

### 3.2.3

## EPIDEMIOLOGICAL STUDY OF SCHISTOSOMA HAEMATOBIIUM INFECTION

IN

COASTAL AREA, KENYA

### 2. Comparison of different units of egg count in urine

to express

the intensity of S. haematobium infection

by single examinations

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### Introduction

The intensity of S. haematobium infection has long been described by the egg output in urine and the egg count per unit of volume (usually 10 ml) has been widely accepted as the unit which express the intensity of infection. Whereas, from the view point of the consistency of day to day egg output, egg count per unit of time has been also recommended for use. Recently, a new reliable technique for quantification of egg count in urine, nuclepore filtration method, has been developed and applied for epidemiological studies. This technique encouraged us to examine to what extent the urinary egg output varies day to day by the different method of expressing the egg count in urine.

## Materials and methods

The present study was carried out at the primary school of the pilot area, Mwachinga village in Kwale district. Among the school children who had been proved to be positive for eggs previously, 25 children between the age of 6 and 15 years were selected by random sampling. They were asked to discharge urine into the containers separately at 11:30 am and 0:30 pm on each examination day for five days within 8 days. After the urine samples were well mixed, an aliquot, 1 ml in most cases, was filtered through the nuclepore system. The filter was placed on a slide and the accurate count of eggs was read later.

To express the egg output, 4 different methods were applied. Method 1 is the one frequently used by many workers. The egg output was expressed as the number of eggs per 10 ml of the first urine collected at 11:30 am; egg count/10 ml of sample. In method 2, egg output was expressed as total number of eggs included in the first urine; egg count/sample. In method 3, egg count was expressed as the number of eggs per 10 ml of the second urine which was discharged one hour after the bladder was emptied of urine; egg count/10 ml of sample/hour. Method 4 is the one recommended by Weber et. al.. Egg output was expressed as total number of eggs contained in the second urine; egg count/hour. For each method, the mean egg count and the standard deviation for the five days and the coefficient of variation of each child was calculated and the results were compared.

## Results and Discussion

Out of 25 school children, only 12 could provide adequate samples for the analysis, although 7 of them were absent and one child could not provide the second urine specimen on certain days

during the period of study.

Table 1 and table 2 show the results calculated according to method 1 (egg count/10 ml) and 4 (egg count/hour). The average of the coefficient of variation was the greatest with method 1 and smallest with method 4.

Using the Kolmogorov-Smirnov test, the difference of coefficient of variation between the data obtained by using method 1 and that by method 4 is statistically significant ( $n = 12$ ,  $0.01 < P < 0.05$ ). The average of the coefficient of variation derived from the data of method 2 and method 3 were 61 and 51 percent respectively and the daily consistency of the egg counts obtained by these methods seemed to be more reliable than that by method 1.

For comparing the data set obtained on each observation day to the data set of average, Kendall's rank test was applied. The pairs of the data of untimed specimen provided very little correlation on day 1, 2 and 3.

The present study revealed that when egg output was expressed as egg count/hour, day to day variation in egg output remarkably reduced, and when egg output was expressed as the number of egg/10 ml of sample, considerable day to day variation was encountered. In our both timed and untimed specimens, a slightly lower day to day variation was also observed, when the egg count per sample rather than egg count per 10 ml of sample was used. This result suggests that the change of urine volume might result in the difference in egg output. As the volume of urine is considerably variable according to seasons or climate, egg count per unit of volume is ill suited for an epidemiological study in which the change of intensity of infection would be followed up for some years.

Table 1. Daily variation of egg count per ten ml..

Case No.	Examination					Mean	S.D.*	C.V.**
	1	2	3	4	5			
1	1540	940	3300	3280	2730	2360	1140	48
2	100	380	60	270	60	170	150	88
3	440	310	630	-	-	460	180	39
4	710	360	380	400	280	430	175	41
5	-	150	1050	730	510	610	410	67
6	240	120	190	-	-	180	70	38
7	180	120	200	190	460	230	140	62
8	740	1230	820	3320	660	1350	1190	88
9	90	-	70	990	2330	870	1150	133
10	850	960	4000	-	26150	7990	11390	142
11	410	60	90	-	400	240	210	87
12	140	310	110	-	2940	880	1500	171
Kendall's Tau	0.49	0.43	0.58	0.81	0.73	Average of C.V.		84

\*Standard deviation

\*\*Coefficient of variation(%)

Table 2. Daily variation of egg count per one hour.

Case No.	Examination					Mean	S.D.*	C.V.**
	1	2	3	4	5			
7	6000	6990	4380	3640	5510	5300	1410	27
14	2150	1790	1680	1300	2550	1890	510	27
21	1710	670	1480	-	-	1280	620	48
28	2060	1150	1040	1720	-	1490	520	35
35	-	1150	490	985	760	850	310	37
63	380	280	180	-	-	280	115	41
70	1140	530	900	1680	910	1030	440	43
84	2500	1720	1760	3150	3880	2600	980	38
91	670	-	610	380	730	600	160	27
119	2890	3090	5950	-	8040	4990	2680	54
126	300	210	380	-	1170	520	480	93
133	3600	2650	4240	-	2740	3310	820	25
Kendall's Tau	0.93	0.84	0.91	0.81	0.72	Average of C.V.		41

\*Standard deviation

\*\*Coefficient of variation(%)

## ビルハルツ住血吸虫症の疫学的研究

### 2. 尿中虫卵数の表現法の比較

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ビルハルツ住血吸虫症では Intensity of infection を表わす示標として単位尿量中の排泄虫卵数が最も一般的に用いられている。しかしこの値は尿量の変動に伴ってたやすく変わり、感染の Intensity を長期間継続的に観察しその微小な変化を把むためには不適當と考えられる。本研究の目的は検査毎の虫卵数ができるだけ一定で変動の少ない方法を見出すことである。

虫卵数を表現する単位として以下の4通りを用い、その日々の変動の大きさを比較した。

単位1) Egg count/10ml

単位2) Egg count/sample

単位3) Egg count/10ml/hour

単位4) Egg count/hour

ケニア人小学生12名から5日間毎日採尿し、上記のそれぞれの単位を用いた場合の平均排泄虫卵数と変動係数を計算した。

その結果、12名の変動係数の平均はそれぞれ、単位1)を使った場合84%、単位2)で61%、単位3)で51%、単位4)の場合41%となり、単位1)と単位4)の間で統計学的に有意の差を認めた。即ち、単位1)を使うと単位4)の場合に比べ、日々の虫卵排泄数の変動が見かけ上大きくなることが明らかになった。

以上のことから、Intensity of infection の変動を長期間継続的に観察する場合、排泄虫卵数は単位時間あたりで表現する方が有利であることが示唆される。

3.2.4

EPIDEMIOLOGICAL STUDY OF SCHISTOSOMA HAEMATOBIIUM INFECTION  
IN  
COASTAL AREA, KENYA

3. Initial parasitological findings  
in pilot area

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Introduction

Mwachinga village was chosen as a pilot area. The village is located in the Hinterland area, 20 km from Kwale town. The main reasons why this area was chosen were: 1. the village represented typical Hinterland conditions, 2. the village has a water pipeline which is inadequately used by the inhabitants, therefore, could be used as a control measure in future, 3. a laboratory is conveniently located in nearby Kwale and, 4. the good cooperation of the villagers and the authorities.

The main purpose of this study was to obtain baseline data on the prevalence and intensity of infection and to determine the initial state of infection of inhabitants living in a particular area in detail as a beginning of epidemiological study. The other information such as water contact behavior of inhabitants, change of snail population and infection rate of snails were also

gathered to determine the mode of infection or the mode of transmission. The results are presented in separate papers. The information obtained will also be useful to monitor changes in transmission, and especially to evaluate the impact of the introduction of any control measure to be applied in the study area.

#### Description of the study area (Fig. 1)

The area is not geographically isolated, and therefore interaction of the inhabitants with people living outside the village. The land is undulating, dotted with scattered houses on the hill areas, and is only partially cultivated. The main crops are corn (maize) and cassava, harvested mainly only for local use. There are no irrigation canals for cultivation in the area and the inhabitants only cultivate in the rainy season. Along the river bank banana trees, coconut palms and sugar cane are planted and cashew nut trees are plentiful on the hills. Fishing has not been established as an industry but the people do fish in the river for their own consumption.

A river named Marere flows from southwest to the north through the village and serves as a limiting boundary to the east. The river also has a dam with an artificial lake located at its upper part. At the lower part, the river joins a branch called Kadingo which seasonally flows through the middle of the village. In the dry season, the Kadingo river branch almost completely dries up leaving random pools in some areas. Although there is a main water pipeline, the villagers are forced to go either to the rivers to collect water because the pipeline was constructed mainly for Mombasa, the second biggest city in Kenya. There are only three taps and no other safe water sources such as



boreholes and wells in the study area.

#### Population census

The census and mapping were carried out in the whole village and in some parts of the environs along the river. Every house and every person was given a serial number and information such as name, sex, date of birth, birthplace and tribe etc. was collected.

The total population was approximately 1200 consisting of 550 males and 650 females respectively. The population pyramid showed a typical pattern for a rural area, namely relatively fewer males than females in the younger adult group.

Duruma and Digo are the main tribes living in this area.

Population movement does not seem to be frequent except for younger male adults who tend to move to towns for employment. adults who tend to move to town for employment.

#### Materials and Methods

A quantitative examination of urine was carried out by using the nucleopore method. For counting numbers of eggs, egg count per unit of time was applied to determine the intensity of infection, based upon the data obtained from our recent research. The collection of urine was carried out between 10:30 a.m. and 1:30 p.m..

#### Results

##### -Prevalence-

Of the 1217 inhabitants registered by census, 851 suitable urine specimen were collected successfully to detect the ova of S. haematobium. Five hundred and eighty one(68%) was positive in this initial study.

Figure 2(upper part) shows these results. The overall

prevalence is higher in females than in males, being 71 and 65 percent respectively. However there was no statistically significant difference between them.

The age-distribution showed a different pattern between males and females. The infection rate of males increased with age in the first decade of life, showing a clear peak of 100 percent at 10-15 years of age. After the peak, the prevalence dropped down rapidly and remained at about 60 percent consistently in the older group. However this pattern of age-prevalence was not observed in females. Although the prevalence of females also increased rapidly, showing a peak of 98 percent at the same age group, it declined gradually in contrast to that of males.

#### -Intensity-

The overall geometric mean egg count was 50.2 eggs per hour, that of males was 52.4 eggs per hour and that of females was 47.4 eggs per hour. There is no statistical difference between these values.

The age-intensity distribution is shown in Fig. 2(lower part) showing almost the same patterns in curve as that of age-prevalence distribution in both males and females, respectively. The excretion of eggs increased in number with age and reached a peak at age 10-14. In males, after reaching a peak of 1470.9 eggs per hour, egg count declined rapidly to a stable state at about 20 eggs per hour, and in females, the intensity decreased gradually after showing a peak of 985.5 eggs per hour.

#### -Estimation of false negative in the examination-

A total of 156 negative cases were reexamined about one month after the first examination. Out of 86 adults, 8 cases

were detected to be positive. However no children under 15 years of age who convert from negative to positive were observed. Because transmission hardly occur in the rainy season, it might be proposed that the 8 cases among adults could possibly be used as indications of false negative rate in this study. False negative seems negligible among children.

#### Discussion

It has been reported that the peak prevalence and intensity of S. haematobium infection usually occur in the same age group of 10-14 years and that the peak is followed by steep slope and decline in both prevalence and intensity of infection by age 30 or earlier. In our study in the coastal area in Kenya, the age-prevalence distribution of males practically showed this typical pattern of S. haematobium infection usually reported in other studies. However, the profile of the curve of age-prevalence in female did not follow this findings and was rather similar to that of S. mansoni infection.

The difference of age-prevalence and age-intensity distribution pattern by sexes may be due to the difference of water contact behaviour and/or of immunological response between males and females.

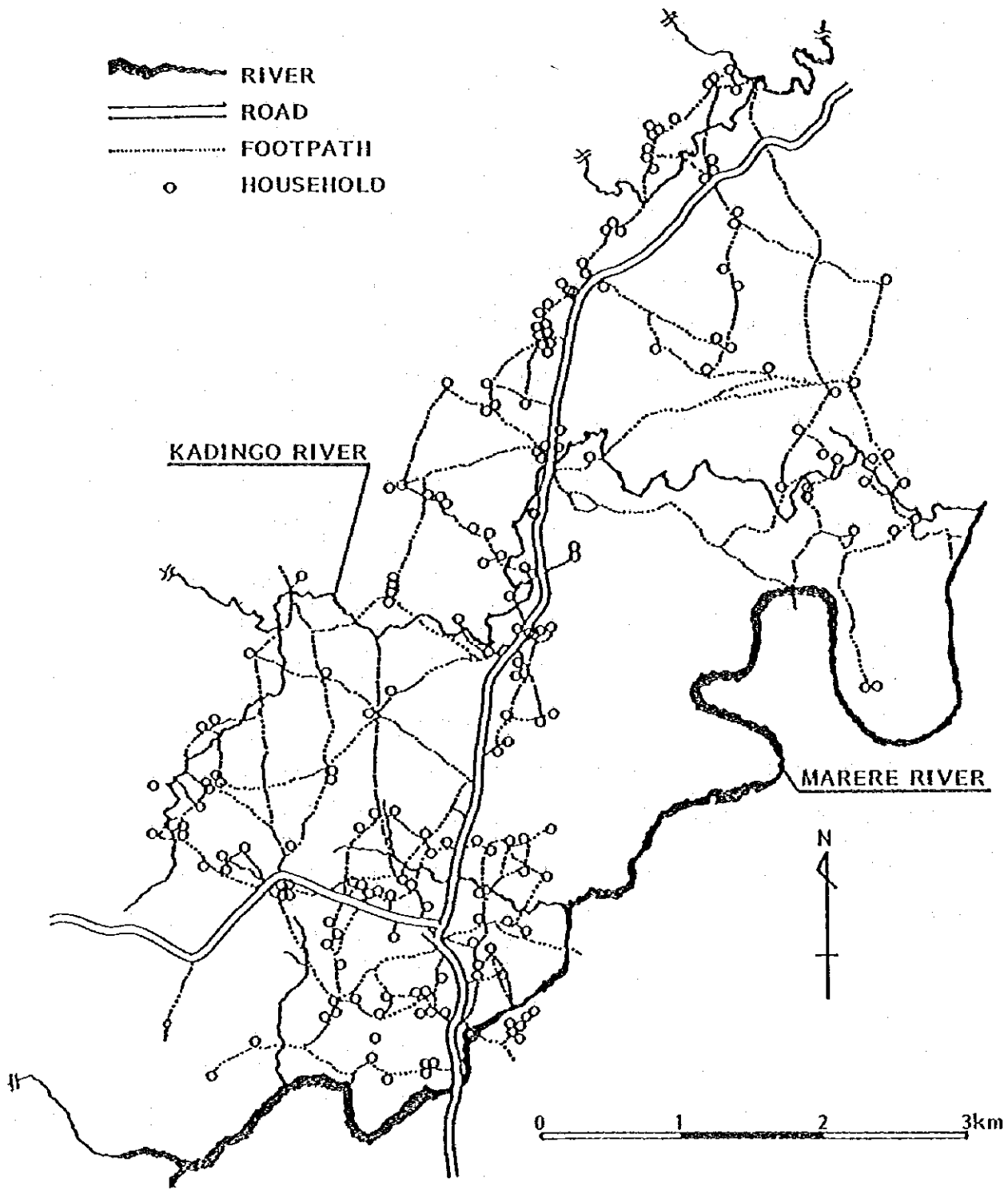


Fig. 1. Map of Mwachinga village (pi:bot area).

