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Urinary schistosomiasis on Zanzibar: application of two novel assays for the detection of excreted albumin and haemoglobin in urine

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Abstract

As part of a urinary schistosomiasis control programme on Zanzibar, an aged cross-sectional survey of 305 children from three schools on Unguja was conducted to investigate the relationships between levels of excreted albumin and haemoglobin in urine and *Schistosoma haematobium* infection status. Diagnosis was determined by standard parasitological methods, dipstick reagents for microhaematuria, visual inspection for macrohaematuria as well as collection of case-history questionnaire data for self-diagnosis. Prevalence of infection as determined by parasitology was 53.9% and approximately, one quarter of the children examined were anaemic ($<11\,g\,dl^{-1}$). A statistically significant negative association of blood haemoglobin levels of boys and *S. haematobium* infection intensity status was observed ($r_s = -0.23$, $P = 0.005$). Through sensitivity analysis of urine-albumin values it was determined that a concentration of above $>40\,mg\,l^{-1}$, as measured with the HemoCue urine-albumin photometer, had sensitivity, specificity, positive and negative predictive values of 0.90, 0.83, 0.86 and 0.89 respectively against 'gold-standard' parasitology. There was a clear association of reported pain upon micturition for children with elevated urine-albumin levels, with an odds ratio of 20 to 1. Levels of excreted blood in urine were quantified with the HemoCue Plasma/Low Hb photometer. However, dipsticks remain the method of choice for urine-haemoglobin of $0.1\,g\,l^{-1}$ and below. Urine parameters over a 24-h period were assessed in a small sub-sample. Reductions in both albumin and haemoglobin excretion were observed in 11 children 54 days after praziquantel treatment. It was concluded that these rapid, high-through-put, portable HemoCue assays could play a role in better describing and monitoring the occurrence, severity and evolution of urinary schistosomiasis disease. The urine-albumin assay has particular promise as a biochemical marker of *S. haematobium* induced kidney- and upper urinary tract-morbidity.

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Introduction

Urinary schistosomiasis has long been recognized as a persistent public health problem on the islands of Zanzibar off the East Coast of Tanzania. On the two main islands of Unguja and Pemba high prevalence levels of *Schistosoma haematobium* in school-aged children have been recorded and in the past various efforts have been directed towards detection and control (see Stothard *et al.* (2002a) and references therein). Recently a new schistosomiasis control programme 'Kick out Kichocho' ('Piga Vita Kichocho' in Kiswahili) has been launched on Unguja. The programme aims to reduce prevalence and intensity of infection in school-aged children by the use of chemotherapy with praziquantel, to provide better health education and increase awareness of the disease and aims to investigate longer-term possibilities for transmission control.

Snail species of the genus *Bulinus* are present on both Unguja and Pemba and play a role in transmission of *S. haematobium* (Rollinson *et al.*, 2001; Stothard *et al.*, 2002b). Fortunately, as *Biomphalaria* has not colonized the islands, only occasional imported cases of *S. mansoni* are seen and transmission cannot occur. Compatibility studies in the laboratory and observations on natural snail populations have revealed that *Bulinus globosus* is the main intermediate snail host and that *B. nasutus* is refractory to infection with local strains of *S. haematobium*. Molecular methods clearly differentiate the morphologically similar *B. globosus* and *B. nasutus* and this has allowed accurate mapping of their distribution. On Unguja, *B. globosus* is restricted to northwestern and central areas whereas *B. nasutus* is confined mainly to the south. On Pemba, *B. globosus* is widespread and *B. nasutus* is more localized on the eastern border of the central region.

The proven efficacy and low cost of praziquantel make mass chemotherapy aimed at morbidity reduction a feasible strategy; however, the 'focal' distribution of disease transmission within endemic areas means that targeted chemotherapy is essential, to both minimize costs and maximize benefits. On Unguja, prevalence of *S. haematobium* infection is closely linked with the proximity of *B. globosus* habitats (Stothard *et al.*, 2002c). This immediately helps to focus control strategies to areas associated with active transmission. In addition, questionnaires to schoolchildren have provided details of behaviour, water contact and general awareness of schistosomiasis (Stothard *et al.*, 2002c). An initial survey of 400 schoolchildren from ten schools in Unguja using both diagnostic tests and questionnaires has been followed up in more detail in 24 schools in the endemic area to provide baseline data on prevalence and intensity prior to the commencement of control. Prevalence of infection determined by microhaematuria and egg counts ranged from 3 to 75%.

