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Original Research Article

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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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ABSTRACT:

9 Schistosomiasis is a chronic parasitic disease caused by a digenetic trematode blood fluke of the
10 genus *Schistosoma*. The disease remains a serious public health problem in endemic countries
11 and affects at least 207 million people worldwide, with 85% in Africa. In Kenya more than 3.5
12 million are infected. A definite diagnosis of the disease plays a key role in the control of
13 schistosomiasis. The detection of Schistosome Circulating antigens is an effective approach to
14 discriminate previous exposure and current infection. Simple, non-invasive tools for detection *S.*
15 *mansoni* would be highly valuable with the view of post intervention assessment in programmes
16 but also for treatment/re-treatment of the individual patient. The study was conducted in
17 Makueni, a low infection area (6 to 90 years old). The prevalence was 56.4% after three
18 consecutive Kato-Katz stools. A population sample of n= 521 individuals were selected from an
19 initial study cohort in an epidemiological follow-up of Schistosome infections in this foci. All
20 the subjects in the sub-sample had been treated with praziquantel 40mg/kg. The study was to
21 determine the prevalence of *S. mansoni* using Kato technique and CCA (Circulating Cathodic
22 Antigen) urine Elisa strips at baseline and 24 hours then also at 2 years later. The cohort study
23 population at baseline had a prevalence of 62% of the schistosomiasis infection. The detection of
24 schistosomiasis CCA at baseline using the CCA Elisa strip on urine samples gave a prevalence
25 of 71.9%. The CCA was determined 24 hours after treatment with PZQ and the prevalence was
26 44.1%. In conclusion, Kitengei village is low endemic for schistosomiasis. Finally, circulating
27 cathodic antigen can be used as a diagnostic tool, for the follow-up of chemotherapy and re-
28 infection and as a field test and/or screening tool in control programmes.

29 **Key Words:** Circulating cathodic antigen (CCA), *Schistosoma mansoni,* Diagnosis, Prevalence.

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INTRODUCTION:

32 Schistosomiasis is a chronic parasitic disease caused by a digenetic trematode blood fluke of the
33 genus *Schistosoma*. It is more predominant in the rural riverine areas and in areas where
34 improvements of ventures to encourage irrigation and deliver hydroelectric power are being
35 undertaken (Akinwale *et al*., 2008; Akogun and Akogun, 1996). The disease remains a serious
36 public health problem in endemic countries and affects at least 207 million people worldwide,
37 with 85% in Africa, (Fenwick *et al*., 2009). Schistosomiasis is one of the largely extensively
38 spread bloodsucking infections, second only to malaria in socio- economic and public health

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39 significance in tropical expanses where it is a severe, incapacitating and occasionally serious
40 disease (WHO,2002; Chitsulo *et al.,*2000; WHO, 1993). Subsequent to a worldwide trend,
41 schistosomiasis predominantly involves school children aged between 5 and 15 years equally in
42 the Western and Coast counties of Kenya (Kahama *et al.,* 1998; Brown, 1994; Sturrock *et al.,*
43 1990; Kinoti, 1971), similarly in Central and Eastern Kenya (Siongok *et al.,* 1976; Mutinga *et*
44 *al.,* 1971). In the rural regions of many developing countries, schistosomiasis is a significant
45 work-related danger (Doumenge *et al.,* 1987) and is a main public health interest in Kenya
46 (Ouma and Waithaka, 1978). The incidence of schistosomiasis in Kenya has gradually increased
47 over the last three decades. Presently more 3.5 million Kenyans are infected with either one or
48 both species of the parasites and nearly 10 million are at risk of infection (Muchiri *et al*., 1996).
49 A definite diagnosis of the disease plays a key role in the control of schistosomiasis.

50 Infection with *Schistosoma mansoni* is one of the major parasitic diseases in the tropics and its
51 socio- economic and public health importance based on indications and incapacity linked
52 morbidities such as anaemia, chronic pain, diarrhoea, exercise intolerance and under nutrition is
53 obvious (King *et al.,* 2005). The foremost public health effect is due to prolonged granulomatous
54 host reaction around dispersed parasite eggs since many of the eggs are transported to the liver
55 where they become lodged in the sinusoids and is a major cause of the morbidity of the infection
56 (Cheever *et al.,* 2000). Spread of schistosomiasis is generally correlated with poor socio-
57 economic circumstances essentially lack of safe water and sanitary facilities; consequently,
58 schistosomiasis is mainly a disease of the poor (WHO, 1999). Parasite spread and the resultant
59 risk of human infection are intensely associated to local geographic environments because the
60 parasite undergoes numerous developmental phases that must ensue in fresh water, embracing an
61 interval of growth within certain species of intermediate snail hosts (King, 2009).

