Evaluation of reagent strips in urine tests for detection of *Schistosoma haematobium* infection: a comparative study in Ghana and Zambia

K. E. MOTT, ¹ H. DIXON, ² E. OSEI-TUTU, ³ E. C. ENGLAND, ³ K. EKUE, ⁴ & A. TEKLE ⁴

The presence of haematuria and proteinuria, detected by reagent strips, was compared with Schistosoma haematobium egg counts in the urines of human subjects from two epidemiologically distinct areas in Ghana and Zambia. In children and adults in both areas, the individual or combined semiquantitative levels of proteinuria and haematuria were related directly to increasing urinary egg counts. In both areas the presence of blood in the urine was highly specific (greater than 85%) and sensitive, being positive in 97% of urine specimens with more than 64 eggs per 5-ml sample of urine. The sensitivity of the protein indicator was also high, but its specificity was less than the blood indicator. The specificity of combined proteinuria and haematuria was higher than either alone; on the other hand, the sensitivity was lower than either alone. At each level of proteinuria and haematuria, the geometric mean urinary egg count was higher in Ghana than in Zambia. This study confirms the necessity to evaluate indirect diagnostic techniques in each endemic country, in order to establish criteria for their interpretation, before wide-scale use.

Urinary schistosomiasis due to Schistosoma haematobium infection affects over 90 million persons, principally in Africa. Haematuria and proteinuria among infected children has long been recognized as an early sign of infection, and it has been shown that the degree of haematuria and proteinuria in children is related to the intensity of S. haematobium infection (1).

New urine-filtration techniques for the detection of S. haematobium infection, although useful under field conditions, require microscopic examination by trained personnel. The lack of trained field personnel in endemic areas is a serious constraint on the development of schistosomiasis control programmes (2). Simple indirect diagnostic techniques that could be used efficiently by minimally trained health workers to identify heavily infected persons, particularly children, would therefore aid the implementation of

such control programmes. Rapid diagnosis of heavily infected persons followed by treatment with new, safe, and highly effective antischistosomal drugs (at a cost acceptable to endemic countries) could be expected to reduce the morbidity related to schistosomiasis.

The prevalence, intensity, and related morbidity of urinary schistosomiasis in Africa vary according to the epidemiology, transmission patterns, and ecology of each endemic area. The present study describes an evaluation of reagent strips in the detection of urinary blood and protein in relation to S. haematobium infection in two geographically separated endemic areas that were epidemiologically distinct.

MATERIALS AND METHODS

Study areas

Ghana. The Epidemiology Unit of the Ministry of Health is responsible for an applied field research project (3) on Lake Volta, 180 km from Accra. The present study was carried out in five settlements in Adawso, near this project area, where no previous diagnosis or treatment programmes had been under-

¹ Chief, Schistosomiasis and other Snail-borne Trematode Infections, Parasitic Diseases Programme, World Health Organization, 1211 Geneva 27, Switzerland.

² Epidemiological and Statistical Methodology, World Health Organization, Geneva, Switzerland.

³ Schistosomiasis Unit, Division of Epidemiology, Ministry of Health, Accra, Ghana.

⁴ WHO Regional Office for Africa, Brazzaville, Congo. Formerly, Tropical Disease Research Centre, Ndola, Zambia.

Table 1. Prevalence and intensity of *S. haematobium* infection in the study population, according to age and sex, in Adawso, Lake Volta, Ghana

Age (years)	Males				Females		Both			
	No. examined	Prevalence (%)	Egg count"	No. examined	Prevalence (%)	Egg count ^a	No. examined	Prevalence (%)	Egg count	
0-4	30	30.0	4.6	14	50.0	3.8	44	36.4	4.3	
5-9	64	85.9	53.0	58	82.8	28.8	122	84.4	39.9	
10-14	69	94.2	50.0	51	92.2	61.8	120	93.3	54.6	
15-19	34	85.3	39.6	29	69.0	24.8	63	77.8	32.7	
20-24	24	62.5	36.6	21	33.3	14.3	45	48.9	27.2	
25-34	23	65.2	8.5	34	47.1	4.4	57	54.4	6.1	
35-44	24	66.7	10.2	31	35.5	4.1	55	49.1	7.1	
45-54	21	66.7	7.0	11	54.5	6.2	32	62.5	6.7	
55-64	14	50.0	7.5	10	10.0	1.0	24	33.3	5.9	
Total	303	74.3	29.0	259	62.9	21.2	562	69.0	25.4	

^a Geometric means of egg counts per random 5 ml urine sample from infected persons.

taken. The prevalance and intensity of *S. haemato-bium* infection among residents of this area are among the highest reported in Africa.

