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RELATIONSHIPS BETWEEN CERCARIAL INDICES OF *SCHISTOSOMA HAEMATOBIUM*, *S. BOVIS* AND *S. CURASSONI* FROM SENEGAL AND THE ISOENZYME GENOTYPES OF THE ADULT WORMS[1]

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SUMMARY. *S. haematobium* and *S. curassoni* appear to consist of three and two G6PDH genotypes respectively whereas only a single genotype has been observed in *S. bovis*. This genotype corresponds to one of those exhibited by *S. curassoni*, but the two species may be distinguished using the AcP enzyme system.

The antero-dorsal cercarial indices for *S. haematobium* gave a mean of 0.78 ranging from 0.67 ± 0.03 to 0.90 ± 0.11; *S. curassoni* gave a mean of 1.11, ranging from 1.00 ± 0.05 to 1.23 ± 0.14 and *S. bovis* a mean of 1.30 within the range 1.01 ± 0.25 to 1.67 ± 0.18.

From these data it is apparent that there is some correlation between antero-dorsal Cl and enzyme genotype: nevertheless the variation in Cl is somewhat greater than that observed in enzyme genotypes.

Generally, Cl values lower than 0.90 can be considered to be due to *S. haematobium*, those above 1.15 to be *S. bovis* and intermediate values of 0.90-1.15 indicate *S. curassoni*.

Key-words: *Schistosoma haematobium*. *S. curassoni*. *S. bovis*. Chaetotaxy. Cercarial indices. Isoenzyme genotypes.

Corrélations entre les indices cercariens de *Schistosoma haematobium*, *S. bovis* et *S. curassoni* au Sénégal et les génotypes enzymatiques des Vers adultes.

RÉSUMÉ. Pour l'enzyme G6PDH, il semble que *S. haematobium* soit constitué par trois génotypes, *S. curassoni* par deux et *S. bovis* par un seul. Ce dernier est commun avec l'un des deux génotypes de *curassoni*, mais les deux espèces peuvent être distinguées par le système enzymatique AcP.

Les indices cercariens des soies antéro-dorsales (Cl) donnent pour *S. haematobium* une moyenne de 0,78 variant de 0,67 ± 0,03 à 0,90 ± 0,11 ; pour *S. curassoni* une moyenne de 1,11 variant de 1,00 ± 0,05 à 1,23 ± 0,14 ; pour *S. bovis* une moyenne de 1,30 variant de 1,01 ± 0,25 à 1,67 ± 0,18.

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Ces données montrent donc une corrélation entre les indices des soies antéro-dorsales des cercaires et le génotype enzymatique ; la variation des indices cercariens étant cependant plus marquée que celle observée dans les types enzymatiques.

D'une manière générale, les valeurs de l'indice (CI) inférieures à 0,9 correspondent à *S. haematobium ;* celles supérieures à 1,15 à *S. bovis* et les valeurs intermédiaires de 0,90 à 1,15 à *S. curassoni.*

Mots-clés : Schistosoma haematobium. S. curassoni. S. bovis. Chétotaxie. Indice cercarien. Génotypes isoenzymatiques.

Introduction

It has been demonstrated that it is possible to distinguish *Schistosoma curassoni, S. haematobium* and *S. bovis* using isoenzyme analyses (Southgate *et al.*, 1985). Such analyses have proved particularly useful for characterising sibling species and examining the genetic structure of populations of schistosomes. The World Health Organization has for some time recognized the need to identify species of schistosome cercariae emanating from naturally infected snails, for this is most important in epidemiological studies. Chaetotaxy offers a relatively simple inexpensive procedure compared with either isoenzyme analyses or recombinant DNA technology both of which require sophisticated laboratory equipment. Another potential advantage of chaetotaxy is that relatively few cercariae are required for analyses, however the value of chaetotaxy as a taxonomic tool for the identification of schistosome cercariae has still to be fully evaluated, especially with closely related species.

A study of chaetotaxy of ten species of schistosome cercariae led Bayssade-Dufour (1982) to conclude that variation existed in this character in the cercariae of schistosomes belonging to the terminal spined egg complex, and this created difficulties in the identification of species within this complex using this criterion. The complex included *Schistosoma haematobium,* and subsequent studies on *Schistosoma curassoni* and *Schistosoma bovis* from Senegal demonstrated that the cercariae of these two species exhibit analogous variation (Albaret *et al.*, 1985).