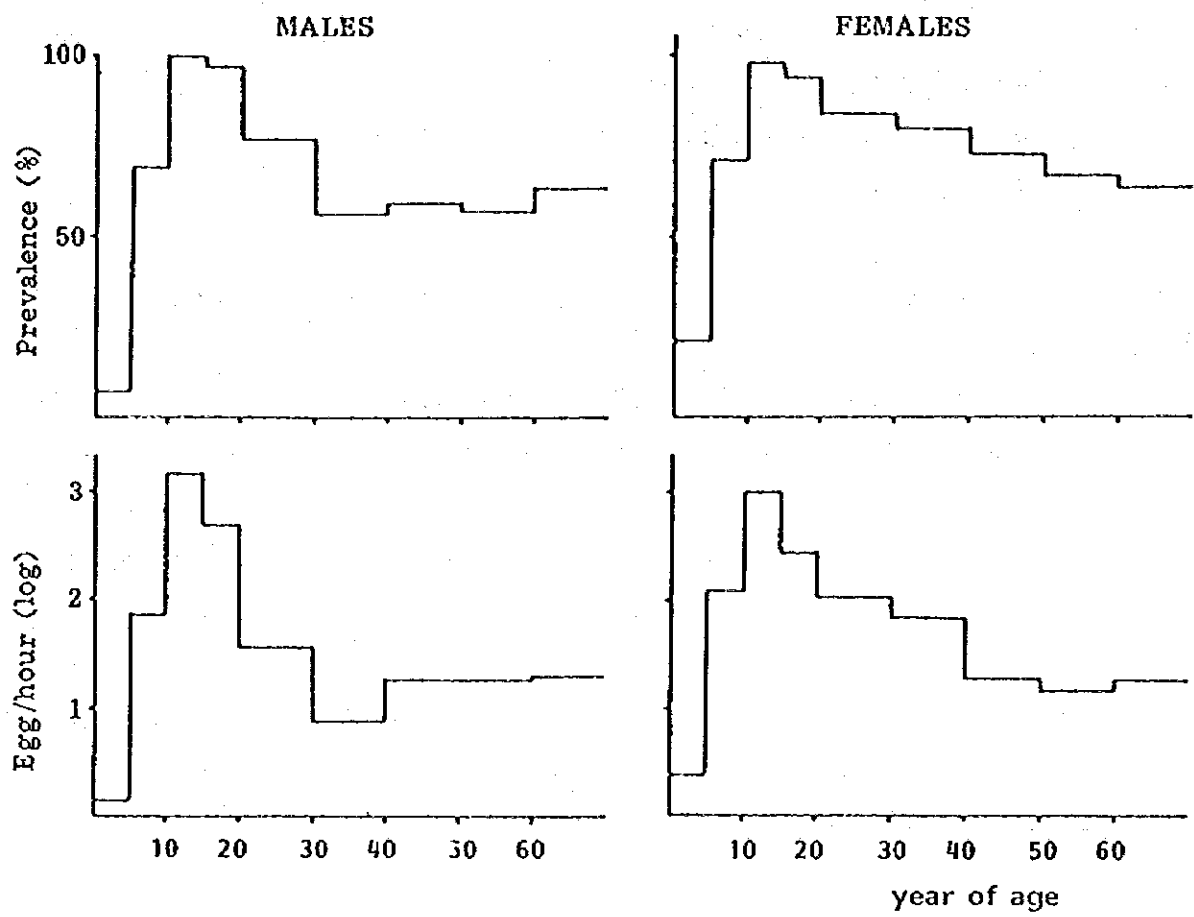


Fig. 2. Age distribution of prevalence and intensity of infection.

## ビルハルツ住血吸虫症の疫学的研究

### 3. パイロット地区住民の感染状況

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クワレ地方のよりくわしい感染動態の特徴を明らかにする目的でムワチンガ村をパイロット地区として選定した。

本村はちょうど海側から丘陵地帯を越えて後背地域にはいる所、そこからサバンナの景観が開けるあたりに位置し、ビルハルツ住血吸虫の浸淫度は極めて高い地域に属する。土地は高原状で起伏に富み乾燥しているので主な作物は雨期を利用したとうもろこしとキャッサバくらいのもので、その他換金作物としてのカシューナッツの木が多く植えられている。川の周辺にはバナナ、さとうきび、ココナッツが見られ、川では小魚もとれるがいずれも産業と言える程のものではない。

水系は比較的大きなマレレ川とその支流のカディンゴ川が村の東南と北西を走り、住民は日常の生活用水をこれにたよっている。マレレ川は上流にダムを持ち年間を通じて比較的水量が豊かだが、カディンゴ川は水量の季節的変動が激しく通常乾期には所々に水溜りを残すだけになる。

住民の若干の移動はあるものの人口はおよそ1200名(男550,女650)で、成人男性の都市への流出が目立つ典型的農村と言える。ドウルマ、ディゴ、ギリヤマなどが主な部族で、ほとんどは回教徒である。

ここに報告するのはこの村での1982年5-6月に行った第1回の検尿結果で、1217名の登録者の内851名から検体が得られた。採尿は午前10時半から午後1時半の間に行い、尿中の虫卵をヌクレポアフィルターを用いたろ過法で定量的に検査し、単位時間あたりの排虫卵数で表現した。

その結果、851名中581名(68%)の尿中に虫卵を認め、全体としては男女の間に陽性率の差は認められなかった。これを年齢別にみると、男女共10-14オグループで最も陽性率が高く、15才以上では陽性率は低下してゆくという住血吸虫症流行地に特有なパターンが得られた。しかし、女性では15才以降の陽性率の低下は緩慢で、ビルハルツ住血吸虫症に

特徴的と言われている急激な低下はみられなかった。排泄虫卵数を各年齢別に幾何平均で表わしてもほぼ同様の傾向を認めた。

このように男性と女性では15才以降の陽性率、排泄虫卵数の低下の程度に差が見い出されたことは、その原因を考える上で極めて興味深い。

検尿で陰性であった156名についておよそ1ヶ月後に再検した結果、15才未満の者で陽性者はひとりもなく、新たに陽性とされた8名はすべて15才以上であった。この時期の前二ヶ月は雨期で感染の機会はほとんどないことを考えあわせると、15才以上の者については偽陰性の可能性を常に念頭に置く必要があると考えられる。

3.2.5

EPIDEMIOLOGICAL STUDY OF SCHISTOSOMA HAEMATOBIMUM INFECTION

IN

COASTAL AREA, KENYA

4. Water contact patterns in the pilot area

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As schistosomiasis is transmitted by contact of people with infested water, it has been accepted that human water contact behavior may play a crucial role in maintaining the disease in an endemic area. It is, therefore, essential to conduct research on human water contact patterns to understand the dynamics of the disease. This study is also important in order to monitor any changes in transmission, especially to evaluate the impact of the introduction of any control measures.

The study was carried out by the methods introduced by Dr. Dalton. Briefly, human water contact was observed at 16 out of 32 major sites (Fig. 1) which had initially been chosen as busy sites by two local observers who had lived there for many years.



Activities were observed at each site for 4 days per month for a duration of 4 month from 6:00 a.m. to 6:30 p.m. on each observation day. Information such as name, type of activities, frequency and duration of contact was collected.

The categories for activities and degree of water contact are shown in Table 1. The degree of water contact of a person in a single water contact was expressed as the duration of contact (minutes) multiplied by the rate of wet area to the total body surface. In order to estimate the degree of water contact in a population, the geometric mean of all the individual water contacts was calculated.

A total of 616 people were observed at the observation sites in the first one year from June 1982 to May 1983. Of these, 308 were males and 308 were females.

- Degree -

The overall geometric mean of degree of contact was 13.66 in males and 12.05 in females.

Among both males and females, the children of 5-9 years of age showed the highest level of water contact with a peak of 43.93 min. in males and with a peak of 29.12 min. in females. There was a slight difference in the pattern of age distribution after adolescence between males and females. The contact rapidly decreased in degree after the peak in both sexes, but another peak was observed at age 40-49 in males and a dip at 15-19 years of age in females. The geometric mean of degree of contact was revised by the proportion of observed people and is shown in Figure 1 according to age and sex.

- Frequency - (Fig. 2)

The frequency of visiting water changed according to age and

sex. In males, visiting frequency per person increased with age showing a peak at age 10-14, after the peak gradually decreased and showed another peak at 40-49 years of age. Visiting frequency of females increased gradually with age and showed one clear peak at age 30-39 where the frequency of males was very low.

- Site -

The most busy site among the 16 sites chosen for observation was Site No. 6 with a frequency of 438 in total. Site Nos. 5, 10, 18C, 23 and 29 were also busy sites.

- Activities - (Table 2)

The main activities were bathing and washing a part of their body through all of the age groups. However, at the age group of 5-14 years, swimming, playing and fishing in the water were also observed to be important activities of water contact especially among males. In the 30-39 years of age where females were found to contact water frequently, drawing water and washing clothes were also major activities in addition to bathing.

- Diurnal variation of visiting frequency - (Table 3)

The children under 15 years of age were likely to visit water in the early afternoon. On the other hand, the adults were observed to contact water in the early morning or the evening more frequently.

As has been suggested, the difference in degree of water contact by age may be responsible for the age-intensity distribution of infection. However, some small discrepancies were observed between the age distribution pattern of the degree of water contact and the intensity of infection. It might be

necessary to postulate another factor such as worm life span in order to understand the relation between water contact and the intensity of infection by age.

The emergence of S. haematobium cercariae from Bulinus globosus showed a diurnal periodicity with a peak at midday in the pilot area (in separate report). Thus, more reliable estimation of degree of exposure to infection may be obtained when the data of cercariometry in study area is taken into consideration, since the favorite visiting time to the water contact sites seemed to be different among age groups.

Table 1. Categories of activities and degree of contact

1. Washing clothes in river with soap	1. One hand
2. Washing clothes in river without soap	2. Two hands
3. Washing clothes in buckets with soap	3. One foot
4. Washing clothes in buckets without soap	4. Two feet
5. Rinsing in river	5. One arm
6. Rinsing in buckets	6. Two arms
7. Washing utensils and other things	7. One leg
8. Bathing (whole body)	8. Two legs
9. Washing body (part)	9. Hands and feet
10. Drawing water	10. Hands and legs
11. Swimming and playing	11. Arms and feet
12. Crossing river	12. Arms and legs
13. Watering animals	13. Whole body
14. Fishing	14. Others
15. Drinking	
16. Others	

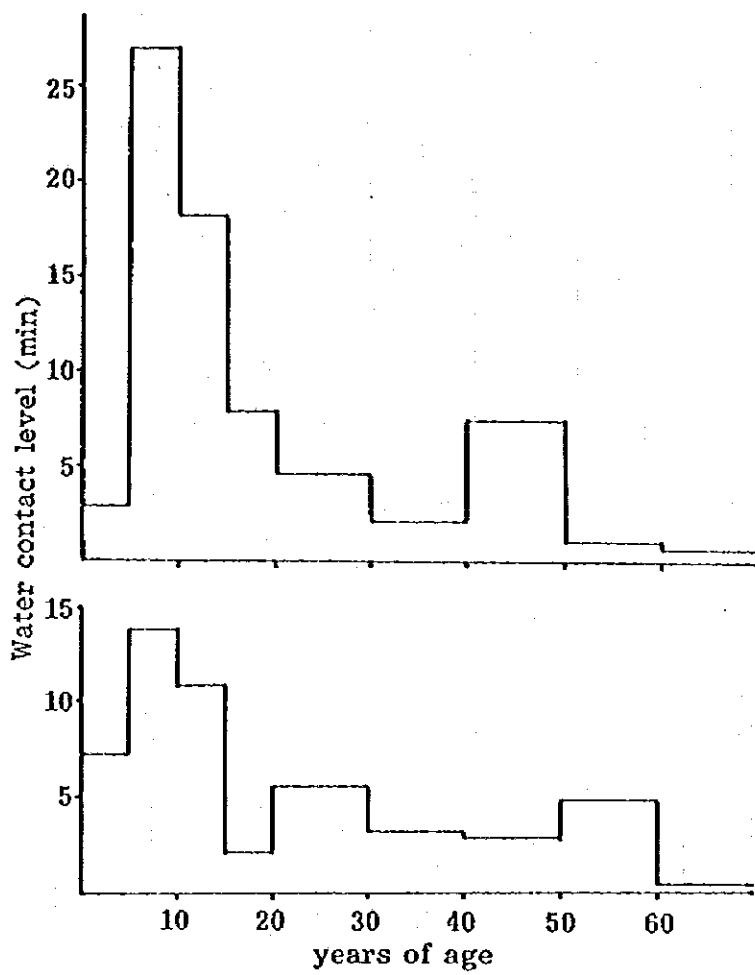


Fig. 1. Age-distribution of water contact level.

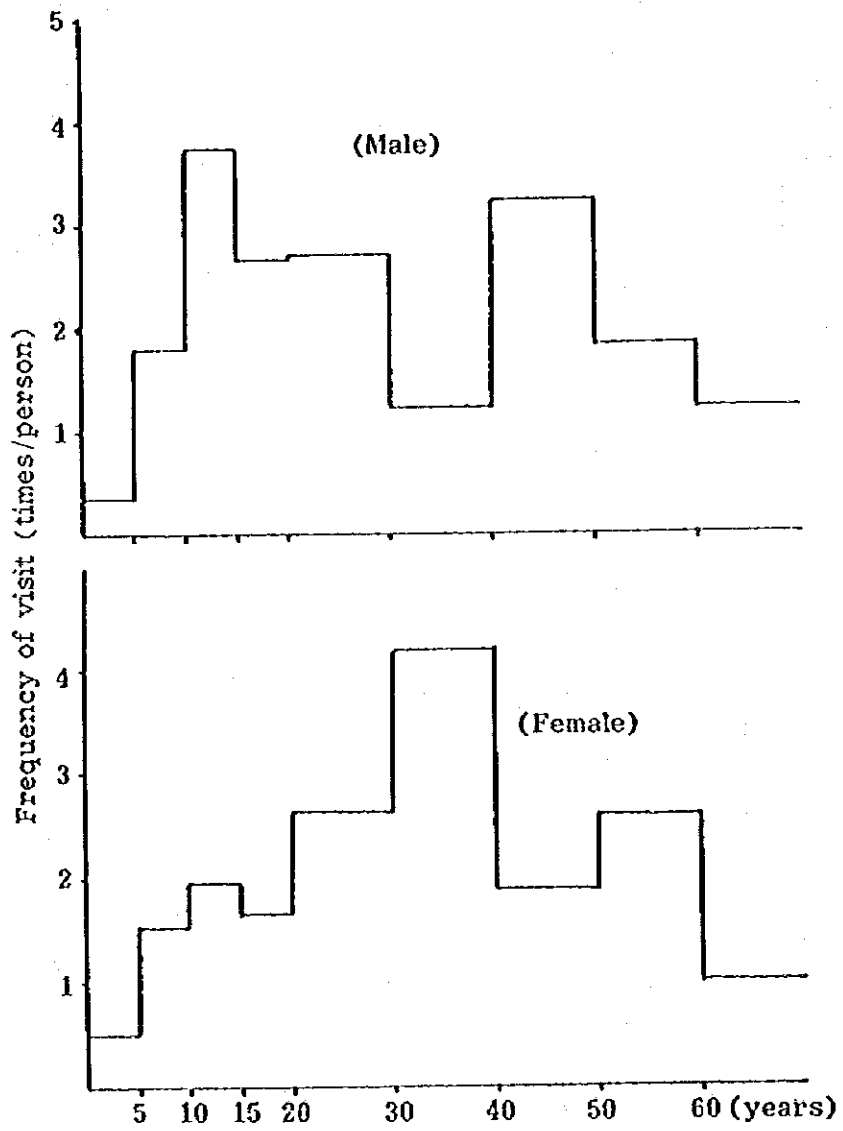


Fig. 2. Age-distribution of frequency of water contact activities.