Zambia. The Tropical Disease Research Centre in Ndola, supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, undertakes field testing of antischistosomal drugs in areas near Ndola. The present study in Mutenda was part of a screening programme to identify infected schoolchildren. None of the children examined had been treated previously.

Methods

In the study areas, 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 protein content in the urine 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 blood in the urine was recorded as negative, +, + +, or + + +.

After testing with the reagent strips, the urine specimen in the polyethylene bag was labelled 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)

were examined microscopically using a Millipore filtration technique (3).

The data from Ghana refer to 5 ml urine samples, i.e., half the volume of the urine samples examined in Zambia. Currently, 10 ml aliquots of urine are recommended for urine filtration techniques requiring the Nuclepore, Nytrel, or paper filters. However, because of the blockage of the paper filters with sediment and the high intensity of S. haematobium infections in the Lake Volta region, small volumes (5 ml) of urine were examined. Thus, in order to compare the egg counts between Zambia and Ghana, the data from Ghana should be multiplied by 1.5-2 times (3).

The data from the questionnaires and laboratory records were processed independently and analysed in WHO headquarters, Geneva. The results from the two study areas are described below separately and are compared in the discussion.

RESULTS

Adawso, Ghana

Study population. A total of 562 persons were interviewed and their urine specimens tested (Table 1). The age and sex distribution of the study population was similar to that reported before for the

[&]quot; See article on pages 135-142 in this issue of the Bulletin.

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

^c Nuclepore polycarbonate filters, 12 or 14 µm pore size, from Nuclepore Corporation, Pleasanton, CA, 94566 USA.

^d Nytrel polyamide filters, 20 μ m mesh, from Union des Gazes à Bluter, 42360 Pannisières, France.

entire project area (3). The peak prevalence and intensity of S. haematobium infection was observed in the 10-14-year age group. These results have been reported previously (1).

Presence of urinary blood and protein in relation to egg count levels. Blood was detected in nearly all (97%) urine specimens that had more than 64 eggs per 5 ml of urine and in 86% of urine specimens with more than 16 eggs per 5 ml of urine. These specimens were from persons in both the 5-14-years (children) and ≥ 15-years (adult) age groups. The specificity of the urinary blood determination was high; at least + blood was observed in 15% of urine specimens from children and 11% of urine specimens from adults in which S. haematobium eggs were not found.

The sensitivity of urinary protein determinations was high. Protein was detected in urine specimens from 94% of infected children and 84% of infected adults. Protein levels of at least 100 mg/100 ml of urine were found in 62% of infected children and 39% of infected adults. On the other hand, the specificity of urinary protein determinations was low. Proteinuria was detected in the majority of urine specimens that had no S. haematobium eggs—from children (81%) and from adults (65%). Protein concentrations of at least 100 mg/100 ml of urine were observed in 26% of egg-negative specimens from

children and 12% of egg-negative specimens from adults.

The combined criteria of trace proteinuria or more and + blood was more specific than either indicator alone. The combined criteria of at least + + protein ($\geq 100 \text{ mg}/100 \text{ ml}$ of urine) and + blood showed even higher specificity only in adults (Table 2). However, the sensitivity of the combined criteria was lower than that for either indicator alone.

Urinary blood levels and S. haematobium prevalence and egg counts. Among both children and adults, a positive correlation was observed for males and females between semiquantitative urinary blood levels and the prevalence of infection and geometric mean S. haematobium egg counts. The geometric mean egg counts in children were generally higher than those in adults for the same urinary blood levels.

