Rev Soc Bras Med Trop 50(6):848-852, November-December, 2017
doi: 10.1590/0037-8682-0097-2017

Short Communication

Schistosomiasis in the Amazon region: is the current diagnostic strategy still appropriate?

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Abstract

Introduction: This study analyzed the performance of the Kato Katz technique in detecting intestinal schistosomiasis in the State of Pará. **Methods:** Of three stool samples provided by each of 380 participants, a total of 16 Kato Katz slides were examined to define the reference value (RV) of positives for comparisons. **Results:** The RV revealed 37 (9.7%) infected participants in contrast to 10 (2.6%) according to a single slide. **Conclusions:** This significant underestimation of the infection rate gives reason to discuss if the current classification of prevalence levels reflects the real situation, principally in low transmission areas, like the Amazon region.

Keywords: Schistosomiasis. *Schistosoma mansoni*. Kato Katz. Diagnostics. Prevalence. Amazon region.

Schistosomiasis is one of the most neglected tropical diseases and a public health problem mainly affecting emerging countries in Asia, Sub-Saharan Africa, and Latin-America[1]. In Brazil, the Schistosomiasis Control Program (PCE) registered 22,434 egg-positives out of 709,169 examined in the year 2015[2]. According to the Brazilian Ministry of Health, endemic areas are classified into three categories. Areas with a positivity rate of less than 5% are considered as low prevalent, between 5 and 25% as medium, and above 25% as highly prevalent[4]. It is estimated that the actual number of infected individuals may be considerably higher than that notified[1,2].

The diagnosis of schistosomiasis is based on the detection of parasite eggs in stool samples using the Kato Katz technique[3], which is the recommended procedure by the World Health Organization (WHO) and the Brazilian Ministry of Health[1,4]. This technique stands out due to its easy applicability under field conditions with limited laboratory infrastructure, low costs, and the possibility of quantifying the individual worm burden by defining the number of eggs per gram of feces. However, the principal limitation, namely, a significantly low detection of positives in areas of low prevalence and in patients with a low parasitic load, is long known and well documented in the scientific literature[5,6]. Consequently, the low sensitivity of this diagnostic method in circumstances mentioned above may underestimate the true prevalence of schistosomiasis and negatively impact the control efforts. In this context, the present study evaluated the variability in the detection of *S. mansoni* infection related to the number of slides and samples examined and assessed the possible impact on disease transmission and control in areas of low prevalence.

The study was conducted in the county of Primavera with an estimated population of 10,458 inhabitants[7], located in the North-eastern region of the State of Pará, Brazil, which is part of the Eastern Amazon basin. Within the municipality of Primavera, two communities, Pedrinha and Canaã, were selected due to their low population migration rate and a prevalence of 2 to 3%, recorded by the local schistosomiasis control team during the last five years. From both communities, 422 residents were invited to participate, while a total of 380 eligible individuals formed the study participants.

All participants provided three stool specimens, which were collected on three consecutive days. The Kato Katz (KK) method was used for the preparation of fecal thick smears. A total of 16 slides per individual were prepared, consisting of 12 slides from the first, two from the second, and two from the third samples. The sum of all the egg positives, detected in the 16 slides, yielded the reference value (RV) and the overall positivity rate. All slides were analyzed by experienced technicians of

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Received 3 April 2017
Accepted 24 August 2017

the Laboratory of Intestinal Parasites, Schistosomiasis, and Malacology (LPIEM), Section of Parasitology, Evandro Chagas Institute (IEC/SVS/MS). Ten percent of the slides were re-examined to ensure quality control.

In order to compare the positivity rates obtained from the different numbers of smears and samples to the RV, the following combinations were selected: a) 12 slides from the first sample, b) two slides, one from the first sample and one from the second sample, c) four slides, two from the first sample and two from the second sample, d) three slides, one from the first, one from the second, and one from the third samples and e) six slides, two from the first sample, two from the second sample and two from the third sample. Different combinations of slides and samples were also compared among each other, including the following sets: f) two slides from the same sample compared with two slides from different samples, g) three slides from the same sample with three slides from three different samples, h) four slides from the same sample with four slides from two different samples, and finally, i) six slides from the same sample with six slides from three different samples, two smears for each sample.