While the presence of blood in urine is a well-accepted marker of *S. haematobium* infection (Lwambo *et al.*, 1997) and has led to the widespread use of Hemastix dipstick reagents, there still is a need for exploration of other more sensitive, rapid and high-through-put screening methods to better describe the occurrence, burden and evolution of disease especially within the context of ongoing disease control. It is also desirable, ideally, that such methods are low-cost and applicable in the field. As part of the disease-monitoring component within the Piga Vita Kichocho programme, we have assessed the potential value of two novel, portable, diagnostic assays for the detection and quantification of urine-albumin and urine-haemoglobin using the HemoCue Urine Albumin and Plasma/Low Hb photometers respectively. The urine-albumin assay was initially developed for use in European first-point-of-care health centres for rapid detection and quantification of microalbuminuria, which can be a biochemical marker of kidney dysfunction. Similarly the Plasma/Low Hb assay was originally developed to better estimate the associated blood loss and transfusion requirements of patients undergoing surgery through the rapid quantification of haemoglobin lost in bodily fluids collected during operation. In the present study we report on the use of the two assays by comparison with parasitology and other proven field diagnostic methods for detection of urinary schistosomiasis within a cross-sectional sample of schoolchildren from three schools on Unguja.

Materials and methods

Parasitological survey

Cross-sectional parasitological surveys and administration of structured case-history questionnaires detailing water contact patterns and self-assessed signs and symptoms of disease were carried out at three primary schools Chaani, Kitope and Muyuni (fig. 1), between 23 and 27 February 2004 following Stothard *et al.* (2002c). Data from parasitological examinations and questionnaires were collected from a random sample of 305 students enrolled at the three participating schools, two of which were in the northern part of the island where *S. haematobium* is endemic (Chaani and Kitope) and one (Muyuni) acted as a control to assess existing patterns of microalbuminuria, if any, in the general absence of schistosomiasis. To assess the levels of putative anaemia (Hb $< 11\,\mathrm{g\,dl^{-1}}$), finger-prick blood was collected for each child and assessed using the HemoCue Blood Haemoglobin photometer following manufacturer's instructions.

Study participants were asked to provide a mid-morning urine sample in a 100 ml plastic beaker. Urine was visually inspected by the investigators for gross haematuria and assigned a number using a urine colour reference chart which graded macrohaematuria into light, medium and heavy categories. Urine was also visually inspected for turbidity by placing the container on top of a black and white line barcode. If the barcode could be still be seen through the urine solution, the sample was judged to be clear, if it could not then the sample was judged to be turbid. Further details of the charts can be obtained from the corresponding author. Parasitological diagnosis was performed by 10 ml syringe filtration of urine using established methods (WHO, 1991) to quantify the number of *S. haematobium* eggs present in the urine. Hemastix (Bayer) reagent dipstick tests were used to semi-quantitatively determine levels of microhaematuria.

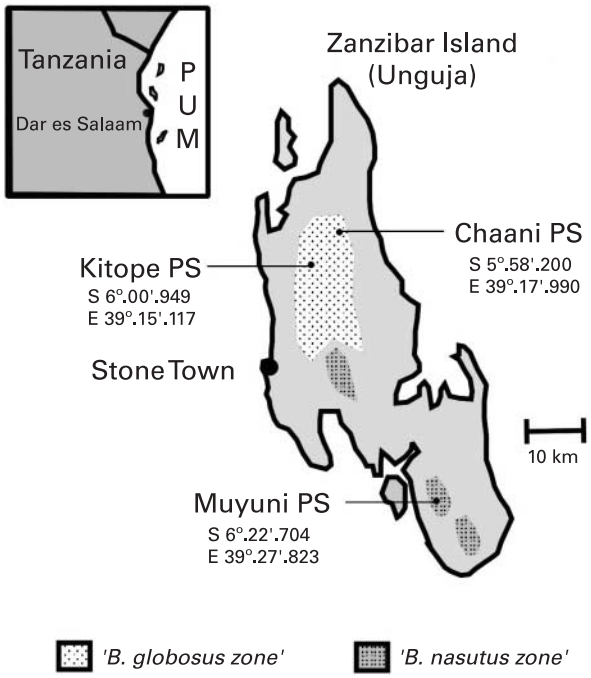

Fig. 1. Sketch map of eastern Tanzania and coastal islands. Inset shows the three major coastal islands Pemba (P), Unguja (U) and Mafia (M). The locations of the three schools included in this survey are shown as well as the zones where *Bulinus globosus* and *B. nasutus* are found.

