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CERCARIAL DENSITY IN THE RIVER OF AN ENDEMIC AREA OF SCHISTOSOMIASIS HAEMATOBIA IN KENYA

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Abstract. The cercarial density in natural water and number of infected *Bulinus globosus* were monitored over a one-year period to identify the transmission foci in an endemic area of schistosomiasis haematobia in Kenya. Overall prevalence and intensity of infection of the study community were 59.2% and 10.9 eggs/10 ml of urine. Cercariometry was carried out on 456 occasions at 20 study sites while snail sampling was done on 465 occasions at the same sites over a one-year period. Cercariometry was exclusively done at flowing water habitats. The results showed the focality and seasonality of transmission. Cercariae were detected on 44 occasions at 11 sites. The detections were made on seven occasions at two study sites, six occasions at one site, four occasions at four sites, three occasions at one site, two occasions at two sites, and one occasion at one site. Densities of 1–4 cercariae/100 liters of water were found on 31 occasions. Five to nine cercariae/100 liters of water were found on seven occasions, 10–19 cercariae/100 liters of water were found on two occasions, and high cercarial densities greater than 20 cercariae/100 liters of water were found on four occasions. The highest count was 52 cercariae/100 liters of water. The presence of cercariae in natural water was shown to depend on the water temperature, but the intensity and duration of sunlight did not affect the presence of cercariae in water. The monthly variability of cercarial density was proportional to the number of infected snails. Cercarial density was highest in March and April, in the middle of the rainy season, whereas no cercariae were detected in cool dry season. The snail population peaked late in March, the beginning of the long rainy season, remained high for two months, and decreased rapidly late in May when heavy rain occurred. The overall infection rate of snails was 7.3% and the majority of infected snails were collected from March to May. There was no definite correlation between the presence or absence of cercariae and infected snails. Cercariae were frequently found where infected snails were absent and cercariae were sometimes absent where infected snails were present. Cercariometry and snail sampling remain quite complementary in identifying the transmission foci of schistosomiasis.

Several technologies have been proposed for identifying water bodies that are transmission sites for schistosomiasis. The large-scale use of sentinel rodents to detect transmission foci has already been shown to be logistically difficult, expensive, and too slow for an effective response.[1] Snail collection shows the general pattern of transmission for individual sites and for the area as a whole, but it provides information about the intensity of the parasite flux from the vertebrate to the snail vectors and thereafter the rate of deposition of schistosome eggs in the sites.[2] Cercariometry allows direct evaluation of the cercarial density depending on the volume of filtered water, although the presence of cercariae other than those of human schistosomes, such as *Schistosoma bovis,* is a problem. Recently, Theron and Prentice demonstrated the advantages of the direct filtration technique.[2, 3] The method showed a high recovery rate and is easy to use in the field.

Cercariometry can be used in a hydrographic network map in an endemic area of schistosomiasis over long periods to evaluate contamination risk to human beings as a function of sites and time.[2] However, this approach has had little application in epidemiologic studies.

In the present study, the number of *S. haematobium* cercariae in natural habitats was monitored over a one-year period by cercariometry supplemented with sampling of field snails. Prevalence and intensity of infection in this study area is also described.

MATERIALS AND METHODS

Study area and population. The study was done in Mtsangatamu village, Kwale District in Coast Province, Kenya during the period 1985–1987. Mtsangatamu is approximately 10 km southwest of Kwale town and has an area of approximately 5–6 km.[2] It is bordered by Shimba Hills forest on the eastern side from which originate four rivers that flow through the village. The Mtsangatamu, Tswaka, and Mwale rivers join the Mbadzi river (Figure 1), and the flow of these rivers is perennial except for certain parts of the Mwale river, which dry up in the hot dry season. The climate of the area may be divided into four seasons: a long rainy season (April–June), a cool dry season (July–October), a short rainy season (November), and a hot dry season (December–March). The maximum and minimum air temperature were recorded daily in the shade, and rainfall was measured on a daily basis with a rain gauge located at the center of the study area. Population census and mapping were carried out in the entire village in 1987. The total population registered was 1,250 (623 males and 627 females).

