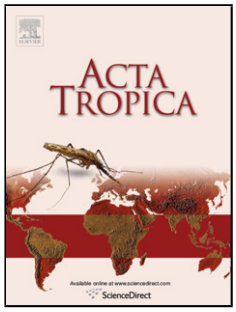

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Validation of a urine circulating cathodic antigen cassette test for detection of *Schistosoma haematobium*in uMkhanyakude district of South Africa.

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Abstract

Circulating cathodic antigen (CCA) tests for schistosomiasis are fast and less complicated allowing making them good candidates for routine qualitative screening for schistosomiasis at point of care. The urine-CCA has been evaluated for detection of *S. mansoni* with promising results. Its specificity and consistency in detecting *S. haematobium* infection in different endemic regions has been variable. This study validated a rapid urine-CCA cassette test for qualitative detection of *S. haematobium* infection in an *S. haematobium* endemic area with low *S. mansoni* prevalence. Microscopic examination for the standard urine filtration technique was used to validate the commercially available urine-CCA cassette test (rapid medical diagnostics ®). The validation was done in a sample of primary school pupils (n=420) aged 10-15 years in schools in the Jozini Municipality, KZN. There was a relationship between infection intensity and a positive urine-CCC test. Using the urine filtration method as the gold standard, the prevalence for *S. haematobium* was 40%, the accuracy of the CCA kit was 54.8%, sensitivity was 68.1% while the specificity was 45.8%. The positive predictive value was 45.82 % while the negative predictive value was 68.05%. Both the urine filtration and the urine-CCA methods detected heavy (≥ 50 eggs/10 mL urine) and light infections at statistically significant levels. The overall accuracy, sensitivity and specificity of the urine-CCA cassette test were low. The urine-CCA cassette test performed much better for heavy infections than low infections ($p < 0.05$) implying that the kit may not be suitable for low endemic areas.

Keywords

Schistosoma haematobium; *Schistosoma mansoni*; urine circulating cathodic antigens; point of care diagnosis.

1. Introduction

Schistosomiasis is a major parasitic disease, afflicting about 240 million individuals in the tropics (WHO, 2006). Resources for control are often limited necessitating rapid and accurate diagnostic tools that do not require highly specialised skills to identify and map high-risk communities in order to target interventions in a cost-effective manner (Brooker et al., 2009).

Presence of parasite eggs in urine or faeces indicates the presence of worms or that the individual harboured the parasites at some stage. The 'gold' standard for the detecting eggs in urine is the filtration method (for *S. haematobium*) or the Kato-Katz technique or other direct methods in the case of *S. mansoni* (Bergquist et al., 2009). However the reliability of these methods is affected by day-to-day and intraspecimen variation in egg output and low infections can easily be missed (De Vlas and Gryseels, 1992; J Utzinger et al., 2001).

Detection of parasite antigens such as circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) by the enzyme linked immunosorbent assay (ELISA) as a means of diagnosis has been shown to have many advantages, such as being able to detect active infections, determine efficacy of treatment and being highly specific. CCA and CAA are both genus-specific glycoconjugates associated with the gut of the adult worm (Kremsner et al., 1994).

While the gold standard Kato-Katz technique and urine filtration techniques require expert microscopy, it has been shown that trained school teachers (usually within half a day) are able to use the reagent strips (Brooker et al., 2009).

A genus specific test does not discriminate between urinary and/or intestinal schistosomiasis. While this is not critical for purposes of treatment interventions, the disadvantage is failure to provide information that may influence particular interventions like controlling faecal contamination, which is specific for *S. mansoni*. Development of a user friendly test that only needs a drop of urine and buffer to detect CCA has been recommended for use in the field (Van Dam et al., 2004). The lateral flow assay (LFA) design used by the CCA rapid kits evaluated in this study offers several advantages especially in developing countries as the kits have extended shelf life and do not need to be refrigerated (Sharma et al., 2015). Furthermore, visual interpretation of the results provides satisfactory results thus making the technique a vital tool for primary screening at point of care/need.

The manufacturers of the urine-CCA cassette kit used in this study point out that although the method was originally designed for the detection of *S. mansoni*, it can detect *S. haematobium* and *S. japonicum* in mid to high level infections.

