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Major Article

Performance of POC-CCA® in diagnosis of schistosomiasis mansoni in individuals with low parasite burden

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Abstract

Introduction: Schistosomiasis, caused by *Schistosoma mansoni*, is a public health concern in Brazil. However, the most popular diagnostic method, the Kato-Katz technique, exhibits low sensitivity in low-endemicity areas. We aimed to compare the performance of an immunological assay, the point-of-care circulating cathodic antigen (POC-CCA®) test, with that of two parasitological techniques in a low-endemicity population. **Methods:** Our study included 141 individuals living in Estreito de Miralta, Minas Gerais, Brazil. Fecal samples were obtained from all participants and analyzed for schistosomiasis using two parasitological techniques: the Kato-Katz technique and the saline gradient technique. Additionally, POC-CCA® strips were utilized for testing urine samples. The results obtained by the different techniques were compared. **Results:** Analysis of two or 24 slides using the Kato-Katz technique resulted in a positivity rate of 10.6% (15/141) or 19.1% (27/141), respectively. The saline gradient technique yielded a positivity rate of 17.0% (24/141). The prevalence according to both parasitological techniques was 24.1% (34/141). The POC-CCA® test yielded a positivity rate of 22.7% (32/141); however, the positivity rate was merely 2.1% if trace results were considered negative. The agreements observed between POC-CCA® and the parasitological techniques were good (Kappa indexes > 0.64). The POC-CCA® test was more sensitive than the two-slide Kato-Katz technique ($p < 0.05$) in detecting cases of *S. mansoni* infection when trace results were considered positive. **Conclusions:** These findings reinforce the importance of using multiple diagnostic techniques in low-endemicity areas for effective control of disease.

Key-words: *Schistosoma mansoni.* Schistosomiasis. Diagnosis. Low parasite burden.

INTRODUCTION

Schistosomiasis, caused by *Schistosoma mansoni*, remains a significant public health problem in Brazil, with an estimated 1.5 million people infected (below 1%), according to data obtained by the national prevalence survey (2011-2014)[1]. Control measures, which were implemented several decades ago and include parasitological surveys, specific chemotherapies, and basic sanitation, have led to a sharp reduction in the prevalence and morbidity of the disease in most Brazilian endemic areas[2].

Proper diagnosis of *S. mansoni* infection enables accurate prevalence estimation, evaluation of drug and control program efficacy, and better patient management. Due to its specificity, convenience, and low cost, the Kato-Katz technique[3] is the most commonly used tool in the parasitological survey for the diagnosis of schistosomiasis and soil-transmitted helminths in stool samples from individuals living in endemic areas[4]. However, in low-endemicity areas, where most individuals excrete fewer eggs in their feces, the limited sensitivity of the Kato-Katz technique compromises accurate estimation of the prevalence of the disease[2][5][6][7]. Therefore, the development of improved diagnostic methodologies is essential for overcoming the limitations of the Kato-Katz technique. The ideal diagnostic test should be sensitive, field-applicable, inexpensive, and involve non-invasive procedures to obtain samples.

Circulating cathodic antigen (CCA) and circulating anodic antigen (CAA) are *Schistosoma* markers that can be detected in the serum and urine of infected individuals, and the levels of these antigens represent sensitive and specific biomarkers for the intensity of infection[8][9]. Furthermore, in a study performed in an endemic area in the State of Minas Gerais, 100% of the individuals evaluated 30 days after schistosomiasis treatment resulted negative for serum CCA[10]. This indicates that levels of serum and urine CCA and CAA decrease significantly some weeks after treatment, constituting a biomarker for schistosomiasis cure. Additional studies have found that detection of CCA and CAA offers some advantages for schistosomiasis diagnosis, such as the identification of active infections and a good diagnostic performance, particularly in highly infected individuals[11][12].