Children found to be positive for infection with *S. haematobium* were treated with a standard dose of praziquantel (40 mg kg^{-1}), and treatment was offered to students not examined in the study but who wished to receive treatment. The Ministry of Health, Zanzibar and Imperial College of Science Technology and Medicine granted ethical approval for this study.

HemoCue urine assays

Urine specimens were analysed to detect microalbuminuria using the HemoCue Urine Albumin assay following manufacturer's instructions. A 1.5 ml aliquot of each urine sample was centrifuged for 2 min at 12,000 rpm in a bench top microcentrifuge prior to application, by capillary action, into the urine-albumin cuvette. This cuvette contains lyophilized antibodies specific to human albumin, which form an immunoturbid flocculate. The level of turbidity is directly proportional to the levels of albumin, which is measured by the urine-albumin photometer after incubation/agitation for 90 s and an absorbance reading at 610 nm is subsequently taken. The concentration of urine albumin is expressed in mg l^{-1} units. The normal reading range of the photometer is usually between 15–200 mg l^{-1} levels of albumin; otherwise the machine will record LLL (values $<$15 mg l^{-1}) or HHH (values $>$200 mg l^{-1}). For HHH values it was necessary to perform a doubling-dilution series of the urine sample in normal saline until a reading within the range of 15–200 mg l^{-1} was obtained. Random spot replicate readings were taken to ensure that values were consistent, typically each reading was within $\pm$5 mg l^{-1}. Urine was analysed for levels of haemoglobin using the HemoCue Plasma/Low Hb assay following manufacturer's instructions and random spot replicate readings were taken to ensure consistency.

A subset of children at each school was selected to collect urine for 24 h following treatment with praziquantel. Egg counts were performed on the total volume of urine collected, and total number of eggs was quantified. Dipsticks and the HemoCue Urine Albumin were used to quantify proteinuria and microalbuminuria. The 24-h urine collection was conducted primarily to assess feasibility and acceptability of the collection method, but at Muyuni it functioned as a negative control to see how much albumin values fluctuated in egg negative individuals who were presumed to be free of infection or had light infections based upon egg counts. Eleven children from Chaani were re-examined 54 days post-treatment with praziquantel to determine any changes in the urine parameters. All data were entered and analysed using Microsoft EXCEL XP and STATA Version 8.0.

Results

Questionnaire data and parasitological survey

Questionnaire data were recorded from an age-cross sectional sample of a total of 305 children. Of these, 113 were female and 191 were male, the average age of 15 years, ranging from a minimum and maximum of 10 to 22 years. The majority of students (73.6%) were between 13 and 16 years of age. Questionnaire data revealed that few children (5%) had a precise understanding of the exact nature of *kichocho* while (37.4%) reported on having had some form of received treatment for the disease. Health complaints at the time of the study were widespread, and included reported headache (85.5%) and general abdominal pain (83.2%) while over a quarter (29.3%) reported pain upon micturition.

A water contact score was calculated per school, by summing for all subjects their 'yes' responses to questions about water contact activities most closely associated with disease transmission sites, and then dividing this number by the total possible sum. Students were asked about playing in ponds and streams around the home, tending rice paddies, and washing clothes in ponds. Water contact scores were 0.96 for Kitope, 0.79 for Chaani, 0.26 for Muyuni with 0.75 for the aggregated data mean. Boys generally reported playing in ponds near their homes more frequently than girls (75.9% vs. 47.8%), but water contacts associated with tending rice paddies and washing clothes were reported with similar frequency among both sexes, 88.5% vs. 85.0% and 77.5% vs. 62.8% respectively.

Urine filtration and direct parasitological examination were used to determine infection status and intensity on single urine specimens collected from 280 students. Absence of eggs was classified as infection-negative by parasitological analysis. The presence of 1–10 eggs was considered to be a 'light' infection; 11–49 eggs a 'medium' infection; and, a 'heavy' infection was defined as 50 or

more eggs present in the 10 ml urine sample. A total of 151 specimens (53.9%) were positive for *S. haematobium* eggs. Of these, 29 (19.2%) were classified as light infections, 41 (27.2%) were medium infections, and 81 (53.6%) were considered heavy infections. All but one of these egg positive infections were detected in students from Chaani and Kitope in the north, where *S. haematobium* transmission is known to be highly endemic; the only egg positive student at Muyuni was also the only student from this school to report visiting the northern part of the island, where infection was likely to have been acquired.

