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Evaluation of Urine CCA Strips for (Field) Diagnosis of Active *Schistosoma mansoni* Infection in a Low Endemic Kenyan Community

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EOA and MTM managed the analyses of the study. Author GOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Schistosomiasis is a chronic parasitic disease caused by a digenetic trematode blood fluke of the genus *Schistosoma*. The disease remains a serious public health problem in endemic countries and affects at least 207 million people worldwide, with 85% in Africa. In Kenya, more than 3.5 million are infected. A definite diagnosis of the disease plays a key role in the control of schistosomiasis. The detection of Schistosome Circulating antigens is an effective approach to discriminate previous exposure and current infection. Simple, non-invasive tools for detection of *S. mansoni* would be highly valuable with the view of post-intervention assessment in programmes but also for treatment/re-treatment of the individual patient. The study was conducted in Makueni, a low infection area (6 to 90 years old). The prevalence was 56.4% after three consecutive Kato-Katz stools. A population sample of n= 521 individuals was selected from an initial study cohort in an epidemiological follow-up of Schistosome infections in this foci. All the subjects in the sub-sample had been treated with praziquantel 40 mg/kg. The study was to determine the prevalence of *S.*

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mansoni using Kato technique and CCA (Circulating Cathodic Antigen) urine Elisa strips at baseline and 24 hours then also at 2 years later. The cohort study population at baseline had a prevalence of 62% of the schistosomiasis infection. The detection of schistosomiasis CCA at baseline using the CCA Elisa strip on urine samples gave a prevalence of 71.9%. The CCA was determined 24 hours after treatment with PZQ and the prevalence was 44.1%. In conclusion, Kitengei village is low endemic for schistosomiasis. Finally, circulating cathodic antigen can be used as a diagnostic tool, for the follow-up of chemotherapy and re-infection and as a field test and/or screening tool in control programmes.

Keywords: Circulating Cathodic Antigen (CCA); Schistosoma mansoni; diagnosis; prevalence.

1. INTRODUCTION

Schistosomiasis is a chronic parasitic disease caused by a digenetic trematode blood fluke of the genus *Schistosoma*. It is more predominant in the rural riverine areas and in areas where improvements of ventures to encourage irrigation and deliver hydroelectric power are being undertaken [1,2]. The disease remains a serious public health problem in endemic countries and affects at least 207 million people worldwide, with 85% in Africa [3]. Schistosomiasis is one of the largely extensively spread bloodsucking infections, second only to malaria in socio-economic and public health significance in tropical expanses where it is severe, incapacitating and occasionally serious disease [4,5,6]. Subsequent to a worldwide trend, schistosomiasis predominantly involves school children aged between 5 and 15 years equally in the Western and Coast counties of Kenya [7, 8,9,10], similarly in Central and Eastern Kenya [11,12]. In the rural regions of many developing countries, schistosomiasis is a significant work-related danger [13] and is a main public health interest in Kenya [14]. The incidence of schistosomiasis in Kenya has gradually increased over the last three decades. Presently more 3.5 million Kenyans are infected with either one or both species of the parasites and nearly 10 million are at risk of infection [15]. A definite diagnosis of the disease plays a key role in the control of schistosomiasis.

Infection with *Schistosoma mansoni* is one of the major parasitic diseases in the tropics and its socio-economic and public health importance based on indications and incapacity linked morbidities such as anaemia, chronic pain, diarrhoea, exercise intolerance and undernutrition is obvious [16]. The foremost public health effect is due to prolonged granulomatous host reaction around dispersed parasite eggs since many of the eggs are transported to the liver where they become lodged in the sinusoids and is a major cause of the morbidity of the infection [17]. The spread of schistosomiasis is generally correlated with poor socio-economic circumstances essentially lack safe water and sanitary facilities; consequently, schistosomiasis is mainly a disease of the poor [18]. Parasite spread and the resultant risk of human infection are intensely associated to local geographic environments because the parasite undergoes numerous developmental phases that must ensue in freshwater, embracing an interval of growth within certain species of intermediate snail hosts [19].

