Indirect screening for *Schistosoma haematobium* infection: a comparative study in Ghana and Zambia

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Four indirect approaches, based on inquiry into a past history of haematuria, visual inspection for blood in the urine specimens, and the use of reagent strips to detect haematuria and proteinuria, were evaluated to identify persons with Schistosoma haematobium infection. These approaches were applied individually and in three different screening sequences on two populations in Ghana and Zambia in order to identify infected children and adults for subsequent treatment in both areas. Detection of haematuria using reagent strips was the single approach with the highest sensitivity and specificity. The observation of gross haematuria (bloody urine), followed by detection of blood by reagent strips, identified 87% of infected children in both areas. This screening sequence showed the highest combined sensitivity and specificity in the identification of infected children as well as adults for treatment in both areas. Differences in the results between the two countries are discussed. This study emphasizes the need for evaluation of indirect screening procedures for the diagnosis of S. haematobium infection in each endemic area so as to establish criteria for their interpretation, prior to large-scale field application.

A method of rapid identification of persons with Schistosoma haematobium infection, especially school-age children, for subsequent treatment with antischistosomal drugs would facilitate operations of schistosomiasis control programmes. The use of reagent strips to test urine specimens has been suggested as a suitable indirect screening technique for determining S. haematobium infection. Previous studies have shown that haematuria and proteinuria, individually or both together, as well as leukocyturia are positively associated S. haematobium infection in children and adults (1-4). The present study was designed to evaluate the efficiency of four indirect approaches: (1) a questionnaire regarding a past history of haematuria, (2) inspection of the urine specimen for blood, (3) use of reagent strips to detect proteinuria, and (4) use of reagent strips to detect haematuria. These approaches were tested individually, as well as in combination in three different screening procedures, and compared with a quantitative urine filtration technique to detect S. haematobium infection (indicated by egg counts).

MATERIALS AND METHODS

The study areas in Ghana and Zambia have been described already (4).^a In Adawso, Ghana, 562 persons were consecutively interviewed and their urine specimens checked, as described below. The age and sex distribution of this study population have also been published (4).^a In Mutenda, Zambia, 656 persons, mostly school-age children, were interviewed and their urine specimens examined by the same techniques.

For urine examination, the total bladder content of the participants was collected between 10 h 00 and 14 h 00 into polyethylene bags; at the same time, a simple questionnaire was completed (results will be reported elsewhere). With the aid of reagent strips, the urinary protein content was recorded as negative (< 10 mg/100 ml of urine), trace (10-30 mg), + (30-100 mg), + + (100-300 mg), + + (300-1000 mg), or + + + + (> 1000 mg/100 ml). Presence of urinary blood was recorded as negative, +, + +, or + + +.

After testing with the reagent strips, the urine specimen in the polyethylene bag was labeled and fixed with a few drops of 10% formaldehyde and transported to a designated central laboratory. In the laboratory two aliquots (5 ml each in the highly endemic area of Ghana and 10 ml each in Zambia)

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^e See pages 125-133 of this issue of the Bulletin.

^b Neostix-3 from Ames-Miles Laboratories, Elkhart, IN, USA.

were examined microscopically using a Millipore filtration technique (5).

The questionnaire was pretested in Ghana to ensure that the questions could be properly phrased in the local language. On the basis of the pretest evaluation it was decided that only one question should be asked: "Have you ever noticed blood in the urine?" Women were also asked if they were menstruating at the time of the examination. The appearance of the urine was observed and recorded as: (a) clear, (b) cloudy yellow, (c) cloudy brown, (d) blood red, (e) any other appearance (with details).

Design of screening procedures

The following three screening sequences were adopted and the results analysed by computer.

Screen I: for use in identifying the largest proportion of infected persons with minimal use of reagent strips. As a first step, the participant should be asked if he or she had ever passed blood-stained urine. Subsequently, the urine specimen is inspected and gross haematuria is diagnosed if the urine colour is blood red or cloudy brown. Only the urine of persons without a previous history of haematuria or who present a bloody urine specimen should be examined by reagent strips.

