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Notes on the use of urine-CCA dipsticks for detection of intestinal schistosomiasis in preschool children

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ABSTRACT

Urine-dipstick diagnostic tests that detect schistosome circulating cathodic antigen (CCA) have the potential to provide more sensitive and rapid testing for intestinal schistosomiasis in field-based surveys; this is especially so when examining preschool children, from whom it may be difficult to obtain consecutive stool samples. To assess the performance of urine dipsticks, 569 preschool children from four villages along the shore of Lake Albert, Uganda, were screened for *Schistosoma mansoni* by Kato-Katz (K-K) examination of a single stool sample and CCA urine dipsticks. The prevalence of infection was 32.2% by K-K and 40.0% by CCA tests. Sensitivity and specificity were influenced by whether 'trace' results from the CCA test were characterised as positive or negative for infection with *S. mansoni*; ambiguities around this issue need to be resolved. Nevertheless, the CCA test showed particular promise for routine epidemiological screening in this setting.

1. Introduction

Detecting infection with the trematode flatworm *Schistosoma mansoni*, during epidemiological mapping and routine surveillance, has usually relied upon Kato-Katz (K-K) preparation of single stool samples, as recommended by the WHO.[1] Although K-K testing is deemed to be the 'gold standard' because of its high specificity and relatively low cost (the latter is estimated to be as low as $1.93 per test in Tanzania for example), the procedure is time consuming and requires trained personnel and rather cumbersome equipment.[2] Its sensitivity depends on several factors, including infection intensity (egg excretion may be sporadic), user variability and the number of stool samples used per person.[2] Because of the high day-to-day variation in host egg excretion, especially when infections are of light intensity, multiple stool sampling on consecutive days is required to increase the technique's sensitivity. The influence of this factor on the sensitivity of K-K testing is much greater than that of any variation associated with different observers or different slides.[1,3] To overcome these issues, alternative and more rapid diagnostic tests have been developed; these include the use of circulating cathodic antigen (CCA) immunochromatographic dipsticks, which indicate the presence of feeding adult worms by detecting excreted schistosome antigens in host urine.[4]

The CCA dipstick allows a typical location to be surveyed in less than 1 h, but like K-K examination it can also indicate the level of infection intensity of an individual.[5] Its application in determining prevalence of *S. mansoni* among school-aged children has yielded sensitivities (SS) between 76.7% and 99.1% and specificities as high as 74.2%.[6–8] There is little evidence of its use in preschool children, although a

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study of a small sample population has shown CCA to be at least as sensitive as K-K in this group.[9] In the present study we compared the accuracy of CCA testing to K-K (duplicate thick smears from a single stool) as a rapid diagnostic test for identification of *S. mansoni* infection in preschool children along the shores of Lake Albert, Uganda.

2. Methods

The study took place in June and July 2010 in four villages in Buliisa district: Sonsio (lat 1°52′49″N, long 31°23′44″E), Nyamukuta (1°52′0″N, 31°23′28″E), Piida A (1°49′13″N, 31°19′30″E) and Kawebanda (1°48′18″N, 31°19′27″E). Preschool children aged 1–5 years were recruited by the villages' local health assistant and community medicine distributors, who informed parents and guardians about the study's objectives the day before it took place. In each village, the study site was situated in easily accessible locations (churches or health centres), and consent obtained from children's parents or carers.

Each child provided one stool sample, which was used for duplicate 41.7 mg K-K thick smear preparation, and a urine sample in a 50 μl aliquot for CCA testing (Rapid Medical Diagnostics, Pretoria, RSA). For the K-K test, infection intensity was categorised using eggs per gram of stool (epg) as: negative (0 epg), light (1–99 epg), moderate (100–399 epg) or heavy (>400 epg).[10] Results from the CCA tests were recorded according to the visual intensity of the CCA reaction band: trace, single positive, double positive or triple positive. All children received a single oral dose of the anthelmintic praziquantel (40 mg/kg), regardless of infection status.