Identification of schistosomiasis, one of the foremost parasitic diseases in tropical areas, is mostly accomplished by parasitological, microscopic eggs recognition, and/or immunological procedures (antibody and antigen detection) [20]. The establishment of parasite eggs in urine or faeces unswervingly designates the existence of the worms, but the shortcomings of this methodology embrace a high variation in egg counts, certainly missed low infections and a relatively time- consuming approach. The detection of Schistosome Circulating antigens is an effective approach to discriminate previous exposure and current infection. Simple, non-invasive tools for detection *S. mansoni* would be highly valuable with the view of post-intervention assessment in control programmes but also for treatment/re-treatment of the individual patient. Schistosomiasis identification may serve two functions that are the medical care for individual patients and screening of communities or school children for mass treatment or epidemiological studies. The diagnosis of schistosomiasis in individual cases is composed probably by a blend of the clinical presentation, and a history of residence in a prevalent area. The study was to determine the prevalence of *S. mansoni* using Kato technique and CCA (Circulating Cathodic Antigen) urine Elisa strips at baseline and 24 hours then also at 2 years later.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Kitengei (Mtito-Andei)- Kenya. This is an area with low transmission of schistosomiasis. There is seasonality in rainfall that influences the transmission of *S. mansoni*. This study was undertaken on a community from low intensity, morbidity area. The study was conducted in Makueni County, Kenya. This is an area with *S. mansoni* and soil-transmitted helminth infections. The site is based in Kitengei sub-location in Kibwezi Sub-County. Kibwezi is one of the sixteen administrative divisions of Makueni County and is located approximately 200 kilometres south-east of Nairobi. It lies latitude 2° 40'60 S and longitude 38°10'0 E at an altitude of 769 metres above sea level. The area experiences two rainy seasons, the short rains in March – April and the long rains in November – December and an extended dry season in June – October. Mean annual rainfall is just over 600 mm [21]. Because of rainfall patterns, seasonality is an important characteristic of life and therefore, this influences the transmission of *S. mansoni* and other neglected tropical diseases. The County has shallow, low fertility soils composed of brown/red sandy loam of volcanic origin [22]. The main crops for the region include sorghum, millet, cowpeas, green grams, beans, pumpkins, and other dryland crops. But there are also soil management challenges such as erosion, moisture conservation, fertility constraints and crop production [23].