Table 2. Frequency of activities observed in the study area.

AGE	SEX	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0-4	M	0	1	1	0	1	1	1	23	2	0	8	5	0	2	4	0	49
	F	0	3	1	0	2	0	4	41	0	18	20	4	0	3	9	1	109
5-9	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	4	2	0	3	1	5	67	2	18	28	9	0	5	13	1	158
10-14	M	3	0	0	0	0	0	0	92	20	8	49	25	7	25	29	0	257
	F	6	2	6	0	5	0	6	65	25	47	44	27	2	11	25	0	271
15-19	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	9	2	6	0	5	0	6	157	45	55	92	52	9	39	51	0	531
20-29	M	10	2	3	0	4	0	0	129	49	11	41	58	14	53	28	1	403
	F	8	2	4	0	3	1	7	60	20	54	10	19	4	21	9	1	221
30-39	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	18	4	7	0	7	1	7	189	69	65	51	78	18	74	35	2	624
40-49	M	7	0	3	0	4	1	4	82	30	4	6	38	6	27	8	2	222
	F	7	1	3	0	5	0	5	43	39	66	1	21	1	10	0	0	205
50-59	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	14	4	6	0	9	1	9	125	69	70	7	59	7	37	8	2	427
60-69	M	4	1	0	0	4	0	2	73	26	5	5	66	2	27	3	0	218
	F	27	10	18	0	22	0	16	100	62	148	1	61	1	12	0	1	479
70-79	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	31	11	18	0	26	0	18	173	88	153	6	127	3	39	3	1	697
80-89	M	4	1	0	0	3	0	0	36	10	2	1	28	0	8	2	1	96
	F	14	8	6	1	11	0	10	66	62	125	0	66	0	4	0	2	375
90-99	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	18	9	6	1	14	0	10	102	72	127	1	94	0	12	2	3	471
Unkown	M	1	0	1	0	1	0	0	61	15	4	0	45	2	17	0	0	147
	F	3	2	3	0	4	0	4	34	29	43	0	27	0	2	0	0	150
Total	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	4	2	4	0	5	0	4	95	43	47	0	72	2	19	0	0	297
Total	M	0	0	1	0	1	0	0	23	11	1	0	25	0	1	1	2	66
	F	9	3	1	0	4	0	4	36	27	58	0	25	1	0	0	0	168
Total	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	9	3	2	0	5	0	4	59	38	59	0	50	1	1	1	2	231
Total	M	1	0	0	0	1	0	0	21	9	0	0	26	1	1	2	2	54
	F	0	2	0	0	1	0	0	13	10	29	0	9	0	0	0	0	61
Total	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	1	2	0	0	2	0	0	34	19	29	0	35	1	1	2	2	128
Total	M	1	0	0	0	1	0	0	2	3	0	1	3	0	0	0	0	11
	F	1	0	1	0	0	0	0	3	1	5	0	0	0	0	0	0	11
Total	M	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	5
	F	1	0	1	0	1	0	0	0	5	6	1	3	0	1	0	0	26
Total	M	31	5	9	0	20	2	7	542	175	35	110	319	32	161	77	8	1533
	F	75	36	43	1	57	1	56	464	274	593	76	258	9	66	42	5	2056
Total	M	0	0	0	0	0	0	0	0	2	1	0	0	0	1	0	1	5
	F	106	41	52	1	77	3	63	1006	451	629	186	577	41	228	119	14	3594

Table 3. Frequency of visit according to the time of day.

AGE	SEX	Hour of Day						Total
		- 8	8-10	10-12	12-14	14-16	16-	
0-4	m	0	9	3	6	9	10	37
	f	1	7	9	9	19	27	72
	u	0	0	0	0	0	0	0
	s	1	16	12	15	28	37	109
5-9	m	14	18	22	50	35	53	192
	f	12	23	29	29	45	41	179
	u	0	0	0	0	0	0	0
	s	26	41	51	79	80	94	371
10-14	m	41	22	33	71	57	83	307
	f	16	13	19	21	23	43	135
	u	0	0	0	0	0	0	0
	s	57	35	52	92	80	126	442
15-19	m	20	16	21	37	23	53	173
	f	17	21	14	17	18	43	130
	u	0	0	0	0	0	0	0
	s	37	37	39	54	41	96	303
20-29	m	25	33	17	24	26	67	192
	f	40	70	43	38	38	76	305
	u	0	0	0	0	0	0	0
	s	65	103	60	82	64	143	497
30-39	m	6	6	15	13	15	27	82
	f	42	47	33	29	31	70	252
	u	0	0	0	0	0	0	0
	s	48	53	48	42	46	97	334
40-49	m	19	21	15	16	26	29	126
	f	12	21	12	14	13	30	102
	u	0	0	0	0	0	0	0
	s	31	42	27	30	39	59	228
50-59	m	6	17	7	8	8	13	59
	f	15	28	15	14	16	24	112
	u	0	0	0	0	0	0	0
	s	21	45	22	22	24	37	171
60-	m	12	6	7	7	7	13	52
	f	6	10	11	6	3	10	46
	u	0	0	0	0	0	0	0
	s	18	16	18	13	10	23	98
Unknown	m	2	2	0	1	1	0	6
	f	1	2	0	0	0	2	5
	u	1	1	0	0	2	1	5
	s	4	5	0	1	3	3	16
Total	m	145	150	143	233	207	348	1226
	f	162	242	195	177	206	366	1338
	u	1	1	0	0	2	1	5
	s	308	393	328	410	415	715	2569



## ビルハルツ住血吸虫症の疫学的研究

### 4. 住民の水接触行動について

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住血吸虫症の感染はヒトが水と接触して初めて成立する。従ってヒトと水との接触行動を量的、質的に明らかにすることが感染の危険度を推定する一手段として極めて重要と考えられる。そこで調査期間を通じて住民の水接触行動を観察したが、最初の一年間の結果をここで報告する。

方法はDaltonに準じて直接観察法によった水系の中からヒトがよく利用する16ヶ所を選び、観察者によって朝から夕方までの間そこを訪れた住民の水との接触行動を記録するというものである。記録内容は各個人の水接触時間、回数、体のどこまで侵ったか、利用目的などで、それぞれの観察場所は1ヶ月に1度観察されるよう配置した。

1982年6月から1983年5月までの1年間に観察された住民の数は616名、男女それぞれ308名で、延べ観察回数は2569回となった。この期間中観察された者の水接触時間の幾何平均は男性で1人あたり13.66分、女性では12.05分で有意の差はなかった。また水に最もよくつかる年齢層は男女共5-9才であった。水との接触頻度についてみると、必ずしも接触時間と並行していないことが年齢別分布から明らかになった。

水と接触する時の活動内容についてみると各年齢を通じて水浴、体の一部を洗う行動が大きな比重を占めるが、5-14才では特に男子で水泳、水遊び、魚とりが重要な活動となる。女性では30-39才で水汲み、洗濯の回数が増加しているのがみられた。一日の内では子供は真昼に、成人は朝、夕に主に水に接触する傾向がみられた。

以上の結果から、水との接触行動は年齢や性別によって一様ではなく、大きく異なっていることが明らかになった。住民の住血吸虫症陽性率、Intensity of infectionとの密接な関係が予想されるが、そのためには虫の寿命等の他の因子を加えた分析が必要であろう。水中のセルカリア濃度などを更にくわしく把握することによって、住民の感染に対する危険度をより正確に推定できるかもしれない。

### 3.2.6

#### DETECTION OF SCHISTOSOME CERCARIAE IN NATURAL WATERS

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Cercariometry is the method to detect and count cercariae in a known volume of water in an endemic area of schistosomiasis. This method is indispensable to the study of transmission potential in the epidemiology of schistosomiasis.

By using a filtration technique of Prentice ( personal communication ), cercariometry was carried out in and around Mwachinga village, the study area.

To examine the daily fluctuation in density of cercariae, 10 liters of water were taken every 2 hours for 8 hours in the daytime, at 4 sites of Kinango dam, one of the heaviest infested water sources in Kwale. The result was shown in Fig. 1. S. haematobium cercariae apparently showed daily periodicity. Although the number of cercariae observed varied from site of site, the highest density was counted at 11:00 a.m. at all study sites, with few cercariae present at 9:00 a.m. or after 4:00 p.m.

Cercariometry was carried out hourly in daytime at site 6 and 19 in Mwachinga village, to determine the level of contamination of water by cercariae. At site 6, where water is running, cercariae were detected only in the sample of water

collected at 11:00 a.m. and 12:00 p.m. but the number of cercaria recovered were very low ( 0.5 cercariae/liter ). On the other hand, at site 19 where water is stagnant, cercariae were detected at any of the daytime except at 9:00 a.m., with the highest density ( 17.0 cercariae/liter ) at 12:00 p.m.. ( Fig. 2 )

It has already known that schistosome cercariae show the marked daily periodicity in natural water. Kloos et al. ( 1982 ) reported that the highest densities of S. haematobium cercariae were between 7:00 and 9:00 a.m. in Egypt. In our study on S. haematobium in Kenya, the peak was observed about noon, with few cercariae at 9:00 a.m. or after 4:00 p.m.. This difference suggests that the daily fluctuation in density of cercariae is probably influenced by several factors, such as the condition of water source examined, climate, the strain of schistosome etc.

Our report is the first to describe direct measurement of risk of transmission in an endemic area of S. haematobium in Kenya and showing that water contact around noon constitutes, the greatest danger of infection in Kwale. At the site 19 of our study area, where water is stagnant, cercariae were recovered at anytime of the day. Water contact observations also revealed that this place is frequently visited by many villagers. These facts indicate that the site 19 is the one of the main source of infection in our study area. At the site 6, where water is running, a few cercariae were recovered only around noon. Site 6 is also the place where many villagers visit for collecting drinking water, washing, bathing and swimming as well. Further studies would be necessary for the interpretation of the risk of transmission at site 6.

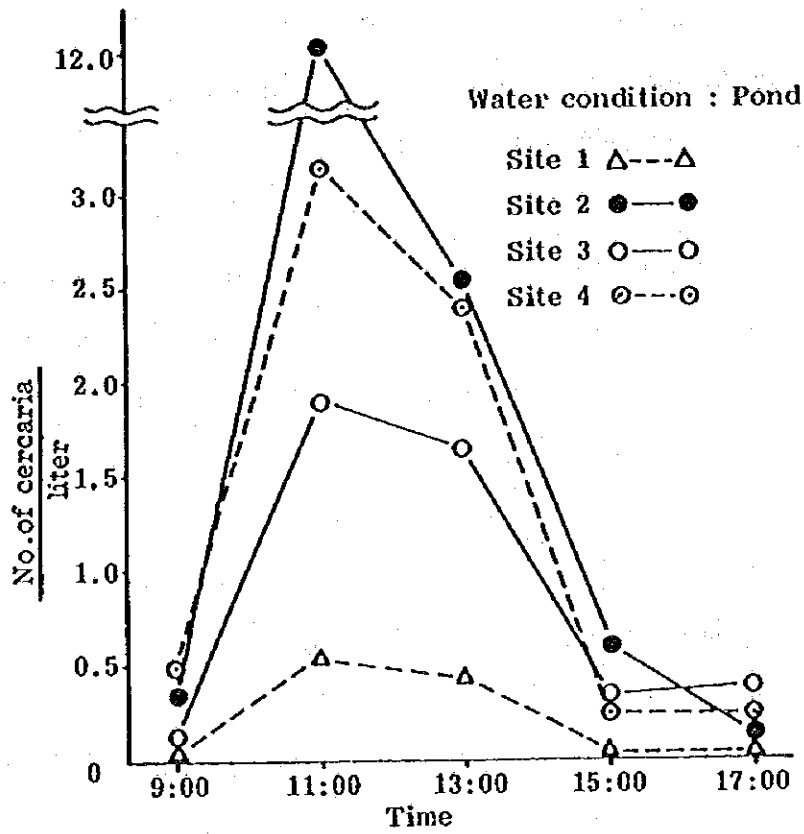


Fig. 1 Results cercariometry at Kinango dam.  
November, 1983.

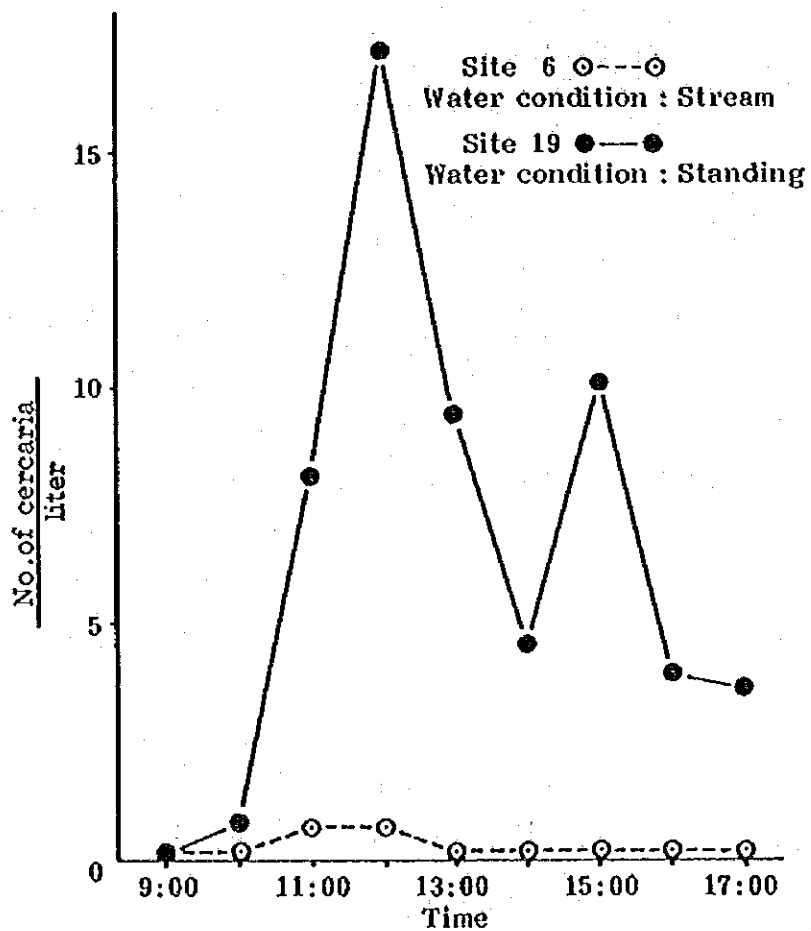


Fig. 2 Results of cercariometry at Mwachinga.  
November and December, 1983.