The level of endemicity in various regions have been cited as contributing to the varying sensitivity of the CCA detection method (Stothard et al., 2006). Following earlier promising results of a CCA cassette test (Coulibaly et al., 2011), the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) initiated a multi-country study to assess the accuracy of a commercially available CCA kit for the diagnosis of *S. mansoni*. To the best of our knowledge such work has not been extended to South Africa. It is against this background that this study evaluated a commercially available (rapid medical diagnostics, USA) urine-CCA cassette test developed for qualitative detection of *S. haematobium* infection in an area with low *S. mansoni* prevalence.

2. Materials and Methods

2. 1 Ethics Statement

The study protocol was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Reference number BE182/13). District health and education authorities, village chiefs, parents/legal guardians, and participating children were informed about the purpose and procedures of the study. Parents/legal guardians provided written informed consent for their children to participate. Additionally, all children assented orally. All parasitological results were coded and treated confidentially. All children found infected with either *S. haematobium* or *S. mansoni* were immediately treated with praziquantel (single 40 mg/kg oral dose).

2.2 Study sites

Four hundred and twenty (420) children at 10 schools in Mgedula, Ndumo and Makhanisi villages located in the Jozini Municipality of the uMkhanyakude District of Kwa Zulu Natal province of South Africa donated urine samples in September 2014. Of the 420 children, only 380 were able to provide stool samples. The area is endemic for *S. haematobium* (Saathoff et al., 2004) and non-endemic for *S. mansoni* (Daniel, 2009; Malan et al., 2009). At the time the study was done there had been no recent schistosomiasis mass treatment programme in the area.

2.3 Study design and sampling

The study was cross-sectional involving school-going children aged 10–15 years from 10 primary schools in the Ndumo area. The study population was recruited as part of a bigger study looking at the general prevalence in uMkhanyakude district whose sample size was calculated as described by Naing et al (2006); N = Z2P (1 − P)/d2 where Z statistic = 1.96 for 95% confidence level; P the expected proportion in the study area = 0.8 (12); d the precision = 0.05 for 95% confidence interval. To cater for attrition N was multiplied by 2.5. School-going children were systematically sampled using school registers in a manner that equally represented males and females in each school.

2.4 Urine filtration method.

Urine filtration was carried out for each urine sample to detect *S. haematobium* eggs as described by Mott et al (1982). Urine samples from the primary school children were collected between 10:00hrs and 14:00hrs to correspond with diurnal peak egg output (Utzinger et al., 2011). About 50 mL of urine was collected in 100 mL screw cap bottles and 10 mL of urine was filtered through a 13-mm diameter nylon Nucleopore^{TM} filter with a 12 μm pore size (Costar Corporation, USA). The filter was placed on a labelled slide. A drop of 10% Lugol's iodine was placed onto the filter to stain the eggs. The slides were viewed for *S. haematobium* eggs under a light microscope and infection intensity expressed as eggs/10 mL of urine.

2.5 Urine-CCA cassette test.

The urine circulating cathodic antigen test was carried out according to the manufacturer's instructions (rapid medical diagnostics, USA®). Briefly, one drop of urine was added to a well on the cassette and allowed to absorb. Once fully absorbed, one drop of mobile phase buffer (provided with the CCA test kit) was added. The test results were read 20 min after adding the buffer and scored as either negative or positive. The test was carried out by the same technician to avoid bias. A second person confirmed the result.

2.6 Stool examination

Faecal samples were processed and examined for the presence of *S. mansoni* and other soil transmitted helminths' eggs by the quantitative Kato-Katz parasitological method (Katzet al, 1972). Forty-two milligrams of stool smears were prepared on microscope slides and covered

by gently pressing a cellophane cover slip stained with malachite green. The slides were allowed to clear for 24 hrs, and then examined for *S. mansoni* eggs under a light microscope. The number of eggs detected was expressed as eggs per gram of faeces.