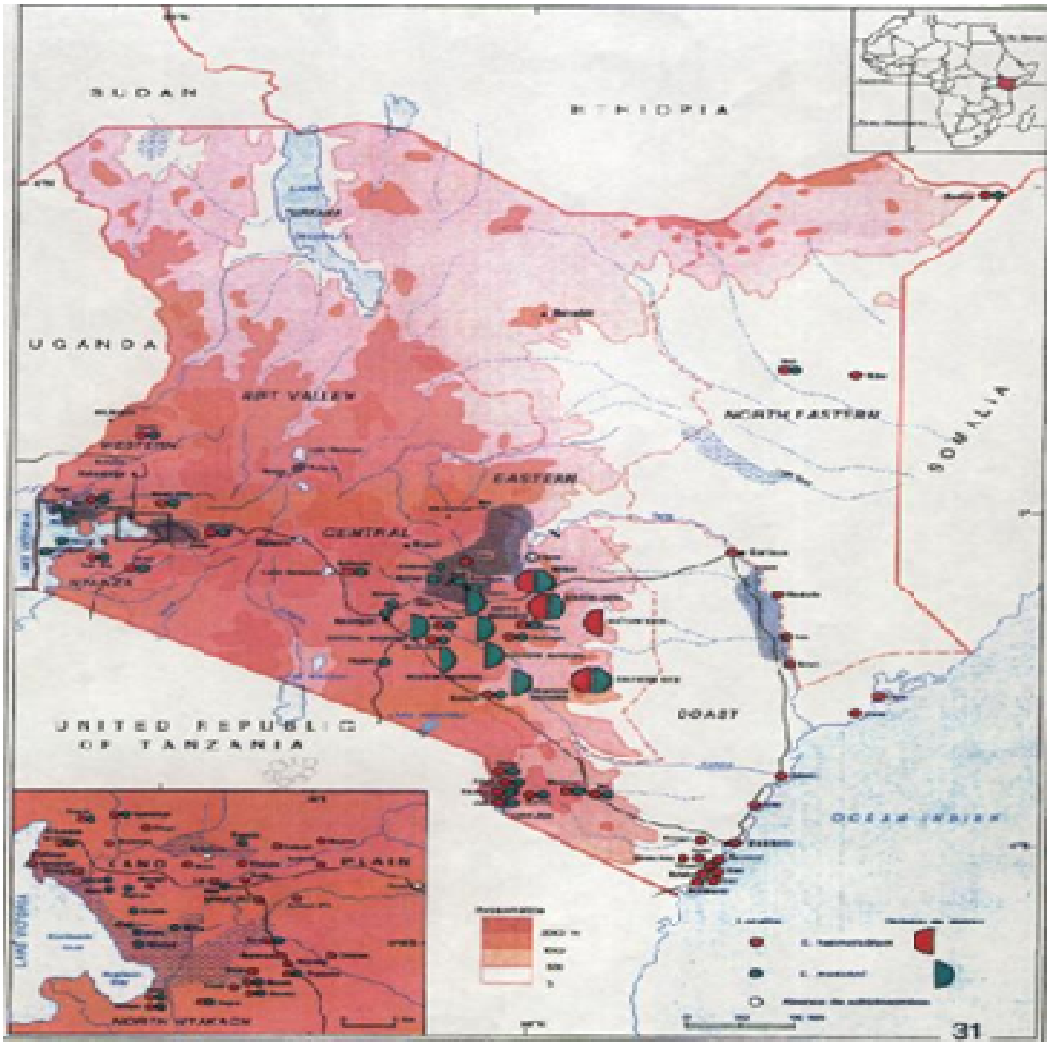

Fig. 1. Map showing the study location of Makueni County, Kenya

2.2 Study Population

With the available data on schistosomiasis from the Division of Vector-Borne and Neglected Tropical Diseases field station, this area had a populace with a low prevalence of *S. mansoni* and other helminth infections. The people in the community whose ages ranged from 6 years to above 40 years were randomly selected. The prevalence and intensity of *S. mansoni* were determined from the stool samples for all the people that participated in the study.

2.3 Study Design

This was composed of 521 people from the study area with no egg counts for *Schistosoma*, helminth infections and those with egg counts in their stools. It was a cross-sectional and randomized study that involved examination of stool for *S. mansoni* using Kato-Katz technique and urine-based dipstick for Circulating Cathodic Antigen (CCA) at baseline and 24 hours later and 2years after treatment.

2.4 Collection of Samples and Parasitological Examination

The people participating in the study were given plastic containers between 10.00 am and 2.00 pm to coincide with the peak of *Schistosoma* egg excretion [24] and asked to provide their urine specimen. Urine was collected from all the people. Aliquots of well-suspended urine were collected for CCA dipstick analysis. After collection, the urine samples were kept cold in a cooler box, transported to DVBNTD field laboratory within four hours and stored at -20°C for further analysis. Personal information on the participants, which comprised identification number, name, sex, and age, parasitological examination and CCA were entered on a data form.

2.5 Methodology

CCA strips were prepared based on immunochromatographic principle [25]. Unbacked nitrocellulose (PRIMA 125, Whatman, Dassel, Germany) was fixed on a GL-45569 (G and L, San Jose, CA, USA), with a sample pad and an absorbent pad. As the test line, 0.75 mg/ml Mab 54-5c 10-A in 5mM borate plus 1% ethanol pH 8.8 was sprayed on the card, and as the control line, goat anti-mouse (Sigma, San Jose, CA, USA), 0.2mg/ml in 5 mM borate pH 8.8 was used. The carbon conjugate was prepared using Mab 54- 4C2-A following the same standard protocol. After conjugation, 0.75 µl of this solution per 25 µl of a Sucrose- containing drying buffer was added to microtitre plate wells and dried overnight in a 37˚ Incubator. To perform the test, 75 µl of running buffer and 25 µl of urine sample was added to wells, mixed well with the colloidal carbon, after which the strip was placed into the wells. Strips were read wet after 30 mins and scored against a series of five standards.

The urine samples were mixed with the carbon-conjugate containing buffer and the *Schistosoma* Circulating Cathodic Antigen (CCA) bound to the antibody-carbon particles. Lateral flow transports the CCA-carbon complex through the strip, where the CCA present attached to the anti-CCA antibody at the test line and shown black carbon precipitation while the excess carbon conjugate was caught by the control line.

