Evaluating a point-of-care circulating cathodic antigen test (POC-CCA) to detect *Schistosoma mansoni* infections in a low endemic area in north-eastern Brazil

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ABSTRACT

Schistosomiasis is still a public health problem in Brazil. The Kato-Katz test is the most frequently used diagnostic method for *Schistosoma mansoni* infection. However, it lacks sensitivity in areas of low prevalence. We have assessed the positivity rate of *S. mansoni* infection in Bananeiras, a village on Capistrano, Ceara, Brazil by performing a point-of-care test in urine to determine the circulating cathodic antigens (POC-CCA), and we compared the findings with those of the Kato-Katz technique for egg detection in stool and an enzyme-linked immunosorbent assay for specific antibodies against adult worms (SWAP-ELISA) in serum before treatment (baseline). Additionally, the POC-CCA and Kato-Katz test results were compared at one and two years post-treatment, and only POC-CCA strips were utilised for follow-up testing on urine samples at 3–6 weeks. Only one sample of stool and urine was collected per event. Overall, 258 individuals were investigated at the baseline. The POC-CCA test detected 10 (3.9%) positive cases; however, this amount increased to 30 (11.6%) when considering trace readings as positive (t +), whereas the Kato-Katz method found only 4 (1.6%) positive cases and the SWAP-ELISA detected 105 (40.7%) positive cases. The consistency observed between a single POC-CCA (t +) or (t-) and the Kato-Katz (three slides) was poor (Kappa indexes < 0.20). The highest positivity rate as determined by CCA and Kato-Katz was found in adults. At the baseline, a praziquantel treatment was administered to all individuals regardless of their infection status. According to the POC-CCA test, 93% of the previous positive cases became negative by the third week after the treatment; this rate reached 100% at the sixth week assessment. The follow-up showed that of the 175 individuals evaluated at one year post-treatment, only one (0.6%) showed 'trace' results, and all the individuals were negative for eggs in the stool. At two years, all 185 examined individuals were negative by the Kato-Katz method, and 11 (5.9%) presented traces by POC-CCA. Our results indicate that a single POC-CCA test reveals a significantly higher number of positive cases than the Kato-Katz technique for diagnosing *S. mansoni* in a low endemic setting, when trace results are considered as positive cases. Nevertheless, the true significance of the trace is not clear. These findings reinforce the need to associate different tools for improved schistosomiasis diagnosis in individuals with low parasite burdens.

1. Introduction

According to data obtained by the national prevalence survey (2010–2015), an estimated 1% positive rate of *Schistosoma mansoni* was found in Brazil, where schistosomiasis remains a significant public health problem (Katz et al., 2017). The parasitological detection of *S. mansoni* by Kato-Katz faecal microscopy is used extensively for diagnosis due to its practicability, efficacy, and low cost. However, this method has some disadvantages. It has low sensitivity in areas of low endemicity, and consequently, stool analyses must be repeated several times (Cavalcanti et al., 2013; Enk et al., 2008; Pinheiro et al., 2012; Siqueira et al., 2011).

Alternatively, antibody detection has been suggested to complement the parasitological method and to increase the diagnostic sensitivity. In fact, in low endemic areas and/or after large-scale chemotherapy, the combination of stool microscopy with serological methods has been proposed for the detection of *Schistosomiasis mansoni* cases in low transmission areas (Alarcón de Noya et al., 2007; Carneiro et al., 2012; Espirito-Santo et al., 2014; Gomes et al., 2014).

