# MEKONG SCHISTOSOMIASIS: 2. EVIDENCE OF THE NATURAL TRANSMISSION OF *SCHISTOSOMA JAPONICUM*, MEKONG STRAIN, AT KHONG ISLAND, LAOS

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## INTRODUCTION

Previous reports (Sornmani *et al.*, 1971, 1973) suggested that the principal source of infection with the Mekong strain of *Schistosoma japonicum* at Khong Island was the river itself. The evidence, however, was indirect: the heaviest worm burdens were reported from residents of Khong Town and its immediate vicinity beside the river.

Direct evidence of transmission was sought, using two methods: 1) Sentinel mice were exposed in cages on the river in or near areas suspected of heavy contamination, and subsequently examined for the presence of schistosome infections, and 2) efforts were increased to collect naturally-infected snails *(Lithoglyphopsis aperta)* at Khong Island.

The present report gives details of the results of these efforts.

## MATERIALS AND METHODS

### Description of exposure cages

The literature contains several descriptions of cages devised for exposing mice to schistosome-contaminated waters (Radke *et al.*, 1961; Pitchford and Visser, 1962; Dazo, 1965). In the present work, a cage was developed by a carpenter following verbal descriptions of what was wanted and the use to which the contraption would be put. The result (Figs. 1, 2) proved somewhat unwieldy but essentially serviceable. The cages were equipped with $\frac{1}{4}$-inch galvanized screen on five sides, the screen protruding about $\frac{1}{2}$-inch below the level of the bottom frame. Teak was used for the frame, with a plywood lid made with an overhang to protect mice from the sun. Outside measurements, not including the lid, were 9 by 6.5 by 6.5 inches. The inside volume was approximately 300 cubic inches. Because the screen did not extend inside beyond the top horizontal frame, mice were unable to crawl into the top of the cage. Adjustable clamps on two sides supported styrofoam floats and the depth of

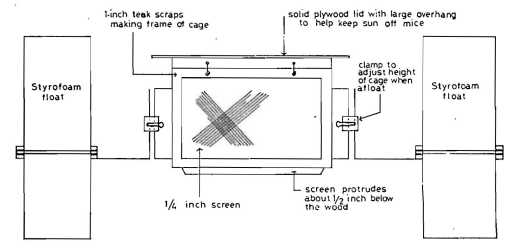


Fig. 1—Diagram of mouse exposure cage used in 1971 and 1972.

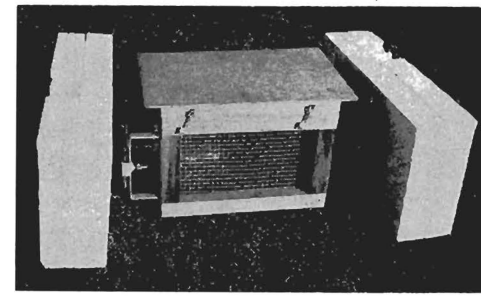

Fig. 2—Photograph of the cage diagrammed in Fig. 1.

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immersion of the floating cage could be controlled by raising or lowering these floats. When the floats were removed, the heavy cage could only float totally submerged.

Four such cages were constructed in Bangkok and were flown directly to Khong Island where the following experiments were performed.

### Exposure of mice on the Mekong River: 1971

Three sites were selected near the Ban Xieng Wang section of Khong Town, where transmission was thought to be active. The cages were anchored in position on short lines in one to two feet of water. Ten laboratory-raised white mice were placed in each cage and exposed at sites A, B and C for 3 hours a day (0900 to 1200 h.) during 5 consecutive days (April 14-18). Approximate locations are shown in Fig. 3. The floats were adjusted to allow the bottom $\frac{1}{2}$-inch of the cage to remain submerged. The current at site A was judged to be somewhat slower