## 住血吸虫症流行地河川からのセルカリア検出について

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セルカリオメトリーは、住血吸虫症流行地の河川より検出する方法で、これは住血吸虫症の伝播を調べるためには、不可欠のものである。

今回、本プロジェクトにおいて我々は、Prenticeの河過法によるセルカリオメトリーを用いて、クワレ地区ムワチンガ村及びその周辺で、セルカリアの検出を試みた。

まず自然環境下におけるセルカリア数の日内変動を調べるために、ビルハルツ住血吸虫による汚染が顕著なクワレ地区のキナンゴダムにて、午前9時より午後5時まで2時間毎にセルカリオメトリーを行なった。その結果検出されたセルカリア数には、明らかな日内変動が見られた。(表-1) 各時刻のセルカリア数は、場所毎に異なっているが、いずれの場所でもピークは午前11時に観察された。午前9時、あるいは午後4時以後は、わずかしか認められなかった。

次に、パイロットエリア内の2ヶ所を選び、それぞれ午前9時から、午後5時まで1時間毎にセルカリオメトリーを行なった。66では、午前11時と正午にそれぞれ一隻ずつのセルカリアが認められただけで、その他の時刻にはセルカリアは見られなかった。しかし、69では、午前9時を除いたすべての時刻でセルカリアが検出され、正午にピークが観察された。(表-2)

住血吸虫セルカリアが、自然環境下で明らかな日内変動を示すことはよく知られており、Kilooosらはエジプトにおいて、ビルハルツ住血吸虫セルカリアは、午前7時から午前9時にかけて最も多く見られたとしている。しかし、今回の調査によればピークは正午に観察され、午前9時、あるいは午後4時以降はセルカリアはわずかしか見られなかった。おそらく自然環境下におけるセルカリア数は、河川の状況、天候、住血吸虫の糸など諸々の要因によって影響を受けるのかもしれない。

また、クワレ地区のビルハルツ住血吸虫症流行地においては、正午前後の河川での水利用が最も危険であることがわかった。特に619は、多くの住民によって利用されている場所であり、おそらくここがムワチンガ村におけるビルハルツ住血吸虫症の主な感染源のひとつであることが推察される。

### 3.2.7

Population studies on intermediate hosts in relation to transmission of Shistosoma haematobium in Kwale District, Coast Province, Kenya

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The Marere River runs through the Mwachinga Village, and has a dam with an artificial lake located at its upper part. At the lower part, the river joins a branch called the Kadingo River which seasonally runs through the middle of the village.

Snail survey was carried out at 13 water contact observation sites of Mwachinga Village. Snails were collected 2 times a month at each site by continuous scooping for 10 minutes. All collected snails were identified and measured for size at the field station. Then each of Bulinus globosus, intermediate host of S. haematobium, was put into small petri dish for observation of shedding of cercariae. The petri dish was kept at a lighted place more than 2 hours,

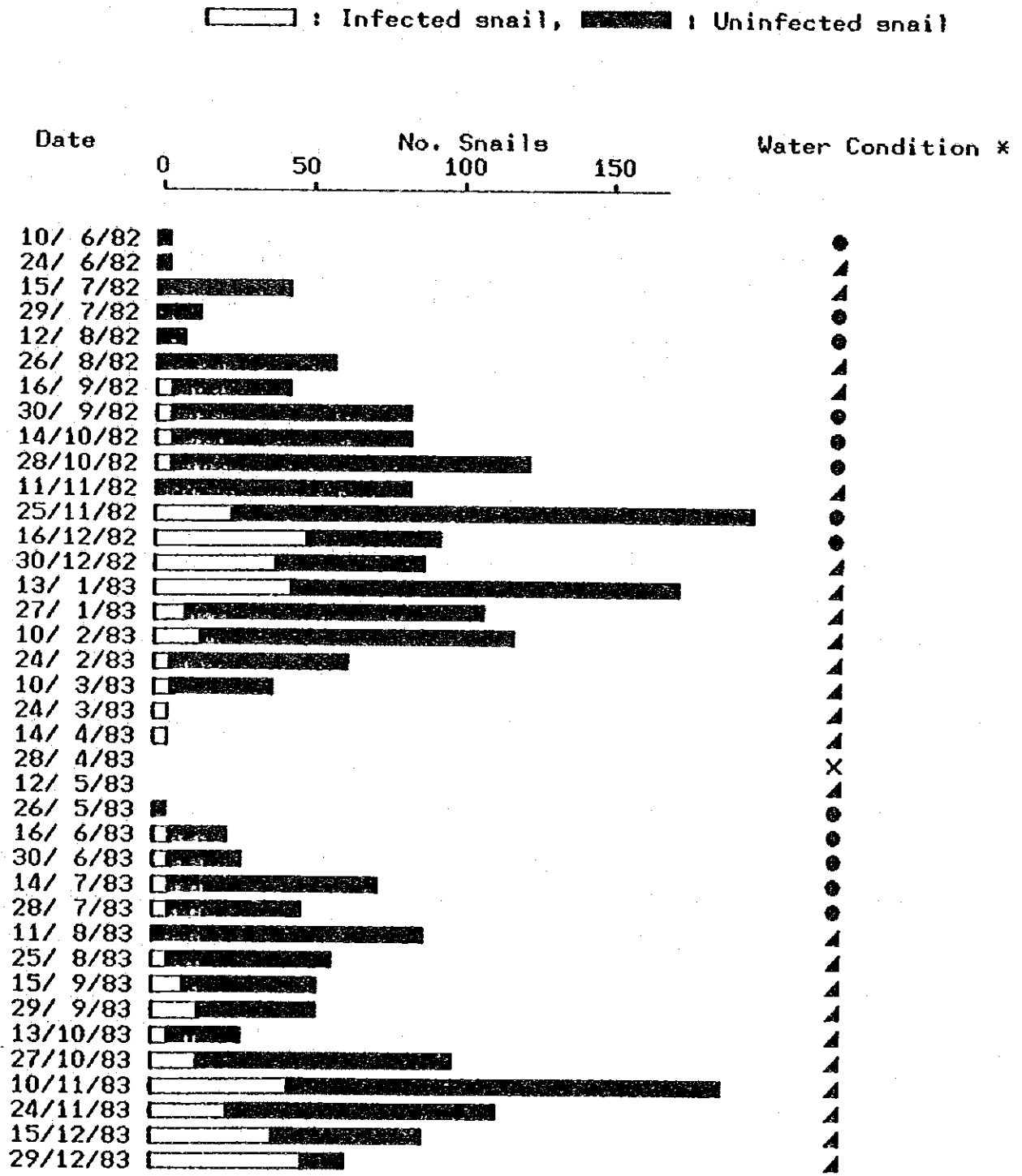
and examined for cercariae under a stereoscopic microscope. After examination, all the snails were returned to source.

At the Marere River ( main stream ), infected snails were collected from only one site out of 4 sites, and infection rate was low. On the other hand, at the Kadingo River ( branch stream ) infected snails were collected from all sites, and infection rate were more than 10 % at 3 sites. Seasonal change of snail population and infection rate at site 19 are shown in Fig.1. The population of snail started to increase after the long rainy season (April - June), reached the peak at short rainy season ( November ), and then decreased. In the period of 3 months of November, December and January, a number of snail were collected and about 30% of them were proved to be infected with cercariae. For the other 9 months of the year, infected snails were scarcely collected. The same seasonal change of snail population and infection rate was observed at other sites.

From this study, it can be concluded that the villagers probably get infection of S. haematobium in November, December and January. The result of snail survey will be useful for a control trial run.



Fig. 1 Survey of intermediate host snail, Bulinus globosus, at water contact site No. 19.



\* Water condition -- ● : Flowing, ▲ : No flow, X : Dry

ケニア国のクワレ地区におけるビルハルツ住血吸虫の中間宿主貝における個体数および感染率の周年変化

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調査地ムワチンが地区では上流にダムがあるマレレ川が流れており、この川に地区中央部を流れるカディンゴ川が合流している。このカディンゴ川は乾期には乾燥し、いくつかの水溜りを残すだけとなる。

中間宿主貝の調査は住民が水を利用する地点のうち13地点を選んで実施した。貝は10分間のスタンプ法により2回採取した。これらの貝はフィールド・ステーションに持ち帰り、貝の種類と大きさを調べ、ビルハルツ住血吸虫の中間宿主貝 Bulinus globosus については小型シャーレに入れ、セルカリアの遊出の有無を観察した。

主流であるマレレ川では感染貝は4地点のうち1地点のみで採取され、感染率は低かった。しかし、支流であるカディンゴ川では感染貝は全ての地点で採取され、その感染率は3地点において10%以上であった。619地点における中間宿主貝の個体数と感染率の変化を図1に示した。貝の個体数は大雨期(4~6月)の後から増加し始め、小雨期(11月)に最大となり、その後減少する。11・12・1月には多くの貝が採取され、約30%が感染していた。しかしその他の時期にはほとんど感染貝が採取されなかった。同じような変化は他の地点においても観察された。

この調査から、当地区住民は主に11・12・1月にビルハルツ住血吸虫の感染を受けているものと考えられる。

3.2.8

**TRIAL RUN OF CONTROL OF SCHISTOSOMA HAEMATOBIIUM INFECTION BY  
TREATMENT WITH METRIFONATE AND PIPED WATER SUPPLY IN KWALE  
DISTRICT, COAST PROVINCE, KENYA**

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The research programme on schistosomiasis started in 1981 in Kwale, Kenya, with the object of determining the mode of transmission of *S. haematobium* infection and the implementation of a trial run of control of the disease. The results of epidemiological survey suggest that children are potentially responsible for the transmission of the disease in the study area Mwachinga, and the villagers are likely to get infection when they play, wash clothes, and bath at the river especially in November, December and January. In our project, the treatment of patients by metrifonate and the piped water supply were selected as measures of control of the disease.

Mass-treatment was done in February and March, 1984. The population of the study area is approximately 1,200. Eight hundred thirteen villagers, including patients passing schistosoma eggs in the urine and negative persons aged 5-15 years, were selected as the subjects for treatment with metrifonate. The drug was scheduled to be given three times at a dose of 7.5mg/kg of body weight with a 2-week interval between treatment. Four hundred thirteen subjects completed a third dose. However, 159 had only two dose and 118 only the first dose. One hundred twenty three refused the treatment. Sixty five pupils of the primary school were examined for egg count and colour of the urine for the periods of 42 days after the start of treatment. The egg count decreased quickly and no haematuria was noted.

Piped water supply has been provided since February, 1984. Five water supply facilities (water Kiosks) were constructed in the study area, and one shower shed was constructed at the primary school. The followup study for evaluation of the effect of the treatment and piped water supply is now under way.

ケニア国クワレ地区におけるメトリフォネイトと安全水供給によるビルハルツ住血吸虫感染のコントロール

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国際協力事業団（JICA）のケニア国における医療協力プロジェクトの住血吸虫に関する計画は、ビルハルツ住血吸虫症の感染動態の解明と病気のコントロールを目的として1981年に始められた。調査により子供達が病気の伝播に重要な役割をしており、住民は主に11・12・1月に水浴・衣類と家庭用品の水洗や川の中での遊びなどにより感染していることが明らかになった。当プロジェクトでは病気のコントロール方法としてメトリフォネイトによる治療と住民への安全水供給が選ばれた。

治療は1984年2月に始められ、パイロット地区の住民は約1,200人で、ビルハルツ住血吸虫卵が尿中に認められた者と5～15才の陰性者の合計813名が投薬の対象者として選ばれた。これらの住民にメトリフォネイト（7.5 mg/kg）3回投与が予定されたが、対象となった813名のうち413名は3回投与、159名は2回投与、118名は1回投与を受けた。しかし残り123名は全く投与を受けなかった。虫卵排出と尿の色に関して治療開始後42日間、65名の小学生について追跡調査を行った。その結果、尿中に排出される虫卵数は急速に低下し、また血尿も消失した。

安全水供給も1984年2月に始められた。パイロット地区には“Water Kiosk”と呼ばれる安全水供給施設が5ヶ所に、さらに小学校にはシャワー設備が作られた。

パイロット地区において、治療効果と安全水供給の効果を確認する為の追跡調査が現在行なわれている。

3.2.9

**PRECIPITATES FOUND AROUND SCHISTOSOMA HAEMATOBIIUM EGGS FROM  
HUMAN URINE PRIOR TO CIRCUMOVAL PRECIPITIN TEST**

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S. haematobium eggs were recovered in urine from school children in Mwachinga Village, Kwale District, Kenya. Direct examination of eggs showed a pattern similar to that observed in a circumoval precipitin (COP) test. Observed precipitates were removed by treatment of eggs with pepsin. However, there was no effect when treated with trypsin. Similar precipitates were observed after incubating the pepsin-treated eggs with urine supernatants from the same children. Attempts were made to identify what components could contribute to observed bleb (precipitate) formation. Use of fluorescent antibody test showed the presence of both IgG and IgM in the precipitate; and urine examination by immunodiffusion revealed the presence of IgG, IgM, IgA and C<sub>3</sub>. It is very likely that these components may have participated in the bleb formation. This possibility was further strengthened by the presence of anti-egg antibodies in the immunoglobulins identified in the urine, and agrees well with the expected activity of pepsin on these immunoglobulins. These results, therefore, suggest that S. haematobium eggs recovered in urine contain precipitates formed mainly by immunoglobulins, and is necessary to treat these eggs with pepsin prior to carrying out a COP test. The relevance of these findings to the immunodiagnosis of Schistosomiasis haematobium in man is also discussed.

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### 3.2.10

#### A modification of slide preparation in the circumoval precipitin (COP) test for field survey

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A simplified slide preparation for the field circumoval precipitin (COP) test was studied. To a role of PVC electrical insulating tape (19mm width, 10m length, 0.2mm thick, Nichiban CO. Ltd, Japan) , two holes were drilled with heated 11mm and 5mm tubular cork borers, which were to be opened each other. A strip of the perforated tape was adhered on a microscope slide cleaned with alcohol, and then air bubbles between the tape and the slide were pressed out by an edge of another slide. The slide was left in a room at least 2 days to ensure tight adhesion of the tape to the slide. Ten  $\mu$ l of *S. japonicum* egg suspension containing approximately 100 eggs was placed in the large hole using a micropipette. The eggs were obtained from a 8 week-infected rabbit according to Yokogawa et al. (1966). The suspension



was spread all over the large hole with a tip of the micro-pipette and was left to dry. The prepared slides were used after 2 weeks, 1 month and 2 months.

One drop ( $25\mu\text{l}$ ) of test serum was placed at a corner of the large hole near the small hole. Subsequently a strip of cellulose tape (24mm width, 35m length, 0.02mm thick, Sekisui Co. Ltd, Japan) was adhered over the holed insulating tape, pressing with balls of an index and a middle finger. On touching with the tape, the serum entered into the space between the tape and the slide by capillary phenomenon. A part of a small hole remained empty if serum volume is appropriate. After all the holes were covered, the surface of the tape was tightly pressed with the back of a marking ink. the completed slide was incubated usually at room temperature for 48 hrs. Microscopical observation of the COP reactions through the cellulose tape was satisfactory, and the reactivity of the prepared eggs on the slide 2 months previously showed no difference from that of lyophilized eggs stored in a refrigerator. Since the tapes are apparently cheaper than a coverslip, approximately one-fifth, and are easily available in rural areas, the present method is suitable especially for field surveys.

## 疫学調査の為の虫卵周開沈降反応法の簡易化

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虫卵周開沈降反応の術式を簡易化する為、スライドガラス上で塗沫、乾燥した虫卵を用い、カバーガラスの代わりに安価な絶縁テープ、セロファンテープを用いる方法を考案した。

絶縁テープに2つの大小の穴(直径, 11 mm, 5 mm)を互に一部重なる様に熱したコルク穴開器で開けた。穴開テープの一片をスライドガラス上に張付け、テープが密着するように別のスライドガラスの一端でこすりつけ、更に2日間放置して使用した。

1000コの虫卵を含む浮遊液10  $\mu$ l を大穴におき、均等に拡げ自然乾燥させた。2週間、1カ月、2カ月後虫卵の反応性を調べた。

被検血清1滴(25  $\mu$ l)を大穴に置いた後、セロファンテープで大小の穴を被った。その時、最初大穴の端から張付け、液がもれない様に人差指、中指で穴の周囲を押えながら小穴の方へ進めた。小穴は液量が多過ぎる時洪水防止の予備として有効である。最後にマジックインクの後端で強く押しテープを確実に密着させた。

完了した標本は通常条件下(温度18~26°C, 湿度40~60%)及び高温乾燥下(温度37°C, 湿度20~30%)でセロファンテープからの水分蒸発について検討したが、少なくとも前者で48時間、後者で24時間障害になる変化を認めなかった。

スライド上に塗沫、乾燥した虫卵の反応性は通常条件下で2週間から2カ月後、冷室内保存凍結乾燥虫卵と比較し、差を認めなかった。

3.2.11

RELATION BETWEEN LUNCH TIME AND HOURLY OUTPUT PATTERN OF  
SCHISTOSOMA HAEMATOBIIUM EGGS IN URINE

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We studied the hourly urinary output of *Shistosoma haematobium* eggs in four Kenyan schoolboys. If they ate no midday meal, the hourly output peaked between 10 : 00 AM and 2 : 00 PM. If they took an early lunch (10 : 45 or 11 : 45 AM), the egg output peaked within one hour of mealttime. Similar observations were made when lunch was taken late (0 : 45 or 1 : 45 PM), and in some instances a second, smaller peak was noted. Neither breakfast nor supper affected the diurnal output of *S. haematobium* eggs. There was no evidence that the urine volume affected the diurnality of egg output under conditions in which the water intake was controlled.