Assays for the detection of circulating *Schistosoma* antigens (adult worm gut-associated antigens) were described. The circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA) have both been used to diagnose active infections (Corstjens et al., 2014). To improve the techniques used to diagnose schistosomiasis in the field, van Dam et al. (2004) developed a lateral flow strip immunoassay to detect CCA in urine, and they declared that this test has high sensitivity and specificity for the study of schistosomiasis. The laboratory format of the urine CCA strip test has been developed into a commercially available rapid Point-of-Care test (POC-CCA), and various studies have been conducted in endemic areas of the African and Asian continents to determine the efficacy and field applicability of this test for schistosomiasis diagnosis. A summary of 5 countries' evaluations of the POC-CCA test, which was funded by the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE), demonstrated that this method is valuable for detecting *S. mansoni* in endemic areas (Adriko et al., 2014; Colley et al., 2013; van Dam et al., 2015).

A systematic review indicated that when the Kato-Katz prevalence is below 50%, the POC-CCA test is much more sensitive than the Kato-Katz test, and thus the prevalence determined by the POC-CCA test is consistently much higher (Kittur et al., 2016).

Until the present, only a few studies have been published to show the results of POC- CCA trials in Brazil. A study performed in a low prevalence area (Estreito de Miralta, state of Minas Gerais, Brazil) compared the results of two parasitological techniques, the Kato-Katz and Saline Gradient and the POC-CCA. An analysis of two and 24 slides using the Kato-Katz technique showed positive rates of 10.6% (15/141 individuals) and 19.1% (27/141), respectively. The Saline Gradient technique yielded a positive rate of 17.0%. Considering the results of both parasitological techniques, the positive rate was 24.1%. The POC-CCA test showed a positive rate of 22.7% (32/141- trace as positive), and, when trace results were considered negative, the positivity rate was only 2.1% (Siqueira et al., 2016).

Other studies evaluating the POC-CCA test in low-endemicity settings are essential, because in these settings, the infection intensity is less than 100 EPG and the diagnosis fails when parasitological stool sample tests are used.

The present study aimed to evaluate the performance of the POC-CCA under field-based conditions in a population from a low endemic area located in the north-eastern region of Brazil for the diagnosis of *S. mansoni* before and after treatment.

2. Material and methods

2.1. Ethics statement, recruitment, and treatment

The study protocol was approved by the Universidade Federal do Ceará ethical committee (application No. 302.204). It was conducted according to Resolution No. 466/12 of the Brazilian Health Council. District health authorities and the entire community were informed about the purpose, procedures, and potential risks and benefits of the study. Before enrolment, informed consent was obtained from the volunteers. Parents/legal guardians provided informed consent for their children to participate.

At the baseline, praziquantel (PZQ) treatment (Farmanguinhos, Ministry of Health, Brazil) was administered to all individuals regardless of their infection status. This treatment consisted of a single dose of 60 mg/kg for children ($\leq$15 years old) and 50 mg/kg for adults, as recommended by the Brazilian Health Ministry (Ministério da Saúde, 2014).

2.2. Study area and population

The study was conducted between 2013 and 2015 in the community of Bananeiras, a rural locality in the Capistrano municipality, in Ceará state, Brazil (geographical co-ordinates 4° 28′ 20″S latitude, 38° 54′ 14″ W longitude). Capistrano extends for 222.6 km^2 and is located at 155 m altitude, approximately 93 km south of Fortaleza, the capital of Ceará. The area has been known to be endemic forhave endemic *S. mansoni* since 1976. In a region with a semi-arid climate, the river that crosses the village (Aracoiaba River) remains dry for most of the year. The flood season runs between the months of December and March. Our door-to-door census was performed in March of 2013, and it identified 297 people aged 2 or older in the Bananeiras community. The prevalence rate of schistosomiasis reported in 2010 was 1.6% and there has been no specific treatment for this disease in the past two years.

2.3. Inclusion criteria

To be included in this study, all the inhabitants of Bananeiras had to meet the following criteria at the baseline: 1) aged $\geq$ 2 years at recruitment; 2) informed consent; 3) provided stool, urine, and serum samples at sufficient/suitable amounts for triplicate Kato-Katz thick smears, for a single point-of-care circulating cathodic antigen (POC-CCA) cassette test and for a serology test; and 4) no recent treatment for schistosomiasis (at least within the past two years). All the people who had been recruited for the study at the baseline (n = 258) were considered for follow-up if the treatment was performed. The flowchart of the study population is shown in Fig. 1.