Prevalence of infection at Chaani as determined by parasitological examination was 55.6% (90/162). Of these cases of infection, 24.4% were light, 37.8% were medium, and 37.8% were heavy. Egg count data available from 66 study subjects at Kitope showed that the vast majority of students harboured heavy infections. Only 7/66 students (10.6%) did not have *S. haematobium* eggs detectable in their urine; 7/66 students (10.6%) had mild infections, 6/66 (9.1%) had medium infections, and 46/66 (69.7%) harboured heavy infections. At Muyuni, the only egg positive infection was of medium intensity.

Anaemia status

Table 1 displays the observed haemoglobin status across the sample. The average haemoglobin (Hb) level among the children studied, using blood obtained by finger-prick, was 11.6 g dl^{-1} for females and 11.8 g dl^{-1} for males, however, over a quarter of the children in the sample could be considered to be anaemic (Hb <11 g dl^{-1}). A bivariate plot of Hb scores against *S. haematobium* egg intensities showed there to be statistically significant negative correlation ($r_s = -0.18$, $P = 0.004$); however, after disaggregating the data by gender, the correlation was maintained only in males ($r_s = -0.23$, $P = 0.005$) and not females as also shown by mean haemoglobin levels in table 2.

Diagnostic methods

Visual inspection of gross haematuria

Urine specimens were visually inspected for red colour by at least three different persons, including the student presenting the urine, a teacher at the respective school and a laboratory technician/investigator. All study participants were asked to grade the colour of their urine specimen that they provided by matching against the urine colour chart. Only 47 students (15.5%) reported visibly red urine while teachers recorded 81 (26.6%) urine samples to be visually red. Children tended to under-report macrohaematuria in comparison with school-teachers' assessments. Infection intensity as measured by egg counts was correlated with both self-assessed haematuria ($r_s = 0.238$, $P = 0.0001$) and teacher-assessed haematuria ($r_s = 0.396$, $P < 0.0001$). The sensitivity, specificity, negative and positive predictive values for visual inspection methods versus parasitological diagnosis is shown in table 3.

Table 1. Anaemia status of school children on Zanzibar in 2004.

Haemoglobin levels	Males ($n = 155$)	Females ($n = 107$)
Normal ≥ 11 g dl^{-1}	66.6%	72.9%
Anaemic <11 g dl^{-1} and >8 g dl^{-1}	29.5%	25.2%
Severely anaemic <8 g dl^{-1}	3.9%	1.9%

Table 2. Venous haemoglobin levels and *Schistosoma haematobium* infection status in schoolchildren on Zanzibar in 2004.

Schistosome infection status	Mean haemoglobin values (g dl^{-1}) Males	Females
Egg negative	12.2 ± 0.5	11.8 ± 0.4
Egg positive intensity		
<10 eggs per 10 ml	12.4 ± 0.8	11.2 ± 0.7
>10 and <50 eggs per 10 ml	12.0 ± 0.9	11.7 ± 0.8
>50 eggs per 10 ml	11.0 ± 0.6	11.2 ± 0.6

Inspection of microhaematuria

Hemastix dipsticks were used to assess microhaematuria in 304 study subjects. Inclusive of trace blood detected in the urine by this method and not including four girls at Muyuni with menses at the time of diagnosis, 150 (49.3%) specimens were positive for presence of blood. Of these specimens, 2 (1.33%) were positive with trace amounts of blood, 23 (15.3%) were graded +, 19 (12.7%) were graded ++, and 106 (70.7%) were graded +++. Inclusive of trace blood, prevalence of microhaematuria was 54.9% in these combined schools. The high prevalence of microhaematuria as diagnosed by this method stands in contrast to the relatively few urine specimens that were red upon visual inspection. The sensitivity, specificity, negative and positive predictive values for inspection of microhaematuria versus parasitological diagnosis is shown in table 3.

Table 3. Comparison of diagnostic methods against microscopy.