Urinary protein levels and S. haematobium prevalence and egg counts. Among both children and adults, a positive correlation between urinary protein levels and prevalence of infection and geometric mean S. haematobium egg counts was observed for males and females. In this highly endemic area, children had higher mean egg counts than adults with similar urinary protein levels, but the differences were not statistically significant (P > 0.14).

Table 2. Relationship between *S. haematobium* egg counts and results of urinary protein and blood determinations by reagent strips in the study population in Adawso, Ghana

			Perce	entage of specime	ens with	
		≥ Trace	≥ + +	Protein reading neg.	. ^b ≽ trace	≥ + +
Age group and egg count*	Number examined	neg.	neg.	Blood reading: ≥ +	<i>b</i> ≥ +	≥ +
5–14 years						
0	27	81	26	15	11	11
1-4	20	80	25	35	35	15
5-64	106	93	57	74	72	49
65-256	53	98	68	94	92	66
≥ 257	36	100	89	100	100	89
Total infected	215	94	62	80	78	57
≥ 15 years						
0	119	65	12	11	9	4
1-4	53	70	19	34	23	8
5-64	65	89	40	54	52	29
65-256	29	93	66	90	86	66
≥ 257	10	100	70	100	100	70
Total infected	157	84	39	57	52	31

^{*} Egg counts per random 5 ml urine sample from infected persons.

^b See text (page 126) for equivalent values of trace, ++, etc.

Table 3. Prevalence and intensity of *S. haematobium* infection in the study population, according to age and sex, in Mutenda, Zambia

		Males		Females			Both		
Age (years)	No. examined	Prevalence (%)	Egg count*	No. examined	Prevalence (%)	Egg count"	No. examined	Prevalence (%)	Egg count
0-4	0	_	-	5	60.0	20.0	5	60.0	20.0
5-9	77	59.7	17.7	77	55.8	11.1	154	57.8	14.1
10-14	200	66.5	14.5	124	66.1	19.1	324	66.4	16.1
15-19	58	63.8	13.4	22	95.5	14.1	80	72.5	13.6
20-24	8	100	4.6	8	100	18.1	16	100	9.1
25-34	4	100	22.3	11	100	55.2	15	100	43.4
35-44	4	100	7.1	14	100	30.9	18	100	22.3
45-54	6	83.3	4.6	24	100	41.2	30	96.7	28.2
55-64	6	83.3	10.8	8	100	61.2	14	92.9	31.4
Total	363	66.7	13.8	293	73.0	20.6	656	69.5	16.7

[&]quot; Geometric means of egg counts per random 10 ml urine sample from infected persons.

Table 4. Relationship between *S. haematobium* egg counts and results of urinary protein and blood determinations by reagent strips in the study population in Mutenda, Zambia

		Percentage of specimens with							
				Protein reading	; b				
		Trace	≥ + +	neg.	≥ trace	≥ + +			
Age group				Blood reading:	b				
and egg count*	Number examined	neg.	neg.	≥ +	≥ +	≥ +			
5-14 years	•								
0	174	39	7	5	5	3			
1-4	71	89	34	55	55	20			
5-64	186	95	73	86	86	68			
65-256	33	100	94	100	100	94			
≥ 257	14	100	93	100	100	93			
Total infected	304	94	67	81	81	60			
≥ 15 years									
0	24	29	17	0	0	0			
1-4	34	79	35	76	74	35			
5-64	78	99	69	90	90	64			
65-256	28	100	86	100	100	86			
≥ 257	9	100	89	100	100	89			
Total infected	149	95	66	89	89	63			

⁴ Egg counts per random 10 ml urine sample from infected persons.

^b See text (page 126) for equivalent values of trace, ++, etc.

Combined urinary blood and protein levels and S. haematobium prevalence and egg counts. Among both children and adults, a positive correlation between combined urinary blood and protein levels and the prevalence of infection and geometric mean S. haematobium egg counts was observed. About a 10-fold difference was noted in geometric mean egg counts between urine specimens without detectable protein and blood and urine specimens with at least both + blood and trace protein in children (respectively, 5.7 vs 72.5 eggs per 5 ml of urine) and in adults (3.7 vs 34.7 eggs per 5 ml of urine).