2.7 Data analysis

The results were analysed using IBM SPSS Statistical Package Version 23. The following formulae in which (a) is true positive, (b) is false positive, (c) is the false negative and (d) is the true negative were used to calculate the different parameters; Accuracy (A)= (a + d)/n; Sensitivity (SS) = a/(a +c); Specificity (SP) = d/(b+d); Positive predictive value (PPV) =a/(a+b); Negative predictive value (NPV) = d/(d+c) and Prevalence (P) = (a+c)×100/(a+b+c+d). Egg counts for *S. haematobium* were classified into light (1-49eggs/10ml) and heavy (≥50 eggs/10ml) infection intensities according to the WHO classification. Egg counts for *S. mansoni* calculated as eggs/gram. The results were stratified according to sex, age and school. The school was considered as a proxy of village of origin of the children. A *P* value of less than 0.05 ($p < 0.05$) was considered indicative of statistical significance for Pearson's chi square. The Kappa statistic (K) was calculated to determine the level of agreement between the methods as previously stated (Landis and Koch, 1977). The odds ratio were calculated and binomial regression performed using the SPSS Statistical Version 23. For the urine filtration method three categories were used for calculating the likelihood of having a positive urine-CCA test result; negative (no eggs), light infection intensity (1- 50 eggs/10 mL urine) and heavy infection intensity (≥50 eggs/10mL urine). Odds ratio was calculated using children who provided both urine and stool samples (n = 380).

3. Results

3.1 Study population characteristics and prevalence

The mean age of participants was 12.9 years. Two hundred and seventy three children were female while 160 were male. Only 2 children out of 380 tested positive for *S. mansoni* using the Kato-Katz technique translating to a prevalence of 0.5% and 69 children tested positive for *A. lumbricoides* by the same method translating to a prevalence of 18.2%. One hundred and sixty nine children tested positive by the urine filtration method giving an overall prevalence of 40.23% for *S. haematobium*. The prevalence was higher among females (25%) than males

(15%). At school level, the prevalence ranged from 15.4 to 83.3% (Table 1). Four schools had high a prevalence (≥ 50%) while the other six schools had a medium prevalence (10% and 50%) and none had a low prevalence according to categorization by Stothard (Stothard et al., 2006), (Table 1).

3.2 Overall sensitivity, specificity, PPV and PPV

One hundred and fifteen children tested positive for *S. haematobium* using both the urine filtration and urine-CCA kit (true positive), 136 children tested negative for the urine filtration method and positive for the urine-CCA kit, (false positive), 54 children tested positive for the urine filtration method and negative for the urine-CCA kit, (false negative) and 115 children tested negative for both the urine-CCA kit and urine filtration method, (true negative) (Table 1). The accuracy, sensitivity and specificity of the urine-CCA cassette kit was 54.8%, 68.1% and 45.8% respectively. Positive predictive value was 45.8% while the negative predictive value was 68.1% as calculated from Table 2.

The urine-CCA test gave less false negatives at high infection intensity than low infection intensity. There was an association between the urine-CCA test and intensity of infection ($P<$ 0.05) (Table 3). The urine-CCA detected heavy infection intensities better than low infection intensities. Fifty four (54) children who had heavy *S. haematobium* infection detected by urine filtration technique also tested positive by the urine-CCA method. There were only 12 children who tested negative for the urine-CCA and showed heavy infection intensity by the urine filtration method. In contrast 61 children were positive for the urine-CCA method at low to medium infection intensity (1-49 eggs/10 mL urine) and 42 children gave a false negative in this category (Table 3).

3.3 Agreement between diagnostic approaches

The odds for a positive urine filtration outcome having a urine-CCA positive result was1.60,(95% CI 1.037-2.507), that of the school attended by the child on a positive urine-

CCA outcome was 0.95 (95% CI 0.885-1.023) and that of a positive *A. lumbricoides* result on a urine-CCA positive result was 1.80 (95% CI 0.885-1.023).The Kappa statistic for the agreement between the urine-CCA and urine filtration methods was 0.13.

Schools with high prevalence had higher PPVs while those with low prevalence had higher NPVs. There was generally no relationship between prevalence and SS, SP and accuracy at schools with low prevalence. At schools with high prevalence (> 50%), sensitivity of the urine-CCA method was high.