2.4. Sample collection

Five cross-sectional surveys were implemented. Urine, stool, and blood samples were collected at the baseline from each individual enrolled in the study; at 3 and 6 weeks after the PZQ administration, only a urine sample was collected; and at one and two years post-treatment, both urine and stool samples were collected. Only one sample of stool and urine was collected at each time point.

At the baseline, one day before the collection day, two plastic containers labelled with unique identification numbers (IDs) (stool and urine containers) were delivered to all the study participants. For child participants, the containers were delivered to the children's mothers or guardians; venous blood samples were also taken at that moment. Individual serum samples were obtained after blood sample centrifugation at 3000*g* for 5 min, and these samples were stored at −20 °C. On the following day, the individuals were invited to return the containers filled with a fresh morning urine sample and a lime-sized stool sample to the fieldworkers stationed at Bananeiras Health Center, where the samples were processed on the same day.

2.5. Microscopy

Microscopy was performed using the Helm Test® manufactured by Biomanguinhos-Fiocruz (Rio de Janeiro, RJ, Brazil). Three Kato-Katz thick smear slides were prepared per stool sample using 41.7 mg templates, and they were examined for *S. mansoni* (Katz et al., 1972). The infection intensity was expressed as eggs per gram of stool (EPG) for everyone, using the arithmetic mean of *S. mansoni* faecal egg counts (FECs) and multiplied by a factor of 24. For quality control, 10% of the Kato-Katz thick smears were re-examined by a senior technician from Rene Rachou Research Center, Oswaldo Cruz Foundation, Minas Gerais, Brazil.

2.6. Circulating cathodic antigen (CCA) test

The urine samples were tested using a commercially available POC-CCA cassette test (Batch numbers 33,827: baseline, 3- and 6-weeks after
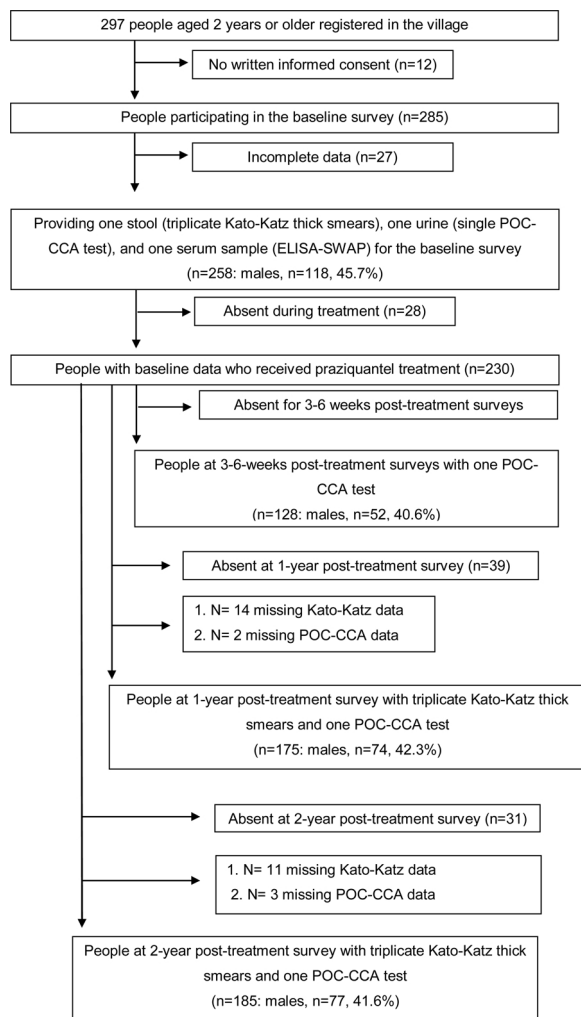
Fig. 1. Flowchart showing study participation in the baseline cross-sectional survey, at 3–6-weeks and at one and two years post-treatment. A flowchart detailing the study participation and individual adherence to providing stool, urine, and blood samples for the diagnosis of *S. mansoni* infections at the baseline cross-sectional survey and 3–6-weeks (urine sample) and at one and two years (stool and urine samples) post-treatment in Bananeiras village, Ceará, Brazil, from 2013 to 2015.

