Comments on "The Routes of Schistosome Egg Passage . . ."

BY

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D. M. Blair, in the preceding paper, has analysed the data from 7,007 individuals with regard to the appearance of the eggs of *Schistosoma haematobium* and *S. mansoni* in stool and urine specimens. There are 16 possible combinations for the appearance of the two types of eggs from two sites, ranging from complete absence of eggs to the appearance of eggs of each species in both stool and urine.

Of the people examined, 2,684 were passing *S. haematobium* eggs in their urine. The proportion of cases in this category equals 2684/7007 or .3830. In other words, if 1,000 cases were selected at random for re-examination, about 383 would be expected to show *S. haematobium* eggs in the urine. Since .383 is the proportion positive for this trait, the proportion negative is 1.000 minus .383 or .6170. More generally, where X is the rate of appearance of positive cases in a category, then (I-X) is the negative rate.

It is possible with this approach to construct a table of rates from the data. Since for any category the sum of the proportion positive and proportion negative must equal one, as one increases the other decreases.

It is interesting at this point to consider whether or not the pairs of rates given above are related or whether they are independent. We might wish to know, for instance, if the presence of *S. haematobium* eggs in urine is related to the presence of *S. mansoni* eggs in stool. That is, we wish to discover the expected numbers of people which would occur in each of the 16 categories if the rates given above are independent. We can then use a statistical test to compare the numbers observed with the numbers calculated mathematically. If the numbers are very similar we can then accept the hypothesis that the rates are not related. If they are not similar we can assume that the rates are not unrelated and that the probability of having (or not having) eggs of a schistosome species passing through a certain route is affected by the presence or absence of eggs in other routes. The proportions positive are in fact probabilities that an individual from the sample will fall into the category in question. Thus the probability that an individual from the total sample will have *S. haematobium* in urine is .3830. Similarly, the probability that an individual will have *S. mansoni* in stool is .2807. The probability that an individual will have both *S. haematobium* eggs in the urine and *S. mansoni* eggs in the stool is the *product* of the separate probabilities or .3830 times .2807.

By following this approach and substituting the appropriate rates from Table I, the probabilities have been calculated for each of the 16 categories. These figures multiplied times the total sample size 7007 give the number of people expected in each. The results are presented in Table II. The categories are rearranged so that they are rank-ordered by size, starting with the largest.

The four categories which are expected to be largest are, in order:

(1) No eggs of either species; uninfected.
(2) *S. haematobium* eggs in the urine only.
(3) *S. mansoni* eggs in the stool only.
(4) *S. haematobium* in urine and *S. mansoni* in stool.

These are the normal, or usual, portals of exit and, as expected, most of the observed cases

Table I

RATES OF APPEARANCE OF EGGS OF TWO SPECIES OF SCHISTOSOME BY NORMAL AND ABNORMAL ROUTES, WITHOUT REGARD TO COMBINATIONS

Species/Portal of Exit	Number Positive Cases	Positive Rate	Negative Rate
S. haematobium in urine	2,684	X = .3830	(I-X) = .6170
S. mansoni in stool	1,967	Y = .2807	(I-Y) = .7193
S. haematobium in stool	158	A = .0225	(I-A) = .9775
S. mansoni in urine	68	B = .0097	(I-B) = .9903

actually fall into one of these categories. There is an excess of observed negative or uninfected cases and also of normal double infections, while there is a deficiency of normal single infections. Also contrary to general expectation under a hypothesis of independence, the category "double infection, normal portals only," contains more observed cases than the category "*S. mansoni* in stool only."

It has been demonstrated (Clarke, in press) that among individuals known to be infected with *S. haematobium* an unexpectedly high proportion also were infected with *S. mansoni*. His work demonstrated that there is little or no interspecific resistance conferred upon humans by either species. Blair's data confirms this.

Disregarding the portals of exit briefly, we find an interesting situation in the distribution of single and double species infections. This is presented in Table III which is compiled from Table II.

The application of chi-square test with three degrees of freedom gives a significant level of 0.005. In other words, the probability of obtaining the observed distribution, if in fact the presence of the two species is random, is less than 0.005. Consequently we may reject that hypothesis, since it is clear that there is an

Table II

The Observed and Expected Numbers of Cases from 7,007 Examined in Each of 16 Categories. Rate Symbols are as in Table I. "H" and "M" are Used to Denote S. haematobium and S. mansoni Respectively.