2.6 Test Principle

The lateral flow assay involved the use of nitrocellulose strip with capillaries through which the mixture of the urine sample and detection conjugate (Monoclonal antibody labelled with Carbon particles) flowed. The presence of the analyte, (CCA) was made visible by the captured immune complex of antigen and labelled antibody by anti- CCA monoclonal antibodies (Mcb) that were immobilized on the strip as a test line. Also, a line of immobilized polyclonal anti-mousee antibodies was used to capture the excess carbon- labelled antibodies that acted as the positive control line. Seventy-five microlitre run buffer was added per tube. Twenty-five microlitre urine samples were added per well and mixed to obtain a homogenous suspension. The dipstick was applied into the 100µl urine/ run buffer/ carbon suspension then incubated for thirty-five minutes at room temperature. The results were read against the quality control samples.

Stool samples were collected in the morning in poly pot containers, transported to the laboratory for processing and microscopic examination within four hours. Stool samples were collected from all the people in the area.

The presence or absence of *S. mansoni* and other helminth eggs in stool was determined by

the Kato-Katz method [26]. A 50 mg Kato-Katz slide was prepared from a fresh stool sample after taking a fixed quantity of sieved stool. This was deposited on to a glass slide covered with glycerine-impregnated cellophane and was left to clear for a minimum of 45 minutes [27] and a maximum of 2 hours [28]. The preparation was examined under a microscope, S*. mansoni* eggs counted and expressed as eggs/gm faeces.

2.7 Statistical Analysis

Data analysis was carried out using the SPSS for Windows (11.0) statistical programme (Jandell Scientific, San Rafael, CA). Chi-square test was used to compare the statistical difference for prevalence between males and females. The Chi-squaree test was used to compare the statistical differences between mean egg counts and CCA levels before and after treatment.

The geometric mean of the egg counts was calculated as antilog [∑LnX+1)/n]-1; where Ln is the natural logarithm, X is the number of the egg counts per gram of faeces and n, is the number infected individuals. One-way analysis of variance (ANOVA) was carried out between the groups to get the differences of the mean geometric egg counts in age groups while the difference in mean Log10 egg counts of *S. mansoni* between each age group was compared using the Tamhane analysis test. In all tests, a probability value of less than 0.05 was considered statistically significant.

3. RESULTS

The prevalence of *S. mansoni* using the Kato-Katz technique was 62% in the study area. There was a significant difference in mean egg counts between males and females (p< 0.001).The prevalence was 71.9% using the CCA Elisa dipstick at baseline and 44.1% 24 hours after treatment with the drug praziquantel. The reduction in CCA prevalence was significant (p<0.001). The cohort population was 521 with a prevalence of 62% of the schistosomiasis infection.

The differences in mean egg count of *S. mansoni* between each age group between the sexes were compared and using the t-test, where significant difference was found in mean egg count between age group 6-11years and age groups;12-18 years (P = 0.023, P> 0.05) and above 40 years old (P= 0.043, P< 0.05) but no difference was found in age groups; 19-29 years (P= 0.868, P> 0.05) and 30-40years (P= 0.166, P> 0.05). The age group 12-18 years were found to be significantly different between age groups 30-40 years (P< 0.05) and also above 40 years (P< 0.05) while there was no difference between age group 19-29 years (P> 0.05). The age group 19-29 years was only significantly different in mean egg count between the age group above 40 years (P< 0.05). There was also a significant difference in mean intensity of infection in the age group 12-18 years old between males and females (P< 0.05, t = 2.3534), the females excreted fewer eggs comparatively. In the rest of the age groups, there was no significant difference in mean egg counts.