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ビルハルツ住血吸虫卵の毎時尿中排泄パターンと昼食との関係

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ビルハルツ住血吸虫に感染した四人のケニア人学童(13~15歳)のボランティア協力の下に、尿中への虫卵排泄に及ぼす昼食摂取の影響をみてみると、昼食を摂らない時には虫卵排泄数のピークは学童毎に、又同人でも日毎に出現時刻は異なるが、午前10時から午後2時の間に集中した。全例の算術平均のピークは正午から午後1時にあり、一般的に知られている午後のピークに一致した。一方、学童に昼食を早期(午前10時45分あるいは11時45分)に摂らせると、それぞれの食事後から1時間以内に非常に多量の虫卵排泄をみる“食後のピーク”が出現した。昼食が遅れると(午後12時45分あるいは1時45分)、既に虫卵排泄数のピークがあったものでは新たに小さな二次ピーク(“食後のピーク”)が現われ、以前にピークが無いと長大な食後のピークが見られた。本観察において学童に過剰な水分摂取を制限させた範囲内で、尿量が虫卵排泄の昼間出現性に関与することは無かった。

寄生虫学雑誌 33巻4号, 297-303頁, 1984年

3.2.12

Drought tolerance of adult Biomphalaria and Bulinus snails  
and their distribution in Kenya

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Fresh-water snail species are involved in the transmission of human schistosomiasis in various areas where the disease is endemic. There are four groups of Biomphalaria and another four groups of Bulinus. Whether these groups belong to the same species complex or not has been the subject of much dispute and speculation among snail taxonomists. This problem in snail taxonomy may be due to the presence of snails in numerous different foci isolated into different habitats and exposed to varying degrees of environmental changes.

This study was conducted for observations made on the ability of the following six species to tolerate prolonged and varying degrees of desiccation. It is well known that Biomphalaria pfeifferi and Bulinus globosus inhabit small canals or streams, whereas Biomphalaria sudanica and Bulinus nasutus live at ponds or in lakes. The other species, Bulinus tropicus and B. forskalii are wide-spread and are found in any kinds of water bodies.

dry 1/2

We recovered live snails from dry concrete-like mud, and observed that the density and distribution of the snails (*Bulinus nasutus*) in a certain pond at Mwachinga, Kwale District in Dec., 1981 (before drought) were almost in the same order of magnitude as those in Apr., 1982 (after heavy rains). This study further revealed that the snails easily dry up and soon die on filter paper, while the same snails withstood the drought and continued to survive for a long time in mud. The rate of weight loss might play an important role in the survival of these snails. Furthermore, pond-snail species (*Biomphalaria sudanica*, survive for 2 weeks - 2 months on mud; *Bulinus nasutus*, 8 weeks - 6 months) can tolerate longer periods of drought, while stream species (*Biomphalaria pfeifferi*, 1-6 weeks; *Bulinus globosus*, 3 weeks - 3 months) tolerate only shorter periods.

Except for what is commonly known about the natural habits of these snails, there is little information on the factors limiting their distribution. Physical barriers are obvious. The degree of drought tolerance in this study is one of the factors that may determine, at least in part, the distribution of vectors and subsequently the epidemiology of schistosomiasis in Kenya and elsewhere. For example, stream-water snails of *Biomphalaria pfeifferi* and/or *Bulinus globosus* are found in Taita Taveta, Kitui, Machakos, Mwea, and the Lake Victoria Basin. This could be due to the fact that most of the streams and dams found in these areas are not seasonal, or if they are, the period of dryness is not long.

Biomphalaria 属及び Bulinus 属の成貝の乾燥抵抗性とケニアにおける分布について

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淡水産貝類は住血吸虫症の流行地であって感染源に連なる。Biomphalaria 属及び Bulinus 属に夫々グループの種類があり、夫々のグループが同じ種かどうかは貝分類学者の中でも混乱が起きている。この分類上の問題は貝が種々の生息環境下に孤立しその環境変化に応じて生きていく多くの生息果があるから生じたのであろう。

本研究は以下の6種類の貝の活性について種々の乾燥程度に耐える様を観察したものである。Biomphalaria pfeifferi と Bulinus globosus は小さな流れに棲み、Biomphalaria sudanica と Bulinus nasutus は池とか湖に棲むことが知られている。更に Bulinus tropicus と B. forskalii はいずれの水系でもみられるものである。

ビルハルツ住血吸虫流行地の Kwale の Mwachinga 村にある小池は丘の頂上であって他地区からの水の流入が全く不可能な所である。しかし年間のその貝の生息調査から乾期前と雨期初期とで成貝の数が類似していて乾期の大部分を乾いた上中に生存し続けたと推定された。事実、その地で乾期で固くコンクリート様になった泥中より生きた貝を検出することができた。このことから引き続き実験室内で貝への乾燥の影響をみると、口紙上では貝は容易に乾燥して死滅するが、土の上では乾燥によく耐え長期間生存できた。それには貝の水分消失の程度が関係あった。更に池に棲む貝 (Biomphalaria sudanica 乾土中にて2週~2ヶ月生息, Bulinus nasutus 8週~6ヶ月) は乾燥に強く、一方小さな流れに棲む貝 (B. pfeifferi 1週~6週, B. globosus 3週~3ヶ月) は弱い傾向がみられた。

これらの貝類の自然生息について普通知られていること以外には、分布を制限する要因はほとんど知られていない。物理的障害があることは明白である。本研究での貝の乾燥抵抗性の程度が少なくとも一部ではその貝の分布、即ちケニアでの住血吸虫の流行地を決定していると思われる。たとえば流水に棲む Biomphalaria pfeifferi や Bulinus globosus は Taveta, Kitui, Machakos, Mwea あるいはビクトリア湖周辺にいるが、これはその地区での流れやダムのはほとんどが季節的でない(いつも水がある)かあるいは涸れてもその期間が短いことと関与するのだから。

### 3.2.13

#### Dependence of hatching of Schistosoma haematobium eggs on physical factors

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Some factors stimulating hatching of schistosome eggs, especially of S. mansoni and S. japonicum, may be known. They are the hypotonicity of the external medium, activity of miracidium, and light. To our knowledge, there were some reports that referred to various effects of physical and chemical conditions by hatching rate and/or hatching pattern of the eggs of S. haematobium as well as S. mansoni and S. japonicum, but little detailed informations are available even on physical factors such as light, osmotic pressure and mechanical agitation. This study has been undertaken to elucidate the roles of the three physical factors.

Urine samples used in this experiment was obtained from patients with S. haematobium in 'complete darkness' so that the eggs may be prevented from light exposure before experiment. The egg sediment was used as sources for light-free eggs.

Under natural light conditions and 1,000 lux of artificial light conditions, eggs began to hatch 5 min after submergence in water and reached their peak in 10-15 min. In this case, the eggs were exposed to natural diffused sunlight or the artificial light



for more than 5 min before experimental submergence in water. They had become light-exposed eggs. The hatching pattern by the light-exposed eggs in submergence with light, was referred as 'standard' in this study.

The light-free eggs reached their peak in 15-20 min after submergence under the artificial light conditions. The peak was found 5 min later than that of the standard.

As for hatching pattern with darkness, although light-exposed eggs could begin to hatch 5 min after submergence with darkness, it took one hour for almost all eggs to hatch. Whereas, light-free eggs began to hatch 15 min after submergence with darkness, and some failed to hatch within one hour.

Miracidia could hatch even in the solution with high osmotic pressure of 340 mOsm. In this case, prolonged hatching patterns were observed in solutions more than 60 mOsm.

Light-exposed eggs having been agitated with several syringe movements could begin to hatch 2 min after submergence with light and reach their peak in 5-10 min. The peak was found 5 min faster than that of the standard.

In this study, three factors (light, agitation and osmotic pressure) affecting hatching pattern of Schistosoma haematobium eggs were obvious. In general, hatching process is understood to be a biological phenomenon induced by the organism. However, physical stimulations, especially agitation, easily influenced the hatching pattern of S. haematobium. This means that the hatching of S. haematobium eggs is of combination between mechanical rupture and biological release from eggs.

## ビルハルツ住血吸虫卵の孵化に及ぼす物理的要因について

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住血吸虫、特にマンソン住血吸虫及び日本住血吸虫の虫卵の孵化を促す要因は幾つか知られている。それらは外液の浸透圧、ミラシジウムの活性あるいは光である。我々の知る所によると、物理的、化学的な種々の影響を虫卵の孵化率あるいは孵化パターンでみてある報告が幾つかある。それらはマンソン住血吸虫や日本住血吸虫のみならずビルハルツ住血吸虫の虫卵についてであるが、光、浸透圧、機械的振動のような物理的要因についてすら詳しい情報はほとんどない。本研究はその3つの物理的要因の役割を明らかにするためになされた。

実験に使われた材料はビルハルツ住血吸虫に感染している患者の尿で採尿時から光が当たらないようにしてある。尿沈査がその light-free の虫卵として使われた。

自然光あるいは1,000ルクスの人工光の下では虫卵は浸水5分後から孵化し10~15分目にその孵化のピークがみられた。この時虫卵は浸水前に光に5分以上暴露した light-exposed のもので、この時の孵化パターンが以下 standard として比較参照された。

light-free の虫卵は人工光下に浸水後15~20分目に孵化のピークを示し、standard と比べて5分遅れている。

暗黒下での孵化についていえば、light-exposed の虫卵は浸水5分後に孵化はみられたものの全ての虫卵が孵化するには1時間を要した。一方 light-free の虫卵はやっと浸水15分後に孵化が開始し1時間以内では一部孵化が完了しないものがあった。

孵化は340mOsmの高い浸透圧の溶液中でも可能であるが、60mOsmより高い浸透圧では孵化が遅れた。

light-exposed の虫卵で物理的振動を注射器のピストン運動で与えたものの孵化をみてみると、浸水2分後に孵化がみられそのピークは5~10分目と standard より5分早まった。

本研究で3つの要因(光、振動、浸透圧)がビルハルツ住血吸虫卵の孵化パターンに影響することが明らかになった。一般的に孵化の過程は生物学的現象とみられるが、振動のような物理的刺激が容易にビルハルツ住血吸虫卵に影響を及ぼすことはその孵化が機械的破裂と生物学的飛び出しの組み合わせで起っていることを示している。

3.2.14

Maintenance of Schistosoma haematobium in laboratory

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It has been very difficult to keep S. haematobium in laboratory, whereas maintenance of S. mansoni is easy and that of S. japonicum is not so difficult. It is likely that the main reason comes from minor interest in urinary schistosomiasis having mild symptoms, among researchers on schistosomiasis. In fact, some barriers are: 1) how to keep vector snails in laboratory, 2) how to get miracidia, and 3) how to get cercariae.

This study has been undertaken to solve problems concerning maintenance of S. haematobium in laboratory.

1. Maintenance of the snails, Bulinus globosus and B. nasutus

It is possible to keep the snails in a shallow container or a large petri-dish, but this requires a lot of man-power. Needless to say, reproduction of eggs in the container is slow and development of young snails is poor because the system is too simple to culture the gastropoda.

We recommend an aquarium with air-circulation and mud-sand filter system. This is easily made, but needs an air-compressor, It is almost maintenance-free except for feeding twice a week. It needs only one person to rear thousands of snails from young ones to adults.

maintain 1/3

## 2. How to get miracidia

It is well known that miracidia of S. mansoni and S. japonicum have positive phototaxis, but those of S. haematobium have negative phototaxis. Further, a hamster is only one of the suitable hosts in common laboratory and it excretes the eggs of S. haematobium in feces. This means that it is impossible to get miracidia of S. haematobium by the same method as S. mansoni and S. japonicum from animal feces and liver including their eggs.

When feces of hamster infected with S. haematobium is available, a feces mass should be put intact into water and then the water is clear during half an hour of hatching. It is easy to get miracidia swimming around the faecal mass. When liver (fresh infection) or colon tissue (clonic) is available, a past mass of homogenized tissue should be left intact in water to keep ambient water clear during the hatching.

This method is only way to get miracidia with negative phototaxis from feces and tissue including S. haematobium eggs.

## 3. How to get cercariae

Cercariae of S. haematobium shed during day-time and the peak is seen around noon. The number of cercariae released from a snail per day is not so much as that of S. mansoni. As our previous paper (1978) reported how to stimulate cercarial shedding of S. haematobium immediately, the interjection of a period of darkness, the duration of which could be more than 5 sec during the photo-

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period, should be introduced to get many cercariae in time for the experiment schedule.

#### 4. Others

We found that young snails B. globosus (below 8 mm) were several times as susceptible to S. haematobium as the adult (over 10 mm). When the snails less than 5 mm were exposed to miracidia, they could hardly grow up to 10 mm of adult size: they released quite a few cercariae. This indicates that 6 to 8 mm of young snails should be used for miracidium infection so that minimum miracidia may give maximum cercariae.

## ビルハルツ住血吸虫の実験室内維持

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マンスン住血吸虫の実験室内維持は容易で又日本住血吸虫のそれはそんなに困難ではない。一方ビルハルツ住血吸虫の実験室内維持は非常に困難である。その主な理由は住血吸虫症の研究者の中に、症状の軽い泌尿器系住血吸虫症への興味が小さいことによる。事実、①媒介虫の実験室内飼育法、②ミラシジウムの採取法、③セルカリア遊出法について幾つかの問題点が横たわっている。

本研究はビルハルツ住血吸虫の実験室内維持に関しての全ての障害を解決するためになされた。

### 1. 貝類 (*Bulinus globosus*, *B. nasutus*) の維持

貝を浅い容器や大きなシャーレで飼育することは可能であるが、手間暇がかかる。言うまでもなく、その容器での産卵や貝の発育は思うように行かずその装置では腹足類を飼育・繁殖させるには余り単純すぎると思われる。

ここで我々の水槽飼育を示すと、それは空気循環と泥-砂の床システムを持ったものである。容易に作れるが空気コンプレッサーが必要である。1週間に2回の給餌以外には飼育管理がほとんど必要でない。数千の貝を飼育するのに1人で充分である。

### 2. ミラシジウムの採取法

マンスン住血吸虫や日本住血吸虫ミラシジウムは走光性であるが、ビルハルツ住血吸虫のそれは負の走光性である。更に唯一の実験動物のハムスターでは人の場合とは寄生部位が異なりその虫卵は尿ではなく糞便中に排泄される。そこで、負の走光性を持つビルハルツ住血吸虫ミラシジウムはマンスン及び日本住血吸虫での糞便あるいは肝臓の中の虫卵のように光で集めて採取することができない。

もし感染したハムスターの糞便を材料とする場合、糞塊をそのまま水に漬け、その周囲で泳いでいるミラシジウムをピペットで吸い取るとよい。もしその肝臓あるいは結腸を使う場合、ホモジナイズした後ペースト状のものをそのまま水に入れて周囲の水を濁さないようにしてミラシジウムが孵化して遊出するのを待つとよい。

この方法は負の走光性を持つビルハルツ住血吸虫卵にとっては唯一のものである。

### 3. セルカリアの採取法

ビルハルツ住血吸虫のセルカリアは昼間に遊出してそのピークは正午頃にある。貝からの1

貝あたりの遊出数はマンスン住血吸虫のセルカリアよりずっと少ない。我々が以前報告したように、ビルハルツ住血吸虫のセルカリアの遊出を促す方法は昼間の遊出途上にて5秒以上に暗黒下に貝を移すことである。大量のセルカリアを一挙に、即ち1時間後にその日の2倍量を採取できる。

#### 4. その他

ビルハルツ住血吸虫の中間宿主貝類（ここでは *B. globosus*）では、10mm以上の成貝は8mm以下の幼貝と比べてはるかに感受性が低く感染させるには多くのミラシジウムを必要とする。もし5mm以下の幼貝に感染させると、容易に感染するがセルカリアを遊出する時期までに充分に貝自身が大きくなり遊出するセルカリアは少なくとどまる。6～8mmの幼貝を使うと貝も充分大きくなり、有効にミラシジウムを使用できる。

New cercariometry

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Cercariometry to be a useful tool should be clear of the following problems (criteria):

1. Water quality:

Natural water including cercaria is of nature from clear to turbid water.