treatment/33,955: 1 year post-treatment/50,173: 2 years post-treatment, Rapid Medical Diagnostics, Pretoria, South Africa) performed at room temperature according to the manufacturer's instructions on the day of the sample collection. In brief, one drop of urine was added to the well of the testing cassette and allowed to absorb. Once fully absorbed, one drop of buffer (provided with the CCA test kits) was added. The test results were read visually 20 min later. In cases when the control bands did not develop, the test was considered invalid. Invalid tests were repeated using the same urine sample. Valid tests were scored as negative, trace (weak band) or positive. All the tests were read independently by two investigators, and in case of discordant results, a third independent investigator examined it until an agreement was reached. Urine samples collected at all the time points in the study were treated as described above.

2.7. Serology

The ELISA assay was performed to detect an IgG antibody against soluble worm antigen preparation (ELISA-SWAP) according to the methodology described by Grenfell et al. (2013). In summary, Max-iSorpTM surface microtitre plates (NUNC, Denmark) were sensitised with 100 μL/well of 1 μg/mL of SWAP diluted in 0.05 M carbonate-

bicarbonate buffer, pH 9.6, for 16 h at 4 °C. The plates were washed three times with 0.15 M PBS, pH 7.2, with 0.05% Tween 20 (LGC Biotecnologia, BR) (washing buffer), and the non-specific sites were blocked with 10% foetal bovine serum (FBS) in washing buffer at 37 °C for 1 h. After another round of washing, 100 μL of sera diluted 1:100 in PBS was added to each well in triplicates and the plates were incubated at room temperature (RT) for 1 h. The plates were then subjected to more washing and then incubated at RT for 1 h with IgG-HRP (Southern Biotech, USA) diluted 1:60,000 in washing buffer. The plates were washed again and 100 μL of TMB/H_2O_2 substrate was added to each well. The reaction was stopped after 20 min of incubation in the dark by adding 50 μL/well of 2 N sulphuric acid. The results were obtained as absorbance values at 450 nm in a microplate reader (Bio-Rad Laboratories 3550, JA). The sensitivity, specificity, cut-off values, receiver operating characteristic (ROC) curves, likelihood ratios and positive predictive values were determined by Prism 5 software based on true individual egg positives and negatives.

2.8. Statistical analysis

Data were entered into Excel (Microsoft Corp., Redmond, WA, USA) spreadsheets and analysed with SPSS software version 22 (IBM Corp.; Armonk, USA). The proportions were tested using chi-square and difference-of-proportions tests. Consistency between binary variables was established by determining the kappa statistics (k) as follows: k = 0 indicating no agreement; k = 0–0.2 indicating poor agreement; k = 0.21–0.4 indicating fair agreement; k = 0.41–0.6 indicating moderate agreement; k = 0.61–0.8 indicating substantial agreement; and k = 0.81–1.0 indicating almost perfect agreement (Landis and Koch, 1977). A 5% level of significance was adopted for all the inferential procedures.