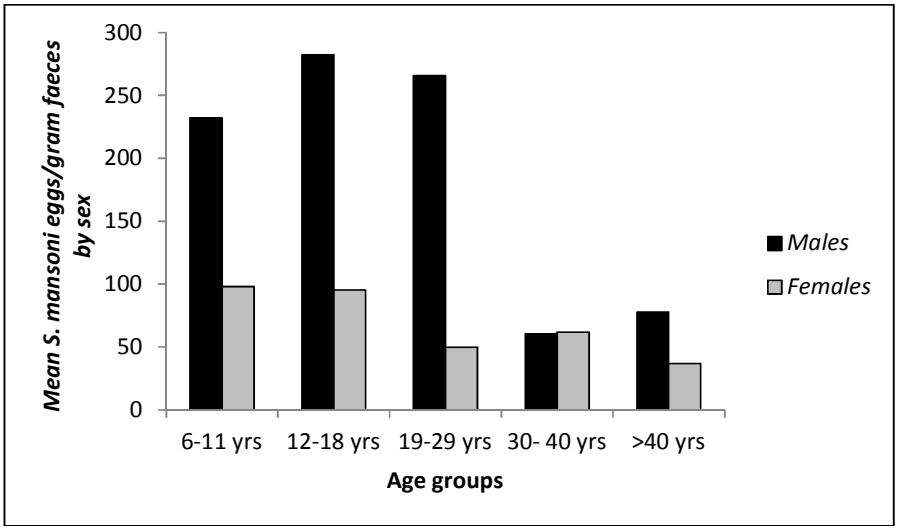

Fig. 2. *S. mansoni* mean egg/gram faeces between males and females in the ages

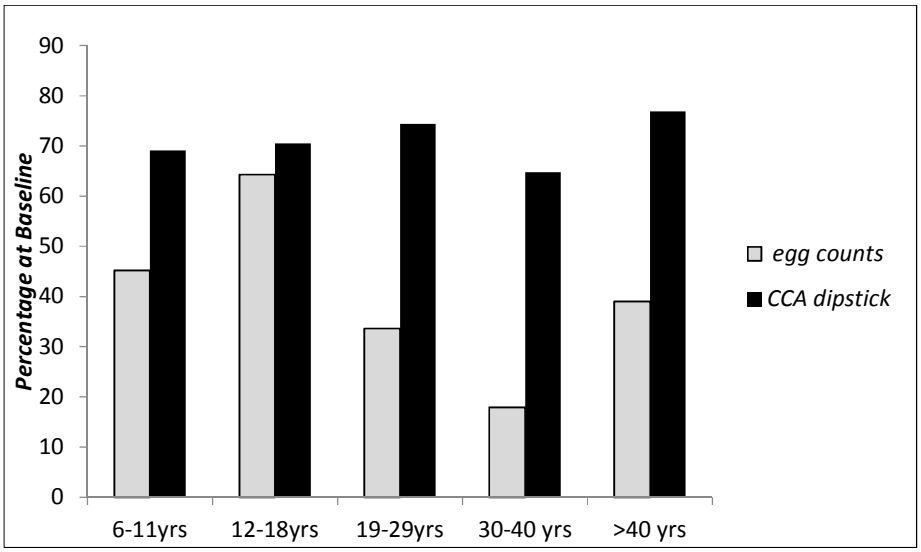

Fig. 3. The prevalence of CCA positivity in age groups at baseline

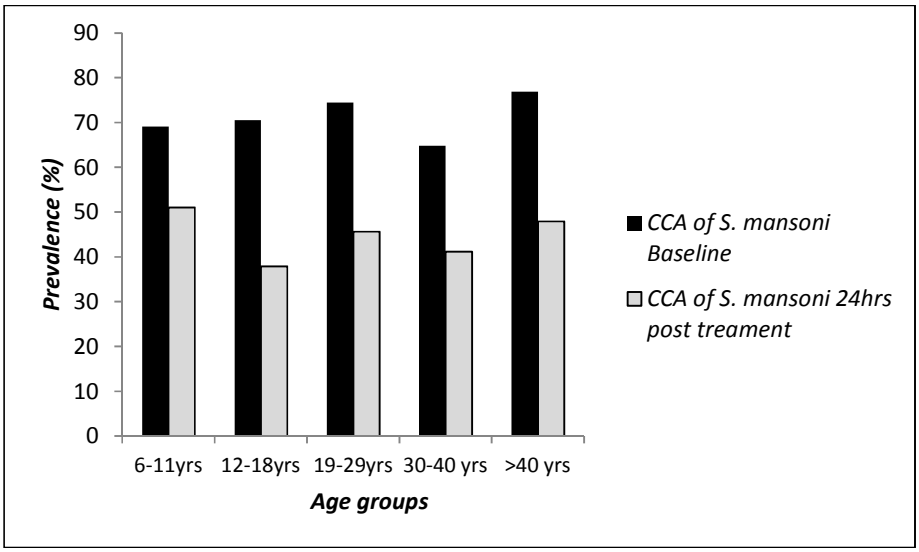

Fig. 4. The CCA prevalence in age groups 24 hrs. Post-treatment with praziquantel