Mutenda, Zambia

Study population. A total of 656 persons were interviewed and their urine specimens tested (Table 3). This population was mainly school-age children; a few adults were also examined. The data from urine examinations of five children below 5 years of age were excluded from this analysis.

Presence of urinary blood and protein in relation to egg count levels. In Mutenda, the presence of either

blood (+ or more) or protein (trace or more) was detected in all the urine specimens that had more than 64 eggs per 10 ml of urine (Table 4). The specificity of the trace protein determination was low, particularly in children (61%). The specificity of the blood determination was high, particularly in adults (100%). Blood was detected in 81% of urine specimens containing S. haematobium eggs from 5-14-year-old children and in 89% of egg-positive urine specimens from those over 14 years of age.

Urinary blood levels and S. haematobuium prevalence and egg counts. A positive correlation was observed between urinary blood levels and the geometric mean S. haematobium egg counts in both children and adults (Table 5). Prevalences of infection for each semiquantitative urinary blood level were similar between both age groups and between men and women. The geometric mean egg counts at specific blood levels were similar between children and adults.

Urinary protein levels and S. haematobium prevalence and egg counts. Among the children a positive

Table 5. S. haematobium prevalence and egg counts, by age group and sex, in relation to semiquantitative urinary blood readings of reagent strips in Mutenda, Zambia

		Presence of blo	od in the urine		
	Negative	+	++	+++	Total
5-14-year age group:					
Males:					
No. of subjects	122	80	36	39	277
% infected	24	96	97	97	65
Egg count	4.6	9.7	26.6	57.4	15.3
Females:					
No. of subjects	102	46	22	31	201
% infected	28	98	96	97	62
Egg count	5.0	11.2	26.9	56.8	15.8
≽ 15-year age group:					
Males:					
No. of subjects	32	27	11	16	86
% infected	28	100	100	100	73
Egg count	4.3	7.2	17.3	23.1	10.5
Females:					
No. of subjects	8	18	28	33	87
% infected	88	100	100	100	99
Egg count	4.7	11.0	42.6	58.0	30.2

Geometric means of egg counts per random 10 ml urine sample from infected persons.

Table 6. S. haematobium prevalence and egg counts, by age group and sex, in relation to semiquantitative urinary protein readings of reagent strips in Mutenda, Zambia

	Presence of protein in the urine						
	Negative	Trace	+	++	+++	++++	Total
5-14-year age group:							
Males:							
No. of subjects	70	29	47	66	50	15	277
% infected	14	38	68	94	98	100	65
Egg count	7.3	6.5	5.4	14.0	27.1	96.5	15.3
Females:							
No. of subjects	54	28	35	42	28	14	201
% infected	15	50	74	91	93	93	62
Egg count	3.8	5.2	9.2	18.9	29.8	65.0	15.8
≽ 15-γear age group:							
Males:							
No. of subjects	21	12	10	23	16	4	86
% infected	24	75	100	87	94	100	73
Egg count	2.0	5.0	7.0	14.7	19.1	24.4	10.5
Females:							
No. of subjects	4	4	20	31	19	9	87
% infected	75	100	100	100	100	100	99
Egg count	5.0	4.1	19.2	25.5	62.2	140.9	30.2

^a Geometric means of egg counts per random 10 ml urine sample from infected persons.

correlation was also observed between the urinary protein levels and the prevalence of infection (Table 6). No difference in geometric mean S. haematobium egg counts in the urine specimens from children without detectable, trace, or + levels of protein was noted (Table 6), whereas in the urine specimens from adults (over 14 years old) the egg counts were higher in urines with at least + protein compared to negative urines or with trace protein (Table 6). Among adults, a high prevalence (75%) of infection was observed at all levels of proteinuria.

Combined urinary blood and protein levels and S. haematobium prevalence and egg counts. Among both children and adults a positive correlation was observed between combined urinary blood and protein levels and the geometric mean S. haematobium egg count. No differences in prevalence of infection and geometric mean egg counts at the specific different combined levels of urinary blood and protein were observed between children and adults (Table 7). In children, a 4-fold difference in egg count was observed between negative urines and urines with at least + blood and trace protein (5.5 vs 20.4 eggs per 10 ml). In adults the difference was nearly 9-fold.