3. Results

3.1. Study group characteristics and adherence

Fig. 1 shows the individuals' adherence at providing stool, urine, and blood samples for a set of diagnostic tests to detect *S. mansoni* at the time points of the study. The village census revealed 297 people aged 2 years or older, all of whom were invited to participate. Of the 285 individuals who participated in the baseline cross-sectional survey, twenty-seven were excluded due to incomplete data A complete specimen set (stool, urine, and blood) was available for 258 (90.5%) of the 285 individuals enrolled in the study at baseline, so all three tests could be performed. Among them, 45.7% (n = 118) were males and the median age of the cohort was 30 years (age range: 2–87 years). At three and six weeks post-treatment, 128 individuals were re-examined using one POC-CCA test. Among them, 40.6% (n = 52) were males and the median age of the cohort was 19 years (age range: 2–87 years).

3.2. Positivity rates of S. mansoni infection before treatment

Table 1 shows the results for the Kato-Katz method, POC-CCA and SWAP-ELISA for *S. mansoni*.

According to the three Kato-Katz thick smears examined per individual, the observed positivity rate for *S. mansoni* was 1.6% (n = 4). The positivity rate of *S. mansoni* increased with the increasing number of examined smears (p = 0.681), but no significant difference was found. All the positive individuals were classified as having a very low-intensity infection with an arithmetic mean of 8 EPG.

When the trace results were scored as positive, the positivity rate of *S. mansoni* based on the CCA test increased from 3.9% to 11.6%. The positivity rate for *S. mansoni* by CCA was significantly higher than that obtained using 3 Kato-Katz smears when the trace results were considered positive (p < 0.001), but this was not the case when the trace results were considered negative (p = 0.176) (Table 1). Of the 258

Table 1
Baseline positivity rates of *S. mansoni* according to each diagnostic method.

Diagnostic approach	NO. of infected Individuals	% positive (95% CI)
Single Kato-Katz thick smear	2	0.8 (0.0–1.8)
Duplicate Kato-Katz thick smears	3	1.2 (0.0–2.5)
Triplicate Kato-Katz thick smears	4	1.6 (0.0–3.1)
Single POC-CCA cassette test (t-)	10	3.9 (1.5–6.2)
Single POC-CCA cassette test (t +)	30	11.6 (7.7–15.5)
SWAP-ELISA	105	40.7 (34.7–46.7)

individuals, just one (0.4%) was *S. mansoni*-positive according to all the tests, and 133 individuals (51.5%) were negative for *S. mansoni* in all the assays. A total of 10 (3.9%) were egg-negative but positive according to the CCA test and the SWAP-ELISA. Ninety-two individuals (35.7%) were positive only by SWAP-ELISA, whereas 19 (7.4%) were positive only by CCA test. Only one individual (0.4%) was positive by Kato-Katz method, but not by any other test. The remaining 2 individuals (0.8%) were positive according to the Kato-Katz method and by the ELISA-SWAP. There were 29 individuals (11.2%) who were cassette CCA-positive, but they were egg-negative. Of those individuals, 10 (34.5%) were positive by SWAP-ELISA.

3.3. Age-dependent positivity rates

The positive cases by microscopy, POC-CCA test and ELISA-SWAP per age group are shown in Table 2. Overall, the highest positivity rate occurred in adults; however, it is worth noting that there were many people under 20 years old who scored as CCA-positive. The ages of the egg-positive individuals ranged from 33 to 47 years. Twenty (66.6%) and 80 (76.2%) positive people were older than 15 years old according to the POC-CCA test and SWAP-ELISA, respectively. The proportion of POC-CCA (t +)-positive tests was higher in the age groups below 40 years old, and all individuals in the 2–9 year-old age group had trace readings. There was no association between infection and gender (19 females vs. 14 males; p = 0.683).

3.4. Consistency among the different diagnostic methods before treatment

Table 3 shows the consistency between the different diagnostic approaches for diagnosing *S. mansoni*. Poor consistency was found between the three Kato-Katz thick smears and a single CCA test regardless of whether the trace results were considered negative (k = 0.12) or positive (k = 0.03).

3.5. Post-treatment readings

Of the 128 individuals who had CCA data records 3 and 6 weeks

Table 2
Number of positive cases for the respective age group, according to different diagnostic approaches.