There were no significant statistical differences for both light and heavy intensities between the males and females. The ratio of positive to negative reaction of the urine-CCA test and urine filtration method were different at school level (Fig. 1).There were more positive cases detected with the CCA test at schools that had a higher parasitological prevalence (Mbadleni, Maphindela, Madeya and Munyuwana). However, this was variable at less than 50% prevalence. At Makhane, Mgedula, Mpolimpholini and Zimphosheni there were more negative cases than positive cases by the urine filtration method and in the opposite, more positive cases than negative cases by the urine-CCA method. At Thelamama and St Phillips there were more negative cases than positive cases for both the urine filtration and urine-CCA cassette test methods.

The egg intensity as calculated by geometric mean per school had low correlation with prevalence at school level. However a box plot of the combined population showed that there was some relationship between the urine-CCA reaction and egg count per individual (Fig.2). Egg intensity also did not have a correlation with accuracy, sensitivity and specificity.

4. Discussion

This study evaluated a urine-CCA cassette test against the gold standard urine filtration for the detection of *S. haematobium* infection in an area with low *S. mansoni* prevalence. Although the CCAs have been validated before (Ayele et al., 2008; Brooker et al., 2009; Knopp et al., 2015; Kremsner and Deelder, 1994; Stothard et al., 2006) it was important to validate its diagnostic performance and effectiveness in South Africa which presents a different endemic setting with low prevalence of *S. mansoni*. As expected, at school level, it was shown that PPV increased with prevalence (Watson and Petrie, 2010) while NPV decreased with prevalence in

this study. In general a high PPV indicates that in case of a positive result, it is a true positive result.

Geometric mean egg intensity did not show any trend indicating that it may not be suitable for evaluating the efficiency of the urine-CCA test. The box plot showed a relationship between urine-CCA positive reaction and high egg counts. It has been demonstrated previously using a box plot (Stothard et al., 2006) that egg counts have a correlation with colour intensity of CCA positive reaction suggesting the urine-CCA can also be used as a semi quantitative method. At the four schools where the prevalence was above 50% the urine-CCA test result showed a similar pattern with the urine filtration method. However, at four of the six schools, when prevalence was below 50%, the urine-CCA method actually showed more positive cases than the negative cases. This demonstrates the weakness of the method at low prevalence underscoring that this method may not be suitable for low endemic settings. Indeed Stothard (Stothard et al., 2009) found no relationship between the number of positive urine-CCA tests encountered and infection prevalence at the unit of the school. Regression analysis showed that the school the learner attended was less likely to influence a positive urine-CCA test result while a positive urine filtration result was shown to be likely to influence urine-CCA test result. A Kappa statistic of 0.13 showed that the two methods had a slight agreement based on the scale of Landis and Kock (1977). A urine filtration odds ratio of 1.6 showed the methods had some agreement. To check if there were any compounding factors, the study also looked at the odds of the geographical area of the children (represented by the school) and that of another infection (*A. lumbricoides*) having an effect on a positive urine-CCA test result. *Ascaris lumbricoides* was used since considerable cases were found in the area. The school attended by the children was less likely to affect the outcome of the test while having an *A. lumbricoides* was likely to affect the test result.

The sensitivity reported in this study is comparable (65 and 85%) to that reported by (Ayele et al., 2008). The Kato-Katz parasitological diagnostic method showed that the population in this study had very low *S. mansoni* prevalence; less than 1% (2 out of 380). It is reasonable to suggest that the urine-CCA cassette test positive results were not due to *S. mansoni*. However, this should be interpreted with caution since it has been suggested that the CCA may be able to detect prepatent infections. (Stothard et al., 2006). In such a case the Kato Katz method would not be able to detect the infection.

Accurate and rapid antigen detection assays are currently available for some neglected tropical diseases like lymphatic filariasis, onchocerciasis and visceral leishmaniasis (Brooker et al., 2009) and less progress has been made in establishing similarly reliable tests for

schistosomiasis although the dipstick version of CCA detection has been commercially available for at least a decade (Van Dam et al., 2004). In areas or communities with low prevalence and intensities of infection, high sensitivity (true positive rate) and specificity (true negative rate) are of great importance (Brooker et al., 2009).