3.1 Detection of the Schistosomiasis Circulating Cathodic Antigen (CCA) at Baseline

A total of 366 individuals were positive of schistosomiasis out of the 509 cohorts available using the Circulating Cathodic antigen ELISA strip on urine samples giving a prevalence of 71.9%. The prevalence rate of Circulating Cathodic Antigen (CCA) in the females was 64.5% (182/262) while in the males the CCA prevalence was 74.5%, this difference was not significant (χ^2 = 1.591, P= 0.207). Among the age groups, there was no difference in the CCA excretion (χ^2 = 3.783, df = 4, P= 0.436). The study also found that 63 individuals were negative for both CCA and eggs while 159 individuals were CCA positive but had negative eggs. A further 207 were positive for both CCA and eggs while 80 individuals being CCA negative but egg positive.

3.2 Detection of the Circulating Cathodic Antigen (CCA) 24 hrs. Post-treatment

A total of 202 individuals were positive for schistosomiasis out of the 458 available using the Circulating Cathodic antigen ELISA strip 24

hours post-treatment in their urine samples giving a prevalence of 44.1% after treatment with praziquantel. The percentage reduction rate of Circulating Cathodic Antigen (CCA) in the populace was 35.8% (164/458), this reduction seen in CCA excretion was significant (χ^2 = 65. 214, P< 0.001).

3.3 The CCA Elisa Strip Score at Baseline and 24 hrs. Post-treatment with Praziquantel

The Circulating Cathodic antigen ELISA strip score at baseline and 24hours post-treatment were also recorded, after testing their urine samples giving a reagent strip index between negative, 1+, 2+ and 3+ before and after treatment with praziquantel. 143/403 (35.5%) had a negative score at baseline and 260/403 (64.5%), 24 hrs. post-treatment. A score of 1+ was 211/403 (52.4%) at baseline and, 164/403 (40.7%) 24 hrs. post-treatment, with a score of 2+ at baseline, was 81/ 403 (20.1%) and 24hrs. post-treatment was 20/403 (5.0%) finally, those who had a score of 3+ was 73/403 (18.0 %) at baseline and 24hrs. post-treatment was 10/403 (2.5%). Circulating cathodic antigen (CCA) in the populace was 35.8% (164/458). A positive association between increasing intensity of the CCA urine Elisa strip test band and faecal egg count was observed. Fig. 5 illustrates the CCA Elisa Strip score at baseline and 24 hrs. Post-treatment with praziquantel.

Living schistosomes excrete several different antigens into a host's circulation. The major circulating antigens belong to the adult worm gut-associated circulating antigens [20]. These antigens are released into the circulation of the host by regurgitation of the undigested contents of the parasites' gut. One of these stable glycoproteins, the Circulating Cathodic Antigen (CCA) was used in this study. The urine CCA Elisa strip detects the presence of schistosome CCA released by the adult worms into the host's urine (15 µl), removing the need for faecal sampling.

The findings showed that more cases of schistosomiasis were captured with CCA than with the gold standard Kato-Katz method for counting eggs in the stool. The sensitivity of a single examination, however, can be very low due to a combination of well- known factors, such as the variation in the distribution of eggs within a stool specimen and day- to –day variation in faecal egg concentrations especially when host egg excretion is sporadic [29,30,31,32]. Obtaining urine from the participants for the CCA dip-stick was much easier than obtaining the stool samples. Furthermore, since CCA dipstick is a non-invasive method, it is more readily accepted by endemic populations than obtaining blood or serum for Circulating anodic antigen (CAA). In this low transmission environment, at both baseline and follow-up, the urine CCA test identified more infections than the Kato-Katz