62 Identification of schistosomiasis, one of the foremost parasitic diseases in tropical areas, is
63 mostly accomplished by parasitological, microscopic eggs recognition, and/or immunological
64 procedures (antibody and antigen detection) (van Lieshout *et al.,* 2000). The establishment of
65 parasite eggs in urine or faeces unswervingly designates the existence of the worms, but the
66 shortcomings of this methodology embrace a high variation in egg counts, certainly missed low
67 infections, and a relatively time- consuming approach. The detection of Schistosome Circulating
68 antigens is an effective approach to discriminate previous exposure and current infection.
69 Simple, non-invasive tools for detection *S. mansoni* would be highly valuable with the view of
70 post intervention assessment in control programmes but also for treatment/re-treatment of the
71 individual patient. Schistosomiasis identification may serve two functions that is the medical
72 care for individual patients, and screening of communities or school children for mass treatment
73 or epidemiological studies. The diagnosis of schistosomiasis in individual cases is composed
74 probable by a blend of the clinical presentation, and a history of residence in a prevalent area.
75 The study was to determine the prevalence of *S. mansoni* using Kato technique and CCA
76 (Circulating Cathodic Antigen) urine Elisa strips at baseline and 24 hours then also at 2 years
77 later.

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81 **MATERIALS AND METHODS:**

82

83 **Study Area:**

84 The study was conducted in Kitengei (Mtito-Andei)- Kenya. This is an area with low
85 transmission of schistosomiasis. There is seasonality in rainfall that influences the transmission
86 of *S. mansoni*. This study was undertaken on a community from a low intensity, morbidity area.
87 The study was conducted in Makueni County, Kenya. This is an area with *S. mansoni* and soil
88 transmitted helminth infections. The site is based in Kitengei sub-location in Kibwezi Sub-
89 County. Kibwezi is one of the sixteen administrative divisions of Makueni County and is located
90 approximately 200 kilometres south-east of Nairobi. It lies latitude 2° 40'60 S and longitude
91 38°10'0 E at an altitude of 769 metres above sea-level. The area experiences two rainy seasons,
92 the short rains in March – April and the long rains in November – December, and an extended
93 dry season in June – October. Mean annual rainfall is just over 600 mm (Jaetzold and Schmidt,
94 1983). Because of rainfall patterns, seasonality is an important characteristic of life and
95 therefore, this influences the transmission of *S. mansoni* and other neglected tropical diseases.
96 The County has shallow, low fertility soils composed of brown/red sandy loam of volcanic origin
97 (Musimba, 1994). The main crops for the region include sorghum, millet, cowpeas, green grams,
98 beans, pumpkins, and other dry land crops. But there are also soil management challenges such
99 as erosion, moisture conservation, fertility constraints and crop production (Gichuki *et al.,* 2000).