Screen II: for use if sufficient reagent strips are available for testing all the specimens from the entire population. If no blood is detected by the reagent strip, then the urine's colour should be noted. Bloody urine specimens with a high ascorbic acid content are known not to react with the reagent strip, so observation of the colour is important. Lastly, the participant should be asked if he or she had ever passed blood-stained urine.

Screen III: for use in situations where no reliable history can be obtained and where use of the reagent strip in selected cases is necessary. First, the colour of the urine should be inspected; only those that are not blood red or cloudy brown should be tested with the reagent strips.

RESULTS

Children 0-4 years old

Ghana. Of 44 children in this age group, 16 (36%) were infected with S. haematobium, all with less than 64 eggs/5 ml of urine. A history of haematuria in 5 infected children was reported by their mothers. Of 9 children with haematuria detected by reagent strips, 6 had eggs in the urine. No gross haematuria was observed.

Zambia. Of 5 children in this age group, 3 were infected, all with less than 257 eggs/10 ml of urine. Only these 3 infected children were found to have haematuria by the reagent strips. Three children, two of whom were infected, had a history of haematuria.

Children 5-14 years old

Ghana. Among the 5-14-year-old children, urinary blood detected by the reagent strips and a history of haematuria were the single screening procedures with the highest individual specificity (85% in both instances) and sensitivity (69% and 80%, respectively), as shown in Table 1. Urinary blood was detected by reagent strips in 87% of infected children who had more than 64 eggs/5 ml of urine. Altogether 32% of infected urine specimens were blood red or cloudy brown. The simple visual observation of bloody urine was nearly as specific and as sensitive as the presence of + + + proteinuria by the reagent strips. Proteinuria was strongly associated with the presence of S. haematobium eggs in the urine; however, the specificity was low, ranging from 19% (with trace proteinuria or greater) to 74% (with + +proteinuria or greater).

Zambia. Among children from this low endemic area, the sensitivity (81%) and specificity (95%) of the reagent strips for detecting urinary blood was high (Table 1). Blood was found in the urine of 100% of the children with more than 64 eggs/10 ml of urine; 17% of infected urine specimens from children were blood red or cloudy brown. A history of haematuria and presence of trace or + proteinuria were sensitive indicators of S. haematobium infection, but the specificity of these indicators was low. The presence of + + proteinuria correlated well with S. haematobium infection (67%) and the specificity was high (93%).

Screening procedures in 5-14-year-old children

Table 2 presents the results from Ghana:

Screen I. This procedure detected 86% of children with S. haematobium eggs in the urine and 98% of those with more than 64 eggs per 5 ml of urine. Of 27 uninfected children, 23% had a positive indicator.

Screen II. The sensitivity and specificity of this screening procedure were similar to those of screen I.

Screen III. This procedure identified 82% of the urine specimens with S. haematobium eggs and 98% of urine specimens with more than 64 eggs per 5 ml of urine. The specificity (81%) was the highest of the three screening procedures in this highly endemic area.

Table 1. Frequency of haematuria and proteinuria among 5-14-year-old children, in relation to *S. haemotobium* egg counts, ^a in Ghana and Zambia

	No. of children	Percentage of specimens with						
			Haematuria		Prote	inuria		
		History	Visible	≥ + ^b	≥ Trace	≥ + + ^b		
Ghana								
No eggs	27	15	4	15	81	26		
Eggs present	215	69	32	80	94	62		
64 eggs/5 ml	79	73	41	87	89	79		
Zambia								
No eggs	174	47	2	5	39	7		
Eggs present	304	85	17	81	94	67		
64 eggs/10 ml	47	98	36	100	100	94		

^a Egg counts per random 5 ml or 10 ml urine sample.

Table 2. Screening procedures for detection and presumed treatment of *S. haematobium* infection in 5–14-year-old children in Adawso, Ghana

Egg count*	No. in group	Screen I ^b		Screen II ^b		Screen III b	
		% treated	% missed	% treated	% missed	% treated	% missed
0	27	23	77	23	77	19	81
1-4	20	55	45	55	45	50	50
5-64	106	81	19	82	18	75	25
65-256	53	96	4	96	4	96	4
≥ 257	36	100	0	100	0	100	0

[&]quot; Egg counts per random 5 ml urine sample.