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Recently, a lateral flow cassette assay to detect CCA in patient urine for diagnosis of *S. mansoni* infection, the point-of-care circulating cathodic antigen (POC-CCA®) test, has become commercially available and has been evaluated under field-based conditions in endemic areas in Africa and Asia[13] [14] [15] [16] [17] [18] [19] [20] [21] [22] [23] [24]. Currently, the ability of POC-CCA® to diagnose schistosomiasis mansoni in different settings in Brazil is being evaluated. Our aim was to compare the performance of POC-CCA® in a Brazilian population from a low-endemicity area with that of two traditional parasitological techniques, the conventional Kato-Katz technique[3] and the saline gradient technique[25]. The results of this study can provide technical evidences to recommend the incorporation of POC-CCA® in the Brazilian Schistosomiasis Program Control.

METHODS

Study area and population

This population study was conducted in 2013 in the community of Estreito of Miralta, a rural locality belonging to the Montes Claros municipality that is considered an endemic area for schistosomiasis mansoni. This locality is situated in northern Minas Gerais, Brazil, approximately 500km from capital Belo Horizonte. This area was chosen because the prevalence rate of schistosomiasis reported in 2008 was 10.3%, there has been no specific treatment for schistosomiasis in the past two years, and the area exhibits a low migration rate. Individual residents of either sex in this locality who were older than one year of age, agreed to participate in the study, and signed a consent form were included. Those individuals who did not agree to participate or did not complete all diagnostic tests were excluded. Thus, from a total population of 163 individuals, the study population consisted of 141 participants.

Sample collection

Participants were given two plastic containers and instructions to collect stool and urine samples and were invited to return the filled containers the next day. The urine samples were stored at -20°C until being tested, and both urine and fecal samples were analyzed according to the protocols of each parasitological technique.

Laboratory procedures

The Kato-Katz[3] analysis was performed using the Helm-Test® produced by Biomanguinhos-Fiocruz (Rio de Janeiro, RJ, Brazil). In order to perform a quantitative comparison between the parasitological techniques, a total of 1,000mg of each individual stool sample was assessed on 24 slides, with each slide containing 41.7mg. The intensity of infection was calculated by determining the mean number of *S. mansoni* eggs found on each slide and multiplying by 24 to determine the number of eggs per gram (epg). According to the World Health Organization (WHO)[4], *S. mansoni* infection intensity can be categorized as light (1-99 epg), moderate (100-399 epg), or heavy (≥400 epg).

For the saline gradient technique[25], feces were filtered through a nylon screen (150μm), and quantification was performed using a metal plate. For each stool sample, two aliquots of 500mg were each homogenized with 3mL of 0.85% saline and poured into separation columns. A continuous-flow drip system was used to passage a 3% saline solution in the main reservoir through the stripping column. This promotes a continuous flow of the light debris suspension and its subsequent disposal. After clarification and sharp separation of the suspension from the sediment, the system was closed and all remaining material transferred to a Falcon® 15-mL tube and 20% formaldehyde was added to preserve the material until analysis. Slides were prepared with a drop of the sediment and a drop of saline, and a glass cover slip was affixed. Helminth eggs were counted under a microscope and all material was examined.

The results of the Kato-Katz and saline gradient tests were considered the reference for discrimination of positive and negative *S. mansoni* infection.

Urine samples were transferred to the Schistosomiasis Laboratory at the *Centro de Pesquisas René Rachou/Fundação Oswaldo Cruz* (CPqRR/FIOCRUZ) and subjected to POC-CCA® (Rapid Medical Diagnostics, Pretoria, South Africa). The test was performed at room temperature, following the manufacturer's instructions. In brief, one drop of urine was added to the well of the testing cassette and after being fully absorbed, one drop of buffer was added to the same well. After 20 min of incubation with the testing cassette in the horizontal position, the result was visually read. The test was only considered valid when the control band was visualized. Invalid tests were repeated using the same urine sample. Valid tests were scored as negative, trace (weak band), or positive.