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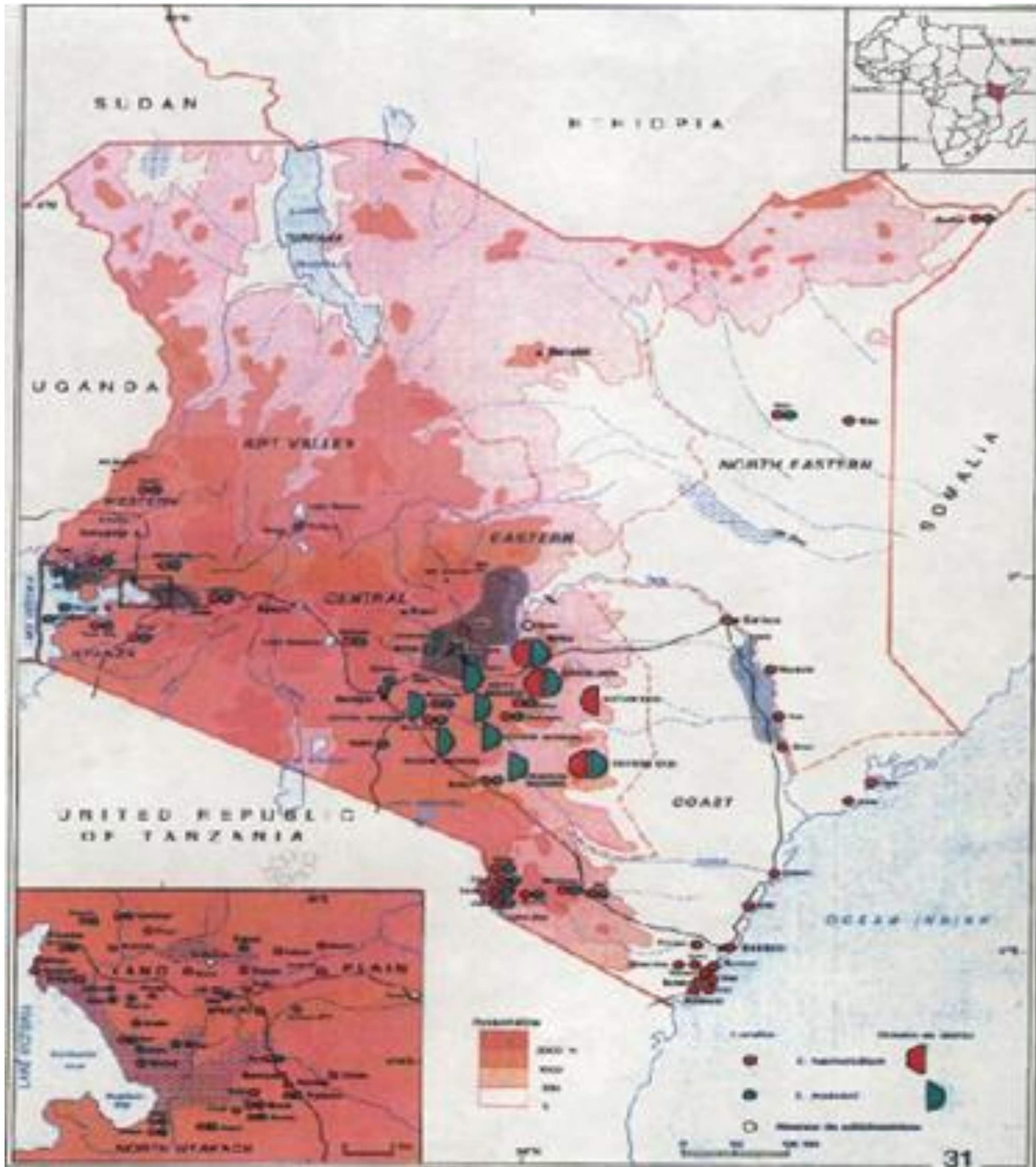

Fig. 1. Map showing the study location of Makueni County, Kenya

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

107 above 40 years were randomly selected. The prevalence and intensity of *S. mansoni* were
108 determined from the stool samples for all the people that participated in the study.

109 **Study Design**:

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

115 **Ethical consideration**

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

120

121 **Collection of Samples and parasitological examination:**

122 The people participating in the study were given plastic containers between 10.00 am and 2.00
123 pm to coincide with the peak of *Schistosoma* egg excretion (Doehring *et al.,* 1985b) and asked to
124 provide their urine specimen. Urine was collected from all the people. Aliquots of well-
125 suspended urine were collected for CCA dipstick analysis. After collection, the urine samples
126 were kept cold in a cooler box, transported to DVBNTD field laboratory within four hours and
127 stored at -20°C for further analysis. Personal information on the participants, which comprised
128 identification number, name, sex, and age, parasitological examination and CCA were entered on
129 a data form.