Evaluating the diagnostic performance of the urine-CCA strip over a range of local levels of *S. haematobium* endemicity has been suggested to represent the different stages which ensue from chemotherapy-based control (Stothard et al., 2009). Variable performance of the urine-CCA has been reported in previous studies. The dipstick version of CCA kits was shown to have limited or no use for *S. haematobium* detection (Stothard et al., 2006) in field evaluations carried out in an *S. mansoni* endemic area in Uganda as well as in an *S. haematobium* endemic area in Ethiopia (Ayele et al., 2008) where low sensitivity (52%) and specificity (64%) were shown. However, high sensitivity and specificity of urine-CCA test were shown with *S. mansoni* infections (Legesse and Erko, 2007).

The inherent difficulty with the assay includes difficulty in determining trace and negative results. Different technicians may come up with different conclusions for very light infections. However, it has been shown that there is a relationship between infection intensity and color change intensity (Van Dam et al., 2004). The relationship between infection intensity and colour change intensity was not considered in this study although positive reaction was shown to be related to egg counts per 10 mL (Fig. 2).

It is apparent that the urine-CCA cassette kit needs further improvements to suite various levels of endemicity of *S. haematobium*. It is not clear why the conceptually genus specific antigens are more efficient in detecting *S. mansoni* and not *S. haematobium*. It is therefore important to unravel why circulating cathodic antigens are less reactive in the case of *S. haematobium*.

The sensitivities reported here and elsewhere are lower than those for other methods in the market like PCR, ELISA, sedimentation. However, there are no guidelines for sensitivity and accuracy set by individual countries' health regulatory bodies or even the WHO. .

It is important to note that our gold standards, urine filtration and Kato-Katz methods were based on a single urine or stool sample, and hence some infections could have been missed thus creating false negatives.

5. Conclusion

The overall accuracy, sensitivity and specificity of the urine-CCA cassette test were low. The performance of this test for diagnosis of urinary schistosomiasis was not satisfactory in the

study area. The urine-CCA cassette test performed better for heavy infections than it did for light infections. As shown in the previous studies of the SCORE program, this method may not be suitable especially for low infection intensities. This method may however be used as a complimentary method or in areas with high *S. haematobium* infections..

Competing interests

The authors declare that they have no competing interests.

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References

Ayele, B., Erko, B., Legesse, M., Hailu, A., Medhin, G. (2008). Evaluation of circulating cathodic antigen (CCA) strip for diagnosis of urinary schistosomiasis in Hassoba school children, Afar, Ethiopia. *Parasite, 15*(1), 69-75.

Bergquist, R., Johansen, M. V., Utzinger, J. (2009). Diagnostic dilemmas in helminthology: what tools to use and when? *Trends in parasitology, 25*(4), 151-156.

Brooker, S., Kabatereine, N., Gyapong, J., Stothard, J., Utzinger, J. (2009). Rapid mapping of schistosomiasis and other neglected tropical diseases in the context of integrated control programmes in Africa. *Parasitology, 136*(13), 1707-1718.

Coulibaly, J. T., Knopp, S., N'Guessan, N. A., Silué, K. D., Fürst, T., Lohourignon, L. K., N'Goran, E. K. (2011). Accuracy of urine circulating cathodic antigen (CCA) test for Schistosoma mansoni diagnosis in different settings of Côte d'Ivoire. *PLoS Negl Trop Dis, 5*(11), e1384.

Daniel, A. K. (2009). Spatial analysis of schistosoma haematobium infection among school children in a rural sub-district of South Africa: an application of geographical information. Thesis. *Faculty of Health Sciences.* University of the Witwatersrand, Johannesburg.

De Vlas, S., Gryseels, B. (1992). Underestimation of Schistosoma mansoni prevalences. *Parasitology Today, 8*(8), 274-277.

Katz, N., Chaves, A.,Pellegring, J. (1972). A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni.

Knopp, S., Corstjens, P. L., Koukounari, A., Cercamondi, C. I., Ame, S. M., Ali, S. M., Rollinson, D. (2015). Sensitivity and specificity of a urine circulating anodic antigen test for the diagnosis of Schistosoma haematobium in low endemic settings. *PLoS Negl Trop Dis, 9*(5), e0003752.