Method of diagnosis	Sensitivity	Specificity	PPV	NPV
Macrohaematuria Self assessed	0.26	0.96	0.87	0.53
Macrohaematuria Teacher assessed	0.44	0.91	0.86	0.58
Visual turbidity Bar code chart	0.59	0.95	0.95	0.64
Albumin (conc. >40 mg l^{-1})	0.90	0.83	0.86	0.89
Haematuria Plasma/Low Hb	0.74	0.55	0.64	0.66
Microhaematuria Hemastix	0.83	0.88	0.89	0.81

NPV, negative predictive value; PPV, positive predictive value.

HemoCue Plasma/Low Hb

Using $>0.0\,g\,/l^{-1}$ of urine-haemoglobin (urine-Hb) as a positive criterion, 157/258 urine samples (60.9%) tested with the HemoCue Plasma/Low Hb were positive for urine-Hb. Distribution by age showed peak urine-Hb in urine from children 15 years of age with values of typically of $0.3–0.8\,g\,l^{-1}$ of excreted urine-Hb common. Continuous urine-Hb levels detected by the HemoCue Plasma/Low Hb were significantly correlated with categories of infection intensity ($r_s = 0.228$, $P = 0.0003$); however, when the data were disaggregated by sex, urine-Hb levels were correlated with categories of infection intensity in males only ($r_s = 0.238$, $P = 0.0037$). When continuous Hb levels in urine were plotted against continuous egg counts, the linear regression coefficient was non-significant. Urine-Hb levels were correlated with microhaematuria as measured by Hemastix dipsticks ($r_s = 0.2549$, $P < 0.0001$). There was no statistically significant correlation between excreted urine-haemoglobin and venous finger prick blood haemoglobin levels. The sensitivity, specificity, negative and positive predictive values for inspection of haematuria as detected with the HemoCue Plasma/Low Hb photometer versus parasitological diagnosis are shown in table 3.

HemoCue Urine Albumin

The HemoCue Urine Albumin assay was utilized to analyse 260 urine samples. Albumin levels under $20\,mg\,l^{-1}$ were coded as '0,' given that micro-albuminuria is defined as at least $20\,mg\,l^{-1}$ of albumin present in a morning spot sample of urine. Albumin levels in a single urine sample ranged from under $20\,mg\,l^{-1}$ to $4256\,mg\,l^{-1}$, a level well within the nephrotic range of $>3000\,mg\,l^{-1}$. Boys tended to excrete more albumin ($\mu = 340.8\,mg\,l^{-1}$) than girls ($\mu = 162.7\,mg\,l^{-1}$); the difference between the mean albumin excretion in boys and girls was statistically significant ($t = -2.76$, $P = 0.0062$). Whereas the majority of urine samples from females contained less than $1000\,mg\,l^{-1}$ of albumin, 16/152 males (10.5%) demonstrated albumin levels exceeding $1000\,mg\,l^{-1}$, and ten of these children had visually red urine.

Mean excreted albumin by age and infection intensity is shown graphically in fig. 2a. Mean albumin excretion in heavy infections is divergent from the graphs for the medium and light infections, especially in children 14 years of age or older. Egg negative infections generally show mean albumin excretion under $100\,mg\,l^{-1}$; light infections under $200\,mg\,l^{-1}$; medium infections under $400\,mg\,l^{-1}$; and heavy infections under $1000\,mg\,l^{-1}$. A non-parametric test for trend across ordered categories of infection intensity showed a statistically significant trend in albumin based upon infection intensity ($z = 12.32$, $P < 0.0001$).

A sensitivity analysis showed that a criterion of $>40\,mg\,l^{-1}$ of excreted albumin maximized both sensitivity and specificity of the test when compared to the gold-standard egg counts. Using this as the cut-off for a positive test consistent with a diagnosis of *S. haematobium* infection, 56.2% of the subjects were considered to be infected. Plotting continuous values of albumin, the area