Table 3. Screening procedures for detection and presumed treatment of *S. haematobium* infection in 5–14-year-old children in Mutenda, Zambia

Egg count"	Screen I ^b		en l ^b	Screen IIb		Screen III b	
	No. in group	% treated	% missed	% treated	% missed	% treated	% missed
0	174	48	52	48	52	5	95
1-4	71	83	17	83	17	55	45
5-64	186	96	4	96	4	86	14
65-256	33	100	0	100	0	100	0
≥ 257	14	100	0	100	0	100	0

[&]quot; Egg counts per random 10 ml urine sample.

^b Determined by using reagent strips.

^b In screen I, questioning was first; in screen II, the reagent strip was used first; in screen III, inspection of the urine was carried out first. See text for further details.

b In screen I, questioning was first; in screen II, the reagent strip was used first; in screen III, inspection of the urine was carried out first. See text for further details.

In all three screening procedures, the inclusion of + + proteinuria, or greater, as an additional criterion reduced the specificity by 15% (i.e., 15% more uninfected persons would have been unnecessarily treated) and increased the sensitivity by only 4-5%.

Table 3 presents the results from Zambia:

Screen I. This procedure detected 94% of the children with S. haematobium eggs in the urine and 100% of the children with more than 64 eggs per 10 ml of urine. The specificity was 52%.

Screen II. The sensitivity and specificity of this screening procedure were similar to those of screen I.

Screen III. This procedure correctly identified 81% of the urine specimens with S. haematobium eggs and

all (100%) urine specimens with more than 64 eggs per 10 ml of urine. The specificity (95%) was the highest among the three screening procedures in this low prevalence endemic area, so that very few uninfected persons would have been treated unnecessarily.

In all three of these screening procedures, the inclusion of ++ proteinuria or greater as an additional criterion increased the sensitivity by 3-6% but reduced specificity by 2-4%.

Persons aged 15 years and older

Ghana. In persons 15 years of age and older, the presence of haematuria detected by reagent strips was the most discriminating of the single procedures, although this occurred in only 57% of urine speci-

Table 4. Frequency of haematuria and proteinuria among persons aged 15 years and older, in relation to S. haemotobium egg counts,^a in Ghana and Zambia

	No. of persons	Percentage of specimens with						
			Haematuria		Proteinuria			
		History	Visible	≥ + ^b	≥ Trace	≥ + + ^t		
Ghana								
No eggs	119	18	4	11	65	12		
Eggs present	157	50	22	57	84	39		
64 eggs/5 ml	39	77	92	95	95	62		
Zambia								
No eggs	24	38	0	0	29	17		
Eggs present	149	84	24	89	95	66		
64 eggs/10 ml	37	92	51	100	100	86		

[&]quot; Egg counts per random 5 ml or 10 ml urine sample.

Table 5. Screening procedures for detection of *S. haematobium* infection in persons aged 15 years and older in Adawso, Ghana

_		Screen I ^b		Screen II b		Screen III b	
Egg count*	No. in group	% treated	% missed	% treated	% missed	% treated	% missed
0	119	22	78	22	78	14	86
1-4	53	38	62	38	62	34	66
5-64	65	66	34	66	34	56	44
65-256	29	93	7	93	7	93	7
≥ 257	10	100	0	100	0	100	0

[&]quot; Egg counts per random 5 ml urine sample.

^b Determined by using reagent strips.

^b In screen I, questioning was first; in screen II, the reagent strip was used first; in screen III, inspection of the urine was carried out first. See text for further details.

mens that had S. haematobium eggs. Blood red or cloudy brown urine was observed in 22% of infected urine specimens and in only 4% of urine specimens without S. haematobium eggs (Table 4). Though less sensitive, bloody or cloudy brown urine or + + + proteinuria by reagent strips were highly specific criteria (96% and 98%, respectively).