130 **Methodology:**

131 CCA strips were prepared based on immunochromatographic principle by (Van Dam *et al.,*
132 2004). Unbacked nitrocellulose (PRIMA 125, Whatman, Dassel, Germany) was fixed on a GL-
133 45569 (G and L, San Jose, CA, USA), with a sample pad and an absorbent pad. As the test line,
134 0.75 mg/ml Mab 54-5c 10-A in 5mM borate plus 1% ethanol pH 8.8 was sprayed on the card,
135 and as the control line, goat anti-mouse (Sigma, San Jose, CA, USA), 0.2mg/ml in 5 mM borate
136 pH 8.8 was used. The carbon conjugate was prepared using Mab 54- 4C2-A following the same
137 standard protocol. After conjugation, 0.75 µl of this solution per 25 µl of a Sucrose- containing
138 drying buffer was added to microtitre plate wells and dried overnight in a 37˚ Incubator. To
139 perform the test ,75 µl of running buffer and 25 µl of urine sample were added to wells, mixed
140 well with the colloidal carbon, after which the strip were placed into the wells. Strips were read
141 wet after 30 mins and scored against a series of five standards .

142

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

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148 **Test principle**
149 The lateral flow assay involved the use of nitro- cellulose strip with capillaries through which the
150 mixture of the urine sample and detection conjugate (Monoclonal antibody labelled with Carbon
151 particles) flowed. The presence of the analyte, (CCA) was made visible by the captured immune
152 complex of antigen and labelled antibody by anti- CCA monoclonal antibodies (Mcb) that were
153 immobilized on the strip as a test line. In addition, a line of immobilized polyclonal anti- mouse
154 antibodies was used to capture the excess carbon- labelled antibodies that acted as the positive
155 control line. Seventy-five microlitre run buffer was added per tube. Twenty -five microlitre urine
156 samples were added per well and mixed to obtain a homogenous suspension. The dipstick was
157 applied into the 100µl urine/ run buffer/ carbon suspension then incubated for thirty-five minutes
158 at room temperature. The results were read against the quality control samples.
159 Stool samples were collected in the morning in polypot containers, transported to the
160 laboratory for processing and microscopic examination within four hours. Stool samples were
161 collected from all the people in the area.

162 The presence or absence of *S. mansoni* and other helminth eggs in stool was determined by the
163 Kato-Katz method (Katz *et al.,* 1972). A 50 mg Kato-Katz slide was prepared from a fresh stool
164 sample after taking a fixed quantity of sieved stool. This was deposited on to a glass slide
165 covered with glycerine-impregnated cellophane and was left to clear for a minimum of 45
166 minutes (Peters *et al.,* 1980) and a maximum of 2 hours (Gryseels *et al*., 1991). The preparation
167 was examined under a microscope, S. *mansoni* eggs counted and expressed as eggs/gm faeces.

168

169 **Statistical analysis:**
170 Data analysis was carried out using the SPSS for Windows (11.0) statistical programme (Jandell
171 Scientific, San Rafael, CA). Chi- square test was used to compare the statistical difference for
172 prevalence between males and females. The Chi- square test was used to compare the statistical
173 differences between mean egg counts and CCA levels before and after treatment.
174 The geometric mean of the egg counts was calculated as antilog [∑LnX+1)/n]-1; where Ln is the
175 natural logarithm, X is the number of the egg counts per gram of faeces and n, is the number
176 infected individuals. One-way analysis of variance (ANOVA) was carried out between the
177 groups to get the differences of the mean geometric egg counts in age groups while the
178 difference in mean Log10 egg counts of *S. mansoni* between each individual age group was
179 compared using the Tamhane analysis test. In all tests, a probability value of less than 0.05 was
180 considered statistically significant.

181

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

189 The differences in mean egg count of *S. mansoni* between each individual age group between the
190 sexes was compared and using the t- test, where significant difference was found in mean egg