Data were compiled into a Microsoft Excel 2007spreadsheet and analysed using the statistical package R 2.11.1 (2010) to calculate the Wilson Score interval (95% CI) for sensitivity (SS), specificity (SP), positive predictive values (PPV) and negative predictive values (NPV).[11]

3. Results

A total of 569 preschool children aged 1–5 years (mean age 3.38 years; 290 boys) each provided samples for K-K and CCA testing. K-K and CCA testing were in broad agreement with one another, demonstrating a prevalence of infection of 32.2% (n=183, CI 28.3–36.2%) and 40.0% (n=227, CI 35.8–44.0%) respectively. Overall, 20.7% (n=118, CI 17.5–24.3%) of children were positive for *S. mansoni* on both CCA testing and K-K analysis (Table 1), with 'trace' being interpreted as a negative result. Among the egg-patent individuals, 18.6% (n=34) were negative for *S. mansoni* on CCA (Table 1).

On K-K testing, most infection intensities were 'light' (59.0%, n=108), with 23.5% (n=43) being 'moderate' and 17.5% (n=32) 'heavy'. Urine testing with CCA dipsticks required visual assessment of the intensity of the CCA test band colour; 18.3% (n=104) of results were categorised as 'trace'. Of the positive results, 66.1% (n=150) were 'single positive', 22.3% (n=53) 'double positive' and 11.5% (n=26) 'triple positive'. Of the 227 CCA-positives, 48.5% (n=111) were not egg patent on K-K examination and of these individuals 77.5% (n=86) were 'single positive' on CCA testing. One infant with a heavy-intensity infection on K-K (≥400epg) had a negative CCA result and six children who were non-egg patent on K-K were 'triple positive' on CCA.

As there is ambiguity in the interpretation of a 'trace' CCA test band, i.e. it can be read as positive or negative, we used two categories for our diagnostic analysis, using the K-K method as reference: 1. 'trace' was considered as positive; 2. 'trace' was considered as negative. For the first category, using the K-K method as the reference, sensitivity (SS) was found to be 81.4% (CI 75.1–86.4%) and specificity (SP) 53.2% (CI 48.2–58.1%). Positive predictive value (PPV) and negative predictive value (NPV) were 44.7% (CI 39.5–50.1%) and 86.0% (CI 81.1–89.8%), respectively. For the second category, SS was 64.4% (CI 57.3–71.1%), SP 71.8% (CI 67.1–76.0%), PPV 51.5% (CI 45.1–57.9%) and NPV 81.3% (CI 76.8–85.0%).

4. Discussion

Prevalence estimates obtained from both diagnostic methods (K-K and CCA) were broadly similar in this epidemiological setting, demonstrating a medium prevalence of intestinal schistosomiasis among preschool children in the study area, further confirming embedded disease within these younger children.[12] Although prevalence determined by CCA was higher than that derived from K-K, the difference is similar to that found among school-aged children in a similar setting in Lake Victoria.[6,7] The discrepancy between the CCA and K-K results can be partly explained by the fact that, while schistosomal egg excretion typically begins about 6 weeks after initial cercarial exposure and after maturing worms have begun to feed (and release CCA), many eggs fail to be voided in the faeces, which increases this discrepancy between the methods.[13] Furthermore, the sensitivity of K-K depends on infection intensity, which implies poor detection in a population where 59% of infants have

Table 1
Proportion of 118 children aged 1–5 years and infected with *Schistosoma mansoni* who fell within each infection intensity category, according to two diagnostic tests: K-K duplicate smear testing and CCA urine dipstick, with 'Trace' being interpreted as a negative result

		CCA results			
		Trace	+	++	+++
K-K infection intensity categories	Light (≤99 epg)	23	36	14	6
	Medium (100–399 epg)	4	13	12	10
	Heavy (≥400 epg)	4	14	9	4

n=118; CCA: circulating cathodic antigen; K-K: Kato-Katz; epg: eggs per gram of faeces.

light intensity infections as might be expected when early infections are still accrued.[14] Although the comparison of single K-K testing with CCA dipstick lacks a rigorous diagnostic gold standard, this study compares the accuracy and practicality of the two methods as rapid diagnostic tests. Comparison of multiple stool sampling with CCA testing has yielded similar SS and SP to the results of the present study.[7] It can therefore be interpreted that there is a higher 'true' prevalence than observed prevalence, rather than that CCA testing yields a high number of false positives.