Zambia. In persons 15 years of age and older, haematuria detected by the reagent strips was present in 89% of infected urine specimens; specificity was 100% (Table 4). Proteinuria, at +++ level or greater, was highly specific (96%) but sensitivity was low (32%). Blood red or cloudy brown urine was observed in 24% of infected urine specimens and was not observed in urine specimens without S. haematobium eggs.

Screening procedures in persons aged 15 years and older

Table 5 presents the results from Ghana:

Screen I. This procedure identified 64% of infected

urine specimens and 95% of urine specimens with more than 64 eggs/5 ml of urine. The specificity was 78%.

Screen II. The sensitivity and specificity of this screening procedure were similar to those of screen I.

Screen III. This procedure identified 58% of the urine specimens with S. haematobium eggs and 95% of the specimens with more than 64 eggs/5 ml of urine. The specificity (86%) was the highest of the three screening procedures.

In all three screening procedures, the additional evaluation of proteinuria greater than a + reading reduced the specificity by 7% and increased the sensitivity by 8% only.

Table 6 presents the results from Zambia:

Screen I. This procedure identified 96% of all the infected urine specimens and 100% of urine specimens with more than 64 eggs/10 ml of urine. The specificity was 63%.

Table 6. Screening procedures for detection of *S. haematobium* infection in persons aged 15 years and older in Mutenda, Zambia

Egg count"	No. in group	Screen Ib		Screen II b		Screen III b	
		% treated	% missed	% treated	% missed	% treated	% missed
0	24	38	62	38	62	0	100
1-4	34	89	11	88	12	77	23
5-64	78	98	2	98	2	91	9
65-256	28	100	0	100	0	100	0
≥ 257	9	100	0	100	0	100	0

[&]quot; Egg counts per random 10 ml urine sample.

Table 7. Percentages of the study population in Ghana and Zambia, by age group, whose urines were tested with reagent strips according to the prevalence and intensity of *S. haematobium* infection and the three screening procedures

	Gha	ana	Zan	nbia
	5-14 years	≥ 15 years	5-14 years	≥ 15 years
Prevalence (%)	88.8	56.9	63.6	86.1
Egg count"	47.0/5 ml	13.1/5 ml	15.5/10 ml	19.3/10 ml
Screen I (%)	33	61	28	21
Screen II (%)	100	100	100	100
Screen III (%)	71	86	89	79

[&]quot; Geometric mean of egg counts per urine sample from infected persons.

^b In screen I, questioning was first; in screen II, the reagent strip was used first; in screen III, inspection of the urine was carried out first. See text for further details.

Screen II. The sensitivity and specificity of this screening procedure were similar to those of screen I

Screen III. This procedure identified 90% of the urine specimens with S. haematobium eggs. Neither visible blood nor haematuria by reagent strips was present in any specimen without S. haematobium eggs.

In all these screening procedures, the additional evaluation of proteinuria greater than a + reading reduced the specificity by 4-17% and increased the sensitivity by only 1-3%.

Reagent strip requirements

In an evaluation of the probable requirements for reagent strips, the three different screening procedures were compared (Table 7). Since screen II utilizes the reagent strip as the first step, urine specimens from 100% of the survey population require testing in this way. If a school-age population were to be screened initially by history and visual inspection of the urine for blood, then urine specimens from only about 30% of the population will require to be tested using reagent strips.

DISCUSSION

Detection of urinary blood using reagent strips was shown to be the single most specific and sensitive indirect screening technique for *S. haematobium* infection in children. About 80% of all infected children in both endemic areas in the present study had blood in the urine. More significantly, nearly all heavily infected children with more than 50 eggs per 10 ml of urine had haematuria.

Gross haematuria in school-age children is a widely recognized clinical manifestation of S. haematobium infection. Mere visual inspection of the urine specimens submitted by the children identified up to 32% of the infected children. No studies of entire communities in endemic areas are available to indicate the proportion of cases of haematuria which may be due to other causes. The low frequency of bloody urine from apparently uninfected children indicates that other causes may be relatively infrequent. The effectiveness of control programmes to reduce morbidity due to S. haematobium infections can be adequately evaluated by a combination of simple morbidity indicators such as haematuria and quantitative parasitological diagnosis.