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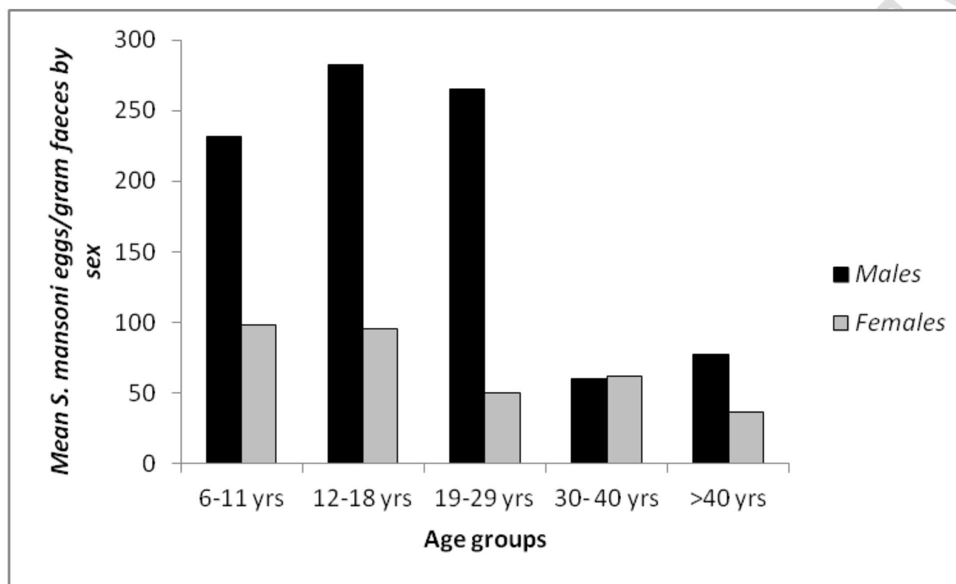
191 count between age group 6-11years and age groups;12-18 years (P = 0.023, P> 0.05) and above
192 40 years old (P= 0.043, P< 0.05) but no difference was found in age groups; 19-29 years (P=
193 0.868, P> 0.05) and 30-40years (P= 0.166, P> 0.05). The age group 12-18 years was found to be
194 significantly different between age groups 30-40 years (P< 0.05) and also above 40 years (P<
195 0.05) while there was no difference between age group 19-29 years (P> 0.05). The age group
196 19-29 years was only significantly different in mean egg count between age group above 40
197 years (P< 0.05). There was also a significant difference in mean intensity of infection in age
198 group 12-18 years old between males and females (P< 0.05, t = 2.3534), the females excreted
199 less eggs comparatively. In the rest of the age groups, there was no significant difference in mean
200 egg counts.



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

203 **Detection of the schistosomiasis Circulating Cathodic Antigen (CCA) at baseline**
204 A total of 366 individuals were positive of schistosomiasis out of the 509 cohorts available using
205 the Circulating Cathodic antigen ELISA strip on urine samples giving a prevalence of 71.9 %.
206 The prevalence rate of Circulating Cathodic antigen (CCA) in the females was 64.5% (182/262)
207 while in the males the CCA prevalence was 74.5 %, this difference was not significant (χ^2 =
208 1.591, P= 0.207). Among the age groups, there was no difference in the CCA excretion (χ^2 =
209 3.783, df = 4, P= 0.436). The study also found that 63 individuals were negative for both CCA
210 and eggs while 159 individuals were CCA positive but had negative eggs. A further 207 were
211 positive for both CCA and eggs while 80 individuals being CCA negative but egg positive.

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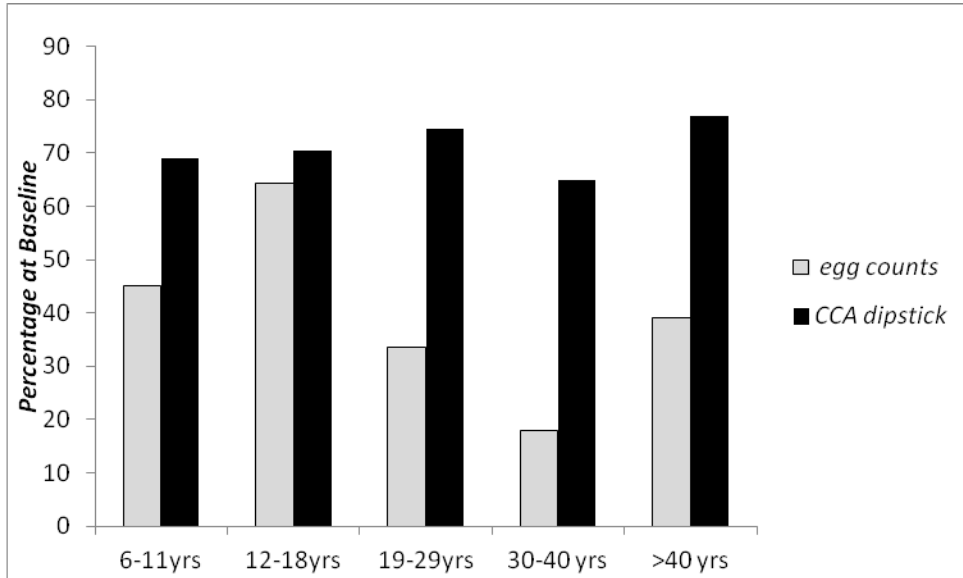
212 