Statistical analysis

Data were analyzed with OpenEpi, version 3.03[26]. Co-positivity, co-negativity, sensitivity, specificity, and diagnostic accuracy of the POC-CCA® were calculated based on the reference test results and using 2 × 2 tables with 95% confidence intervals (CIs) for each of the three approaches. The McNemar test was used for comparison of the positivity rates obtained by the different techniques and was calculated in R version 3.2.3[27]. The significance level was fixed at 0.05. The level of agreement between different diagnostic techniques was determined by the Kappa coefficient. According to Landis & Koch[28], concordance is bad when lower than 0.20, weak at 0.21-0.40, moderate at 0.41-0.60, good at 0.61-0.80, and excellent when higher than 0.81.

Ethical considerations

The study protocol was approved by the Ethics Committee for Research in Humans of the *Centro de Pesquisas René Rachou/Fundação Oswaldo Cruz* (CEPSH 03/2008). All participants and parents/legal guardians were informed of the purpose and procedures of the study and they signed a written informed consent. Samples were coded, and the results were treated confidentially. All participants found to be positive for *S. mansoni* or other helminths were clinically examined by a physician and treated with praziquantel (60mg/kg for children and 50 mg/kg for adults) for schistosomiasis and albendazole (400mg) in a single oral dose for helminths, as recommended by the Brazilian Health Ministry.

RESULTS

Out of the 163 individual residents in Estreito de Miralta, 141 (86.5%) residents, including 74 women and 67 men, aged 1-86 years, participated in this study. In this population, the positivity rate obtained by the Kato-Katz technique was 10.6% (15/141) or 19.1% (27/141) after examination of two or 24 slides, respectively. Using the saline gradient technique, 24 individuals positive for *S. mansoni* were detected, reflecting a positivity rate of 17% (**Figure 1**). The POC-CCA® test presented a positivity rate of 22.6% (32/141) in the population when all trace observations were considered as positive results (**Table 1** and **Figure 2**). When excluding trace results, the positivity rate was only 2.1%. The highest frequency of positive individuals based on parasitological and immunological tests was found in the 10-19 years age group, followed by 20-29 years (**Table 1**). All individuals found positive by both parasitological techniques presented with low parasite burdens (<100 epg). The intensity of infection was 5 epg, calculated by the geometric mean of the number of epg of the positive individuals obtained by the reference tests. This confirms the characterization of Estreito de Miralta as a low transmission area.

The prevalence of schistosomiasis in the study area was 24.1% (34/141) in both parasitological evaluations, considered in this study to be the reference test (**Table 2** and **Figure 1**). The sensitivity, specificity, and diagnostic accuracy of the POC-CCA® test were calculated in comparison with the reference test to discriminate positive and negative *S. mansoni* infection. The POC-CCA® test exhibited a sensitivity of 73.5% (CI: 56.9-85.4%), specificity of 93.5% (87.2-96.8%), and diagnostic accuracy of 88.6% (82.4-92.9%). In a cross-table analysis between the results of the POC-CCA® test and those obtained using the two-slide Kato-Katz technique, 24-slide Kato-Katz technique, or saline gradient test, the co-positivity rates were of 86.6%, 77.7%, and 83.3%, respectively, and the co-negativity rates were 84.9%, 90.3%, and 89.7%, respectively (**Table 2**). The agreements between the parasitological techniques and the POC-CCA® test were good, with Kappa indexes of 0.64 for the Kato-Katz/POC-CCA® comparison and 0.65 for the saline gradient/POC-CCA® comparison.