2. Water quantity:

Once cercarial densities in natural waters are very low or scanty, it should be possible to detect cercaria from more than 20 liters of water.

3. Apparatus in field:

It should be easily constructed, easily used and portable.

Up to now, we have not found ideal methods fulfilling the criteria mentioned above. Some basic barriers are noted in concentration of cercariae by filtration and/or sedimentation, such as the following:

1. Since live cercariae are swimming and hanging in water, it



is impossible to sediment them.

2. Since specific gravity of both live and fixative-killed cercaria is near to that of water (1.0), they are easily flying up and hanging in water sample due to physical phenomenon (convective water current). The current physically continues while water temperatures are different in parts. Therefore, it is impossible to sediment formalin-fixed cercariae in field conditions in which the water temperature differs from air temperature and the wind cooling upper part of water is blowing.
3. Dead cercariae killed by the fixative are very sticky.

Our new method for cercariometry involve direct sedimentation the tails as well as the whole bodies of cercaria, using of a wide flat tray. The method eliminates convective current in water to sediment cercariae. As far as water samples ranging from 10°C to 40°C, the convective water current hardly occurs in flat water body with less than 25 mm depth in the tray, even if the volumes are various (no limit in quantity). The brief procedure are as follows:

1. Fix cercariae in water sample in a bucket by formalin (0.15 %).
2. Ten min later, detach cercariae resting on the side and botton of the bucket by a soft painting brush.
3. Remove the water into a wide flat tray (60 X 40 cm; any sizes are available) up to about 20 mm depth.
4. Settle the water for 10 min to sediment all of cercariae

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and tails on the botton.

5. Suck slowly the surface water by an aspirator (300 cc per min) down to 8-10 mm depth. Return to Step 4 by using the same tray or another smaller tray, if it is needed to repeat.
6. While brushing and flashing clean water in the container, remove all the water (less than 300 cc) into a bottle.
7. Add formalin up to 2 % of concentration for the water sample and take it to laboratory.
8. Count both the whole bodies and the tails for the total number of cercariae under a dissecting microscope.

It is easy to detect the cercaria number in the formalin-fixed water by counting both whole bodies and the characteristic forked tails under dissecting microscopy, even without staining. Around 50 % of cercaria number comes from the tail number. Instead of sucking by aspirator, it is effective to overflow surface water by slowly declining the tray with keeping 20 mm depth at a side hole in a corner of the tray. To decline the tray, it needs a trivet table with three screws.

This technique fulfills all the criteria and is recommended as ideal for cercariometry.

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## 新しいセルカリア検出法

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セルカリア検出法が有効であるためには次の問題点をクリアしなければならない。

### 1. 水質の問題

セルカリアを含む水は透明なものから濁ったものまで様々である。

### 2. 水量の問題

自然水のセルカリアの密度は非常に低いので20ℓ以上の水からセルカリアを検出しなければならない時もある。

### 3. 野外で使う装置であること

装置は容易に組み立てられ、容易に使用でき、携帯に便利である必要がある。

現在まで上記の問題点を解決した理想的な方法は全くない。幾つかの基本的な障害としてセルカリアの濃縮をフィルターの使用あるいは沈澱法を行う際に次のようなものがある。

1. 生きたセルカリアは水中に泳ぎながら漂っているので沈澱させることは不可能である。
2. 生きたセルカリアでも固定液で殺したセルカリアでも、その比重は水(1.0)に近いので対流という物理的現象によって水中に容易に舞い上がり漂う。対流は物理的に水中で水温の異なるところがある間は継続して起こる。それ故、水温が大気温度と異なったり、水を急速に冷やす風が吹いているような野外条件での(ホルマリン)固定死滅したセルカリアを沈澱させることは不可能である。
3. その固定セルカリアも非常にくっつき易いという性質をもつ。

今回の新しいセルカリア検出法はセルカリア自体のみならずその尾も沈澱させるもので、広い平担なトレーを使い水中での対流を無くしてセルカリアを沈澱させることができることに特色がある。10～40℃の間の水において、対流はトレーにて25mmの深さに広げた平面的な水では生じない。そしてその水量は平面が広がる程に無限に多くなってもよい。簡単な手順は次のようになる。

1. バケツの水の中のセルカリアをホルマリン固定(0.15%)する。
2. 10分後、ハケでバケツの壁・底にくっついたセルカリアを浮遊させる。
3. 広い平担なトレーに20mmの深さまで注ぎ込む。
4. セルカリア自体とその尾が沈澱するのを待つ(10分)。

5. 表層の水を吸引器で8~10mmの深さになるまでゆっくり(毎分300cc)取り除いていく。検査する水が多ければステップ4から繰り返す。更により小さなトレーで最終段階のセルカリア濃縮を行う。
6. トレーをハケでブラシしながら水を吹きつけ、全ての水を標本ビンに移し入れる。
7. その水を2%のホルマリン固定液にして実験室に持ち帰る。
8. 実体顕微鏡下にセルカリア自体とその尾を検出して数える。

このようにして染色しなくても実体顕微鏡下に特徴のあるセルカリアとフォーク状の尾を検出してセルカリア数を求めるのは容易である。セルカリア数の50%は尾の数で占められる。尚吸引器を使わないでトレーの前の一端にある穴(20mmの高さ)からオーバーフローするよりにトレーを少しづつ傾ける方法で代用し得る。トレーを傾けるには三脚ネジ付台を用いるとよい。

この方法は先の問題点を解決し、理想的なセルカリア検出法として推奨される。

**SUMMARY AND RECOMMENDATION**

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Of the several parasitic diseases found in Kenya, schistosomiasis haematobium is one of the major health problems. For this reason schistosomiasis haematobium was therefore identified as the one of the research subject for technical cooperation with Japan International Cooperation Agency (JICA) within the communicable diseases research and control project (CDRCP). Since CDRCP had a major objective to find an effective and practical model for the prevention and/or control of communicable diseases, a high priority was given to the epidemiological studies of schistosomiasis. Profound understanding of the epidemiology of the disease is essential for successful control measures. In the present project, the epidemiological information gathered included prevalence and intensity of the infection, the intermediate snail hosts, and human ecology (human water contact). Beside field research, it was also found necessary to emphasize immunology and the basic science research on the parasite and the intermediate snail host. Presearch in these areas promoted not only the search of the useful diagnostic technique but also the understanding of the disease background.

Although the epidemiological information obtained in our study might not be adequate to discuss the transmission dynamics of *S. haematobium*, a trial run of control started in February, as our project was drawing to an end.

### Epidemiology:

Based on the preliminary survey on the geographical distribution of S. haematobium in Kwale district, Coast Province, the study area was confined to Mwachinga village with a population of about 1,200. In the initial survey done in 1982, out of 1,217 residents, 851 individuals provided urine for examination. The overall prevalence of a S. haematobium was 68%. Intensity of infection was expressed by the number of eggs excreted per one hour at midday in order to follow up the change of Intensity precisely. The prevalence and intensity of infection increased in young children up to a peak at age 10 - 14 years, and then declined. The rate of decline was greater in males than in females. The water contact study showed that children have more and longer water contact than adults, and that such water contact is often greatest during the middle of the day. Females aged 20-40 years frequently arrived at water points for household needs: Cercariometry done by the filtration method revealed that the cercarial density in the infested water in and around the study area was highest around noon. The results of water contact and cercariometry probably reflect the age- and sex- related difference in infection level. The epidemiological information probably suggests that children act as the major source of transmission by urinating indiscriminately in or near fresh water. The snail population and its infection rate with S. haematobium showed seasonal fluctuation synchronizing seasonal climatic changes. Seasonality of transmission is most likely in this area.

The integrated study on the spread of infection from man to snail, ecology of snail (movement and aestivation), and acquired human resistance to the infection should be encouraged.

### Immunology:

Priority for research was given to the immunodiagnostic methodology which would be suitable for field use, with special reference to circumoval precipitin test (COPT). S. haematobium eggs from urine of patients were first reported to frequently have the precipitate similar to those formed in COPT.

Fluorescent antibody test revealed the presence of IgG and IgM in the precipitate. It is therefore important to treat S. haematobium eggs with pepsin prior to a COPT, when the eggs from patient are used as the egg antigen.

A simplified slide preparation of COPT was developed. Field testing of this procedure is recommended as one of the methods for seroepidemiological and immunological studies.

#### Basic Science:

Emphasis was put on the biological studies on parasites and intermediate snail hosts. The highlight of the studies are as follows.

The timing of the midday meal affects the diurnal output of S. haematobium eggs in urine. This new finding probably leads to studies on the mechanisms and circadian rhythm of egg excretion. The application of this phenomenon to epidemiology is now under way with the purpose of detecting the light-infection cases.

S. haematobium eggs were stimulated to hatch by physical factors, such as light, mechanical agitation and low osmotic pressure. These factors may be taken into account, when miracidial hatching test is used for evaluation of efficacy of chemotherapy.

Tolerance of snail to drought differed much among the different species. The snails which inhabit a pond are likely to tolerate drought, while snails living in a stream seem to be sensitive to the dryness. The difference in the tolerance for dryness may be one of the factors which are responsible for characteristic geographical distribution of the vector snails.

The new technique for cercarial concentration (cercariometry) was developed in the laboratory. Evaluation of this method for field use is now under way.

### **Control of schistosomiasis:**

As control of schistosomiasis is unlikely to be satisfactory using any one single method, the control measure adopted in our programme was a combination of chemotherapy and safe water supply. Six hundreds and ninety villagers were treated with Metrifonate and the study area also provided with the piped water supply. The efficacy of treatment on patients, the infection rate of snails, and the change of human ecology are now being investigated.



## まとめと助言

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ケニアではまだ多くの寄生虫病が蔓延し、なかでも住血吸虫症はその対策の必要性が叫ばれている疾患の一つである。そこで国際協力事業団のケニア国への医療技術協力プロジェクト（伝染病研究対策プロジェクト）においては、ビルハルツ住血吸虫症が寄生虫学分野での研究対策としてとりあげられることとなった。このJICAプロジェクトは、ケニア国の伝染病の予防およびコントロールに寄与することを基本的な目的としたので、住血吸虫症のプログラムでは、疫学的研究に重点が置かれた。疾病の対策を計画し、また遂行するために、まず疾病の疫学を十分理解する為である。本プロジェクトでは住民の感染率、感染の強さ、中間宿主貝の種類、密度、感染率、およびヒトの水との接触様式等について詳しい多くの情報が集められた。一方野外研究活動以外にも、本プロジェクトでは、寄生虫体、虫間宿主についての基礎的研究および免疫学的研究の必要性も叫ばれ、これらの研究も精力的に行なわれた。これらの研究は、ビルハルツ住血吸虫症の診断法の開発に役立つのみならず、本症の本態をより深く理解する上に大いに役立つものである。

野外調査研究プログラムを遂行する場合、出来れば、対象とする疾患のコントロールのための適当な対策を講じることが望ましいことはいうまでもない。我々が現在まで得ることが出来た疫学的情報は、対象地区におけるビルハルツ住血吸虫症の伝搬を論ずるには十分とはいえないかも知れないが、プロジェクトの終息をむかえた1984年2月に、コントロール対策の試行が始められた。

### 疫学的研究

沿岸州クワレ地区におけるビルハルツ住血吸虫症の分布に関する予備調査の成績を参考に、1981年ムワチンガ村（人口約1,200）が本プロジェクトの研究対象地区に選ばれた。1982年の第一回の調査では1217名の住民中、851名について検尿を行った。感染率は68%であった。感染の強さは、出来るだけ正確にその変化を追跡できる様に、昼間1時間当りに排泄された虫卵数で表わした。ムワチンガ村では、感染率も感染の強さも年齢とともに高くなり、10～14才でピークに達し、その後減少する。減少の程度は女性に比し男性で強い。ヒトの水との接触行動の観察より、成人に比べ子供は頻回にまた長時間水と接触すること、子供の水との接触頻度は昼間に高いこと等が明らかにされた。また20～40才の女性は家事のためによく水場を訪れる。一方対象地区およびその周辺で行ったセルカリ

アメトリー（濾過法）により、水中のセルカリア濃度が真昼にピークに達することも明らかにされた。年齢および性による感染率と感染の強さのちがいは、上記のヒトの水との接触様式、水中のセルカリア濃度の成績よりも説明出来そうである。子供は見境もなく水系近くで排尿し、ビルハルツ住血吸虫症の伝搬の主役であることが十分想像できる。貝の密度と、その感染率は季節によって変化することが明らかにされた。このことは対象地区では感染を受ける時期が限られている可能性を暗示している。

伝搬様式をさらに詳しく知るには、今後、貝がどの様に感染するか、貝の生態（移動や夏眠）、ヒトの再感染抵抗性等について観察が行われることが必要であろう。

#### 免疫学的研究

野外調査での使用に適した免疫診断法の開発、特に虫卵周囲沈降反応（COP T）の改良に重点がおかれた。患者尿より得たビルハルツ住血吸虫卵にはCOP Tで形成される沈降物に類似する沈降物がみられることが注目され、蛍光抗体法により上記沈降物 IgG、IgM が存在することが報告された。報告は、さらに患者尿より得た虫卵を抗原として用いてCOP Tを行う場合は、虫卵をペプシンで前処理する必要性を述べている。

野外でも行える簡便なCOP T法が開発された。これを用いることで、ビルハルツ住血吸虫症の血清疫学的研究も可能であろう。

#### 住血吸虫卵・セルカリア、中間宿主に関する基礎的研究

住血吸虫卵・セルカリア、貝についての種々の生物学的研究が精力的に行われ、下記のような成果が得られた。

ビルハルツ住血吸虫卵の尿中への日内排泄パターンは昼食をとる時刻によって影響を受けることが明らかにされた。この所見は虫卵排泄機序および生体リズムの研究の進展に寄与するとともに、軽症者の発見にも貢献するものと考えられる。

ビルハルツ住血吸虫卵の孵化は光、振動、低浸透圧によって促進される。孵化試験を治療の効果判定に用いる場合、上記のような孵化に及ぼす物理的要因を考慮すべきである。

中間宿主貝は種によって乾燥に対する抵抗性がことなることが報告された。池に棲む貝は乾燥に対して抵抗性が強い傾向があり、一方小さな流れに棲むものは弱い傾向がある。このような乾燥に対する抵抗性の違いは伝搬貝の特長なる分布様式を定めている要因の一つかも知れない。

新しいセルカリア法（セルカリアメトリー）が実験室で開発され、現在、野外でその効用が試されている。

#### コントロール

患者を治療するのみでは、十分な成果があげられないため、本プロジェクトではメトリホネートによる3患者の治療と対象地区への水道水供与を行った。690名が投薬を受けた。治療効果の判定、コントロール開始後の貝の感染率およびヒトの水との接触行動の変化の観察が現在行われている。今後、住血吸虫症の伝搬に関与する疫学的要因の継続観察、水道設備の維持が望まれる。

3.3

**Bacteriological and virological studies  
at the Communicable Diseases Research  
and Control Project, 1979 - 1984**

**Reviewed by**

**Pster G. Waiyaki  
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## A. Introduction

The Communicable Diseases Research and Control Project (CDRCP) was set up through an agreement between the Governments of Kenya and Japan with the objective of carrying out research on communicable diseases in Kenya. Diarrhoeal diseases which afflict millions of people were identified as a priority and it was decided that their aetiology should be determined. To this end in the CDRCP laboratory, bacteriological, and virological investigations were initiated. The studies done by the parasitology group will be reviewed elsewhere by others and the discussion which follows is a brief overview of the bacteriological and virological studies which were carried out by Japanese scientists and their Kenyan Counterparts.