Electrophoretic studies on the variation of enzymes within populations of *S. haematobium,* have been reported elsewhere (Wright and Ross, 1983).

This paper reports the results of experiments designed to ascertain whether correlation between cercarial chaetotaxy and enzyme genotype of the adult worm exists.

Materials and methods

Eggs of *S. bovis* were collected from one *Bos indicus,* St-Louis, Senegal and hatched. The miracidia were used to expose 7 *Bulinus forskalii* collected from an artificial concrete pool in the gardens of the University of Dakar, Senegal of which 4 become infected. A sheep was exposed to the resulting cercariae in Paris,

and then 38 laboratory bred *B. forskalii* were exposed to 2 miracidia each hatched from eggs from the sheep liver. Of the snails exposed, 16 became infected and *Mastomys erythroleucus* was exposed to cercariae from 2 snails.

S. haematobium was isolated either from eggs from 15 patients (8 from Sintiou Malem, 3 from Ouro Sogui, 1 from Sare Sara, 1 from Sare Keita, 1 from Orkadiere, 1 from Mali) or from naturally infected *Bulinus umbilicatus* from human water contact points.

S. curassoni was isolated from naturally infected snails and *Mastomys erythroleucus*, *Ovis aries*, *Mus musculus* and *Mesocricetus auratus* were exposed to cercariae in Paris.

Wild caught snails which showed no signs of natural infection after a minimum observation of three months (two months in the case of one individual snail) were used as intermediate hosts for experimental infections in the laboratory.

Cercarial indices were calculated from measurements of distances between paired papillae A_ID, $A_{II}D$ and $A_{III}D$ using the formula for the A_D index (Richard, 1971):

$$\text{Cercarial Index (Cl) } A_D = \frac{A_ID - A_{II}D}{A_{II}D - A_{III}D}$$

The figures quoted in the text and *table I* represent the mean values of Cl for a number of cercariae from the same shed with coefficients of confidence. Generally, at leat 30 measurements were used to calculate the Cl value although in excess of fifty were used whenever possible.

Adult worms were stored in liquid nitrogen before transfer to London for examination for isoenzymes; these were separated from aqueous tissue extracts by isoelectric focusing in polyacrylamide gels (Ross, 1976). Although glucose-6-phosphate dehydrogenase (G6PDH) was used as a marker *(fig. 1)*, identification of species was confirmed by use of phosphoglucomutase (PGM) for *S. curassoni (fig. 2)*, acid phosphatase (AcP) for *S. bovis (fig. 3)*. The bases for G6PDH genotypes are those for *S. haematobium* types I, II and III (Wright & Ross, 1983), a single genotype common to *S. bovis* and some *S. curassoni* and a further type observed in some individual *S. curassoni* worms (Southgate *et al.*, 1985). For convenience during this study these have been termed A, B, D, E *(S. bovis)*, E *(S. curassoni)* and F respectively. The missing C in this sequence resembles a hybrid of *S. haematobium* types I and II (Wright & Ross, 1983). The genotypes are illustrated in *figure 1*.

Results

Results of all examination are summarised in *table I*. Of the thirty one entries relating to infections of man *(S. haematobium)*, the lowest cercarial index was 0.67 ± 0.03 and the highest was 0.90 ± 0.11 from *B. umbilicatus:* a mean value of 0.78 represents the measurements from 712 individual cercariae. G6PDH geno-

TABLE I. — List of experiments where genotypes and antero-dorsal Cl are known.