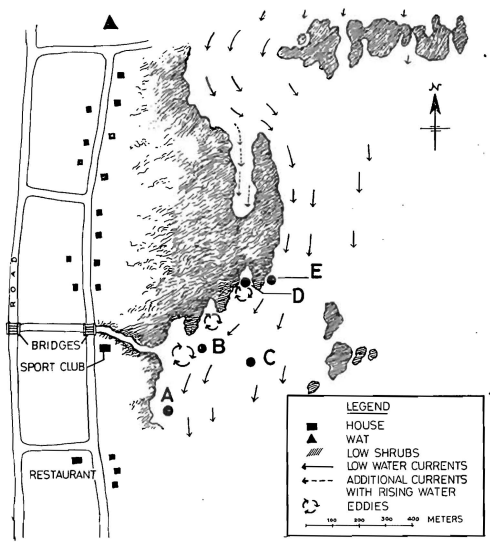


Fig. 3—Detail of the shoreline of Khong Town, Ban Xieng Wang area, showing sites where sentinel mice were exposed to detect schistosomes in 1971 (sites A, B, and C) and 1972 (sites D and E).

that at sites B and C but there was a gentle movement of water at all three sites. True velocity was not measured. Most of the mice got thoroughly wet at the beginning of the exposure but subsequently climbed up the screening, leaving only their tails in the water. A fourth cage of 10 mice, placed too close to shore, was attacked by a dog which broke it open and killed or released the mice: these animals are not considered in this report. In June and July, 1971, another 30 mice were exposed in the water adjacent to Ban Xieng Wang but, because of technical difficulties, none survived long enough to permit inclusion in this report.

### Exposure of mice on the Mekong River : 1972

When the team members returned to Khong in April, 1972, they found that, in addition to the cage destroyed by the dog, two others had disappeared. Moreover, the styrofoam floats on the remaining cage had fragmented to a degree where they were unable to support the cage in the water. Accordingly, this cage was propped in shallow water on rocks for the following experiments. Two groups of 10 mice each were exposed for a total of 40 hours on alternate days at sites D and E (Fig. 3). Exposures at site D were made on April 22, 24, 26 and 28 while exposures at site E were made on April 23, 25, 27 and 29. The two sites were in about a foot of water, separated by a low spit of sand. Water currents were dissimilar at these sites. Site E had a detectable, although gentle, current whereas site D was protected from the main current and water movements were by means of eddies. The cage was submerged in about $1\frac{1}{2}$ inches of water; the mice stood up on their hind legs at first, getting completely wet, but later crawled up on the screen letting their tails hang in the water.

## Snail collections

In previous work, a snail assigned to *Lithoglyphopsis aperta* Temcharoen, 1971, was found experimentally capable of serving as an intermediate host of the Mekong schistosome (Harinasuta *et al.*, 1972; Sornmani *et al.*, 1973). A search for this snail was made during a two-month period (April-May) in 1972, collecting at sites around the perimeter of Khong Island with emphasis on inhabited localities (Fig. 4). Snails were collected by removing submerged branches, twigs and rocks and shaking them over a bucket, or they were taken individually from submerged rocks, twigs, leaves, beer cans, and other solid objects to which they adhered.

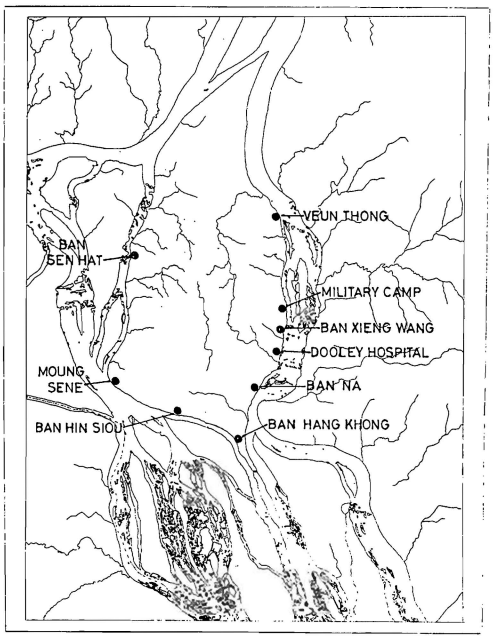


Fig. 4—Map of Khong Island, Laos, showing populated sites around perimeter where *Lithoglyphopsis aperta* were collected in 1972.