213 Figure: 3. The prevalence of CCA positivity in age groups at baseline

214 **Detection of the Circulating Cathodic antigen (CCA) 24hrs post-treatment**
215 A total of 202 individuals were positive for schistosomiasis out of the 458 available using the
216 Circulating Cathodic antigen ELISA strip 24 hours post-treatment in their urine samples giving a
217 prevalence of 44.1 % after treatment with praziquantel. The percentage reduction rate of
218 Circulating Cathodic antigen (CCA) in the populace was 35.8% (164/458), this reduction seen in
219 CCA excretion was significant (χ^2 = 65. 214, P< 0.001).

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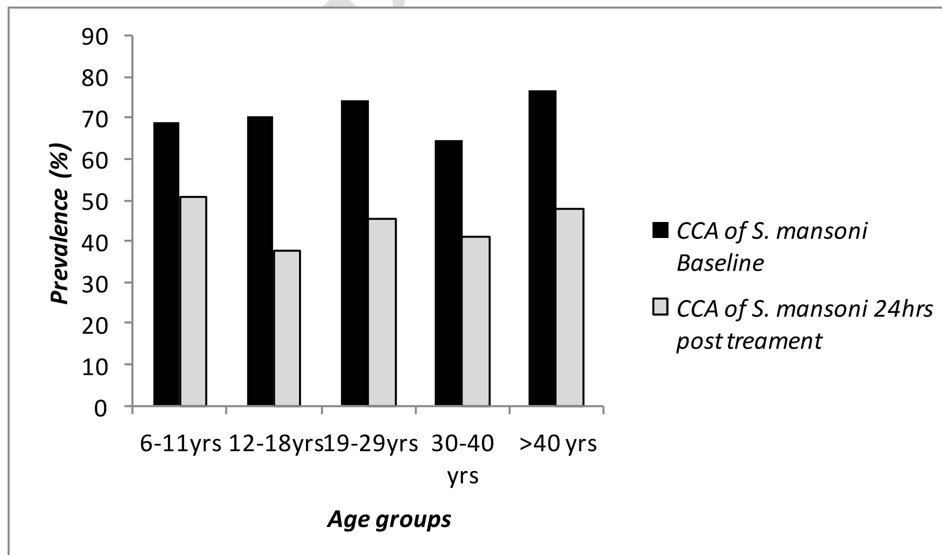
221 

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222 **Figure: 4. The CCA prevalence in age groups 24 hrs. Post-treatment with praziquantel**

223 **The CCA Elisa Strip score at baseline and 24 hrs. Post-treatment with praziquantel**

224 The Circulating Cathodic antigen ELISA strip score at baseline and 24hours post-treatment was
225 also recorded, after testing their urine samples giving a reagent strip index between negative, 1+,
226 2+ and 3+ before and after treatment with praziquantel. 143/403 (35.5%) had a negative score at
227 baseline and 260/403 (64.5%), 24 hrs. post-treatment. A score of 1+ was 211/403 (52.4%) at
228 baseline and, 164/403 (40.7%) 24 hrs. post- treatment, with a score of 2+ at baseline was 81/ 403
229 (20.1%) and 24hrs. post-treatment was 20/403 (5.0%) finally, those who had a score of 3+ was
230 73/403 (18.0 %) at baseline and 24hrs. post-treatment was 10/403 (2.5 %). Circulating cathodic
231 antigen (CCA) in the populace was 35.8% (164/458). A positive association between increasing
232 intensity of the CCA urine Elisa strip test band and faecal egg count was observed. **Figure.11**
233 illustrates the CCA Elisa Strip score at baseline and 24 hrs. Post-treatment with praziquantel