Description	Blair's Category Number	Calculation x 7007 Using Rates from Table I	Observed	Expected	Observed Minus Expected
Uninfected	1	(I-X) (I-Y) (I-A) (I-B)	3,450	3,010	+ 440
H. in urine	2	X (I-Y) (I-A) (I-B)	1,488	1,907	— 419
M. in stool	5	(I-X) Y (I-A) (I-B)	834	1,174	— 340
H. in urine; M. in stool	8	X Y (I-A) (I-B)	1,013	729	+ 284
H. in stool	4	(I-X) (I-Y) A (I-B)	21	69	— 48
H. in stool and urine	7	X (I-Y) A (I-B)	60	43	+ 17
M. in urine	3	(I-X) (I-Y) (I-A) B	4	29	— 25
H. and M. in stool	11	(I-X) Y A (I-B)	12	27	— 15
H. and M. in urine	6	X (I-Y) (I-A) B	15	18	— 3
H. in both; M. in stool	14	X Y A (I-B)	61	17	+ 44
M. in both	10	(I-X) Y (I-A) B	2	12	— 10
H. in both; M. in stool	13	X Y (I-A) B	43	7	+ 36
H. in stool; M. in urine	9	(I-X) (I-Y) A B	0	0.68	—0.68
H. in stool; M. in both	15	(I-X) Y A B	0	0.26	—0.26
Both in both ("full house")	16	X Y A B	2	0.16	+1.84
H. in both; M. in urine	12	X (I-Y) A B	2	0.04	+1.96

excess of double infections and negative cases (which are *double* negatives). Since the laboratory staff who examined the specimens work by number codes, it is unlikely that the results are due to biased procedure. In fact, the stool and urine from a given person are often examined by different technicians and the results correlated later. Appendix A should be consulted for the calculation of the chi-square test.

Three possible explanations present themselves (Clarke, in press). First, people who have contracted an infection with one species might in some fashion become more vulnerable or attractive to cercariae of the other; however, this seems unlikely. Second, people who are infected with one species may be more susceptible to successful infection by the other owing to altered circumstances of nutrition or resistance. Finally, people who come into contact with water frequently are more likely to be exposed to cercariae of both species, whereas people with rare contacts are less likely to be exposed to both types of cercariae.

Returning to Table II, we find that from the fifth to the thirteenth categories listed reflect the relatively high rate of *S. haematobium* infection, and also its rate of occurrence in stool, which is more than twice the rate of *S. mansoni* in urine. In a number of cases the rank order of observed and infected is inverted. These instances usually reflect the superabundance of observed double infections mentioned previously.

The last four categories are uncommon and they all require that one or both species appear in an "unusual" portal while being absent from the "normal" route of excretion. It is interesting that the occurrence of eggs of both species in both portals is not the least probable occurrence.

Of 2,684 individuals with *S. haematobium* in the urine, 125 also had eggs of that species in the stool. Of 4,323 people whose urines were negative for this species, only 33 had *S. haematobium* eggs in the stool. The proportion of people with *S. haematobium* in stool is about six times higher among people with the eggs in the normal portal.

Among individuals with *S. haematobium* in stool, about half were infected with *S. mansoni* (76 cases) and about half were not (81 cases). Statistical analysis is not necessary in order to conclude that the straying of *S. haematobium* is uninfluenced by the presence or absence of *S. mansoni*.

The occurrence of *S. mansoni* in urine with regard to *S. haematobium* infection is much more interesting. Of people with *S. mansoni* in urine, 62 out of 68, or 91 per cent., were infected with *S. haematobium*. It is possible to test the probability of this, assuming that there is no relationship, with a chi-square test using "expected" figures derived from the appropriate categories in Table II. A value of 79.7 is obtained with one degree of freedom and the null hypothesis is rejected at the 0.005 per cent. level of confidence. In any case the appearance of stray *S. mansoni* eggs almost exclusively in conjunction with *S. haematobium* infection is striking and demands explanation.

When the number of eggs per *S. haematobium* positive case is matched against the percentage of cases showing the same species in stool (abnormal route) for each epidemiological survey, a weak relationship is evident from inspection. Areas with high average egg productions tend to be the places where *S. haematobium* eggs appear most frequently in stools. Blair agrees that this may represent a "spilling over" of eggs in very heavy or very active infections. Alternatively, as worm abundance and hence egg abundance increase, it is to be expected that eggs would appear in additional sites simply because there are more eggs to be distributed throughout the body.

Table III

TABLE FOR CHI-SQUARE TEST ON SINGLE AND DOUBLE SPECIES INFECTIONS

Category	Observed	Expected	Observed Minus Expected	$\frac{(O-E)^2}{Expected}$
S. haematobium only, any portal	1,569	2,019	— 450	100.3
S. mansoni, any portal	840	1,222	— 382	119.4
Both species, any portals	1,148	799	+ 349	152.4
Uninfected	3,450	3,010	+ 440	64.3

"Expected" figures are rounded to nearest whole number.