Cercariometry and snail sampling. Cercariometry and snail sampling were done at 20 water habitats; 10 sites in the Mwale river, three sites in the Tswaka river, and seven sites along the Mtsangatamu river (Figure 1). The water temperature of the sites was determined using a glass alcohol thermometer (Iuchi, Osaka, Japan). The flow of the river at these sites was categorized as dry, stagnant, flowing, or flood.

Cercariometry. Cercariometry, which preceded snail sampling, was done twice a month over a one-year period from November 1985 to October 1986 using the method of Prentice with a slight modification.[3, 4] The usefulness of the author's apparatus for quantitative survey work in natural water has been established.[4] In that apparatus, the recovery

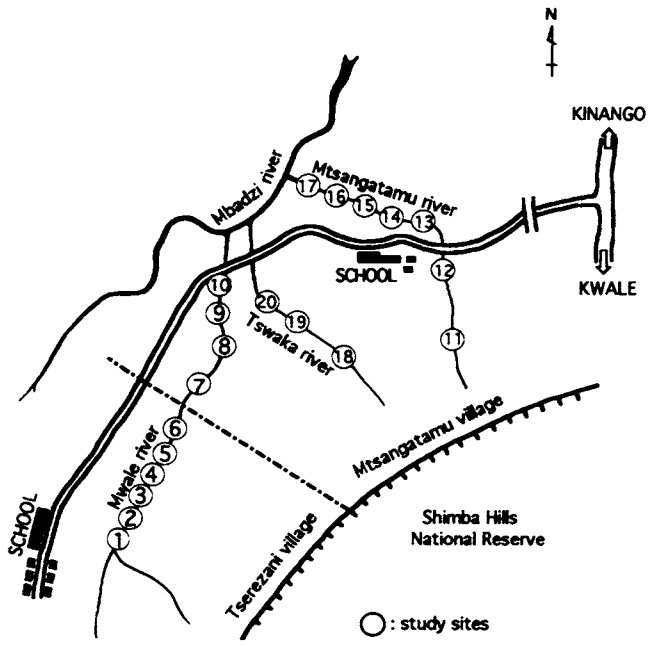

FIGURE 1. Study area showing cercariometry and snail sampling sites, Kwale, Kenya. The **white arrows** indicate the direction to the nearest town.

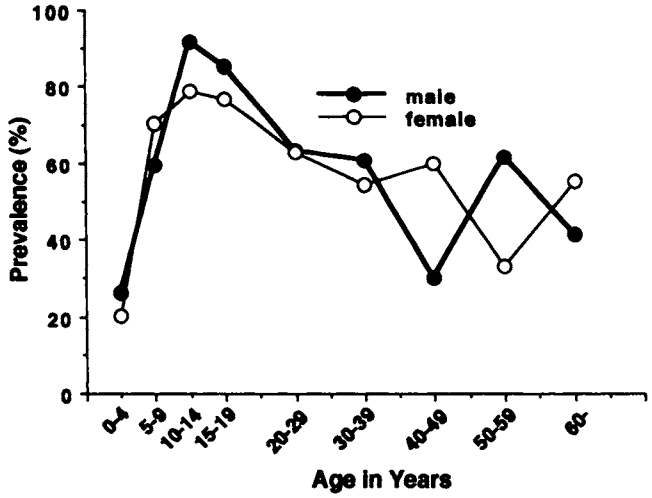

FIGURE 2. Prevalence of schistosomiasis haematobia in Mtsangatamu in relation to age.