DISCUSSION

In both endemic areas of the present study, the levels of proteinuria and haematuria, as detected by reagent strips, were directly related to urinary S. haematobium egg counts in children and adults. These findings are in agreement with observations made in the endemic areas of the Gambia (4), Sudan (5) and Nigeria (6).

Some differences in the results between the two study areas were noted. In both children and adults with similar levels of S. haematobium egg output, for example, the rates of proteinuria and haematuria were higher in Zambia than in Ghana. Furthermore, in children, the geometric mean S. haematobium egg count associated with a specific level of proteinuria or haematuria was generally higher in Ghana than in Zambia. These differences were even more pronounced when combined criteria of proteinuria and haematuria were considered. In children and adults, the geometric mean egg counts for similar levels of proteinuria and haematuria were higher in Ghana than in Zambia.

The inverse relationship between age and the geometric mean S. haematobium egg count at each

Blood reading: ^b Protein reading: ^b	Negative Negative	≥ + Trace	≥ + ≥ +	≥ + ≥ + +	≥ + ≥ + + +
5-14-year age group:					
No. of subjects	124	254	237	189	101
% infected	15	97	97	97	98
Egg count	5.5	20.4	21.4	28.5	41.2
≥ 15-year age group:					
No. of subjects	24	132	121	94	46
% infected	29	100	100	100	100
Egg count	2.7	23.3	26.9	31.5	47.9

Table 7. S. haematobium prevalence and egg counts, by age group, in relation to combined semiquantitative urinary blood and protein readings of reagent strips in Mutenda, Zambia

specific level of proteinuria and haematuria has been discussed elsewhere (1). In Ghana the same levels of haematuria were associated with a higher egg count in children than in adults. This was not observed in Zambia in relation to haematuria or to proteinuria.

Genetic differences in the populations of Ghana and Zambia may have contributed to these findings. Sickle cell trait, for example, is estimated to occur in 10% of the general population in Ghana (7), and in 20% of the population in the study area in Zambia (8). Painless haematuria is frequently associated with sickle cell trait and may have contributed to the rates of haematuria discovered in these endemic areas. No increased risk of morbidity in concomitant sickle cell trait and S. haematobium infection has been reported, as compared with the risk in either clinical state alone.

Differences in the pathogenicity of the S. haematobium strains in Ghana and Zambia may also have influenced these observations but this is less likely. Some differences in morbidity associated with different S. haematobium strains have been shown experimentally (9, 10), but it was felt that host variations rather than strain differences were more significant (9). Wilkins et al. (4) observed the same overall rates of haematuria and proteinuria corresponding to similar egg count levels between populations in Egypt (Nile delta) and the Gambia. Differences in the rates of haematuria and proteinuria between children and adults in these two areas were reported. The rates of haematuria among children and adults with low egg counts were higher in Zambia than among similar age groups in Ghana. The rate of haematuria in Zambia was low in uninfected children (5%) and was not observed in adults.

In contrast to the findings from Ghana, proteinuria at ++ or greater levels was both a sensitive and a specific indicator of S. haematobium infection among

children in Zambia. These observations in Zambia are similar to recent findings in Zimbabwe (11). Low levels of proteinuria (below 100 mg/100 ml of urine) were not specifically related to *S. haematobium* infection in both study areas. This finding is in agreement with observations in Gambia (4) and Nigeria (6).

The present study has shown that regional differences must be considered before establishing criteria for screening for S. haematobium infection. Haematuria was present in 80% of infected children in both Zambia and Ghana, as reported elsewhere (12). On the other hand, haematuria was observed in only 57% of infected adults in Ghana and in nearly 90% of infected adults in Zambia. In both areas, combined ++ proteinuria and haematuria was present in less than 63% of infected persons. It has been suggested that higher levels of proteinuria should be included in the screening procedures to identify heavily infected persons (4, 6). However, the reading of the protein portion of the reagent strips requires additional training for field personnel, and this does not proportionally increase either the sensitivity or the specificity.