### I Bacteriological studies

#### 1. Survey on the causative bacteria of diarrhoea in Kwale District

In bacteriology, Japanese and Kenyan workers (Gatheru et al., 1983) launched a one year survey to determine the seasonal variation of bacteria responsible for diarrhoea in the Kwale District. Kwale was chosen as an example of a rural lowland area. Laboratory work commenced in 1981 at Tiwi Health Centre and at other medical facilities. Fresh stools were collected from outpatients visiting the medical facilities and complaining of diarrhoea with or without abdominal pain. Stools were processed for the isolation and identification of Shigella species, Salmonella species, enteropathogenic Escherichia coli (EPEC), Vibrio cholerae and Vibrio parahaemolyticus. Bacteriological analysis of drinking water was also done on three different occasions in March, June and July. Water sources were chosen at random at Waa, Ngombeni, Likoni and Tiwi. Meteorological data were obtained from the Meteorological Department in Nairobi.

The findings of this investigation were as follows: all water samples were contaminated with human intestinal bacteria i.e. Proteus, Pseudomonas, Klebsiella, Enterobacter, Citrobacter and EPEC. The highest rates of isolation of Shigella, Salmonella and EPEC were obtained

During a cholera outbreak in March 1982, 532 stool specimens were collected from inhabitants in the pilot areas. Two cases from Shirazi camp were positive for V. cholerae and were Ogawa serotype. A survey of the water sources at Shirazi was carried out and no Vibrio or Kappa phage were detected.

Following information that 10 cases of cholera had been found in Vingujini near Msambweni, a fourth surveillance was carried out in April, 1982. There was one positive case detected in Fahamuni. V. cholerae was also isolated from well water. At Vingujini, 200 stool specimens were bacteriologically confirmed. This represented an infection rate of 7%. V. cholerae was also isolated from water taps. All the cholerae strains including the lone case from Fahamuni were serotype Inaba. In the May 1982 surveillance it was not possible for the scientists to reach some of the pilot areas because of the heavy rains. However, of 379 stool specimens and water samples collected, no V. cholerae were isolated. In the follow-up sixth surveillance carried out in June, 1982 attempt was made to examine previously known cholera positive cases and their household contacts. Out of 47 previously positive cases, 17 cases and 65 household contacts examined were all negative for V. cholerae. To check the carrier state after the outbreak, 710 specimens from school pupils in the Vingujini area were examined and all were negative for V. cholerae.

In the seventh and final surveillance carried out in August 1982, the finding in June 1982 that there were few or no cases after May 1982 <sup>was</sup> confirmed. Of 341 specimens examined from outpatients with diarrhoea at all medical facilities (except Msambweni), all were negative for V. cholerae. Thus, it was concluded that the cholera outbreak in 1982 was a relatively minor one and that there were few healthy carriers or actual cases. This was attributed to several factors such as improvement in sanitary and hygienic habits of the inhabitants, the tireless effort made by public health officers to follow up contacts with suspected patients and household contacts, suspicion that only a minor cholera outbreak in Tanzania occurred near the border, economic problems resulting in fewer seasonal workers coming from areas where cholera is endemic and constant surveillance which hastened detection before the infection could spread.

The recommendations made from the findings of this study included: (1) Isolation and treatment of patients, (2) Management of contacts, (3) Environmental sanitation and (4) Surveillance and control at national and international levels.

### 3. Characterisation of Vibrio cholerae isolated in Kenya in 1983

Kaviti et al, (1984) collected 247 strains from cholera patients in Nyanza Province. They found that all the strains were El Tor Celebes original type. All were resistant to phage IV. The majority of the strains were serotype Ogawa but 9 were Inaba. A test to determine the strains resistance or sensitivity to polymyxin B showed that 246 strains were resistant and only one strain was sensitive. The majority i.e. 194 strains were resistant to tetracycline, streptomycin and ampicillin. However, no strains were resistant to gentamicin. In the chicken erythrocyte agglutination test, 246 strains were positive. One strain (No.166) was, however, negative. The same strain was also not sensitive to phage IV and was resistant to polymyxin B. Although all the strains showed lysogenicity, none were sensitive to the Kappa phage. Seventy - three percent of the strains were hemolytic for chicken red cells. In 1975, only 11% of V.cholerae isolated in Kenya were hemolytic. It appears that the hemolytic behaviour of V.cholerae is on the increase and should be monitored regularly. In the fermentation tests using sucrose, mannose and arabinose all the strains tested belonged to Heiberg group 1. A survey on water sources was also done but after the cholera outbreak had subsided. Out of 44 specimens collected from different sources, 14 nonagglutinable Vibrio and 11 strains of Aeromonas were isolated. No V.cholerae was isolated from the water sources. Arising from the results of this study, it was recommended that examination of water sources should continue and should also be carried out during cholera outbreaks in the area. It was also suggested that resistance by V.cholerae to various drugs currently in use should be closely monitored.

### 4. Enteropathogenic Escherichia coli in Kenya

Ehara (1984) compiled data on EPEC isolated in Kenya in 1983. Using a battery of antisera for serotyping EPEC isolated mostly from the Coast and Nyanza Provinces, Japanese scientists and their Kenyan counterparts identified 28 different serotypes. It was found that serotype 027:kt was quite prevalent. Out of 390 E.coli isolates identified as EPEC, 41 (10.5%) belonged to 027:K+. The isolation rates of the other EPEC ranged from a low of 2 (0.5%) for serotype 0144:Kx2 to 29 (7.4%) for serotype 0128:K67. There were 23 (5.9%) cases of double infection. In Kwale, the infection rate was found to peak in the 13-19 year age group. Further and expanded studies need to be carried out to identify the extent to which these organisms cause diarrhoea in Kenya and also their seasonal variation in different parts of the country.

##### 5. Enterotoxigenic Escherichia coli (ETEC) in Kenya

Watanabe (1984) summarised data on ETEC isolates from the Coast and Nyanza Provinces. In the study carried out in the Coast Province during the months of July, September and November 1983, 762 stool specimens were obtained from children with diarrhoea. All the children were under 14 years old. E. coli isolates were checked for the labile toxin (LT) production using the Biken test and for the heat stable toxin (ST) using the suckling mouse assay. There were 47 ETEC isolates. This gave an infection rate of 6%. Twenty one ETEC were LT producers and 24 were ST producers. Two were both LT and ST producers. From the limited data available it appears that ST producers predominate and that the rate of ETEC isolation in Kenya is relatively low compared with that reported in some other countries.

Analysis between the patients age groups and ETEC isolations indicated that ETEC could inflict illness in all age groups. However, the peak incidence was in the 20-25 month age group. There was no significant difference in the sex distribution of ETEC isolates.

In the study done on healthy villagers in South Nyanza and Kwale the ETEC isolation rates were 5.4% and 3.8%, respectively. Out of the 5 ETEC isolates obtained from villagers in Kwale District, 4 were ST producers and 1 produced both LT and ST. In the South Nyanza study stool specimens from a total of 258 people were investigated. Fourteen ETEC were isolated. Eight were ST producers and 6 were LT producers. There were no double producers isolated. In another study carried out in Kwale on 84 healthy high school students, 2 ETEC (1 ST and 1 LT producers) were isolated. The overall isolation rate in healthy people was 4.4%. From the limited data on ETEC isolation from healthy people available, the ST producers predominated (13 out of 21 ETEC isolated). Expanded studies on the epidemiology of ETEC need to be carried out to assess their relative contribution to the aetiology of diarrhoea in this country.

##### 6. Occurrence of Campylobacter jejuni in diarrhoeic stools at the Coast General Hospital and Mvita Clinic in Mombasa

Waiyaki et al. (1984) reported on a Campylobacter jejuni survey done in Mombasa in 1983. The investigation was undertaken to determine the frequency of C. jejuni infection among children with acute diarrhoea. Clinical and epidemiological characteristics of C. jejuni infection were defined. The study



was carried out during the months of July, September and November. The subjects were 779 children ranging in age from 0-8 years. The majority were under 2 years. Most of the children had acute diarrhoea with a history of less than 5 days before they were brought to the clinics for treatment. None had received antibiotic treatment. From the 779 stool specimens processed for bacterial isolation, 90 C. jejuni isolates were obtained. During the July survey (a cool month in Mombasa) the isolation rate was 17%. During the survey in September the isolation rate was 5.4%. In the third survey done in November the isolation rate was 12.2%. The average isolation rate for all the three surveys was 12.6%. Other enteropathogens isolated included 91 EPEC, 26 ETEC which were ST producers, 23 ETEC which produced LT, 17 Shigella species, 13 Salmonella species and 3 Vibrio cholerae non O - 1. During second and third surveys, 3 Vibrio fluvialis and 5 Aeromonas hydrophila strains were also isolated.

From the 98 cases which were positive for C. jejuni; there were 17 cases with mixed infection. In 11 cases EPEC were isolated along with C. jejuni. Two EPEC belonged to serotype O125:K70, 2 were O148:K+ and the rest were one of each of serotype O14:K74, O126:K71, O142:K+, O127:K63(2), O26:K60, O152: K+(1) and O143:Ki. There were also 3 cases of double infections with Shigella. One was Shigella sonnei, 1 Shigella flexneri and the other Shigella serotype B13. There were two cases of mixed infection with Salmonella and EPEC. The age distribution of children with C. jejuni enteritis indicated that children of upto 2 years of age were more frequently infected than older ones. The peak incidence was in the 19 - 24 month age group. There was no significant difference in the distribution on C. jejuni among males and females. Nearly all isolates were biotype 1. One strain was biotype 2. Most Campylobacter infections in man are caused by biotype 1 and the results were in agreement with this general observation.

## II. Virological Studies

### 1. Studies on rotavirus - Monthly survey of rotaviruses in faeces

Makino et al. (1983) carried out a monthly survey on children in Nyeri and Mombasa and found that up to 50% of diarrhoeal cases were due to rotavirus. The infection could be detected almost every month in both areas.

### 2. Analysis of rotavirus genomic ribonucleic acid (RNA) by electrophoresis

Chiba et al (1984) used techniques of polyacrylamide gel electrophoresis on viral RNA segments and studied rotavirus strains and their relative contributions to rotavirus gastroenteritis epidemics in two major districts of Kenya. Their

studies extending from early 1982 to the middle of 1983 showed 18 representative electropherotypes detected in 30 rotavirus specimens obtained from Nairobi. Included in this group were 6 short types. From the Coastal areas, 16 electropherotypes (including 3 short strains) were detected <sup>from</sup> 70 virus specimens. With the exception of 1 strain, there were no identical electropherotypes between the two groups of rotaviruses obtained from the two districts. In early 1983 there was a change in the predominant electropherotype observed in Mombasa. Later, new occurring strains were detected in a small town along the coast when an apparent increase in gastroenteritis was observed in the district. The electrophoretic migration patterns of the genomic RNA of rotaviruses from the two districts showed that there were various rotavirus strains. This was in agreement with the findings of other workers in different parts of the world. A surprising finding was that so many different electropherotypes from the two districts could be identified in such a relatively short period of time. This has not been the general experience of workers in other countries. This work observed that the characterization of rotavirus is important in elucidating the epidemiological nature of the infection <sup>in</sup> tropical areas.

### 3. Epidemiology of Human Rotavirus (HRV) infection in Kenya

Iiyazaki (1984) collected stool specimens in Mombasa, Kilifi and Mariakani and tested for HRV by Elisa and subgrouped the strains by genomic RNA analysis. His findings showed that in Mombasa 25% of infantile gastroenteritis was due to HRV. The highest rate of infection was seen in children less than 1 year old. No correlation between climatological factors and the monthly detection rate was observed. The long electropherotype was common in Mombasa but a change of the prevalent strain from the long type to the short type was observed during the surveillance period.

In Kilifi, 78 (26.8%) out of 291 specimens were positive and in Mariakani 6 (9.5%) out of 63 specimens were positive. The results obtained in Mombasa and Kilifi were similar but in Mariakani the isolation rate for rotavirus was rather low.

Approximately 30% of the gastroenteritis among children age 12 months or less was due to HRV in Mombasa and Kilifi. The incidence of rotavirus infection decreased gradually in children after the age of 12 months in Mombasa. In Kilifi the incidence suddenly came down to 11% in infants aged 2 years.

The monthly detection rate of rotavirus in Mombasa reached a peak of 50% in July, 1982 and the lowest isolation rate of 8% in December. In Kilifi the monthly variation ranged from 0 to 47%.

Genomic RNA of the virus was analysed by polyacrylamide gel electrophoresis. The distance between segments 5 and 6 is wide in the short type virus. Miyazaki (1984) found many minor variants. The long type was predominant (64% in Mombasa and 71% in Kilifi). The short type was rare until August 1983 after which short type strains increased and became predominant.

Although there was no correlation between monthly incidence and climatological in Mombasa there were, nevertheless, some monthly waves when the virus was detected.

### 3. Electropherotypes of human rotavirus in Nairobi

Studies carried out by Miyazaki (1984) showed that 71% of rotavirus electropherotypes were long type and 29% the short type. These results together with those obtained in Mombasa have led to the suggestion that the long type is predominant in Kenya.

### 4. Mixed electropherotypes of human rotavirus in Kenya

Other studies (Miyazaki, 1984) on rotavirus showed the existence of mixed electropherotypes of HRV in Kenya. Two HRV isolates with extra RNA bands were found. One, strain M1438 isolated at the Coast General Hospital was classified as long type according to the electrophoretic mobility of segments 10 and 11. Double bands were observed at the site of segment 1. Another strain, P125 isolated in September 1983 at the Kenyatta National Hospital in Nairobi was also assigned to the long type because of the rapid electrophoretic mobility of its segments 10 and 11. In this strain the distance between segments 5 and 6 was as wide as that observed in the short type. There was also an extra band near segment 5 and another was located at the site of segment 10 of the short type strain. The isolate was a mixture of the long and short types. It was not established whether these mixed electropherotypes resulted from reassortment, mutation or mixed infection of different strains.