The Open Epi program was used to calculate the infection rate, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). It is noteworthy that the PPV results were not considered for the comparisons, as all positives detected in the different combinations were also registered in the RV, resulting in a PPV of 100% in all analyses. The McNemar test was applied to determine statistically significant differences between the evaluated combinations. The level of agreement between slide and sample combinations, and the RV, was calculated according to the kappa coefficient, and classified as poor (<0.20), low (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80), and excellent (0.81-1.00), as proposed by Landis and Koch[8]. All performance measures were presented with their respective 95% confidence intervals (95% CI). Following the common strategy of examining a single KK slide, a total of 10 positive cases were diagnosed, indicating an infection rate of 2.6% (CI 1.3 to 4.6). After increasing the sampling effort to 12 slides from the same sample, 28 infected cases were detected; equivalent to a rate of 7.3% (CI 3.1 to 7.5). **Table 1** shows in detail the relationship between diagnosed positives and the number of slides examined.

Analysis of slides of different samples revealed 16 positive cases that were diagnosed using two slides, one from each sample, indicating a positive rate of 4.2% (CI 2.5 to 6.6). All other combinations of slides and samples are described in **Table 1**. Finally, the RV revealed 37 egg-positive individuals with a positivity rate of 9.7% (CI 7.0 to 13.0), which represents the value closest to the real prevalence.

Regarding the sensitivity of the technique in relation to the number of slides examined, from a single slide, a value of 27% in comparison with the RV was reported. When analyzing six slides, two for each fecal sample, the sensitivity increased to

TABLE 1: Number infected with *Schistosoma mansoni* and the number of infected missed in relation to the reference value obtained by the 16 Kato Katz slides.

Number of slides and samples	Number of positives (%)	Number of positives missed (%)
1SL 1st SA	10 (2.6)	27 (72.9)
2SL 1st SA	13 (3.4)	24 (64.8)
3SL 1st SA	14 (3.6)	23 (62.1)
4SL 1st SA	15 (3.9)	22 (59.4)
6SL 1st SA	19 (5.0)	18 (48.6)
12SL 1st SA	28 (7.3)	9 (24.3)
2SL 1st and 2nd SA	16 (4.2)	21 (56.7)
4SL 1st and 2nd SA	21 (5.5)	16 (43.2)
3SL 1st, 2nd and 3rd SA	20 (5.2)	17 (45.9)
6SL 1st, 2nd and 3rd SA	25 (6.5)	12 (32.4)
16SL 1st, 2nd and 3rd SA, RV	37 (9.7)	0 (0.0)

1SL 1st SA: one slide from the first sample; **2SL 1st SA:** two slides from the first sample; **3SL 1st SA:** three slides from the first sample; **4SL 1st SA:** four slides from the first sample; **6SL 1st SA:** six slides from the first sample; **12SL 1st SA:** twelve slides from the first sample; **2SL 1st and 2nd SA:** two slides from different samples, one slide from each sample; **4SL 1st and 2nd SA:** four slides from two different samples, two slides from each sample; **3SL 1st, 2nd and 3rd SA:** three slides from three different samples, one slide from each sample; **6SL 1st, 2nd and 3rd SA:** six slides from three different samples, two slides from each sample; **16SL 1st, 2nd and 3rd SA:** sixteen slides including twelve slides from the first sample, two slides each from the second and third samples; **RV:** reference value.

67.5%. Sensitivity and NPV, according to the slides and samples combinations, in comparison to the RV are shown in **Table 2**.

Kappa coefficients, comparing different slides and sample combinations with the RV, are shown in **Table 3**, indicating good agreement starting with six slides from the same sample.

Statistically significant differences were observed when the sensitivities of a single slide, six slides, and 12 slides from the same sample were compared with the RV, showing *p* values <0.01. The comparisons of three slides each from the same sample and from different samples, as well as four and six slides from the same sample with the same number of slides from different samples show a *p* value of 0.04. Comparing six slides from three different samples with the RV, a *p* value <0.01 indicates a significant difference. No statistically significant differences were found for comparisons of: a) a single slide, with two slides from the same sample (p= 0.51), b) one slide, with two slides from two different samples (p= 0.24), and c) two slides from the same sample, with two slides from different samples (p= 0.25).