N°	Origin	Location	Mollusc	Indices	no Cerc.	OTP *	Vertebrate	Worms Sex	Worms Age	Genotype	Determination
1	Mollusc	ORKADIERE	senegalensis	0,71 ± 0,06	22	P	Hamster	♂♀	123	B	haematobium B
2	Man	SINTIOU MALEM	umbilicatus	0,67 ± 0,05	22	P	Hamster	♂♀	311	♀A + ♂D	haematobium A+D
3	Mollusc	SARE KEITA	jousseaumei	ND**	-	P	Mouse	♂	134	B	haematobium B
4	Man	SARE SAME	umbilicatus	0,71 ± 0,18	6	P	Hamster	♂	159	B + D	haematobium B+D
5	Man	SARE SARA	jousseaumei	0,68 ± 0,11	15	P	Hamster	♂♀	92	A	haematobium A
6	Mollusc	SORY	umbilicatus	0,77 ± 0,09	20	0	Mastomys	♂	93	B	haematobium B
7	Man	OURO SOGUI	umbilicatus	0,72 ± 0,07	20	0	Mastomys	♂	78	ND	haematobium
8	id	id	id	id	id	id	Hamster	♂	85	B	haematobium B
9	Man	OURO SOGUI	umbilicatus	0,89 ± 0,08	20	0	Mastomys	♂	92	ND	haematobium
10	id	id	id	id	id	id	Hamster	♀	93	A	haematobium A
11	Man	OURO SOGUI	umbilicatus	0,70 ± 0,06	20	0	Mastomys	♂♀	97	D	haematobium D
12	id	id	id	id	id	id	Hamster	♂♀	111	ND	haematobium
13	id	id	id	id	id	id	Hamster	♂♀	142	D	haematobium D
14	Mollusc	SORY	umbilicatus	0,86 ± 0,06	41	0	Hamster	♂♀	112	B	haematobium B
15	Mollusc	SORY	umbilicatus	0,90 ± 0,11	24	0	Hamster	♂♀	67	♂A + ♀D	haematobium A+D
16	Man	SINTIOU MALEM	umbilicatus	0,68 ± 0,12	15	0	Hamster	♂♀	111	B + D	haematobium B+D
17	id	id	id	id	id	id	Hamster	♂♀	124	id	haematobium B+D
18	Man	SINTIOU MALEM	umbilicatus	0,72 ± 0,08	18	P	Hamster	♂♀	125	A + B + D	haematobium A+B+D
19	id	id	id	id	id	id	Hamster	♂♀	111	D	haematobium D
20	Man	MALI	umbilicatus	0,69 ± 0,08	26	0	Hamster	♂	160	A	haematobium A
21	Man	SINTIOU MALEM	umbilicatus	0,78 ± 0,05	45	0	Mastomys	♀	104	B	haematobium B
22	Man	SINTIOU MALEM	umbilicatus	0,89 ± 0,06	50	0	Mastomys	♂♀	77	B	haematobium B
23	id	id	id	0,83 ± 0,05	52	id	Hamster	♂♀	85	B	haematobium B
24	id	id	id	0,88 ± 0,05	52	id	Hamster	♂♀	96	ND	haematobium

N°	Origin	Location	Mollusc	Indices	no Cerc.	OTP *	Vertebrate	Worms Sex	Age	Genotype	Determination
25	Man	SINTIOU MALEM	umbilicatus	0,72 ± 0,04	36	O	Mastomys	♂	78	B	haematobium B
26	id	id	id	0,73 ± 0,05	31	id	Hamster	♂	98	B	haematobium B
27	id	id	id	0,67 ± 0,03	48	id	Hamster	♂	185	B	haematobium B
28	Man	SINTIOU MALEM	umbilicatus	0,80 ± 0,05	44	O	Mastomys	♂	93	D	haematobium D
29	id	id	id	0,77 ± 0,05	65	id	Hamster	♂	185	D	haematobium D
30	Man	ORKADIERE	umbilicatus	0,75 ± 0,09	20	P	Hamster	♂♀	221	B	haematobium B
31	Man	SINTIOU MALEM	umbilicatus	ND	-	P	Hamster	♂♀	83	B	haematobium B
32	Mollusc	PIGNA	umbilicatus	1,09 ± 0,11	30	P	Sheep	♂	134	E curas.+F	curassoni E + F
33	id	id	id	id	id	id	Sheep	♂♀	144	id	curassoni E + F
34	Mollusc	OURO SOGUI	umbilicatus	1,00 ± 0,05	98	P	Mouse	♂♀	128	E curas.	curassoni E
35	id	id	id	id	id	id	Sheep	♂♀	147	id	curassoni E
36	id	id	id	id	id	id	Sheep	♂♀	145	E curas.	curassoni E
37	id	id	id	id	id	id	Hamster	♂♀	112	E curas.	curassoni E
38	id	id	id	id	id	id	Mouse	♂	112	E curas.	curassoni E
39	Mollusc	SORY	umbilicatus	1,06 ± 0,06	40	T	Hamster	♂	105	E curas.+F	curassoni E + F
				1,10 ± 0,05	46						
40	id	id	id	id	id	id	Mastomys	♂	133	E curas.+F	curassoni E + F
41	id	id	id	1,10 ± 0,05	46	O	Sheep	♂	136	F curas.	curassoni F
42	id	id	id	id	46	id	Mastomys	♂	106	F curas.	curassoni F
43	Mollusc	SORY	umbilicatus	1,02 ± 0,06	41	O	Sheep	♀	99	E curas.	curassoni E
44	Mollusc	FETE BOKE	umbilicatus	1,23 ± 0,14	31	P	Mouse	♀	185	E curas.	curassoni E
45	Cattle	ST LOUIS	forskalii	1,15 ± 0,12	32	P	Sheep	♂♀	89	E bovis	bovis
46	id	id	guernei	1,24 ± 0,08	88	P	Mouse	♂♀	71	ND	bovis
47	id	id	forskalii	1,31 ± 0,11	52	P	Calf	♂♀	88	ND	bovis
48	id	id	id	id	id	id	Sheep	♂♀	50	ND	bovis
49	id	id	id	id	id	id	Mastomys	♂♀	72	E bovis	bovis
50	id	id	guernei	1,11 ± 0,14	22	P	Mouse	♂♀	95	ND	bovis
51	id	id	forskalii	1,03 ± 0,05	17	T	Mastomys	♂♀	72	E bovis	bovis
				1,21 ± 0,04	12						
52	Mollusc	SARE KEITA	jousseaumei	1,01 ± 0,11	15	O	Mouse	♂	134	E bovis	bovis

