This new sensitive method is not meant to replace KK as the routine diagnostic method for schistosomiasis, even in low-intensity transmission areas. The KK method remains the first option because of its simplicity and low cost, whereas Helminex analysis would be the final step in a sequential screening algorithm (risk assessment, KK analysis, serology and Helminex analysis). This sequential combination of methods with increasing sensitivity was originally proposed by Andrews (1935) and may represent the most effective approach to detect low-level infections.

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