Age group	Total tested	No. pos/% Positive by microscopy	No. pos/% Positive by POC-CCA (t-)*	No. pos/% Positive by POC-CCA (t +)*	No. pos/% Positive by ELISA-SWAP
2–9	32	0	0	6/18.7	17/53.1
10–19	58	0	2/3.4	7/12.1	18/31.0
20–29	38	0	3/7.9	6/15.8	10/26.3
30–39	43	3/7	2/4.6	7/16.3	15/34.9
40–49	35	1/2.8	0	1/2.8	17/48.6
50–59	23	0	0	0	15/65.2
60 and older	29	0	3/10.3	3/10.3	13/44.8
Total tested	258	4/1.6	10/3.9	30/11.6	105/40.7

*t-, trace negative; t+, trace positive.

Table 3
Consistency between different techniques for the diagnosis of *S. mansoni* infections.

Diagnostic test	Test result	Triplicate Kato-Katz thick smears Positive	Negative	K*	P-value
Single POC-CCA test (t-)	Positive	1	9		
	Negative	3	245	0.12	0.027
Single POC-CCA test (t +)	Positive	1	29		
	Negative	3	225	0.03	0.400

* Kappa indexes.

after treatment, *S. mansoni* eggs were detected by Kato-Katz in 2 (1.6%) individuals during the baseline cross-sectional survey. A single POC-CCA had trace results considered as negative, in 8 individuals (6.2%). When considering trace results as positive, a considerably higher number of individuals were classified as positive (n = 24, 18.7%).

Three weeks after treatment, of those 128 individuals, a single urine CCA cassette test revealed 2 individuals (1.6%) with *S. mansoni*, whereas CCA (t +) found 5 (3.9%). At 6 weeks after treatment evaluation, regardless of whether the POC-CCA trace results were considered positive or negative, all the individuals were CCA-negative.

Of the 33 individuals who were either Kato-Katz or CCA (t +)-positive at the baseline, 31 swallowed PZQ in the presence of the research team, and they were considered for efficacy evaluation. Among those, 29 (93.5%) were POC-CCA-negative 3 weeks post-treatment. There were twenty-eight individuals who tested negative by Kato-Katz, but they were positive by POC-CCA. Of these, 26 (93%) were POC-CCA-negative upon repeat testing when performed 3 weeks post-treatment. The two remaining POC-CCA-positive cases became negative based on follow-up POC-CCA testing at 6 weeks post-treatment.

At one year post-treatment, of the 175 individuals evaluated by triplicate Kato-Katz thick smears and POC-CCA tests, only one (0.6%) was found to be 'trace' and all the individuals were negative upon microscopic examination. At two years post-treatment, 185 were negative in terms of stool examination and 11 (5.9%) were 'trace' by POC-CCA.

4. Discussion

In Brazil, the profile of schistosomiasis has undergone changes over time due to its control programmes. There is a consensus that there are different prevalence and morbidity profiles in endemic African and Brazilian areas (Coelho et al., 2016). However, in Brazil, despite the reduction in mortality and morbidity due to the success of the Ministry of Health control programmes, schistosomiasis was mentioned in 8756 of the 12,491,280 deaths that were recorded between 2000 and 2011; 6319 as the underlying cause and in 2437 cases as an associated cause of death, thus it remains an important public health problem (Martins-Melo et al., 2014). In view of recent discussions and the resolution on schistosomiasis elimination by the 65th World Health Assembly (Rollinson et al., 2013; WHO, 2012), the necessity for highly specific and sensitive diagnostic methods for diagnosing *S. mansoni* after extensive preventive chemotherapy campaigns is imperative.