I was unable to find any relationship between the gross rate of infection (Blair, Table III) and the appearance of *S. haematobium* eggs in stool nor between the gross rate and the number of eggs per positive case. Unfortunately we have no method of quantitatively estimating the production or release of *S. mansoni* eggs and cannot make similar comparisons for that species.

In three instances sites were surveyed both in 1964 and 1965. In all three instances the percentage of people infected increased. Table V shows the actual numbers and percentages involved. The totals and numbers negative are taken from Dr. Blair's Table III. Increases of 24 per cent. at Hippo Valley and 11 per cent. at Triangle Estates indicate the importance of the research and control plans now being implemented in the lowveld. Although this has been pointed out on numerous previous occasions by Clarke and others, it seems worthy of repetition.

Reference to Table IV reveals that the areas which were re-surveyed also showed an increase in the average egg production per positive *S. haematobium* case and an increase in the percentage of individuals with *S. haematobium* eggs in the stool.

SUMMARY

This paper is by no means exhaustive of the data, but merely exploits some of the more evident relationships. In the cases examined, *S. haematobium* was commoner in both usual and unusual routes. As Blair points out, a considerable number of patients carried *S. mansoni* only. Nevertheless, the proportion of double infections and double negative persons is higher than would be expected in the absence of association between the two parasites. The rate of appearance of *S. haematobium* in stools shows a tendency to increase as intensity of infection increases when survey sites are compared. The

Table IV

SURVEY SITES RANK-ORDERED BY THE PERCENTAGE OF CASES WITH S. HAEMATOBIUM EGGS IN STOOL AND THE AVERAGE NUMBER OF S. HAEMATOBIUM EGGS/POSITIVE CASE/HOUR. THE FIRST COLUMN IS CALCULATED FROM BLAIR'S TABLE III AND THE EGG PRODUCTION FIGURES ARE TAKEN FROM THE SURVEY RECORDS OF THE BILHARZIASIS RESEARCH LABORATORY

Epidemiological Survey	% with *S. haematobium* Eggs in Stool	Average No. of Eggs/Hour/Positive Case of *S. haematobium*
Trojan Mine	0	1,106
Triangle, 1964	.19	232
Chirundu Estate	.53	693
Nyamaropa Reserve	.56	223
Bikita area	.57	977
Hippo Valley, 1964	.83	160
Buffalo Range	1.50	519
Arcturus Mine	1.52	371
Mazoe School	1.69	470
Alaska Mine	1.71	1,258
Hippo Valley, 1965	1.80	679
Turk Mine	2.13	577
Msasa School	2.31	806
Triangle, 1965	2.62	682
Premier Estate	2.69	825
R.A.N. Mine	2.92	1,374
Iron Duke Mine	3.85	492
Chipoli Estate, 1964	4.35	1,844
Karoi area	5.38	1,164
Mazoe/Clifton	6.64	1,089
Chipoli Estate, 1965	9.24	1,136

Table V

INCIDENCE OF BILHARZIASIS IN THREE EPIDEMIOLOGICAL SURVEY SITES DURING THE PERIOD 1964-1965

Survey Site	Number Negative	Number Positive	Total	Per Cent. Positive
Hippo, 1964	158	84	242	35
Hippo, 1965	313	453	766	59
Triangle, 1964	280	240	520	46
Triangle, 1965	227	307	534	57
Chipoli, 1964	23	276	299	92
Chipoli, 1965	3	116	119	97

occurrence of *S. mansoni* eggs in urines occurs primarily in people who are also infected with *S. haematobium*.

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Appendix A

THE CHI-SQUARE TEST FOR SAMPLING OF ATTRIBUTES

The chi-square test is widely used as a method of testing for the probability of data obtained concerning happenings or attributes being related to one another or not being related, as the case may be. The examples given below on the information presented in the paper may be of assistance to readers not familiar with the test and its application.

General formula for chi-square:

$$\chi^2 = \Sigma \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$$

Case I.—Single and double species infections (refer to Table III).

$$\chi^2 = \frac{(2019-1569)^2}{2019} + \frac{(1222-840)^2}{1222} + \frac{(1148-799)^2}{799} + \frac{(3450-3010)^2}{3010}$$

$$= 436.4 \text{ with three degrees of freedom.}$$

Probability less than 0.0005.

Case II.—Presence or absence of *S. haematobium* infection in persons passing *S. mansoni* eggs in the urine.

	Observed.	Expected.	Table II Categories.
S. haematobium positive	62	26	6, 9, 12, 13, 15, 16
S. haematobium negative	6	41	3, 10

$$\chi^2 = \frac{(62-26)^2}{26} + \frac{(6-41)^2}{41}$$

$$= 79.7 \text{ with one degree of freedom.}$$

Probability less than 0.005.