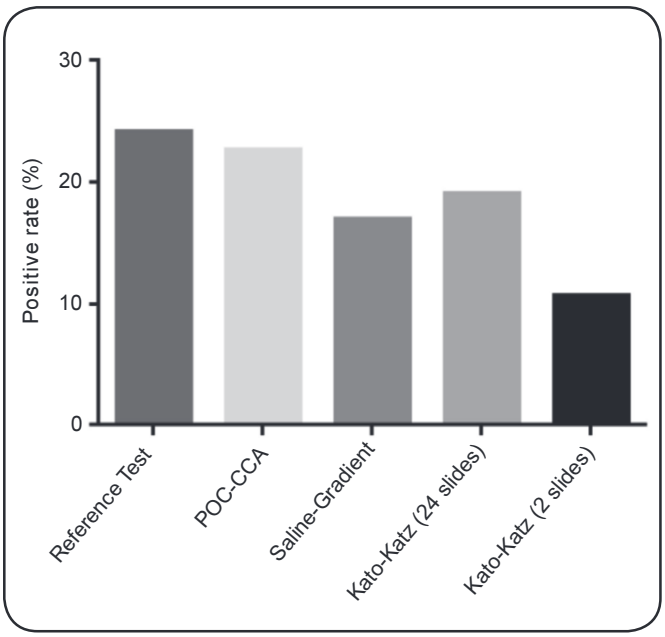

FIGURE 1 - Positivity rates obtained by parasitological and immunological techniques in the Estreito of Miralta population, Minas Gerais, Brazil in 2013. POC-CCA: point-of-care-circulating cathodic antigen.

DISCUSSION

Currently, schistosomiasis is primarily diagnosed using direct parasitological techniques. Specifically, in surveys conducted by

TABLE 1 - Positivity rates by age group according to two- and 24-slide Kato-Katz analysis, saline gradient technique, reference test (see text), and POC-CCA® test.

			Kato-Katz									
			2 slides		24 slides		Saline gradient		Reference test		POC-CCA	
Age (years)	Number	Geometric mean (epg)	%	n	%	n	%	n	%	n	%	n
0-9	21	3.1	9.5	2	9.5	2	9.5	2	9.5	2	33.3	7
10-19	24	4.5	20.8	5	41.6	10	50.0	12	58.3	14	54.2	13
20-29	13	10	15.4	2	23.0	3	46.1	6	46.1	6	30.7	4
30-39	19	16.1	15.8	3	15.8	3	10.5	2	15.8	3	15.8	3
40-49	29	2.1	3.4	1	13.8	4	3.4	1	13.8	4	6.9	2
50-59	8	6.1	12.5	1	37.5	3	12.5	1	37.5	3	25.0	2
60-69	15	1.4	6.7	1	13.3	2	0	0	13.3	2	6.7	1
>70	12	0.0	0	0	0	0	0	0	0	0	0	0
Total	**141**	**5.0**	**10.6%**		**19.1%**		**17.0%**		**24.1%**		**22.6%**	

POC-CCA: point-of-care circulating cathodic antigen; **epg:** eggs per gram.

TABLE 2 - Comparison of positive and negative results as determined by POC-CCA®, saline gradient, or Kato-Katz (two or 24 slides) tests.

		Kato-Katz (2 slides = 83.4mg)			Kato-Katz (24 slides = 1,000mg)			Saline gradient (1,000mg)			Reference test (Kato-Katz + Saline gradient)		
		positive	negative	total	positive	negative	total	positive	negative	total	positive	negative	total
POC-CCA® (Urine)	Positive	13	19	32	21	11	32	20	12	32	25	7	32
	Negative	2	107	109	6	103	109	4	105	109	9	100	109
	Total	15	126	141	27	114	141	24	117	141	34	107	141
		Co-positivity: 86.6% (CI: 62.1 - 96.2)			Co-positivity: 77.7% (59.2 - 89.4)			Co-positivity: 83.3% (CI: 64.1- 93.3)			sensitivity: 73.5% (56.9 - 85.4)		
		Co-negativity: 84.9% (CI: 77.6 - 90.1)			Co-negativity: 90.3% (83.5 - 94.5)			Co-negativity: 89.7% (CI: 82.9 - 94.0)			specificity: 93.5% (87.2 - 96.8)		
		Kappa index: 0.48			Kappa index: 0.64			Kappa index: 0.65			Kappa index: 0.68		
		p < 0.05			p = 0.332			p = 0.08			p = 0.802		

POC-CCA®: point-of care-circulating cathodic antigen; **CI:** confidence interval.