filter is a square sheet of nylon plankton net (105 × 105 mm) with a pore size of 41 um (Type NXXX 25; NBC Industries Co. Ltd., Tokyo, Japan). One hundred liters of clear surface water or 25–50 liters of turbid water was collected in buckets and formalin solution was added to give a final concentration of 0.2%. The samples were gently swirled from time formalin was added until filtration was completed to minimize the problem of cercarial stickiness induced by the addition of formalin. A single recovery filter successively handled 50 liters of clear river water or 5–25 liters of turbid water. When all liquid had passed through the recovery filter, the filter was removed, placed on a filter paper support (15 cm in diameter, GF/A; Whatman, Maidstone, United Kingdom) and put into a transparent plastic bag with an airtight zipper. For staining cercariae, the filter paper support was wetted with 5–6 ml of a 0.05% solution of light green dye in 2% acetic acid. Microscopic observation was done directly through the plastic bags. The cercarial counts were transformed to log (x + 1) to normalize their distributions and variance.[5]

Snail survey. The snail survey was done after cercariometry at the same sites using the method of Noda and others.[6] Snails were collected for 10 min by one searcher using a double-layer steel net shovel. The snails were then identified and *Bulinus globosus*, the vector snail of *S. haematobium* in the study area, was put into small petri dishes filled with dechlorinated tap water. The petri dishes were kept in a lighted place and the water was examined for cercariae under a stereoscopic microscope. No attempt was made to detect nonshedding infection by crushing the snails. The snails other than *B. globosus* were not examined for their trematode infections.

Experimental animal infection. This was conducted to examine the presence of schistosomes other than *S. haematobium* in our study area. The cercariae used were those released from a batch of 13–14 infected snails collected at the snail survey done in April and May 1996. Seven BALB/C mice and seven golden hamsters were anesthetized with pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL), shaved on the abdomen, and exposed to approximately 200 cercariae each for 30 min following the method of Erickson.[7] Since the most reliable and practical criterion for the identification of schistosome species in Africa is the shape of the ova, the animals were autopsied 15 weeks after infection and the livers and intestines were examined for eggs.

Urine examination. Urine specimens of villagers were examined in February 1987 using the filtration method of Peters and others.[8] The study was approved by the Scientific Steering Committee and the Ethical Committee at the Kenya Medical Research Institute. Informed consent from villagers was obtained after explanation of the procedure planned. The intensity of infection was expressed as the number of eggs/10 ml of urine. The geometric mean was obtained by using the n + 1 transformation for a series of egg output including zeros.

RESULTS

Prevalence and intensity of infection in the study area. Urine specimens of 966 villagers were examined. Overall prevalence was 59.2%, 60.2% in males and 57.6% in females. Intensity of infection in the community was 10.9 eggs/10 ml of urine, 12.1 eggs/10 ml of urine for males and 10.2 eggs/10 ml of urine for females. The peak prevalence and intensity of infection occurred in children between 10 and 14 years of age, with a low prevalence and intensity of infection in the older age group (Figures 2 and 3).

Cercarial density in river water. During the one-year program of study, a total of 480 cercariometry tests were intended to be done during the study period. However, tests were not done 15 times at four study sites, habitats 6, 8, 9, and 10, due to low water level at the end of the dry season and nine times at nine study sites, habitats 3–8 and 18–20, due to a flooding river at the end of the long rainy season.

FIGURE 3. Intensity of infection with schistosomiasis haematobia in Mtsangatamu in relation to age.

Subsequently, the number of occasions on which cercariometry was successfully done was 456 times at 20 sites. One hundred liters of water was filtered for 56% of the tests, while 50 and 25 liters of water were filtered for 21% and 23%, respectively, of the tests done. The number of recovery filters used for a test varied from two to five depending on the turbidity of the water. Three types of furcocercous cercariae were observed on the recovery filters. One of them was frequently encountered and was similar to human schistosome cercariae in terms of size and appearance. Since identification of cercariae to the species level is not possible on the basis of morphologic criteria, they were counted as *S. haematobium* cercariae. Two others were only rarely observed. One was long with a longifurcate tail, and the other was short with a brevifurcate tail. Since it is difficult to identify these cercariae morphologically, especially on the recovery filter, the present paper does not deal with these organisms.