The present study attempts to evaluate the performance of the commercially available Point-of-Care Circulating Cathodic Antigen urine cassette test (POC-CCA) for the diagnosis of *Schistosomiasis mansoni* in a low endemic area in Ceará State, Brazil. In fact, the positivity rate increased significantly according to the diagnostic approach; the rate ranged from 1.6% by egg detection to 11.6% by the POC-CCA (t +), meaning there was a seven-fold increase. In fact, studies performed in different African settings (Colley et al., 2013; Coulibaly et al., 2011) where *S. mansoni* is endemic have repeatedly shown that the POC-CCA is capable of detecting many additional positive cases when compared to Kato-Katz thick smears.

In this study, the consistency observed between the POC-CCA test and Kato-Katz technique was poor, regardless of whether the trace results were considered as negative or positive, which could be partly explained by the very low prevalence observed by microscopy. These results support the findings of studies performed in low *S. mansoni* endemic areas in Africa (Adriko et al., 2014) and in Brazil (Coelho et al., 2016), which also identified significant disagreement between these methods. Most of the discordant results were CCA-positive but egg-negative, especially when trace results were considered as positive. As a test that is subject to visual reading, POC-CCA is a qualitative method based on individual interpretations. Considering the ambiguity of interpreting the 'trace' infection readings (light lines), a two-way analysis of CCA data with 'trace' results considered infection-negative and 'trace' results considered infection-positive were performed. The subjectivity and the need for reading standardisation have been discussed (Colley et al., 2013, 2017; Siqueira et al., 2016). Recently, a study in a Brazilian population with a low parasite burden in an endemic area showed that urine samples with trace results were found in individuals with or without *S. mansoni* eggs in their stool, after intensive examinations. After a 10-fold concentration of the urine samples by lyophilisation, the trace became positive in parasitologically positive cases, but it remained a trace result in parasitologically negative cases (Coelho et al., 2016). Thus, trace readings could not be promptly defined as positive or negative. Additionally, this same study showed that hookworm infection influences POC-CCA test results (Coelho et al., 2016), which is inconsistent with previous observations performed in Africa (Shane et al., 2011; Tchuem Tchuenté et al., 2012).

POC-CCA was recently deemed a new tool that could be used in schistosomiasis control strategies (Colley et al., 2017). The authors considered that the results that were Kato-Katz egg-negative/POC-CCA-positive could be explained by the menopause or infertility of *Schistosoma* females, the presence of immature worms, infection caused solely by males and immune fecundity. An important fact, which has not been discussed exhaustively by the authors, is the possibility of a false-positive reaction in the POC-CCA.

Corroborating our data, high prevalence rates of *S. mansoni* infection were found in adults in a study performed in Zambia (Lodh et al., 2013). If we followed the control approach for areas with prevalence levels below 10% using parasitological methods, which are aimed at treating only schoolchildren (WHO, 2006), more than half of the positive individuals in this study would remain untreated and would continue to contribute to the transmission of the infection. Nevertheless, despite the higher positivity rate found in adults, there were too many positive individuals below 20 years of age. Furthermore, our results show that 13% (POC-CCA) and 11% (SWAP-ELISA) of the *S. mansoni*-positive individuals were children younger than 6 years old. This finding reinforces the idea that preschool-aged children are at risk of schistosomiasis and that their treatment is also recommended as described elsewhere (Coulibaly et al., 2013, 2012).

Antibody detection is considered highly sensitive, and it has been recommended as a supplementary tool for schistosomiasis diagnosis in individuals with low infection burdens, which are usually hard to detect by parasitological methods (Alarcón de Noya et al., 2007; Grenfell et al., 2013). Our results show that one out of the four egg-positive cases and 19 (63.3%) of the POC-CCA positive (+) cases were antibody-negative. Antibodies can remain in the body for several years after treatment, and individuals with helminth infections can also show cross-reaction. Nevertheless, the negativity of the SWAP-ELISA in 63.3% of CCA-positive (t +) cases must be further investigated.