In April, 1971, small numbers of *L. aperta* were found in collections consisting predominantly of *Manningiella* species on the east side of Khong Island in the vicinity of Khong Town (Sornmani *et al.*, 1973). However, in April and May, 1972, there was a virtual population explosion of *L. aperta* near Khong Town, particularly in the neighborhood of Ban Xieng Wang, the same site collected in 1971 (Fig. 4). Relatively large numbers of *L. aperta* were taken here, as well as near the Military Camp at the north end of town, the Dooley Hospital at the south end, and Ban Na (Table 3, Fig. 4). Smaller numbers of this species were collected at other villages around the island. *L. aperta* were not found on the sandy shores of the north end of Khong Island.

In examining snails for the presence of schistosome infections, crushing was not used because large numbers of live snails were required for subsequent life-cycle studies planned for the laboratory in Bangkok. Instead, snails were isolated in plastic shell vials in groups of 20, left for three hours under a lamp or in the sunlight, and then examined under a dissecting microscope for the presence of naturally-shed cercariae. Vials containing cercariae were set aside and the snails in them re-isolated into individual vials.

Positive snails shed eyeless, brevifurcocercous cercariae which resembled those of *Schistosoma japonicum* in appearance and behaviour. A pool of cercariae from all of these snails was used to infect 3 mice and a weanling puppy on Khong Island. The number of cercariae available at a given time was small. Infected snails shed from 2 to 3 to 20 cercariae per day. There were never more than 20 snails shedding at one time. Each mouse was anaesthetized and exposed to 40 cercariae by looping. The puppy was anaesthetized with Nembutal and 550 cercariae were applied with a loop to a shaved portion of the abdomen.

Animals exposed in the field were brought directly to the laboratory in Bangkok for further investigation.

## RESULTS

### Mouse exposures

Of the mice exposed on the river in April, 1971, prepatent survival was relatively high (26 survivors out of 30, or 87 per cent). No eggs appeared in the faeces of any of these mice, however, and no worms were recovered when mice were killed and perfused 60 days after the initial exposure on April 14 (Table 1).

In Groups D and E, exposed April 1972, a total of 17 mice out of 20 (85 per cent) survived the 45-day prepatent period which had previously been observed in mice exposed to the Mekong schistosome (Sornmani *et al.*, 1973).

In Group D, one mouse was found dead on June 9, 48 days after its initial exposure on April 22; when tissues were examined microscopically, eggs were found in liver and in the mucosa of the small intestine, but none were seen in simple smears of the faeces. The tissues were too macerated to recover or count adult worms. Three other mice in this group were found dead on June 16, 55 days after initial exposure; eggs were found in liver and in faeces but again the tissues were macerated and the adult worm load could not be determined. The six remaining mice in Group D were killed and perfused 55 days after initial exposure. Five proved to contain male and female worms which appeared morphologically indistinguishable from *Schistosoma japonicum*. However, of 8 mice surviving in Group E, only 2 proved to be infected when killed on June 22, 60 days after initial exposure on April 23, and these had only male worms (Tables 1, 2).

### Snail collections

Forty-eight snails were found to be positive during the 1972 field work on Khong Island, and 18 more positives were detected when surviving collections of *L. aperta* previously recorded as negative were re-examined by shedding later in the Bangkok laboratory.

Table 1

Natural infections with *Schistosoma japonicum*, Mekong strain, in sentinel mice exposed on the Mekong River at Khong Island in areas suspected of affording heavy human transmission.

| Group[1] | Date of exposure | Number of mice | Time of exposure (hours) | Number of mice alive 45 days after initial exposure | Number of mice positive for adult worms |
|---|---|---|---|---|---|
| A | April 14-18, 1971 | 10 | 15[2] | 10 | 0 |
| B | April 14-18, 1971 | 10 | 15[2] | 7 | 0 |
| C | April 14-18, 1971 | 10 | 15[2] | 9 | 0 |
| D | April 22, 24, 26 28, 1972 | 10 | 40[3] | 10 | 9/10 |
| E | April 23, 25, 27, 29 1972 | 10 | 40[3] | 8 | 2/8 |

(1) For locations of Group sites, see Fig. 3.
(2) Exposed 3 hours per day for 5 consecutive days.
(3) Exposed 10 hours per day on 4 alternate days.