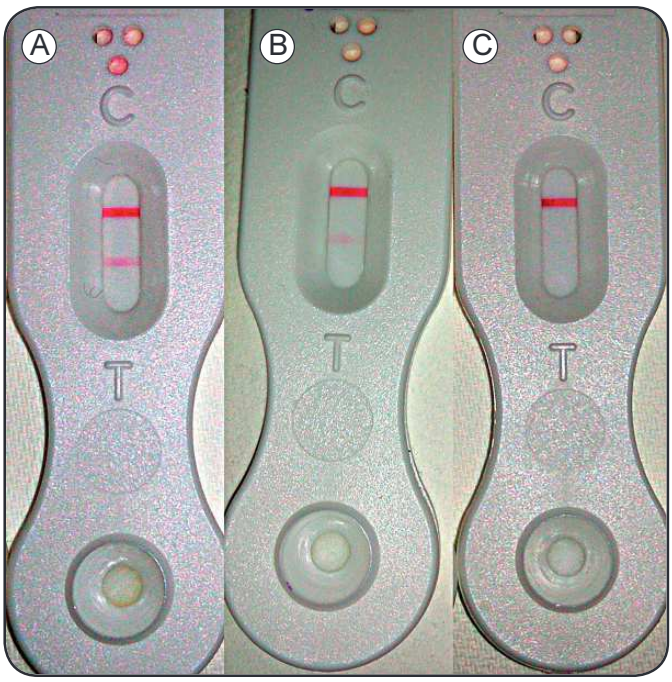

FIGURE 2 - POC-CCA® test results. A. Positive. B. Trace and C. Negative. POC-CCA: point-of-care-circulating cathodic antigen.

the Brazilian Schistosomiasis Program Control, the Kato-Katz technique is used to analyze two slides from a single stool sample to detect positive *S. mansoni* infection, according to the WHO recommendation[4]. In spite of the advantages of this parasitological technique, including high specificity, quantitative measurement of infection, and concomitant detection of geohelminths[29] [30], it fails to detect *S. mansoni* infection in individuals with a low worm burden, which may comprise a majority of individuals living in low-endemicity areas. These areas are characterized by prevalence rates <10%, positive individuals with parasite burden of <100 epg, and a majority of infected individuals presenting as asymptomatic or oligosymptomatic for the disease[31]. In these low-endemicity areas, diagnostic strategies must often be combined to obtain a better estimate of the infection rate of *Schistosoma* spp. in at-risk populations and to evaluate the effectiveness of interventions such as chemotherapy[32].

Assays for the detection of antibodies and antigens offer promising complementary strategies to traditional parasitological examination. In this study, we evaluated the performance of the POC-CCA® rapid test for the laboratory diagnosis of schistosomiasis mansoni in a Brazilian low-endemicity area. We observed that the positivity rate increased from 10.6% to 19.1%, when the Kato-Katz technique was used to evaluate two or 24 slides, respectively. Corroborating our data, an increase in prevalence was found in studies from Brazil[2] [6] [7] [33], Ethiopia[34], and China[35] when more slides were examined. However, this strategy is not viable in epidemiological surveys because repetitive fecal examinations may generate logistical problems and increased costs. The prevalence rate determined by saline gradient was slightly below that determined by the Kato-Katz technique with 24 slides and

that of the POC-CCA® test, although there were no statistically significant differences among tests (p = 0.08). The saline gradient is a new technique that was developed to detect *S. mansoni* and soil-transmitted helminths and should be considered a viable tool for application in parasitological surveys. The highest prevalence of *S. mansoni* infection was found by both parasitological and immunological techniques among children and teenagers aged 10-19 years. This supports previous findings and may be due to cumulative infections and increased water contact[36].

The agreements observed between the immunological (POC-CCA®) and parasitological techniques (Kato-Katz and saline gradient) were good. However, it is worth mentioning that this was achieved when all trace CCA results were considered positive. Moreover, disagreements in positive results obtained for the parasitological techniques and the POC-CCA® test can reach 20%. One of the major drawbacks of the CCA test is a frequent trace result on the test strip. In an attempt to understand the presence of these trace results better, a study to improve the sensitivity of the POC-CCA® test concluded that the color intensity of positive samples with trace results could be increased by using urine ten times concentrated by lyophilization. In contrast, the color intensity of false-positive samples did not change with the increased concentration of urine (Coelho et al., manuscript in preparation).