The recommended time for *S. mansoni* assessment after treatment is 15–20 days (Scherrer et al., 2009), but our data and those of others (Coulibaly et al., 2012, 2013; Lamberton et al., 2014; Legesse and Erko, 2007) still detected CCA from 3 to 6 weeks post-treatment, including or excluding 'trace' results as positive. Regarding cure controls through stool examinations, 20 days is considered a too-short period. Brazilian researchers suggest at least one month, with 4 months being considered

the optimal time after treatment, with several stool examinations (Ministério da Saúde, 2014). It is noteworthy to consider the strength of transmission in the area to define the follow-up time after treatment. Once again, African countries show different epidemiological settings when compared to what is found in the Americas, i.e., areas of high transmission for the former and low transmission for the latter.

A lower percentage of individuals showed positive results according to the POC-CCA test at the post-treatment follow-up. This finding could be due to the presence of juvenile worms that were not affected by treatment (Sabah et al., 1986) and/or worms that survived treatment, because the effectiveness of praziquantel is lower than 100% (Danso-Appiah et al., 2013; Katz et al., 1979, Stothard et al., 2013; Vinkeles Melchers et al., 2014).

Of the 28 cases that showed POC-CCA positivity but were egg-negative before the treatment, 26 (93%) became POC-CCA-negative at 3 weeks and the remaining 2 cases became negative 6 weeks after praziquantel administration. Studies are still necessary to determine when exactly CCA disappears from the urine after treatment.

The follow-up of the population after one and two years showed some positive POC-CCA reactions that are difficult to interpret. In view of the absence of water in the streams during the observation period, these few positive cases (t +) could be due to individuals who were infected in other areas, who relapsed after treatment, or who had false-positive results.

The logistical and financial implications of using each method have been discussed in several studies (Adriko et al., 2014; Colley et al., 2013; Coulibaly et al., 2013). The POC-based CCA is a user-friendly test that can be performed and read in the field. No additional equipment is needed for its use, and only a minimum of training if required. Thus, it can be used in remote rural areas that do not have access to a minimal infrastructure and trained staff. The time needed to perform the POC-CCA test is approximately 25 min, which is less time-consuming than the other methods currently employed for *S. mansoni* screening. Another advantage is that the POC-CCA test can detect prepatent infections (de Water et al., 1986), whilst the Kato-Katz method can only detect infections when the adult worms release eggs and the ELISA tests detect only the host's immunoactivity against the worm, which cannot differentiate between current and past infections. The disadvantages of POC-CCA compared to the Kato-Katz techniques is that the former is not quantitative, its reading is subjective, trace results remain a problem for interpretation and it does not disclose the presence of other helminths.

The current lack of a definitive standard reference test to detect all *Schistosoma* infections indicates the necessity of combining diagnostic methods to identify individuals with low parasite burdens, particularly in areas with low *S. mansoni* infection prevalence. Moreover, further investigations to determine the accuracy of the urine CCA cassette test using highly specific and sensitive diagnostic methods (i.e., polymerase chain reaction (PCR)) (Carneiro et al., 2013; Meurs et al., 2015; Pontes et al., 2002), the detection of Circulating Anodic Antigen by an up-converting phosphor technology-based lateral flow (UPT-LF) assay (Corstjens et al., 2014) or Helmintex (Pinheiro et al., 2012; Favero et al., 2017) is highly recommended in areas with a low prevalence and intensity of infection.

5. Conclusions

The present study showed that the positivity rate for *S. mansoni* in a low-endemic setting according to the urine-based POC-CCA test was significantly higher than that obtained using 3 Kato-Katz smears, when the trace results were considered positive. Nevertheless, the true significance of the trace remains unclear. It is crucial to use different tools to improve the schistosomiasis diagnoses in individuals with low parasite burdens. Finally, an adequate diagnostic approach that includes the characteristics as defined by the World Health Organization (i.e., sensitive, specific, rapid, and robust, affordable, deliverable to users and equipment-free) is urgently required.

Acknowledgements

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