Table 2

Results of exposing sentinel mice to possible schistosome infections on the Mekong River next to Khong Island, April, 1972.

| Group | Mouse | Infection | Worms recovered 55 days after initial exposure | | | |
|---|---|---|---|---|---|---|
| | | | Single males | Single females | Pairs *in copula* | Total worms |
| D[1] | 1 | + | 2 | 1 | 2 | 7 |
| | 2 | + | 7 | 3 | 6 | 22 |
| | 3 | + | 5 | 1 | 3 | 12 |
| | 4 | + | 9 | 8 | 3 | 23 |
| | 5 | + | 4 | 4 | 1 | 10 |
| | 6 | +[3] | | | | |
| | 7 | +[4] | | | | |
| | 8 | +[4] | | | | |
| | 9 | +[4] | | | | |
| | 10 | – | | | | |
| E[2] | 1 | +[5] | 2 | - | - | 2 |
| | 2 | +[5] | 5 | - | - | 5 |
| | 3 | – | | | | |
| | 4 | – | | | | |
| | 5 | – | | | | |
| | 6 | – | | | | |
| | 7 | – | | | | |
| | 8 | – | | | | |

(1) Exposed April 22, 24, 26 and 28, 1972. Cf. Fig. 3.
(2) Exposed April 23, 25, 27 and 29, 1972. Cf. Fig. 3.
(3) Found dead on June 9 (48 days after initial exposure). Mature eggs in liver, immature eggs in mucosa of intestine; no eggs in faeces. Tissues too macerated to recover adults.
(4) Found dead on June 16 (55 days after initial exposure). Mature eggs in liver and faeces. Tissues too macerated to recover adults or count worms.
(5) Worms recovered 60 days after initial exposure.

The largest population of *L. aperta* was found adjacent to the Ban Xieng Wang area, which yielded 15,790 specimens. The Military Camp produced 1,750, whereas immediately downstream, at the Dooley Hospital, 4,132 specimens were collected. The village of Ban Na yielded 6,342 specimens. Elsewhere around the island, populations of *L. aperta* appeared to be much smaller and fewer individuals were collected (Table 3, Fig. 4).

Natural infections detected by shedding were found only in snails collected near Ban Xieng Wang, the prevalence being 0.30 per cent (48 of 15,790).

Multiple collections were made at the Khong Town sites and at Ban Na but the other localities around the island were visited only once, in May. For this reason the numbers of snails collected and reported in Table 3 do not represent accurate differences in population density. However, the collec-

Table 3

Sites where *Lithoglyphopsis aperta* were collected at Khong Island in April and May, 1972, and examined for cercariae resembling *Schistosoma japonicum*. (Cf. Fig. 4).

| Site | No. of snails collected | No. of snails positive | Per cent positive |
|---|---|---|---|
| Ban Veun Thong | 185 | - | - |
| Moung Khong: | | | |
| Military Camp | 1,750 | - | - |
| Ban Xieng Wang | 15,790 | 48(1) | 0.30 |
| Dooley Hospital | 4,132 | - | - |
| Ban Na | 6,342 | - | - |
| Ban Hang Khong | 150 | - | - |
| Ban Hin Siou | 185 | - | - |
| Moung Sene | 375 | - | - |
| Ban Sen Hat | 750 | - | - |
| Total | 29,659 | 48(1) | 0.16 |

(1) When surviving *L. aperta* were returned to Bangkok they were re-examined for cercarial infections by shedding; 18 more positives were found among snails previously recorded as negative. However, in the absence of exact records of snail deaths, it is not possible to include these positives in the natural infection rate detected in the field.

ting team, comprising the same personnel throughout the work and utilizing the same methods throughout, were aware of the relative abundance and ease of collecting from place to place and acquired the strong impression that snail population densities at Ban Veun Thong and on the western shore of the island were indeed considerably lower than those at Khong Town and Ban Na.