The number of the tests done at each study site and results of cercariometry are shown in Table 1. Of 456 tests, schistosome cercariae were detected on 44 occasions at 11 sites. Cercariae were detected seven times in 24 tests at one study site, seven times in 23 tests at one site, six times in 24 tests at one site, four times in 24 tests at two sites, four times in 23 tests at two sites, three times in 24 tests at one site, two times in 23 tests at one site, two times in 21 tests at one site, and one time in 19 tests at one site. The number of cercariae in natural habitats was frequently low. Of 44 occasions on which cercariae were detected, 31 showed low densities of 1–4 cercariae/100 liters of water, seven showed 5–9 cercariae/100 liters, and two showed 10–19 cercariae/100 liters. High densities of more than 20 cercariae/100 liters of water were detected four times. The highest counts were 46 and 52 cercariae/100 liters of water observed at habitats 1 and 2 within the Mwale River on in November 1985 and April 1986, respectively.

Based on the mean annual cercarial density, five sites within the Mwale River showed relatively high cercarial densities (0.370–0.639 cercariae/100 liters). Along the Mtsangatamu River, there were two sites with relatively high densities (0.281–0.425 cercariae/100 liters). At the other sites, no cercariae were detected during the study period or at best densities less than 0.161 cercariae/100 liters of water

TABLE 1
Results of cercariometry and snail sampling in the Mwale, Mtsangatamu, and Tswaka rivers, Kwale, Kenya

Study sites	No. of occasions		No. of cercariae in 100 liters of water		No. of occasions			No. of *Bulinus globosus*	
	Cercariometry done	Cercariae detected	Geometric mean	Range	Snail sampling done	Snails detected	Infected snails detected	Collected	Infected
Mwale River									
1	24	4	0.405	0–46	24	10	3	37	3
2	24	6	0.435	0–52	24	14	4	76	9
3	23	4	0.394	0–28	24	5	2	7	3
4	23	7	0.639	0–11	24	6	0	8	0
5	23	2	0.125	0–4	24	8	1	20	1
6	19	0	0	0	21	5	1	71	1
7	23	4	0.370	0–7	23	8	1	14	1
8	20	0	0	0	21	4	0	6	0
9	21	2	0.089	0–2	21	1	0	1	0
10	19	1	0.037	0–1	19	2	0	3	0
Mtsangatamu River									
11	24	0	0	0	24	0	0	0	0
12	24	3	0.161	0–5	24	12	5	154	13
13	24	0	0	0	24	6	1	15	1
14	24	7	0.281	0–3	24	23	14	431	43
15	24	4	0.425	0–28	24	20	4	267	13
16	24	0	0	0	24	3	0	4	0
17	24	0	0	0	24	11	0	89	0
Tswaka River									
18	23	0	0	0	24	0	0	0	0
19	23	0	0	0	24	1	0	1	0
20	23	0	0	0	24	0	0	0	0

TABLE 2
Correlation between water temperature and presence of cercariae

Water temperature (°C)	No. of occasions: Cercariometry done	No. of occasions: Cercariae detected	%
≤24.9	89	0	0
25.0–25.9	60	1	1.7
26.0–26.9	97	7	7.2
27.0–27.9	123	16	13.0
28.0–28.9	70	16	22.8
≥29.0	17	4	23.5

were observed. Of 456 cercariometry tests in the study area, 444 were carried out in flowing habitats, seven in static habitats, and five in habitats in flood conditions. At flowing water habitats, cercariae were detected 42 times at 11 sites; 28 times at eight sites within the Mwale River and 14 times at three sites within the Mtsangatamu River. At static water habitats, sites 5, 7, and 9, cercariae were detected two times at site 7. Cercariae were detected frequently at habitats with high water temperature (Table 2). The correlation between water temperature and presence of cercariae is significant ($P < 0.001$, by chi-square test for trend). At water temperatures lower than 25°C no cercariae were detected. The presence of cercariae was not influenced by sunlight. Cercariae were detected 24 times in 275 tests on sunny days and 20 times in 160 tests on cloudy days. No cercariae were detected in 21 tests done on rainy days.