Kremsner, P. G.,Deelder, A. M. (1994). Rapid Diagnosis of Schistosomiasis by Antigen Detection in Urine with a Reagent Strip. *Journal of clinical microbiology*, 2404-2406.

Kremsner, P. G., Enyong, P., Krijger, F. W., De Jonge, N., Zotter, G. M., Thalhammer, F., Deelder, A. M. (1994). Circulating anodic and cathodic antigen in serum and urine from Schistosoma haematobium-infected Cameroonian children receiving praziquantel: a longitudinal study. *Clinical Infectious Diseases, 18*(3), 408-413.

Landis, J. R., Koch, G. G. (1977). The measurement of observer agreement for categorical data. *biometrics*, 159-174.

Legesse, M., Erko, B. (2007). Field-based evaluation of a reagent strip test for diagnosis of Schistosoma mansoni by detecting circulating cathodic antigen in urine before and after chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 101*(7), 668-673.

Malan, H., Appleton, C., Day, J., Dini, J. (2009). Wetlands and invertebrate disease hosts: are we askin for trouble. *Water Sa, 35*(5), 753-768.

Saathoff, E., Olsen, A., Magnussen, P., Kvalsvig, J. D., Becker, W., Appleton, C. C. (2004). Patterns of Schistosoma haematobium infection, impact of praziquantel treatment and re-infection after treatment in a cohort of schoolchildren from rural KwaZulu-Natal/South Africa. *BMC infectious diseases, 4*(1), 40.

Sharma, S., Zapatero-Rodríguez, J., Estrela, P., O'Kennedy, R. (2015). Point-of-care diagnostics in low resource settings: present status and future role of microfluidics. *Biosensors, 5*(3), 577-601.

Stothard, J. R., Kabatereine, N. B., Tukahebwa, E. M., Kazibwe, F., Rollinson, D., Mathieson, W., . . . Fenwick, A. (2006). Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. *Acta tropica, 97*(2), 219-228.

Stothard, J. R., Sousa-Figueiredo, J. C., Standley, C., Van Dam, G. J., Knopp, S., Utzinger, J., Deelder, A. M. (2009). An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. *Acta tropica, 111*(1), 64-70.

Utzinger, J., Booth, M., N'goran, E., Müller, I., Tanner, M., Lengeler, C. (2001). Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of Schistosoma mansoni before and after treatment with praziquantel. *Parasitology, 122*(05), 537-544.

Utzinger, J., N'Goran, E. K., Caffrey, C. R., Keiser, J. (2011). From innovation to application: social–ecological context, diagnostics, drugs and integrated control of schistosomiasis. *Acta tropica, 120*, S121-S137.

Van Dam, G., Wichers, J., Ferreira, T. F., Ghati, D., Van Amerongen, A., Deelder, A. (2004). Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *Journal of clinical microbiology, 42*(12), 5458-5461.

Watson,P.F., Petrie,A. (2010) Method agreement analysis: A review of correct methodology. Theriogenelogy, 1167-1179,

WHO. (2006). Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions*: a manual for health professionals and programme managers*: World Health Organization.