*O = 1 Mollusc ; T = 2 Mollusc ; P = Pool of Mollusc.

** ND = Non determinated

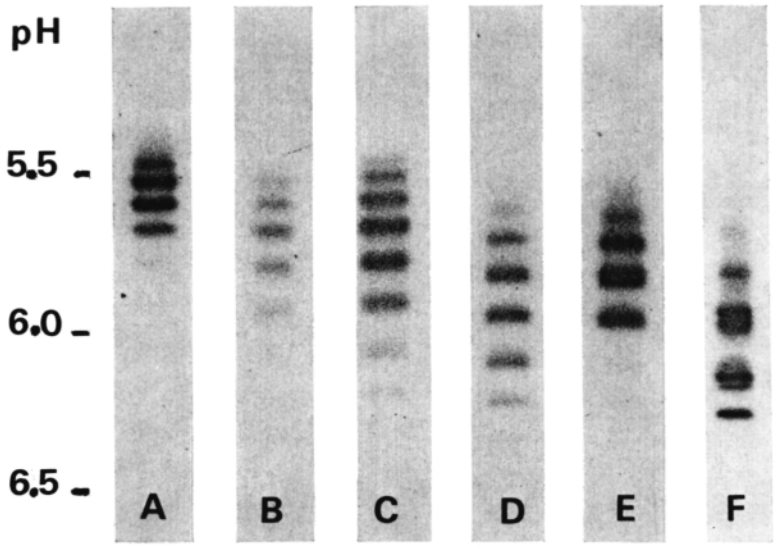

FIG. 1. — G6PDH isoenzyme genotypes A, B, C, D, E and F.

A, B, D : *S. haematobium*, single genotype
C : *S. haematobium*, hybrid of genotypes A and B
E : genotype common to *S. bovis* and some *S. curassoni*
F : single genotype of other *S. curassoni*

types A, B and D were observed individually or as any combination of the three in each isolate examined. There appears to be no correlation of Cl with genotypes A, B or D, three experiments producing worms of genotype A from cercariae with Cl 0.68 $\pm$ 0.11 to 0.89 $\pm$ 0.08, eleven giving worms of genotype B from Cls 0.67 $\pm$ 0.03 to 0.89 $\pm$ 0.06 and five worms producing genotype D from cercariae with Cl 0.70 $\pm$ 0.06 to 0.80 $\pm$ 0.05.

Adult worms of the two genotypes E, E *(S. bovis)* and E *(S. curassoni)*, developed from cercariae with correspondingly different indices, eighti nfections of genotype E *(S. bovis)* arose from cercariae with indices from 1.01 $\pm$ 0.25 to 1.35 $\pm$ 0.15 while seven of genotype E *(S. curassoni)* were produced from cercariae with indices 1.00 $\pm$ 0.05 to 1.23 $\pm$ 0.14. Two samples of genotype F worms (also *S. curassoni*) were produced from cercariae with indices 1.10 $\pm$ 0.05, well within the range of the E *(S. curassoni)* figures. Cl values of 1.01, 1.02 and 1.03 were obtained from cercariae from 3 out of 16 *B. forskalii* infected with an E *(S. bovis)* isolate and these results were considered to be anomalous[1]. Two of the sixteen produced cercariae with indices ranging from 1.16 $\pm$ 0.18 to 1.21 $\pm$ 0.04, the eleven remainder

1. The data in the table I are those when both Cls and genotypes have been identified. Some of *bovis*' Cl were obtained after laboratory infection of *B. forskalii*. The Cl resulting from infections with *B. truncatus* are more homogeneous than those from *B. forskalii*, these data will be presented elsewhere.