Since the visual inspection of the urine specimens (appearance and colour) was shown to be a specific and sensitive indicator of infection, inclusion of this screening procedure may be recommended in order to identify heavily infected persons for treatment in endemic areas. Field workers may be easily trained in this simple procedure, which may reduce the need to use reagent strips by 10-30%, and save a substantial amount of time and money.

In endemic areas where no previous intervention has taken place, the answers to the simple question of whether the person has ever had haematuria may also be a valuable indication of current *S. haematobium* infection. The answers given are influenced by the field workers' manner, clarity of questioning, and local bias. Among the Zambian schoolchildren, for example, the high frequency of a history of haematuria without any infection may be due to any of these factors. This approach therefore requires prior evaluation in each endemic area.

Several differences in the outcome of these screening procedures were observed between our two study populations, indicating that thorough evaluation in pilot studies will be necessary in each endemic area. Error in the replies to the question about previous haematuria has been mentioned above. Although haematuria was less frequent in Zambia, the level of haematuria was much greater than in Ghana. In children in Zambia, proteinuria at ++ or greater levels was a fairly sensitive (67%) and specific (93%) single indicator of S. haematobium infection, compared with the study population in Ghana.

The screening procedures concerning haematuria also identified a high proportion of infected adults, particularly in the low endemic area. The combination of + or greater haematuria and + + or greater proteinuria identified most of the infected adults (93%) and was highly specific (83%) in Zambia. As noted above, the addition of the proteinuria reading should be carefully evaluated since it did not greatly improve specificity and sensitivity of the screening procedure in children in either area. The reactivity of the protein portion of most reagent strips is not easily discriminated and workers required extensive training to make proper readings. Other investigators have suggested that a combination of protein and blood readings (1, 3) or a combination of protein, blood and leukocyte reactivity readings (2) may increase the sensitivity and specificity of the detection of S. haematobium infection. The present study indicates that detection of haematuria alone is sufficient for screening purposes to identify a large proportion of persons infected with S. haematobium.

Our study did not include repeated urine examinations and it was not possible to confirm our classification of uninfected children. In the highly endemic Volta Lake region of Ghana, it has been shown that 30% of S. haematobium infections with 2 eggs/5 ml

of urine would go undetected by examination of a single urine specimen, using the diagnostic filtration technique as in this study (5).

The stability of the reagent strips for large-scale use in the high ambient temperatures and humidity of endemic areas has not been thoroughly evaluated. In our study the containers with 50 reagent strips were opened and used within one day.

The cost of the reagent strips has been estimated to be below US\$0.05 each (2). If the strips contain one reagent for the detection of only urinary blood, the cost may be lower. The cost per infected person identified could be far lower than the cost using conventional microscopic diagnostic techniques.

This study has shown that screening procedures which focus on haematuria alone will identify a high proportion (at least 80%) of infected persons in a defined population, and an even higher proportion (above 95%) of those with more than 50 eggs per 10 ml of urine. A single oral dose of an antischistosomal drug could then be given to those identified as infected with S. haematobium without delay following the screening procedure. The simplicity of this screening approach and its potentially low cost will facilitate its use in primary health care and schistosomiasis control programmes.

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RÉSUMÉ

DÉPISTAGE INDIRECT DE L'INFECTION À SCHISTOSOMA HAEMATOBIUM: ÉTUDE COMPARATIVE AU GHANA ET EN ZAMBIE

Une méthode d'identification rapide des sujets atteints d'une infection à Schistosoma haematobium, en particulier chez les enfants d'âge scolaire, en vue de leur traitement par des schistosomicides, faciliterait l'exécution des programmes de lutte contre la schistosomiase. Quatre approches indirectes ont été évaluées au cours de la présente étude: 1) questionnaire portant sur les antécédents d'hématurie, 2) inspection des échantillons d'urine à la recherche de sang, 3) utilisation de bandelettes de papier réactif pour rechercher une protéinurie, et 4) utilisation de bandelettes de papier réactif pour rechercher une hématurie. Ces méthodes ont été testées individuellement et en association dans trois séries d'épreuves de dépistage et ont été comparées avec une technique de comptage des œufs après filtration de l'urine pour déceler l'infection à S. haematobium dans deux populations du Ghana et de Zambie.