### 5. Human rotavirus diarrhoea outbreaks in small units (Miyazaki, 1984)

Out of 5 stool specimens obtained from neonates in a nursery at the Coast General Hospital, 3 were positive for rotavirus. In Nairobi diarrhoea outbreaks occurred in an orphanage. The first outbreak took place in January, 1983. Enteropathogenic Escherichia coli (EPEC) was isolated from a healthy adult member of staff. Six

stool specimens from the children did not yield rotavirus but in 4 specimens virus-like particles were seen. It was not determined what the particles were. In the second outbreak involving 4 cases, EPFC was isolated. In the third outbreak involving 12 cases, no pathogens were isolated from the stools.

#### 6. Rotavirus infections in children of different races

Notanda et al. (1983) investigated rotavirus infections in African, Asian and European children with diarrhoea and who were attending a private clinic. No statistically significant difference in incidences of rotavirus among the three groups of children were found. In all children studied, rotavirus infection reached a peak in the 12-3 month age group. Diarrhoea due to rotavirus continued to occur up to age 11 years in European children and 6 years in African children. Viruses were not detected in faeces of African children aged over 3 years. The infection showed two seasonal clusterings, one in February and March and the other in July and September.

#### 7. Enzyme-linked Immunosorbent assay (ELISA)<sup>in</sup> detection of antibody to rotavirus

Kibua and Makino (1983) used the ELISA technique to detect IgG<sup>class</sup> antibody to rotavirus in sera and to assess its application in field screening. They found that ELISA was more sensitive than the indirect immunofluorescence test. The 68 specimens used in the study were collected in Nyeri from patients aged 1 to 70 years and suffering from different diseases. The majority of the patients had rotavirus antibody by the age of 5 years. Infant groups under 5 months of age showed the lowest rate i.e. 33.3%. The rate increased with age and reached a peak at the age of 9 years and thereafter decreased slightly in the age group 10 years and above.

The study recommended that future research should focus on the measurement of IgA and IgM in mucosal secretions and sera.

#### 8. Presence of antibodies against rotavirus and polio-viruses in the residents of Coast Province of Kenya

Sato et al. (1984) showed that 60% of infants under one year of age (4-12 months) had antibodies to rotavirus. The percentage of sera with rotavirus antibody ran into 100% at 1-2 years of age and then the rate of antibody positive ratio gradually decreased after three years of age. For polioviruses it was shown that a considerable percentage of children under 6 years of age did not possess neutralising antibodies to poliovirus types 1, 2 and 3. The authors recommended that a proper vaccination programme for residents of the Coast Province should be considered.

B. Concluding remarks

From the above brief review, it is apparent that much useful data on the aetiology of diarrhoeal diseases in Kenya have been obtained. Further and expanded studies need to be carried out. Arising from these, it is hoped that appropriate rational measures can be taken to reduce the menace of diarrhoeal diseases.

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3.4 PARASITIC INFECTIONS:

Intestinal Parasites and Schistosomiasis

In Coast Province. Kenya.

JICA/KEMRI PROJECT (CDRC)

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## PARASITIC INFECTIONS: SCHISTOSOMA HAEMATOBIMUM

### INTRODUCTION

Intestinal parasitic infection is one of the major problems plaguing most of the developing countries in the tropics. Control, eradication or prevention of such parasites has been rendered difficult by the social and economical problems. Ignorance and the lack of a good understanding of the epidemiology of the disease.

A joint project was started under Kenya/Japan co-operation in 1979 to establish the prevalence of the human intestinal parasites in Coast Province. Initially this was done as a preliminary study to obtain a base-line data for later intensive epidemiological studies.

The study was centered in Kwale District. Although no intestinal schistosomiasis was found in the District, urinary schistosoma haematobium was endemic and it became the point of an intensive epidemiological study. As a result of this study, measures to control schistosomiasis were implemented.

Schistosomiasis is estimated to affect about two hundred million people in the world. It is one of the most important public health problems in the tropical and subtropical countries, in view of its prevalence, wide distribution and the extent of morbidity.

The rapid development of irrigation in the agricultural methods, hydroelectric power etc. in the developing countries have caused a great spread of schistosomiasis in many countries and has posed serious economic problems. Many projects and studies have been performed as an effort to prevent, control

or eradicate schistosomiasis in various parts of the world. Such studies show that for a successful employment of a control or prevention measures a thorough epidemiological study of the disease is paramount.

Schistosomiasis study program was started in a Joint Kenya/ Japan project in 1981. The objective was to determine the mode of transmission of schistosoma haematobium infection and the implementation of the control measures. Mwaching village in Kwale District Coast Province was selected as a study area following the results of a preliminary study on intestinal parasitic diseases carried out between 1979-1981.

Under the close co-operation between Japanese parasitologist, members of Kenya Medical Research Institute, the Division of Vector Borne Diseases and the Kinango Hospital much new information was obtained.

The epidemiological study indicated that the main source of transmission was the children aged between 10-15 years and that the villagers were likely to get infection in the rivers around noon. It was also noted that transmission did not occur between February and April. From these studies control measures were implemented. This included chemotherapy, supply of clean water and health education.

The aim of treatment is not only to relieve the patient of the disease but also to reduce the rate of passage of the schistosoma eggs into the water. The eggs excreted into the environment are the source of transmission. The treatment of patients was conducted by the staff of Kinango Hospital from February to April 1984. About 82 per cent of the patients were treated with metrifonate during this period.

The water source in the study area was highly contaminated with schistosoma cercariae. Hence a supply of clean water was necessary. Water lines and stands were started in October, 1983 and completed in February, 1984. The water is now being used by the villagers.

Control measures will not be complete without the dissemination of health education to the community. With the co-operation and the assistance of the District Health Officials, health education was given to the villagers and especially to school children. Health education, however, should be a continuous process. A follow-up study is in progress.

The influence of the neighbouring villages in the success of the measures employed for the control of the infection in the study area is also very important. A study in which a buffer zone is being created in order to reduce such influence is underway.

#### Intestinal Parasites and Schistosoma haematobium

Initially the parasitology section in the Communicable Diseases Research and Control Project embarked on a study to establish the intestinal parasites that might be associated with diarrhoea, especially in children in Coast Province, Kenya.

The study was done in a hospital set up (Coast General Hospital). The results indicated giardiasis, amoebiasis and Trichuriasis to be important agents of diarrhoeas.

The second phase of the study was extended to include schistosomiasis and was community based. Kwale District in Coast Province was selected for this study. The district is composed of four Divisions; Kinango, Kubo, Central and Southern.

The rate of infection in school children represent the general distribution of the parasite and the transmission rate in the community. For this purpose, 41 schools out of the 135 primary schools in the District were selected.

In the study of the prevalence and the distribution of parasites the results showed Ascaris Lumbricoides, Hookworm and Trichuris trichiura, and Entamoeba coli predominantly. (Unpublished<sup>2</sup>). A. Lumbricoideasis and Trichuriasis appeared in endemic state in the Central and Southern Divisions which are predominantly muslim areas. Hookworm distribution was highest among the helminths in all four divisions. A high concentration of Entamoeba coli was observed in Kinango and Kubo Divisions. This was largely attributed to the poor distribution of water, hence a high rate of water contamination by the villagers.

No intestinal schistosomiasis was found in the whole District whereas the urinary Schistosoma haematobium was endemic in all four Divisions. The absence of Schistosoma mansoni and the predominancy of S. haematobium was attributed to the inability of S. Mansoni to withstand the prolonged dry and hot climatic conditions in Kwale District<sup>3</sup> S. haematobium indicated a high rate of survival in this harsh environmental conditions.

The results from the 41 primary schools indicated a high S. haematobium infection and prevalence rate in the school children<sup>4</sup>. The highest per cent rate of infection was seen in Kinango Division (70%) followed by Southern Division. The infection rate in Central and Kubo Divisions was relatively low.

The result obtained from this study in schistosomiasis was used as a baseline data to determine the appropriate location for a pilot study for an epidemiological survey and to assess the possibilities of employing a control method for the infection in the community.

Mwachinga village in Kinango Division was selected for the pilot study and epidemiological survey was commenced. This village was selected because it represented the typical hinterland condition, it permitted interaction of the inhabitants with the villages outside, there were no irrigation practices and the area streams are seasonal. The land is dry and hot in the most part of the year, and the villagers are forced to use the contaminated water. A major water pipeline that supplies Mombasa Town passes through the village. However, this water is scarcely used by the villagers partly because of economical reasons and partly because of the restrictions that, until recently prohibited the use of this water.

The initial population in the survey area was about 1340 persons, adults and children included. The parameters considered for the epidemiological survey included, the prevalence, incidence and the intensity of infection, dynamics of transmission, including snail infection and population survey, cercariometry and human activities and contact with the contaminated water.

A quantitative examination of urine was carried out by the micropore-filtration method. Egg counts per unit of time was done microscopically to determine the intensity of the infection.

### prevalence

The results in the epidemiological study as compiled at the end of the joint Kenya Medical Research Institute/Japan Project<sup>5</sup> indicated that 68 per cent of the 1338 persons surveyed harboured schistosoma parasites. The infection rate increased with age in the first decade of life, showing a clear peak of 100% at 10-15 year age group especially in the males. After the peak, the prevalence dropped down rapidly and remained at 60 per cent consistently in the older groups. In the females, although the prevalence also increased rapidly showing a peak of 98 per cent also at 10-15 year age group, it declined gradually in contrast to that one of the male.

A general profile of age-prevalence showed that the high prevalence remained for about ten years after the peak of the infection rate and the decline appeared to start at age 20 in both sexes<sup>6</sup>. However, the infection started earlier in females than in males.

### Intensity

The overall geometric mean egg counts was 50.2 eggs per hour. It was 52 eggs in females and 47 eggs in males per hour. The difference in the two groups was not significant, however.

The curve for the egg intensity showed a more or less similar pattern as that of age-prevalence in both sexes. The average egg excretion was highest in 10-15 year age group with the peak reaching about 1250 eggs per hour.

### Incidence

In order to determine the seasonal change in the incidence,

negative cases were followed every three months. The final results indicated an incidence rate of 39 per cent in children and 47 per cent in adults.

#### Snail Survey

Thirteen sites, busy with human water-contact activities were selected for snail survey<sup>7</sup>. The population of snails started to increase after the long rain season around April to June and reached a peak around November. About 30 per cent of the snails collected after the peak population were infected with *S. haematobium* parasites. Snails were during the larger part of the year.

#### Water Contact

Human activities at the selected busy water sites were studied to determine the probabilities of acquiring infections from the contaminated water. Bathing and washing part or parts of the body for all age groups were the major activities observed. However, for the younger age groups, swimming, playing and fishing were the major activities. Females of middle age group contacted water more frequently, especially for drawing water and washing clothes. The study also showed that the children under 15 years of age visited water sites mainly in the afternoon. The adults were observed to contact water more frequently in the late hours of the morning and in the evening.

#### Cercariometry

Cercariometry was done by filtration method (Prentice 1982<sup>8</sup>). The daily periodicity and the emergency of the cercariae

in the natural water was determined from the schistosoma contaminated water sites. The daily periodicity of the cercarial emergency was detected. The highest density of cercariae was observed at 11.00 a.m. at all study sites, with a few cercariae present at 9.00 a.m. and after 4.00 p.m. A correlation between the cercarial density and the snail population was assessed by determining the population in the cercariometry sites. This study, however, did not show any correlation between the infection rate in the snail population and the cercarial density.

#### Control Measures

The implementation of the control measures was started after the analysis of the epidemiological data. Water lines were extended to six difference points and water kiosks were constructed. Chemotherapy was done using metrifonate in three doses, 7.5mg/Kg each. All positive cases were treated in all age groups and all positive and negative cases in 5-15 year age group. About 82 per cent of the patients had received treatment by the end of the program in April 1984. A follow-up study is in progress to evaluate the effects of the control measures employed.

#### Serodiagnostic Studies

Tests were done to study the possibilities of getting a simple serodiagnostic method for schistosoma haematobium. The curcumoal precipitin (COP) test is common in S. mansoni diagnosis. Although microscopic presence of the schistosome egg in the urine of the patient is the best diagnostic method for S. haematobium the COP test is good for the after-treatment studies.



Previous report suggested the presence of naturally occurring precipitates around the eggs obtained from urine<sup>9</sup>. A study done in our laboratory<sup>10</sup> described the presence of such precipitates and indicated the need to digest them with pepsin before testing them for COP reaction. Further study compared the sensitivity of COP reaction with that of the countercurrent immuno-electrophoresis (CIE) test (comm).

### COP Test

Direct examination of eggs obtained from the school children in Kwale District showed a pattern similar to that observed in the COP test<sup>10</sup>. These precipitates were identified as Ig G and Ig M in origin in a fluorescent antibody test. The presence of Ig G and Ig M in the urine was also detected by radial immunodiffusion test. This suggested that these immunoglobulins contributed in the formation of the precipitates in the eggs. This was further strengthened by the presence of anti-egg antibodies in the urine. The precipitates were removed by treating the egg with pepsin.

The presence of antibodies against eggs in the urine was examined by COP test on the pepsin treated eggs. The intensity of the positive COP reaction correlated well with increased haematuria. Quantitative measurement of Ig G level in the COP positive reaction eggs by radial immunodiffusion assay well correlated with the urine positive for Ig G.

A comparison study was also done between the COP reaction and Countercurrent immunodiffusion (CIE) assay (comm.) Schistosomiasis positive sera, egg antigen and adult worm antigen were used for this purpose. The results showed a positive rate

of 91 per cent in COP test and 79 per cent in CIE test. The CIE test also showed more false negative cases than the COP.

### Discussion

The initial studies in the five year Joint Kenya/Japan project established amoebiasis as the main cause of diarrhoea in children in the Coast. General Hospital and the Hookworm, Askariasis and Trichuriasis as the major intestinal parasites in the school children in a field study in Kwale District.

Although combined schistosomiasis, intestinal and urinary are common in many parts of Kenya, only the urinary form was found in Kwale District and in an endemic state. The climatic conditions seemed to favour only *S. haematubium* which showed an infection rate of 68 per cent of the survey population. The prevalence histogramme was characteristic of many *S. haematubium* studies in males. Females, however, showed an unusual pattern in which the decline after the peak prevalence was much less marked. That may be due to the difference in the water contact behaviour between the two groups. The rate of exposure to the contaminated water in females was higher than in the males and also at the time in which there were higher risks of acquiring infection. That was apparently associated with the female's daily domestic chores.

The 5-15 year age group was largely associated with the bulk of the transmission of the infection. This was understandable in view of the high rate of exposure to the dangerous water. This age group was also noted to be less likely false negative, hence it was more appropriate for the incidence studies. The incidence rate calculated every six months for a

few years might describe the seasonal influence in the transmission. This, however was not done in our study.

Increased snail population was seen after the long rains. This was followed by a rapid increase in the snail infection rate. The decline in the rate of the water flow after the rains favored the breeding of the snails. Most of the water also become stationary, and in large pools in which there were increased human water activities, hence the increased snail infection rate as was indicated by the number of the snails infected and the cercarial density. The hourly emergence of the cercariae agreed with the report of Nojima and Sato (1978)\*\*. The peak cercarial density in the contaminated water was around noon.

Delton and Pole (1978)\*\* reported that the total duration in water contact in younger male group was higher than in females in the same age group, a finding also seen in our study. That meant increased probabilities of acquiring the infection and explained the higher rate of infection seen in the 10-15 year age group.

The water situation in the study area largely determined the establishment of the snails population, while the rate of infection and the water activities in the villagers determined the transmission rate. In the later part of the dry season a rapid decline in the snail population occurred. Some snail population successfully aestivated. This could take a period of four to five months preceeding the rains. Conclusively, this disruption of the snail population and hence a decline in the probabilities of acquiring the infections can be used as the basis of the control measures for the infection and the trans-

mission. Hopefully, this will be realized.

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Communications

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