Haematuria detected by reagent strips identified a high proportion of infected children and adults, compared with those diagnosed by microscopic examination using a filtration technique. Moreover, 97% of heavily infected children (with more than 64 eggs per 5 ml urine) were found to have haematuria by the reagent strips, the use of which has therefore been suggested as an indirect diagnostic technique to identify heavily infected persons in public health programmes, particularly among school-age children (4-6, 11, 12). Because of variations between countries, preliminary evaluation at a country level is necessary to establish the criteria for their use.

Geometric means of egg count's per random 10 ml urine sample from infected persons.

^b See text (page 126) for equivalent values of trace, +, etc.

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RÉSUMÉ

EVALUATION DES BANDELETTES DE PAPIER RÉACTIF DANS LES ÉPREUVES URINAIRES DE DÉTECTION DE L'INFECTION À SCHISTOSOMA HAEMATOBIUM: ÉTUDE COMPARATIVE AU GHANA ET EN ZAMBIE

La prévalence, l'intensité et la morbidité de la schistosomiase urinaire en Afrique varient selon l'épidémiologie, les modes de transmission et l'écologie de chaque zone d'endémie. La présence d'hématurie et de protéinurie, décelée au moyen de bandelettes de papier réactif, a été comparée avec les numérations d'œufs de Schistosoma haematobium dans les urines de sujets provenant de deux régions épidémiologiquement distinctes du Ghana et de Zambie.

A Adawso, Ghana, 562 personnes ont été examinées. La prévalence et l'intensité maximales de l'infection ont été observées dans le groupe d'âge 10-14 ans. On a trouvé du sang dans 97% des échantillons d'urine comptant plus de 64 œufs par 5 ml, et dans 86% des échantillons d'urine comptant plus de 16 œufs par 5 ml. La recherche des protéines était d'une bonne sensibilité mais d'une faible spécificité. En associant les critères de protéinurie et d'hématurie, on obtenait une meilleure spécificité qu'avec chacun d'entre eux, mais la sensibilité était alors plus faible qu'avec l'un ou l'autre pris isolément.

Chez l'adulte comme chez l'enfant, on a observé une corrélation positive entre les taux semi-quantitatifs de sang et de protéines dans les urines et la prévalence de l'infection, de même qu'avec la moyenne géométrique du nombre d'œufs de S. haematobium.

A Mutenda, Zambie, parmi 656 sujets principalement d'âge scolaire, on a décelé du sang et des protéines dans tous les échantillons d'urine comptant plus de 64 œufs par 10 ml.

La recherche de l'hématurie était d'une bonne spécificité, surtout chez les adultes (100%). Comme au Ghana, on a observé une corrélation positive entre les taux urinaires de sang et de protéines et la prévalence de l'infection ainsi qu'avec la moyenne géométrique du nombre d'œufs de S. haematobium.

Chez les enfants et les adultes ayant une oviurie analogue, l'hématurie et la protéinurie étaient plus élevées en Zambie qu'au Ghana. De plus, les nombres d'œufs associés à un taux spécifique d'hématurie ou de protéinurie étaient généralement plus élevés au Ghana qu'en Zambie. La fréquence du trait drépanocytaire dans la zone d'étude étant, semble-t-il, plus élevée en Zambie (20%) qu'au Ghana (10%), cela pourrait expliquer cette différence. Bien qu'il existe aussi des différences entre les souches de S. haematobium en présence, elles ne sont probablement pas responsables des différences entre les taux de morbidité observés.

Avant d'établir les critères de dépistage de l'infection à S. haematobium, il faut examiner les différences régionales. L'hématurie décelée par les bandelettes de papier réactif permet d'identifier une proportion importante d'enfants et d'adultes infectés, par comparaison avec le diagnostic microscopique après filtration de l'urine. Du fait des différences entre pays, une évaluation préliminaire au niveau du pays est nécessaire pour établir les critères d'infection avant de procéder au dépistage à l'aide de bandelettes de papier réactif.

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