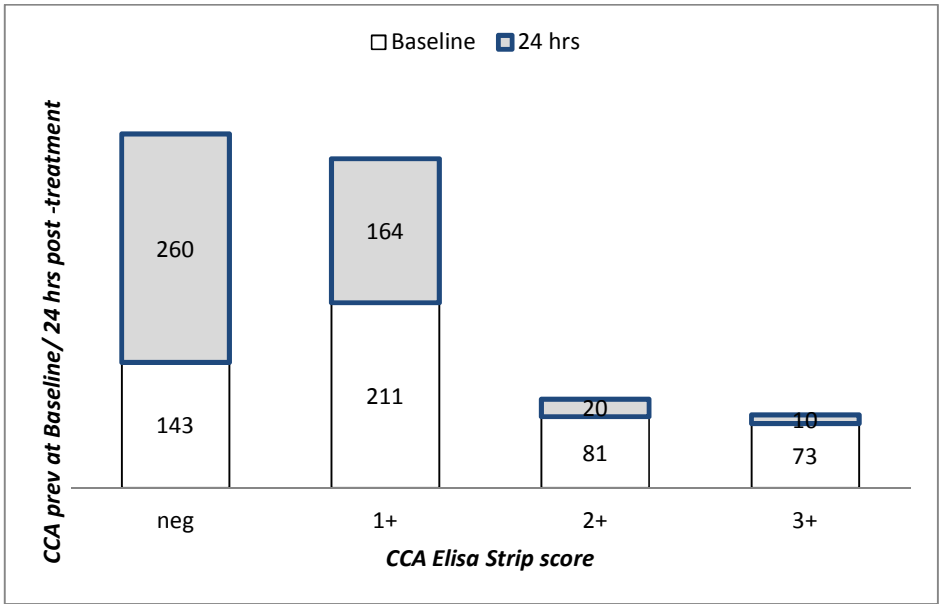

Fig. 5. The CCA elisa strip score at baseline and 24 hrs. post-treatment with praziquantel