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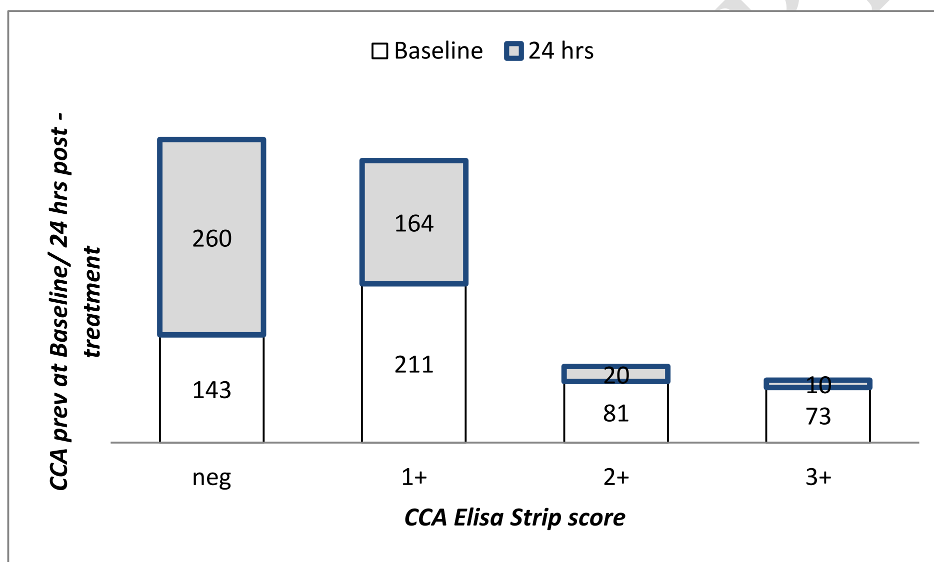

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237 **Figure: 5. The CCA Elisa Strip score at baseline and 24 hrs. Post-treatment with**
238 **praziquantel.**

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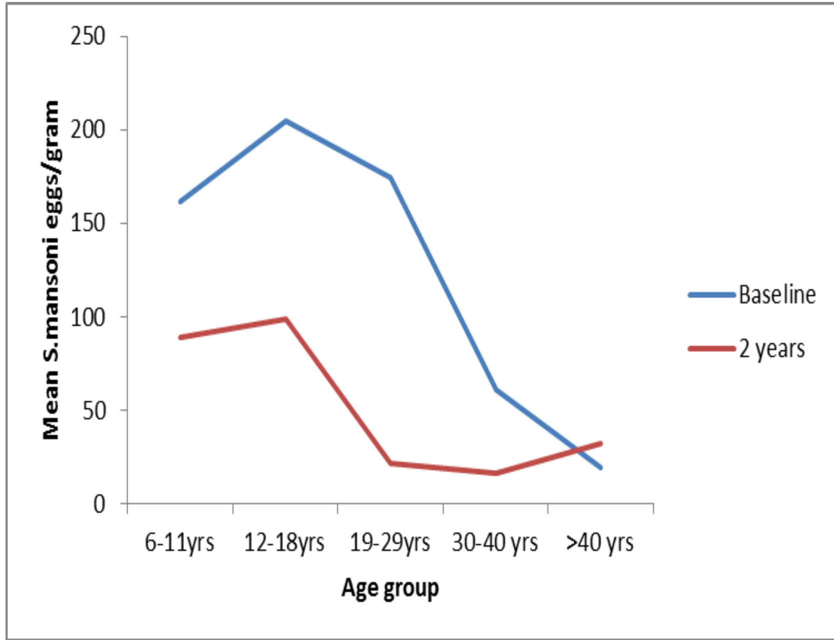