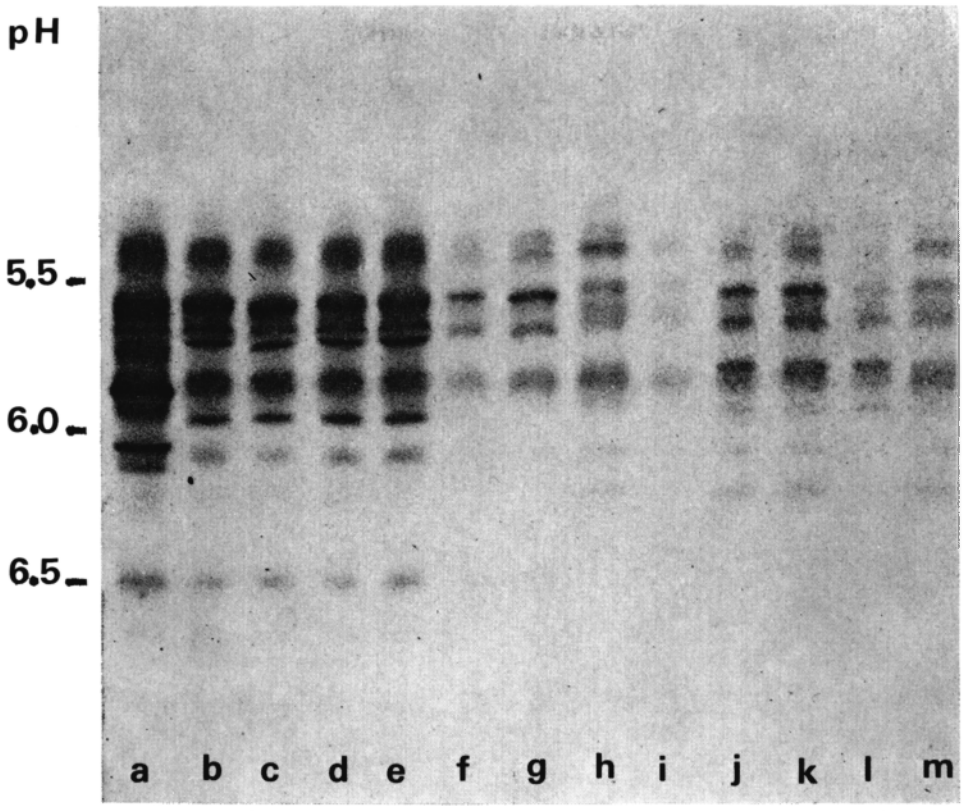

FIG. 2. — PGM isoenzyme patterns.

a-e : *S. curassoni* genotype of male worms
f, g : *S. curassoni*, female worms
h-m : PGM patterns common to *S. haematobium* and *S. bovis*

fell within the range 1.35 ± 0.23 to 1.67 ± 0.18. *Mastomys* infected with cercariae of the 1.03 ± 0.05 and 1.21 ± 0.04 Cl isolates produced miracidia which were used to infect eight *B. forskalii* and fifty six *B. truncatus*. Of these sixty four snails only two *B. truncatus* became patent, producing cercariae with indices of 1.18 ± 0.02 and 1.42 ± 0.04.