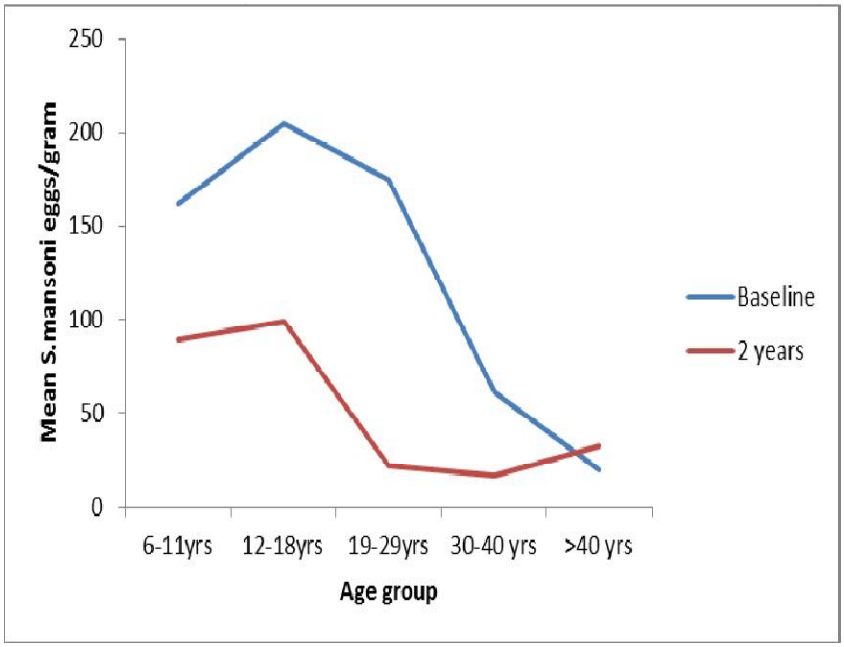

Fig. 6. Mean *S. mansoni* egg/ gram stool at baseline and 2 years

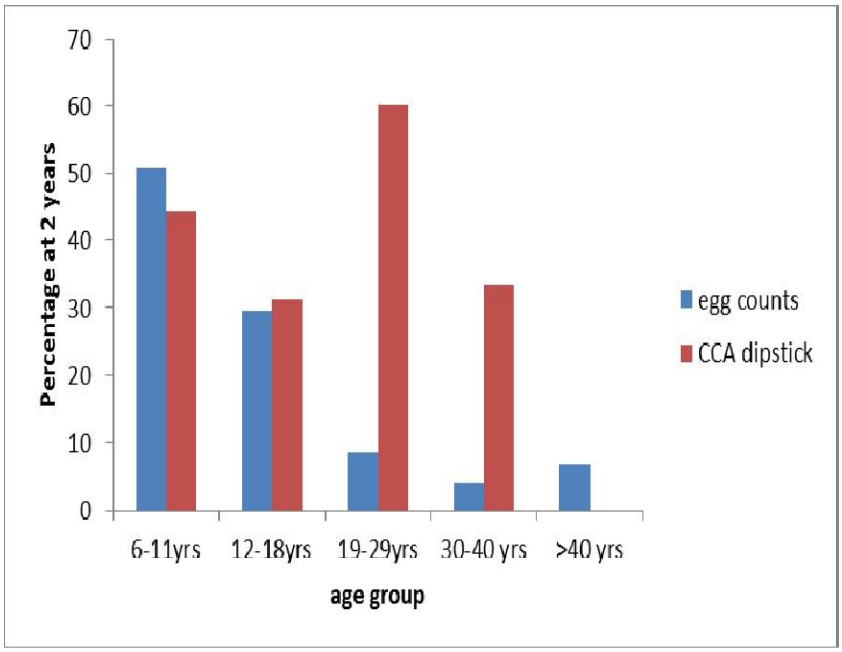

Fig. 7. Prevalence of *S. mansoni* by CCA dipstick and egg counts at 2 years post-treatment

technique used as a gold standard. This is due to the latency between active infection (metabolising worms), as detected by the urine CCA and commencement of egg production detected by the Kato-Katz method. The CCA dipstick was a valuable and sensitive tool for field diagnosis of active *S. mansoni* through its sensitivity should be evaluated further.

Several studies have demonstrated that the Kato-Katz method may miss infections, especially light infections in low endemic areas [33,34], also observed similar findings and demonstrated that due to the low sensitivity of Kato-Katz for detecting the presence of *S. mansoni* infection, an all-embracing sampling effort may still considerably underestimate the

prevalence. 35. Kongs et al. [35] noted that despite the relatively poor sensitivity of a single Kato-Katz slide, the majority of the undiagnosed infections were, in fact, light cases hence light infections are diagnosed with increasingly sensitive diagnostic techniques. Therefore, since the sensitivity of a single Kato-Katz is poor, the unreliability of the method limits its usefulness for evaluating *S. mansoni* infections. These outcomes emphasized the need to examine stools taken on different days when a definitive diagnosis of infection status is required. Hence this recommendation is of particular importance for areas of low endemicity. It is known that prevalence and intensity of infection decrease sharply after treatment with praziquantel thus a single Kato-Katz technique is likely to miss a large proportion of residual infections [32]. These findings reinforce the need to associate different tools for improved schistosomiasis diagnosis in individuals with low parasite burden. For this reason, the detection of *Schistosoma mansoni* circulating antigens for diagnosis of *S. mansoni* would be highly valuable with the view of post-intervention assessment and elimination programmes but also for re-treatment of the individual patient.

4. CONCLUSION

The urine CCA dipstick is a valuable tool for field diagnosis of active schistosomiasis mansoni. CCA urine dipstick should play an important role in assisting in disease surveillance. The CCA is a viable alternative for diagnosis of *S. mansoni* infections, particularly in a community in the endemic areas where the prevalence is quite low. It is further envisaged that if this technique is optimised then it can be used in cases of schistosomiasis infections in travellers and low endemic areas (Lambertucci *et al.,* 2009). Finally, the circulating cathodic antigen technique is a possible candidate for adoption for use in the elimination of Neglected Tropical Diseases programs in line with the recent call by the World Health Organization.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from all those who participated in the study, in line with the National Guidelines of the Ministry of Public Health and Sanitation, whose ethical review committees approved all the protocols used. All the positive cases were treated appropriately with Praziquantel (40 mg/Kg body weight).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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