The KK technique, using a single slide, is the strategy applied by the Brazilian Schistosomiasis Control Program for the diagnosis prior to the treatment of infected individuals, and subsequently for estimating infection rates in endemic areas[4]. It is well known that this method lacks reliability and sensitivity in detecting infections among individuals with low worm burden, living in areas with low disease transmission[4,9,10]. Thus, decisions on individuals or mass treatment campaigns, assessment of cure rates or re-infection after chemotherapy, elaboration of epidemiologic studies and evaluation of schistosomiasis morbidity, identification of risk areas, and monitoring control programs depend to a great degree on accurate and efficient diagnosis[11,12]. In a study carried out in a high prevalence schistosomiasis community in Ethiopia, the increase in the amount of fecal material analyzed resulted in the diagnosis of more positive individuals compared to a single slide[13]. One slide led to the detection of 102 egg positives among 326 individuals examined, indicating a positivity rate of 31.3%. Examining five slides from the same stool sample revealed an increase to 170 infected individuals, implying that 68 (40%) individuals were not correctly diagnosed using the one slide (false negative) examination. Data from Siqueira and colleagues[14], using a similar approach in a medium prevalence area in Brazil, confirmed these findings by identifying 16 participants with schistosomiasis among 201 participants, indicating an approximate positive rate of 8% with a single slide[14]. The increase in the number of slides to six smears detected 25 egg-positive individuals, indicating that 36% of infected individuals were missed using only a single slide. Thus, both studies show that the sensitivity of the method applied in high and medium prevalence settings improves considerably with increasing number of slides, even from the same stool sample.

The present study was carried out in a low prevalence area. **Table 1** shows that 10 (2.6%) egg-positive individuals were diagnosed using a single slide and 19 (5%) using six slides from the same sample, resulting in a loss of 9 (47%) infected individuals. These results corroborate the results of other studies,

TABLE 2: Sensitivity and negative predictive value in accordance with the number of slides and samples obtained by Kato Katz method from individuals infected with *Schistosoma mansoni,* in relation to the reference value

Number of slides and samples	Sensitivity (%) (95% CI)	Negative Predictive Value (%) (95% CI)
1SL 1st SA	27.0 (15.4-42.9)	92.7 (89.5-94.9)
2SL 1st SA	35.1 (21.8-51.2)	93.4 (90.4-95.5)
3SL 1st SA	37.8 (24.0-53.9)	93.7 (90.7-95.7)
4SL 1st SA	40.5 (26.3-56.5)	93.9 (91.0-95.9)
6SL 1st SA	51.3 (35.8-66.5)	95.0 (92.2-96.8)
12SL 1st SA	75.6 (59.8-86.6)	97.4 (95.2-98.6)
2SL 1st and 2nd SA	43.2 (28.6-59.0)	94.2 (91.3-96.2)
4SL 1st and 2nd SA	56.7 (40.9-71.3)	95.5 (92.8-97.2)
3SL 1st 2nd and 3rd SA	54.0 (38.3-68.9)	95.2 (92.5-97.0)
6SL 1st, 2nd and 3rd SA	67.5 (51.4-80.3)	96.6 (94.1-98.0)

95% CI: 95% confidence intervals; **1SL 1st SA:** one slide from the first sample; **2SL 1st SA:** two slides from the first sample**; 3SL 1stSA:** three slides from the first sample; **4SL 1stSA:** four slides from the first sample; **6SL 1st SA:** six slides from the first sample; **12SL 1st SA:** twelve slides from the first sample; **2SL 1st and 2nd SA:** two slides from different samples, one slide from each sample; **4SL 1st and 2nd SA:** four slides from two different samples, two slides from each sample; **3SL 1st, 2nd and 3rd SA:** three slides from three different samples, one slide from each sample; **6SL 1st, 2nd and 3rd SA:** six slides from three different samples, two slides from each sample. The positive predictive value was not included due to the value of 100% in all comparisons.