Monthly fluctuations in the cercarial population in the study area are shown in Figure 4, together with rainfall and temperature. The highest count was observed in March and April, in the middle of the long rainy season. The other peak was seen in November and December (short rainy season). In January, June, July, August, and September (cool dry or hot dry seasons), no cercariae were detected. This variability seems to be proportional to the number of infected snails.

Snail population and rate of infection in *B. globosus*. Snail sampling was not done on 15 occasions at five sites due to the low water level in the hot dry season. The number of occasions on which *B. globosus* was collected and the numbers of *B. globosus* collected and infected are shown in Table 1. Relatively few *B. globosus* were collected less frequently (63 times in 225 tests) from the Mwale River. Based on the number of sites, the *B. globosus* population in the Mtsangatamu River (960 snails, seven sites) was four times that in the Mwale River and snails were collected more frequently (75 times in 168 tests). However, within the Mtsangatamu River, some habitats were better than others for supporting snail populations. Habitats 14, 15, 12, and 17 had 431, 267, 154, and 89 snails, respectively. Habitats 13 and 16 had few snails, and habitat 11 had no snails. Only one snail was collected in the Tswaka River (Table 1).

Monthly fluctuations in the snail population in the study area are shown in Figure 4, together with rainfall and temperature. The variability seems to correlate with rainfall and temperature. All observation sites except for five habitats within the Mwale River had flowing water throughout the year. At the end of the hot dry season when the long rainy season started, populations were augmented in both the Mwale and Mtsangatamu Rivers. The population reached a peak late in March, remained high for two months, and decreased rapidly late in May when heavy rains occurred; the floods probably dislodged snail populations from the habitats. The effect of a short rainy season was insignificant in our study area.

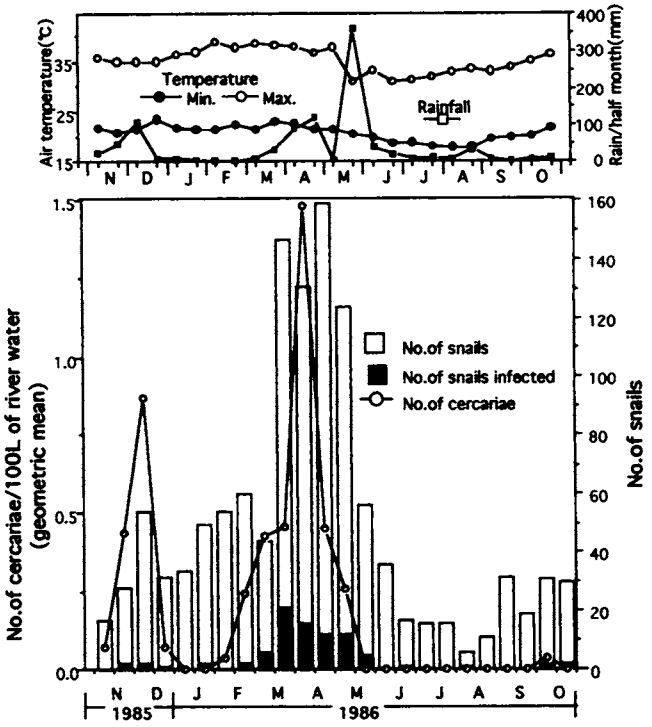

FIGURE 4. Monthly fluctuations in cercarial density, snail populations, and number of infected snails together with rainfall and temperature.

Infected snails were collected at six sites within the Mwale River and four sites within the Mtsangatamu River. The overall infection rate at the sites in the Mwale river (7.4%) was as high as those in the Mtsangatamu River (7.3%). Most of the infected snails (84 of 88) were collected at habitats 1, 2, 3, 12, 14, and 15. No infected snails were collected at habitat 17, although a relatively high population of snails was recorded at that site. Eighty-two percent of the infected snails were collected from March to May.