241 **Figure:6. Mean *S. mansoni* egg/ gram stool at baseline and 2 years.**

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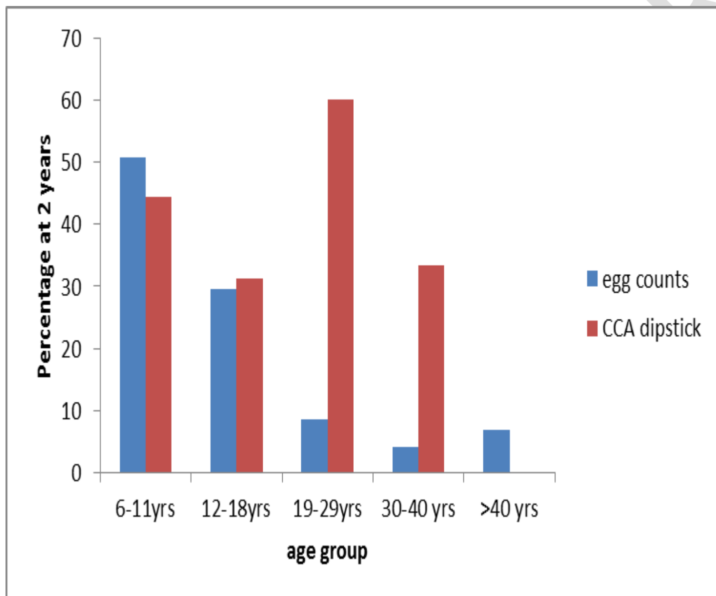

243 **Figure :7. Prevalence of *S. mansoni* by CCA dipstick and egg counts at 2 years post**
244 **treatment.**

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248 Living schistosomes excrete several different antigens into a host's circulation. The major
249 circulating antigens belong to the adult worm gut-associated circulating antigens (L.van Lieshout
250 *et al.,* 2000).These antigens are released into the circulation of the host by regurgitation of the
251 undigested contents of the parasites' gut. One of these stable glyco-proteins, the Circulating
252 Cathodic Antigen (CCA) was used in this study. The urine CCA Elisa strip detects the presence
253 of schistosome CCA released by the adult worms into the host's urine (15 µl), removing the need
254 for faecal sampling.

255 The findings showed that more cases of schistosomiasis were captured with CCA than with the
256 gold standard Kato-Katz method for counting eggs in stool. The sensitivity of a single
257 examination, however, can be very low due to a combination of well- known factors, such as the
258 variation in the distribution of eggs within a stool specimen and day- to –day variation in faecal
259 egg concentrations especially when host egg excretion is sporadic (Hall. A,1981; De Vlas *et al.,*
260 1992; Engels *et al.,* 1997; Utzinger *et al.,* 2001).Obtaining urine from the participants for the
261 CCA dip-stick was much easier than obtaining the stool samples. Furthermore, since CCA
262 dipstick is a non-invasive method, it is more readily accepted by endemic populations than
263 obtaining blood or serum for Circulating anodic antigen (CAA). In this low transmission
264 environment, at both baseline and follow-up, the urine CCA test identified more infections than
265 Kato-katz technique used as a gold standard. This is due to the latency between active infection
266 (metabolising worms), as detected by the urine CCA and commencement of egg production
267 detected by Kato-Katz method. The CCA dipstick was a valuable and sensitive tool for field
268 diagnosis of active *S. mansoni* though it's sensitivity should be evaluated further.

269 Several studies have demonstrated that Kato- Katz method may miss infections, especially light
270 infections in low endemic areas (Zhou *et al.,* 2008). Booth *et al.,* 2003, also observed similar
271 findings and demonstrated that due to the low sensitivity of Kato-Katz for detecting the presence
272 of *S. mansoni* infection, an all-embracing sampling effort may still considerably underestimate
273 the prevalence. Engels *et al.,* 1996, noted that despite the relatively poor sensitivity of a single
274 Kato-Katz slides, the majority of the undiagnosed infections were in fact light cases hence light
275 infections are diagnosed with increasingly sensitive diagnostic techniques. Therefore, since the
276 sensitivity of a single Kato-Katz is poor, the unreliability of the method limits its usefulness for
277 evaluating *S. mansoni* infections (Kongs *et al.,* 2001). These outcomes emphasized the need to
278 examine stools taken on different days when a definitive diagnosis of infection status is required.
279 Hence this recommendation is of particular importance for areas of low endemicity. It is known
280 that prevalence and intensity of infection decreases sharply after treatment with praziquantel thus
281 a single Kato-Katz technique is likely to miss a large proportion of residual infections (Utzinger
282 *et al.,* 2001). These findings reinforce the need to associate different tools for improved
283 schistosomiasis diagnosis in individuals with low parasite burden. For this reason, the detection
284 of *Schistosoma mansoni* circulating antigens for diagnosis of *S. mansoni* would be highly
285 valuable with the view of post intervention assessment and elimination programmes but also for
286 re-treatment of the individual patient.

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289 **Conclusion:**

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

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