By resorting to shedding instead of crushing to detect infections, many immature infections may have escaped attention. The infection rate of 0.3 per cent (Table 3) in the Ban Xieng Wang area must be considered to represent a most conservative figure. Indeed, upon transferring the snails to Bangkok and re-examining them a week later, 18 more positives were detected among snails previously recorded as negative, so that a total of 66 positive snails were obtained on this expedition. Nevertheless, since exact death records of these snails were not available, it is not possible to include these 18 in the overall natural infection rate.

It was demonstrated by animal infection that the cercariae from the wild-caught *L. aperta* represented the Mekong strain of *S. japonicum*. Three mice exposed to 40 cercariae each developed bisexual infections in 43-46 days. Upon perfusion, the adults appeared indistinguishable from *S. japonicum*. A puppy exposed to 550 cercariae began to produce mature eggs in the faeces after the 43rd day. The eggs resembled the short, round eggs of the Mekong schistosome.

## DISCUSSION

With regard to the sentinel mice exposed in April, 1972, it is of interest that 9 of 10 animals in Group D became infected but only 2 of 8 in Group E did so, the latter being unisexual infections with few worms. Althought the D and E sites were close,

the movement of water was different in the two places; at site E there was a detectable, if gentle, current, whereas at site D there was little or no current and the water movement was due to eddying (Fig. 3). Either we must conclude that the infections in Group D were the result of a "lucky strike", or, as seems more likely, that the difference in water movement had something to do with the acquisition of infection. When it is considered that cages exposed where there was detectable current (A, B, C, and E) produced few or no infections, we must conclude that the presence of current may have reduced the chance of infection.

These experiments provided proof that transmission of the Mekong schistosome was occurring in the body of the main river. Yet, the character of the Mekong River during the dry season is such that many quiet, pond-like pockets of still water can be found along the shore. Water currents in such places may be locally reduced or absent. In this regard, the pattern of transmission seems to differ from that of classical schistosomiasis japonica in China, Japan, and the Philippines, where the infection is picked up in seepage areas, wet-grass areas, and paddy fields. Nevertheless, the picture of transmission does not quite approach the situation reported for schistosomiasis mansoni in certain regions of Puerto Rico (Radke *et al.*, 1961) where infections were picked up by sentinel mice from rather rapid water in small streams.

The failure to obtain infections in mice exposed in April, 1971, might be explained in several ways. (1) The water velocity may have been too great; although not measured, it was considered "detectable" close to shore and "substantial" farther away from shore. To avoid misunderstanding, it should be mentioned here that the main stream of the Mekong River, with very fast current even at the height of the dry season, is to be found close to the left bank and several hundred meters away from the vicinity of Khong Town. Still, there are areas close to Khong Town where current is very fast. (2) The mice may have been exposed for too short a time. (3) Cercariae may simply have been absent. Very few *L. aperta* were collected here by team members in 1971 (Sornmani *et al.*, 1973). (4) Cercariae may have been present but may have come from so far upstream that they had lost vitality and/or infective ability by the time they reached the mice in the exposure cages. Radke *et al.*, (1961), working with *S. mansoni* in streams of Puerto Rico, reported two factors that limited their mouse infections: the velocity of the water and the distance the cercariae were carried downstream. In the latter case, the number of worms maturing in exposed animals decreased markedly with increased distance from the infected snails.

We have no doubt that our success in recovering infections from sentinel mice in 1972 was associated with the large numbers of *L. aperta* that appeared in the water adjacent to Khong Town in that year from April onward. The life cycle of these snails has yet to be studied. In previous years, their numbers at Khong Island appear to have been small, but most collections seem to have been made earlier than April or May. In the early dry seasons of 1968 and 1969, Brandt and Temcharoen (1971) found small numbers of *L. aperta* at Khong Town and Ban Na but did not examine them for the presence of cercariae or try to infect them. Lo *et al.* (1971) made extensive mollusk collections in December and January, 1968-69, but did not report *L. aperta* (at that time still an undescribed species) among the snails they examined for cercariae. *L. aperta* were present in small but easily detectable numbers in April, 1971 (Sornmani *et al.*, 1973) and in very large numbers in April and May, 1972 (present work).