Whether 'trace' was interpreted as a positive or a negative result had a strong influence on the SS and SP of the CCA tests. When 'trace' was interpreted as a positive result, CCA testing yielded an SS that was similar to that reported in studies screening school-aged children.[5,6] Although some studies have demonstrated SS to be as high as 87.7% when 'trace' is deemed to be negative, Coulibaly et al. found SS to be 56.3%, similar to 64.4% in this study, in a low endemic area when a single CCA test was compared to a more rigorous gold standard: triplicate-smear K-K testing of 3-day stool samples.[7,15] The interpretation of 'trace' in CCA testing is a contentious issue, as others have reported similar 'trace'-based variations in SS and SP. It has been suggested that 'trace' should be taken as positive if treatment is being given en masse, as it may be an early indicator in *S. mansoni* positive individuals; however, use of an imperfect diagnostic criterion may incur higher costs, as a result of administering unwarranted treatment.[16,17]

The ability of the Kato-Katz test to detect concurrent infection with other parasitic helminth species is an obvious feature that is lacking in CCA testing, a factor that might be problematic in the context of integrated control programmes. However, only a well trained technician can identify species-specific helminth eggs on microscopy, so there is potential for misidentification in poorly established control programmes. An added advantage of CCA is that its accuracy has not been shown to be negatively influenced by concurrent infections, in particular concurrent *S. haematobium* infection.[9,15]

The financial and practical implications of each method must also be compared when considering their suitability as a rapid diagnostic test. Single-smear and double-smear K-K testing has been reported to cost about $1.73 and $2.06 respectively per test in Tanzania, including all financial factors such as staff wages and equipment costs, whereas in this study the cost of each CCA test alone (not including other financial factors) was $1.98.[2] CCA testing may incur higher financial costs, but it is considerably less time consuming and requires less technical training. In comparison to K-K testing, the equipment is more easily portable and therefore there is the potential for a smaller team to map a landscape more quickly, i.e. in fewer days per unit location/area. Salaries and night allowances usually constitute the largest part of the total services cost; the cost of the labour intensive K-K testing will therefore vary, depending on epidemiological settings.[18] Finally, collection of urine samples for CCA testing is more straightforward and convenient for the patients than collecting stool samples, and this is an important factor when considering the compliance of preschool children.

CCA testing has the potential to be a useful rapid diagnostic tool in the mapping of schistosomiasis in preschool children, who are more likely to harbour light (sometimes pre-egg patent) infections. It is a more time-effective test than K-K, making it possible to screen five times as many children in the same time period, using half the number of staff. As rising salaries and night allowances have the biggest impact on total costs, CCA testing could provide a cheaper alternative to K-K testing, and therefore warrants a thorough cost effective analysis.[2] Further investigation is required however as it was strange to observe that a heavily positive infant by K-K, over 400 epg, had a negative CCA result. Therefore future studies are necessary before this rapid diagnostic test can be accepted as a stand-alone alternative to parasitological surveys of preschool children.

Authors' contribution: MM, AMDN and NBK co-ordinated and conducted the field work with the Vector Control Division field team, analysed and interpreted data, and drafted the manuscript. AF and NBK conceived the study and assisted in the drafting of the manuscript, with NBK being the local contact and supervisor of the fieldwork. JRS provided guidance in the field and assisted in drafting the manuscript. JCSF assisted in the analysis and interpretation of the data. All authors revised and approved the final manuscript. NBK is the guarantor of this manuscript.

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