Figures and table

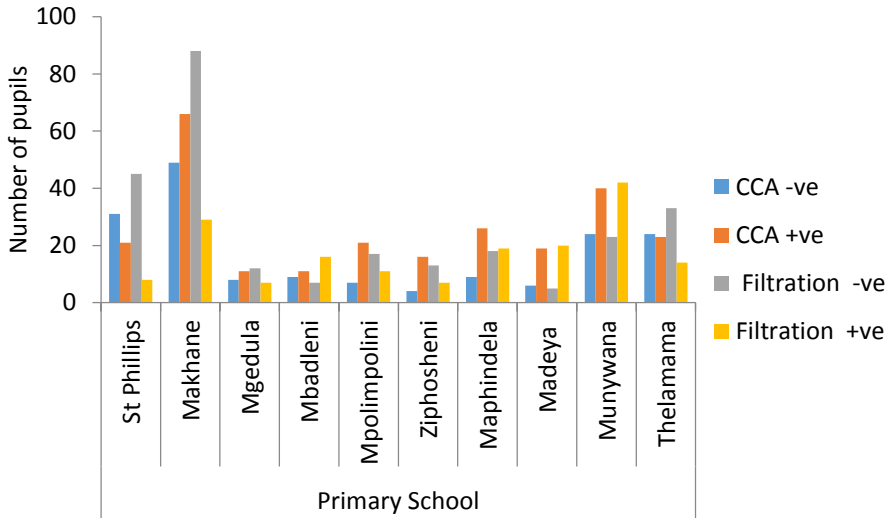

Figure 1. Comparison of the urine filtration and urine-CCA cassette kit by school

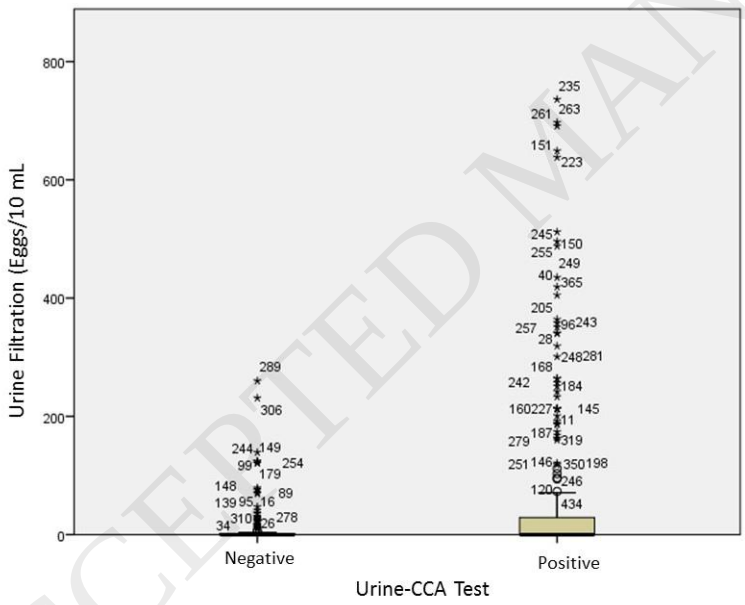

Figure 2. Box plot showing a positive association of egg count and a positive urine-CCA result. (The numbers inside the plot are identities of individuals).

Table 1

School	Prevalence (%)	SS (%)	SP (%)	A (%)	NPV (%)	PPV (%)	Geometric mean (%)
St Philips	15.4	62.5	63.6	63.5	90.3	23.8	31.9
Makhane	23.4	50.0	40.0	42.6	71.4	21.2	8.1
Thelamama	29.8	57.1	54.5	55.3	75.0	34.8	19.9
Zimphosheni	31.6	100.0	30.7	52.6	100.0	40.0	40.8
Mpolimholini	39.3	72.7	23.5	42.9	57.1	38.1	36.4
Mgedula	41.2	71.4	71.4	58.8	71.4	50.0	16.8
Maphindela	51.4	77.8	29.4	54.3	55.6	53.8	70.4
Mbadleni	65.0	61.5	57.1	60.0	44.4	72.7	43.9
Munywana	68.3	70.7	47.4	63.3	42.9	74.4	21.1
Madeya	83.3	85.0	75.0	83.3	50.0	94.4	130.6

Table 2.Diagnostic performance of the urine filtration method compared with the urine-CCA for the diagnosis of *Schistosoma haematobium*

Urine-CCA test	Urine filtration		Chi-square	*p*-value
	Positive	Negative		
Negative	54	115	8.073	0.005
Positive	115	136		*significant at $p < 0.05$

Table 3. Diagnostic performance of urine-CCA in relation to *Schistosoma haematobium* in relation to heavy and light infection intensities.

Urine-CCA test	Urine filtration		Chi-square	*p*-value
	Light infection 1 – 49 eggs/10 ml	**Heavy infection** (≥ 50 eggs/10 ml urine)		
Positive	61	54	9.445	0.002 significant at $p < 0.05$
Negative	42	12		