TABLE 3: Agreement of the Kato Katz method according to the different combinations of slides and samples compared to the reference value used for the detection of infected individuals with *Schistosoma mansoni.*

Combinations	Kappa value (95%CI)	Agreement
1SL 1st SA	0.40 (0.32 – 0.48)	Weak
2SL 1st SA	0.49 (0.41 – 0.58)	Moderate
3SL 1st SA	0.52 (0.44 – 0.68)	Moderate
4SL 1st SA	0.55 (0.46 – 0.64)	Moderate
6SL 1st SA	0.65 (0.56 – 0.75)	Good
12SL 1st SA	0.84 (0.74 – 0.94)	Excellent
2SL 1st and 2nd SA	0.57 (0.48 – 0.67)	Moderate
4SL 1st and 2nd SA	0.70 (0.60 – 0.79)	Good
3SL 1st, 2nd and 3rd SA	0.67 (0.58 – 0.78)	Good
6SL 1st, 2nd and 3rd SA	0.79 (0.69 – 0.89)	Good

95% CI: 95% confidence intervals; **1SL 1st SA:** one slide from the first sample; **2SL 1st SA:** two slides from the first sample; **3SL 1st SA:** three slides from the first sample; **4SL 1st SA:** four slides from the first sample; **6SL 1st SA:** six slides from the first sample; **12SL 1st SA:** twelve slides from the first sample; **2SL 1st and 2nd SA:** two slides from different samples, one slide from each sample; **4SL 1st and 2nd SA:** four slides from two different samples, two slides from each sample; **3SL 1st, 2nd and 3rd SA:** three slides from three different samples, one slide from each sample; **6SL 1st, 2nd and 3rd SA:** six slides from three different samples, two slides from each sample;

confirming a considerable lack of the method´s sensitivity, although each of them was conducted in areas with different epidemiological settings and profiles. It is noteworthy that the observed loss of 47% of infected individuals (false negatives) with the one slide, was even higher under the present low prevalence condition, when compared with 40% in high or 36% in medium prevalence areas[13,14]. A possible consequence of this phenomenon is the less likelihood of detecting infections in individuals with low worm burden from low prevalence areas, due to the decreased disease transmission pressure.

The diversification of stool samples is another important tool for detecting more positives, as already shown in a high and mid-prevalence areas[9,11]. Our data from the current study, investigating a low prevalence area, confirmed these findings. A total of 25 (6.5%) positives were found using six slides, two of each sample, in contrast to 19 (5%) with six slides of the same sample. Thus, six more positives (an improvement of 24%) were diagnosed by diversifying the sample number. The 12 slides from the same sample approach in this setting did not confirm the data described above. In this study, 28 (7.3%) positives were confirmed, three (10.7%) less than with 12 slides of the same sample. The latter result indicates that the decision-making process for the appropriate approach to apply becomes even more difficult in low prevalence areas.

According to the kappa statistics, concordance levels reached values rated as *good* and *excellent*, when six or 12 slides from the same fecal sample were compared with the RV (**Table 3**). The comparison of six slides, two of three samples and four slides, two of two samples with the RV was classified as *good*. These results confirm the hypothesis that the number of detected egg-positive individuals is closely related with the increased number of slides and number of fecal samples examined. The comparison of a single slide to the RV was rated as poor, indicating that a considerable percentage of infected individuals were missed when only one slide is examined.

The poor diagnostic performance of the KK method when examining a single slide in comparison with the RV results in a significant underestimation of the infection rate, leading to at least 73% missed positive cases. The implication of these findings for low prevalence areas, as with the State of Pará, is an adverse effect on schistosomiasis control efforts, which facilitate and maintain the disease transmission under this scenario. This becomes even more important in the light of new efforts for disease elimination, as proposed by the WHO[1] and the Brazilian Ministry of Health[15].

Ethical considerations

Children younger than two years were excluded according to the study protocol, which was approved by the Ethics Committee (CAAE number 21824513.9.0000.5091). All participants signed a consent form before enrolment. Individuals diagnosed as having schistosomiasis and soil-transmitted helminth infection were treated in accordance with the Brazilian Ministry of Health guidelines.

Acknowledgments

The authors would like to thank the Schistosomiasis Control Program team located in Primavera for their support; and the participating communities for their cooperation in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

Financial support

C*onselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq). *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES). *Instituto Evandro Chagas* (IEC)/*Secretaria de Vigilância em Saúde* (SVS)/ *Ministério de Saúde* (MS).

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