In addition to *B. globosus*, some other snail species such as *Cleopatra* spp., *Lanistes* spp., and *Melanoides* spp. were collected. *Cleopatra* spp. were frequently collected at habitats 7 and 8, whereas *Lanistes* spp. were frequently collected at habitats 1, 2, 4, 6, 7, 8, 13, 14, and 17. However, *Melanoides* spp. were seldom collected. These snails were not examined for trematode infection.

Experimental animal infection. *Schistosoma haematobium* eggs were detected in the livers and the intestines of all animals experimentally infected. No eggs other than those of *S. haematobium* were recovered from any of the animals.

Correlation between the presence or absence of cercariae and infected snails. Cercariometry is expected to be superior to snail sampling in detecting the site of transmission because this technique can detect cercariae where infected snails are absent, as well as where they are present.[2] High cercarial density (0.639 cercariae/100 liters of water) was detected at the site where no infected snails were col-

TABLE 3
Correlation between the presence or absence of cercariae and infected snails

Data sets	No. of occasions
Cercariometry (+) Snail infected (+)	14
Cercariometry (+) Snail infected (−)	29
Cercariometry (−) Snail infected (+)	22

lected (Table 1). An attempt was therefore made to determine how effectively cercariometry detected the cercariae where infected snails were absent and how frequently cercariometry detected the cercariae in sites where infected snails were present.

Among the 480 possible observations, 455 yielded both cercariometry and snail sampling data. Many observations (390) showed neither cercariae nor infected snail. The remaining 65 showed some cercariae and/or infected snails. These results are shown in Table 3. Unexpectedly, cercariae were detected only in 14 of 36 tests in which infected snails were collected, and cercariae were frequently detected at the sites where no infected snails were seen.

DISCUSSION

This is the first report that has indicated the actual number of cercariae in water over a period of one year in an endemic area of schistosomiasis haematobia. The study was done in a Kenyan village with a relatively high prevalence (59.2%) and intensity of infection (10.9 eggs/10 ml of urine). Many of the cercariometry tests were done at flowing water habitats. The results show the focality and seasonality of transmission as demonstrated in other areas.[9, 10] Of 20 sites examined, 11 yielded cercariae. Cercariae were detected at two sites on seven occasions, at one site on six occasions, at four sites on four occasions, at one site on three occasions, at two sites on two occasions, and at one site on one occasion. Densities as high as 46 and 52 cercariae/100 liters of water were detected at some sites. However, these were exceptional and cercarial densities in natural water were frequently low. The monthly variability of cercarial density seems to be proportional to the number of infected snails. The cercarial density was highest in March and April, in the middle of the long rainy season. No cercariae were detected in January, June, July, August, and September during the cool dry and hot dry seasons. Kloos and others reported an average density of 0.024 *S. haematobium* cercariae/liter of water in the Nile River and 0.029 cercariae/liter in the irrigation canals.[10] The average density of cercariae in our study area was 0.015 cercariae/liter in April, 0.004–0.008 cercariae/liter in November, December, and March, and less than 0.002 cercariae/liter in February, May, and October. In endemic areas of schistosomiasis mansoni, a density of 0.01–144.2 per liter was reported in St. Lucia,[9] 0.05–21, in St. Lucia,[11] 0.05–35.67 in Puerto Rico,[12] and 0.016–4,244 in Machacos, Kenya.[13]

This study has shown that the presence of cercariae depends on the water temperature, with cercariae likely to be present at high water temperatures. No cercariae were present at water temperatures lower than 25°C. Since a laboratory study showed that *S. haematobium* cercariae emerged from snails at 15°C and 20°C as frequently as at 30°C,[14] the absence of cercariae in lower water temperatures in our study area suggests the absence of infected snails. No infected snails were collected in water habitats where the water temperature was lower than 25°C. Cercarial emergence also appears to be affected by the intensity and duration of the available light.[15] However, in the present study, we did not detect any significant difference in the presence of cercariae between sunny days and cloudy days.