The shore of the Ban Xieng Wang area of Khong Town is flat during the low-water period and there is a heavy growth of shrubby rheophytes (predominantly a euphorb, *Homonoia* sp.) which offer concealment and make the area popular as a defaecation site (Fig. 5). This fact may be related to the recovery of naturally-positive snails in this area.

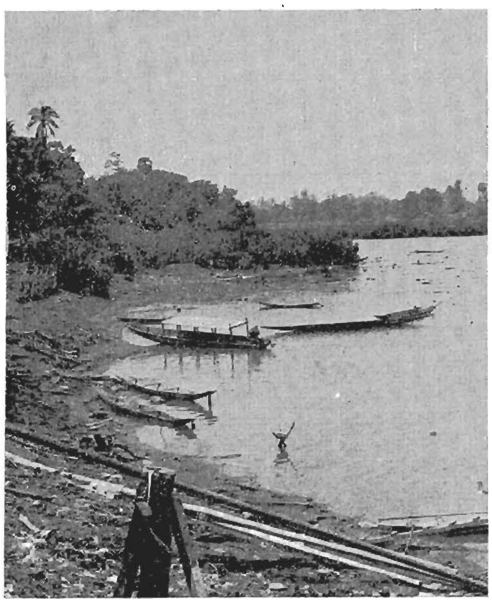

Fig. 5—Photograph of the shore at the Ban Xieng Wang area of Khong Town.

More must be known of the natural life cycle of *L. aperta* and its relatives to be able to predict when and where the snails will appear in abundance. Thus far, the evidence suggests that this species occurs in the waters near Khong Island in large numbers only toward the last months of the dry season. In this part of Southeast Asia, the dry season begins in November or December and ends usually in mid-June. Thus, in April, May and June, river contact at Khong Island, especially in the Ban Xieng Wang section of Khong Town, must be considered extremely risky.

It may be of interest to report here an isolated fact regarding an "accidental" sentinel animal detected on Khong Island. In May, 1971, a 5-month-old puppy was found shedding schistosome eggs. The puppy had been born and weaned in the Ban Xieng Wang area. Assuming that it was born in January, and that the prepatent period was approximately 6 to 7 weeks, and further assuming that it managed to get infected as soon as it was able to leave its mother (probably at 2 months of age), we may conservatively guess that it became infected in late March or early April. If so, this puppy became infected at the time sentinel mice *failed* to pick up the infection in the Ban Xieng Wang area.

It is precarious to attempt to extract too many facts from the few data at hand. Nevertheless, it would appear that the risk of becoming infected with the Mekong schistosome at Khong island is intermittent in time and place and may be limited to the latter part of March, the months of April and May, and possibly mid-June when the rains usually begin. However, confirmation of this hypothesis must depend on further work; thus far the transmitting snails have not been extensively sought during high-water periods (late June to late October).

## SUMMARY

Of 30 sentinel mice exposed for 15 hours in floating cages on the Mekong River at Khong Island, Laos, in March, 1971, in an area thought to be a transmission site for schistosomiasis, 26 mice survived and none acquired schistosomiasis. By contrast, in April, 1972, of 20 mice exposed under somewhat different circumstances for a longer period (40 hours), 18 survived and of these 11 acquired schistosomiasis.

In April and May, 1972, an intensive search for infected specimens of the snail, *Lithoglyphopsis aperta*, produced a total of 29,659 snails from 9 localities around the perimeter

of Khong Island Of these, 15,790 were collected in water adjacent to the Ban Xieng Wang area of Khong Town and they included 48 specimens positive for *Schistosoma japonicum*, Mekong strain, as determined by shedding of cercariae. This represented a natural infection rate of 0.30 per cent, or 0.16 per cent if the snails from the other collecting sites were included.

The data suggest that the risk of acquiring schistosomiasis on Khong Island may be greatest in the Ban Xieng Wang area of Khong Town, may be intermittent in time and place, and may be limited to the months from the end of March to the beginning of the rains in mid-June, when the snails are present in largest numbers.

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