Schistosomes derived from children's urine frequently exhibited fewer genotypes than those derived from wild caught snails where both children and cattle have water contact. For example in Pigna where cercariae emitted from *B. umbilicatus* collected from the lake produced indices in three groups with mean values of 0.68, 0.87 *(S. haematobium)* and 1.09 *(S. curassoni)* (Albaret *et al.*, 1985). Rodents infected with these cercariae produced genotypes A, B, C and D *(S. haematobium)* and genotypes E *(S. curassoni)* and F. Only *S. haematobium* genotypes were produced in material originating from human urine samples. It was also

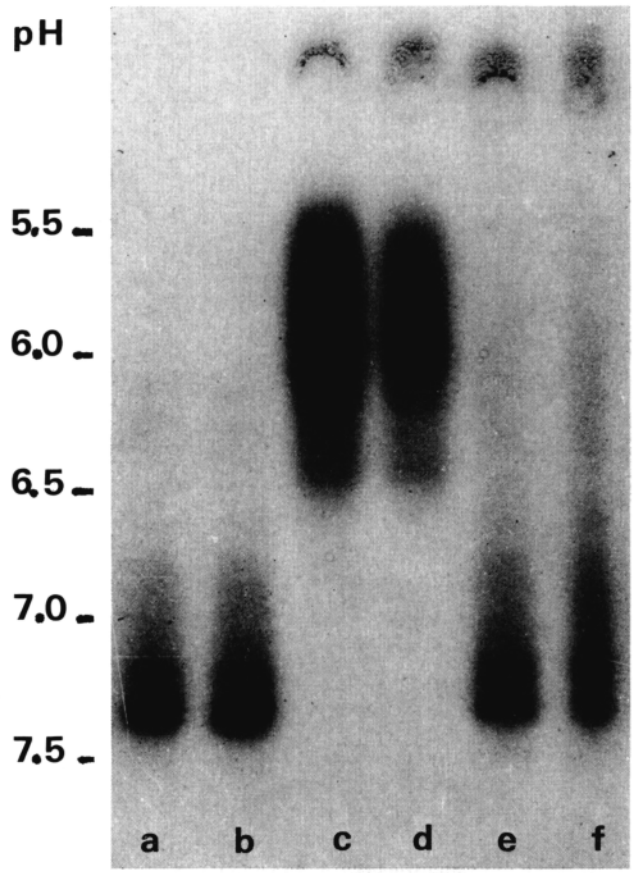

FIG. 3. — AcP isoenzyme patterns from male worms.
a, *b* : *S. curassoni*
c, *d* : *S. bovis*
e, *f* : *S. haematobium*

noted that there is an apparent correlation between the genotypes and the definitive host species; poorly developed worms of genotype A, B and D *(S. haematobium)* originated from infections in *Arvicanthis niloticus;* genotypes B, C, D and E *(S. curassoni)* in mice, genotypes B, D and E *(S. curassoni)* in hamsters, while in sheep only genotype E *(S. curassoni)* and F were noted. Thus, it is clear that the sheep are excluding worms of genotype A, B, C and D i. e. *S. haematobium.*

However Cls from an individual snail appear to be generally constant and little variation has been noted in cercarial indices from different snails infected by miracidia from the same source, although two exceptions to this generalization have been noted. One was from an experimentally infected *B. forskalii* (with miracidia from Zebu, *Bos indicus*) where variation $1.07 \pm 0.09 - 1.35 \pm 0.15$

occurred in the values of the cercarial indices, the other arose in cercariae from 3 out of 16 *B. forskalii* as stated above. These data raised questions (*i*) of the purity of the Zebu isolate: (*ii*) of the possibility that this particular isolate of *S. bovis* has a variable Cl, and (*iii*) of the possibility that *B. forskalii* is not the usual natural host.

Discussion

The data confirm the earlier report of Southgate *et al.* (1985) that it is possible to distinguish *S. haematobium*, *S. bovis* and *S. curassoni* using isoenzymes analyses. Glucose-6-phosphate dehydrogenase is useful in distinguishing *S. haematobium* from either *S. curassoni* or *S. bovis*, but is less useful in separating *S. bovis* from *S. curassoni* where one G6PDH pattern is common to both species. However, the use of acid phosphatase readily distinguishes *S. bovis* from *S. curassoni*.

Interestingly the data of cercarial indices fit into three main groups which correspond to the three species under investigation: values under 0.90 may be considered to be *S. haematobium* those above 1.15 correspond to *S. bovis* and intermediate values (0.90-1.15) correspond to *S. curassoni*. Thus, there appears to be correlation between cercarial indices and enzyme analyses of adult worms. It is suggested that chaetotaxy is a cheap taxonomic tool which may have application in epidemiological studies, especially in those areas where the same intermediate host for example *B. umbilicatus*, is known to be capable of transmitting at least two species of schistosome i. e. *S. haematobium* and *S. curassoni*.

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