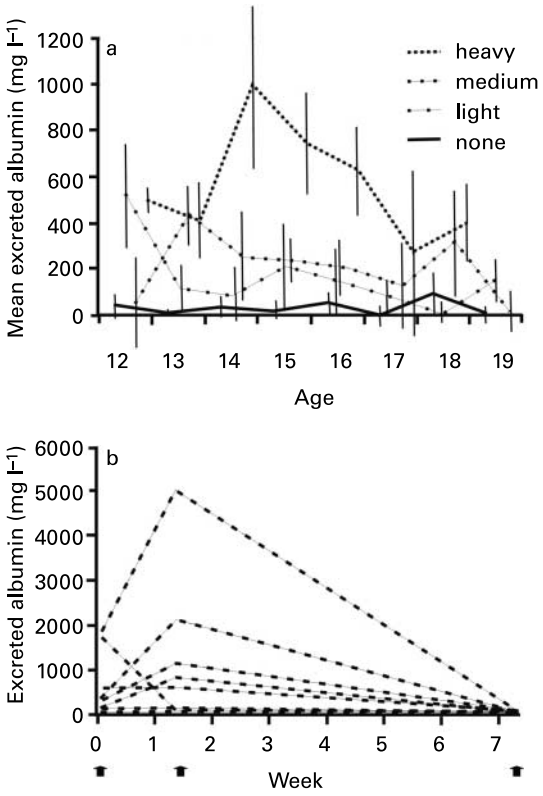

Fig. 2. Patterns of excreted urine-albumin associated with *Schistosoma haematobium* infection status and praziquantel post-treatment changes. a. Mean levels of excreted urine-albumin, with 95% confidence limits, are shown for each age class for each intensity of *S. haematobium* infection by grading into high, medium and low categories. b. Excretion patterns of urine-albumin from 11 children from Chaani school show a clear reduction 54 days post -treatment.

under the receiver-operator curve (ROC) was 0.9053 (fig. 3a) validating the ability of the test to discriminate between true infections and non-infected individuals. When the ROC was generated plotting albumin as a dichotomous variable ($<$ or $>40\,mg\,l^{-1}$), the area under the curve was 0.87 (fig. 3b).

Using this excreted urine albumin cut-off of $40\,mg\,l^{-1}$ derived from the sensitivity analysis, a possible association between egg count, urine turbidity and pain upon micturition with urine-albumin was examined (table 4). Study subjects who reported pain upon urination had statistically higher excreted urine albumin levels than those who did not report pain. Females reporting pain associated with micturition had mean urine albumin levels of $162.0\,mg\,l^{-1}$, whereas the mean urine albumin for males reporting pain was $630.7\,mg\,l^{-1}$. The odds of reporting pain associated with urination were 20.4 times higher in children who had excreted albumin levels greater than $40\,mg\,l^{-1}$. The sensitivity, specificity, negative and positive predictive values for inspection of microalbuminuria versus parasitological diagnosis are shown in table 3.

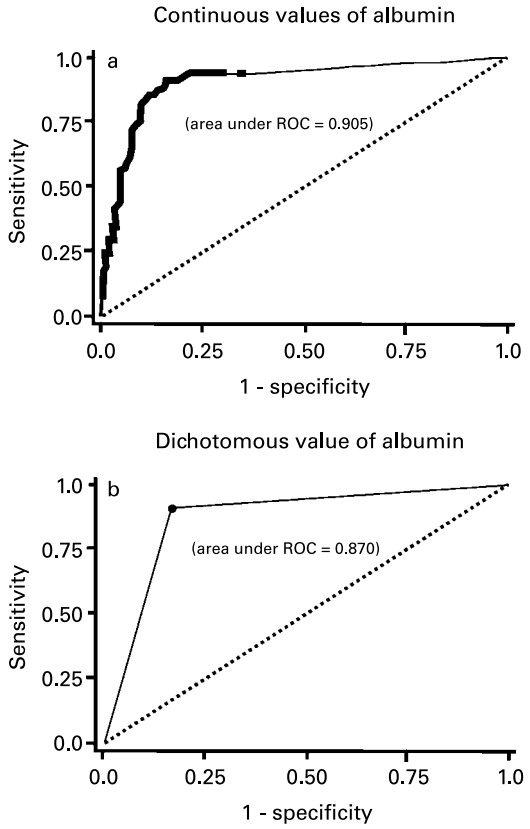

Fig. 3. Receiver operating characteristic (ROC) curves for determining the relationship between excreted urine-albumin and *Schistosoma haematobium* infection status. a. Investigation of the ability of urine-albumin levels (continuous variation) to diagnose *S. haematobium* infection. A value of 40 mg l^{-1} was shown to have optimal cut-off in terms of greatest sensitivity with best specificity. b. ROC analysis of the ability of a dichotomous urine-albumin value (40 mg l^{-1}) to determine S. *haematobium* infection. In comparison to the total area under the curve in fig. 3a. (0.905), a value of 0.870 gives a very good approximation.