There was a significant discordance between the results of the POC-CCA® test and the two-slide Kato-Katz technique (Kappa index = 0.48), which is used by the Brazilian Schistosomiasis Program Control. This discordance was reduced when more slides were examined 24 slides (Kappa index = 0.64) or when the results were cross-tabled with those of the saline gradient test (Kappa index = 0.65) or reference test (Kappa index = 0.68). Based in this cross-table analysis, we observed a higher frequency of false-negative results from the parasitological techniques than those from the POC-CCA® test. This suggests that the POC-CCA® test is more sensitive than the two-slide Kato-Katz technique ($p < 0.05$) in detecting cases of *S. mansoni* infection. These results also support the findings of other studies that compared the performance of the POC-CCA® test with that of the Kato-Katz technique using two[21], four[15], six[13] [14] [37] [38], or nine slides[14] [24]. All these studies also found that the POC-CCA® test is more sensitive than the Kato-Katz technique.

In addition to greater sensitivity, the rapid POC-CCA® test presents some additional advantages over traditional parasitological techniques. These include its ease of use and operation, stability at high temperatures, and short time frame required for testing, as well as the fact that it works on urine samples, which are less invasive and easier to obtain. However, some issues with the test remain, including the occurrence of false-negative and false positive results. In this study, we found seven false-positive and nine false-negative samples when comparing results from the POC-CCA® test to those of the reference test. This means that 20-5% of cases determined as positive by the parasitological techniques are not detected by the POC-CCA® test. In this context, we must consider the day-to-day fluctuation of eggs excreted in the feces and CCA in the urine, two traits that were recently studied in children infected with *S. mansoni* in Ethiopia[39]. In this study, the authors found less variation in the CCA test results than that found in the Kato-Katz results, suggesting that a single CCA test would suffice for screening of *S. mansoni* infection. In special situations, such as for cure assessment and when the elimination of transmission is the aim in endemic areas, more than one urine sample may need to be collected on different days for more reliable diagnosis of *S. mansoni* infection.

Additionally, the cause and interpretation of the trace results of the POC-CCA® test require further study. In the present work, all trace CCA results were considered positive. The distinction between negative and trace readings of the POC-CCA® test is subjective and requires standardization. There is a consensus among experts that there is a need for agreement on what thresholds to use when using CCA. Treating trace results as positive may result in over-treatment; however, in low transmission areas, if there is sufficient praziquantel available, this may be preferable to under-treatment for achieving elimination. Souza-Figueiredo et al.[40] showed that, in a low transmission setting (prevalence below 10%), the urine CCA test performed better if the observer considered trace results as negative cases. If trace results are considered to represent positive cases, the test becomes more sensitive but also, as a consequence, less specific.

A limitation of this study is the use of the results of two parasitological techniques in the reference test. Questions about the specificity and sensitivity of the POC-CCA® test therefore remain unresolved, and additional studies are necessary to evaluate its true performance in diagnosing schistosomiasis mansoni. In this context, a large field trial to evaluate POC-CCA® and other techniques is under way. These findings show that the new parasitological technique for isolation and detection of *S. mansoni* eggs based on their interaction with paramagnetic beads[41] offers higher sensitivity and specificity in the diagnosis of *S. mansoni* infection. In future, this technique may improve the diagnosis of schistosomiasis mansoni in individuals with low worm burdens, contributing to elimination of the disease in low-endemicity areas. The use of a highly sensitive detection method is of fundamental importance for identification of infection and prompt individual treatment. This reduces the opportunity for the disease to evolve into more severe clinical forms, improving morbidity control. From the point of view of transmission control, this study reinforces the need to combine diagnostic techniques to identify individuals with low parasite burdens, especially in low transmission settings, which are more prevalent after many years of disease control.

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ConÀict of interest

The authors declare that there is no conflict of interest.

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