A Adawso, Ghana, 562 personnes ont été interrogées et leurs urines ont été examinées. La présence de sang dans les urines, détectée au moyen de bandelettes de papier réactif, et les antécédents d'hématurie étaient les méthodes de dépistage qui, utilisées séparément, présentaient la plus forte spécificité (85%) et la plus forte sensibilité (80% pour la première et 69% pour la deuxième). La recherche de la protéinurie n'avait qu'une faible sensibilité. La présence de sang dans l'urine a été observée dans 32% des échantillons contenant des oeufs de S. haematobium et dans 4% seulement des échantillons sans oeufs.

A Mutenda, Zambie, 656 personnes, pour la plupart des enfants d'âge scolaire, ont été examinées. La présence de sang dans l'urine, décelée au moyen de bandelettes de papier réactif, était la méthode qui, utilisée séparément, présentait la plus forte spécificité (89%) et la plus forte sensibilité (81%). Les antécédents d'hématurie et la présence d'une faible protéinurie n'étaient pas spécifiques. La présence d'une protéinurie moyenne (++) offrait une bonne corrélation avec l'infection à S. haematobium (67%), avec une bonne spécificité (93%). La présence de sang dans l'urine a été observée dans 17% des échantillons contenant des oeufs de S. haematobium et dans 2% des échantillons sans oeufs.

Le dépistage I commençait par un interrogatoire sur les antécédents d'hématurie, suivi par l'inspection des urines à la recherche de sang; si ces deux étapes donnaient des résultats négatifs, l'échantillon était testé au moyen de bandelettes de papier réactif. Le dépistage II commençait par l'emploi des bandelettes de papier réactif, suivi par l'inspection des urines à la recherche de sang et, enfin, si ces deux étapes donnaient des résultats négatifs, le sujet était interrogé sur ses antécédents d'hématurie. Dans le dépistage III, on examinait la couleur des urines, et celles qui n'étaient ni tachées de sang, ni brunes, étaient testées au papier réactif.

Au Ghana et en Zambie, parmi les enfants de 5 à 14 ans, le dépistage I, commençant par un interrogatoire sur les antécédents, a donné la meilleure sensibilité, avec 86% au

Ghana et 94% en Zambie. Les meilleurs résultats associant une bonne sensibilité à une bonne spécificité ont été observés avec le dépistage III, dans lequel on examinait d'abord la couleur de l'urine, les chiffres obtenus étant respectivement de 82% et 81% au Ghana et de 81% et 95% en Zambie.

L'addition d'une recherche de la protéinurie au moyen de bandelettes de papier réactif avait en général pour effet de diminuer la sensibilité du dépistage plus qu'elle n'en augmentait la spécificité.

Parmi les sujets âgés de 15 ans et plus, au Ghana, seules 57% des urines contenant des oeufs de S. haematobium présentaient une hématurie décelée au moyen du papier réactif, alors que ce chiffre était de 89% en Zambie. Une forte protéinurie (+ + + ou davantage) était un indicateur hautement spécifique, mais peu sensible, de l'absence d'infection à S. haematobium.

Parmi les méthodes de dépistage appliquées dans le groupe d'âge de 15 ans et plus, le dépistage I présentait la meilleure sensibilité, avec 64% au Ghana et 96% en Zambie, avec une spécificité de 78% et 63% respectivement. Le dépistage III, dans lequel on observait d'abord la couleur de l'urine, offrait à la fois la meilleure sensibilité et la meilleure spécificité, avec des chiffres de 58% et 86% au Ghana, et de 90% et 100% en Zambie.

Le traitement des sujets sur la seule base de l'inspection visuelle de l'urine à la recherche de sang est hautement spécifique et est plus économique que le dépistage au moyen de bandelettes de papier réactif.

Les différences géographiques observées dans cette étude soulignent la nécessité d'évaluer chaque séquence de dépistage (I, II et III) dans la zone d'endémie étudiée.

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