24-h urine and post-treatment follow-up

A total of 39 students from Chaani and Kitope and 16 students at Muyuni provided 24-h total urine samples either 1 or 2 days following treatment with praziquantel. Compliance on this exercise was good, however, the 5-litre urine containers given to the children were cumbersome and it was likely that several children failed to collect all their urine output during the 24-h period. Prior to treatment, 34/39 children from Chaani and Kitope were both egg positive and dipstick positive and a total egg counts quantified from the 10 ml of the 24-h urines ranged from 0 to 1013 eggs while excreted urine albumin values ranged from 0 to 800 mg l^{-1}. Only 3/39 children had excreted urine albumin levels less than 20 mg l^{-1} in the survey. Of the 16 students at Muyuni, one female who was previously egg positive was included while the remainder acted as uninfected controls. Egg counts were 0 in survey specimens and 24-h urine specimens from all children except for this single egg positive infection. In the cross-sectional survey, this child's excreted albumin level was 48 mg l^{-1} and in the 24-h urine specimen the level was slightly reduced to 28 mg l^{-1} while all other urine samples were less than 20 mg l^{-1}.

At Chaani school excreted urine-albumin levels of 11 children were monitored at approximately 1.5 weeks and 54 days after praziquantel treatment. The corresponding urine–albumin levels are shown in fig. 2b. Over this period there was an initial increase in urine-albumin levels followed by a general decline. The initial urine-albumin mean of 580 mg l^{-1} reduced to 18 mg l^{-1} and excreted haemoglobin declined from 0.35 g l^{-1} to 0.0 g l^{-1}.

Discussion

The primary focus of this study was to examine the relationship between excreted levels of urine-albumin and urine-haemoglobin for *S. haematobium* infections in schoolchildren on Zanzibar Island (Unguja), using the novel application of two existing rapid assays in the context of an endemic area for urinary schistosomiasis. The urine-haemoglobin assay results clearly showed that quantification of excreted haemoglobin is possible but the assay is much less sensitive than the Hemastix, which can detect as little as 0.15–0.62 mg l^{-1} haemoglobin. The dipstick assay soon saturates (+++) before 0.01 g l^{-1} values of haemoglobin are obtained, which is approaching the limit of detection with the Plasma/Low Hb photometer (~0.1 g l^{-1}). Hence the Hemastix remains the method of choice for the detection of microhaematuria. A maximum observed value of urine-haemoglobin was 4.8 g l^{-1} from a boy from Kitope (the sample had visual haematuria, graded medium). A total of 81 children presented with visually red urine as assessed by teacher and technician/investigator. It is worthy of note that diagnosis of macrohaematuria between teacher and child

Table 4. Relationships between albumin concentration and other urine parameters.

	Urine filtration*		Turbidity of urine*		Pain on micturition*	
Excreted albumin	Egg +ve	Egg −ve	Turbid	Clear	Yes	No
Conc. (>40 mg l^{-1})	123 (47.8%)	20 (7.8%)	73 (28.2%)	72 (27.8)	70 (27.0%)	75 (29.0%)
Conc. (<40 mg l^{-1})	13 (5.1%)	101 (39.3%)	1 (0.4%)	113 (43.6%)	5 (1.9%)	109 (42.1%)

* A Chi-squared test was highly statistically significant, $P < 0.0001$.

was imprecise despite being assisted through the use of a urine colour chart. It has previously been suggested that visual acuity (colour-blindness of children) might play a confounding role (Stothard *et al.*, 2002c). Urine turbidity can be another useful marker (Chippaux *et al.*, 2001) and our measure of turbidity using a black and white barcode has a higher level of sensitivity and specificity than visual colour methods and could be of further value for low cost diagnosis.

Such daily levels of associated blood loss, over a period of time, could constitute a substantial amount of continual haemoglobin loss but despite over one quarter of the children in this survey being judged to be anaemic (Hb $<11\,g\,dl^{-1}$), no clear relationship between excreted urine-haemoglobin and venous haemoglobin levels was found. The finding of a positive association between *S. haematobium* infection intensity and anaemia status in boys strongly suggests this parasite plays some role in determining venous haemoglobin levels (see Stephenson (1993) and references therein).