It is worth noting that many species of schistosomes other than *S. haematobium* are located in Africa. Kimura and others reported coexistence of *S. haematobium* and *S. bovis* cercariae at Kinango Dam in Kwale, 20 km from our study area.[4] However, morphologic differentiation of *S. haematobium* from other cercariae is virtually impossible. In the present study, we counted those resembling human schistosome cercariae in size and appearance as *S. haematobium*. In Africa, *Bulinus* spp. and *Biomphalaria* spp. have been exclusively incriminated as the intermediate hosts of human and animal schistosomes. In our study area, *Biomphalaria* is apparently absent. Moreover, experimental animal infection showed that the cercariae released from *B. globosus* collected from our study sites were only *S. haematobium*. These facts suggested that schistosome cercariae other than *S. haematobium* are unlikely to be present in our study area.

Our study was supplemented by snail sampling done immediately after cercariometry was carried out so as to effectively compare the results of the two tests. The spatial and seasonal variation of cercarial density in natural water were proportional to the number of infected snails. However, the results of cercariometry were not simply the reflection of the results of the snail sampling. The present study has demonstrated that cercariae could be found at sites where infected snails were absent as frequently as at sites where infected snails were present. Also, cercariae were frequently not detected where infected snails were present. Similar results have been reported by Ouma and others with *S. mansoni* cercariae in Kenya.[5]

There are several possible explanations for the apparent mismatch in the results of snail sampling and cercariometry. The first possibility is that cercariometry was not carried out at hours that coincide with the peak of cercarial abundance in natural water. However, to examine for cercariae in our study area, the water samples were collected between 11:00 AM and 1:00 PM. Laboratory and field studies on *S. haematobium* in Kenya have indicated that the peak of cercarial emission from snails and the highest cercarial density in natural water occurred between 11:00 AM and 2:00 PM.[4, 15] Therefore, the first explanation would not be acceptable. Second, furcocercous cercariae were present that could be confused with *S. haematobium*. It has already been mentioned that nonhuman schistosome cercariae are likely to be absent in our study area. However, in our study area, *Cleopatra* spp., *Lanistes* spp., and *Melanoides* spp. were collected. In the certain areas of African countries, these snails release some brevifurcate cercariae that are confused with schistosome cercariae.[16] Since we did not examine whether

these snails were infected with any trematodes and no information is available on the species of furcocercous cercariae in Kwale, Kenya, we can not exclude the possibility that we counted brevifurcate cercariae other than *S. haematobium*. Third, 10-min scooping was inadequate to demonstrate the presence of infected snails in certain large habitats. Cercariae can be found over a wide area of water where only one infected snail is present.[2] A drawback in snail sampling might have partially caused the mismatch in the results of snail sampling and cercariometry. Furthermore, the cercariometry technique is not sensitive enough to detect cercariae in large-sized habitats with low cercarial densities.[1] Fourth, the cercariae were rapidly carried away from their point of release by water currents. There are some reports that provide supporting data for the disapperance of cercariae at their point of release. For instance, Sturrock reported that although one snail may shed many cercariae, those soon become dispersed.[1] Upatham reported that cercariae were carried downstream as far as 195 m in running water habitats,[17] while Radke and others reported that mice were infected 0.6 km downstream from the point of cercarial release.[18] Since our cercariometry tests were exclusively done in flowing habitats, the fourth idea is most probable. The complexity of transmission foci, hydrodynamic conditions, snail distribution, and cercarial emission pattern from the snails probably interferes with the establishment of a precise relationship between cercarial densities and infected snails. It thus appears that both approaches, cercariometry and snail survey, remain quite complementary for identifying the transmission foci of schistosomiasis.

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