Excreted urine-albumin as measured by the HemoCue Urine Albumin assay was useful as a rapid operational field diagnostic tool for determining levels of microalbuminuria and proxy of infection status with *S. haematobium*. Indeed the urine-albumin levels are correlated with infection intensity as measured by egg counts, the current gold-standard test for diagnosing infection with *S. haematobium*. Furthermore, using a positive criterion of microalbuminuria of $>40\,mg\,l^{-1}$, the assay was shown to be both sensitive (0.90) and specific (0.83) and able to characterize infection status correctly 86% of the time. The persistent excretion of urine-albumin must have some clinical significance in relation to the daily protein–energy balance of the child (Stephenson, 1993).

The area under the ROC of 0.905 as shown in fig. 3a demonstrates that continuous albumin data validates the ability of the test to discriminate between true infections and non-infected individuals within this study population. Parasitological measurement is a slight underestimate of the true infection status of the children, as eggs will not always be detectable using a single 10 ml syringe filtration. The specificity of urine albumin might be slightly higher and, if we assume all children with microalbuminuria $>40\,mg\,l^{-1}$ were true schistosome cases, the prevalence of infection would be 56.2% compared to the 53.9% detected by egg filtration. However, other complicating factors, such as menses, other uro-genital infections/dysfunctions or scarification practices, mean that not all raised albumin levels will be solely due to schistosome infections.

From experiences in the field, the albumin assay is robust and reliable. In comparison to urine filtration, the assay is a slightly less laborious way to diagnose infections and the assay has certain advantages as a rapid operational diagnostic in the field setting. The drawbacks, however, include the necessity for an electrical power supply (unlike the HemoCue haemoglobin machines for venous blood and plasma/low Hb which use AA batteries) and, if a precise reading is required, a dilution series of the urine sample may be needed if initial urine-albumin concentrations are in excess of $200\,mg\,l^{-1}$. The technique itself is also rather expensive for initial purchase of photometer, centrifuge and pipette (~£1500 UK) as are the daily overall replenishment costs of reagents and consumables used for each urine sample analysed (~£2 UK).

Morbidity control and infection control have different objectives (Engels *et al.*, 2002), and each must be assessed in its own right, using indicators that adequately capture the phenomenon under investigation. Given the goal of widespread treatment of schoolchildren in endemic areas, new ways of better measuring morbidity associated with schistosomiasis, especially following treatment when egg excretion begins to decline (Vennervald *et al.*, 2000), are needed. A good marker of morbidity will also have the potential to predict recrudescence of morbidity after treatment and should be used to compliment other measures of infection (Richter, 2003). Vennervald *et al.* (2000) set down seven points for consideration during the evaluation of a new marker which should: (i) reflect morbidity as well as intensity of infection, thus providing additional information to egg counts; (ii) vary little with respect to the time of day the sample is collected; (iii) give a reasonable estimate of the prevalence of disease using only one sample collected from individuals within the population under study; (iv) measure a continuous variable; (v) be able to distinguish between early morbidity and later and more severe morbidity; (vi) be non-invasive, and preferably measurable in urine; and (vii) be assayed directly in the field.

Whereas quantitative assessments of albuminuria in urine have been carried out previously by means of Coomassie blue-dye binding tests or by rocket-immunoelectrophoresis of urine supernatant (Doehring *et al.*, 1986; Reimert *et al.*, 1993), no studies have attempted to quantify albuminuria in the field setting as this technology has only recently become commercially available. Whilst it is unlikely that the assay will replace the parasitological diagnosis or Hemastix dipsticks, following Vennervald's guidelines the assay could play an important role in evaluation of control programmes within sentinel schools and communities, especially if a more precise relationship can be found between albumin excretion patterns and pathological changes potentially indicative of serious long-term schistosome-induced sequelae and amelioration following treatment. For example, Burki *et al.* (1986) stated that albuminuria in *S. haematobium*-infected individuals could detect early pathological changes that would otherwise not be picked up using ultrasound methods. Our results show that raised albumin levels are associated with pain upon micturition, table 3. Also albumin excretion patterns reflect infection intensities and reductions of urine-albumin can be seen 7 weeks post-treatment, if not before, fig. 2a and 2b. The clinical significance of raised albumin levels together with low blood in urine values is not clear but might point towards the onset of kidney disease. This might shed some further light on the view of Forsyth (1969) whilst working on Unguja, that urinary schistosomiasis ultimately precipitated the deaths among persons in apparently good health by spontaneous kidney failure. Such an interpretation further strengthens the recent finding of King *et al.* (2005) that urinary schistosomiasis is more of a public health problem than previously documented.

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