MEKONG SCHISTOSOMIASIS: 4. A PARASITOLOGICAL SURVEY OF WILD RODENTS, DOMESTIC PIGS AND CATTLE ON KHONG ISLAND, LAOS

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INTRODUCTION

Morphological similarities between adult worms of the human *Schistosoma* from Khong Island, and of "classical" *S. japonicum* have been noted (Iijima *et al.*, 1971; Sornmani *et al.*, 1971). The Mekong *Schistosoma* also resembles "classical" *S. japonicum* in that dogs are naturally infected (Iijima *et al.*, 1971; Sornmani *et al.*, 1973). The possibility that other domestic and wild animals on Khong Island, such as cow, pig, rat, and water buffalo, might be infected and thus participate in the natural transmission of this parasite merited attention. Faecal examinations of domestic water buffalo on Khong Island, however, failed to provide evidence of infection (Schneider *et al.*, 1975). In the present paper we present (1) the results of a parasitological examination of wild rodents caught near and downstream from the main schistosomiasis transmission site on Khong Island, and (2) the results of single faecal examinations of domestic pigs and cattle from the vicinity of Khong Town.

Since we lack a definitive decision regarding the specific identity of the parasite from Khong Island, we shall, as a taxonomically safe expedient, refer to it herein as the Mekong *Schistosoma*.

MATERIALS AND METHODS

Rodents: The rodents were collected in the vicinity of the known schistosomiasis transmission site in Khong Town, along the nearby shore and on adjacent small islands in the Mekong River. Live-traps were baited with banana and placed beside suspected rat-holes or near trails marked by rodent passage in the grass. In our previous experience, banana proved to be an acceptable bait for field rats. Some traps were baited with glutinous rice mixed with banana or peanut butter; these were set near or inside houses in Khong Town.

Trapped rodents were specifically identified and numbered. Working in an improvised field laboratory, each animal was killed and the portal system was perfused for adult schistosome worms using a perfusion technique modified from that of Pellegrino and Sequeira (1956). Livers were also squashed between two glass plates and searched microscopically for the presence of adult worms and/or eggs. In addition, small intestines were opened lengthwise and the contents examined for worms; then the intestinal wall was crushed between glass slides and examined under the dissecting microscope for the presence of granulomas containing schistosome eggs.

Parasites were preserved in 5% formalin and brought to Bangkok for identification.

Pigs: Most of the pigs examined were located in Ban Na but a few were kept in Khong Town. Fresh faeces were collected either in the evening or the early morning. Because

the pigs were isolated in pens there was no risk of duplicating specimens.

Cows: The cattle examined in this study were transients, having been brought to Khong either from the left bank of the Mekong River or from the inhabited islands between Khong and the Khmer border. Most of the cows were placed in a depot in None Ka Sang, west of Khong Town and away from the river, but some were domiciled in Ban Xieng Wang (in Khong Town) with easy access to the river. Most were destined for early transport to the slaughterhouse in Paksé. Cow owners co-operated willingly in tying adult animals on short ropes to numbered stakes at night. This permitted collection of fresh faeces in the evening or early morning without risk of specimen duplication. Calves, however, characteristically huddled together at night and could not be conveniently separated; as this introduced a risk of specimen duplication, they were omitted from the study.

Parasitological examinations of pigs and cows

Faecal specimens from pigs and cows were examined by two methods: (1) the merthiolate-iodine-formalin concentration (M.I.F.C.) technique of Blagg *et al.*, (1955), and (2) the Faust-Meleney egg-hatching technique recommended by Pesigan and Yogore (1947). No attempts were made to recover adult worms from pigs or cows.

RESULTS

Rodents: A total of 103 rodents were trapped and examined during a 20-day period in March-April, 1974. The collection included 12 *Rattus exulans* (Peale), 81 *Rattus r. molliculus* Robinson & Kloss, and 10 *Bandicota savilei* Thomas (Table 1). *R. exulans* were taken only in houses. The *B. savilei* were not taken on Khong Island but were caught on the left bank of the Mekong River opposite the southern end of Khong Town (Fig. 1).

Table 1

Rodents collected on Khong Island, Laos, during March-April, 1974.

Species	No. skins*	No. in formalin*	Total
Rattus rattus molliculus Robinson & Kloss, 1922	16	65	81
Rattus exulans (Peale, 1848)	4	8	12
Bandicota savilei Thomas, 1916	7	3	10

* Accessioned by the Centre for Thai National Reference Collections, Applied Scientific Research Corporation of Thailand, Bangkok.

Nematodes were recovered from 27 *R. r. molliculus* and 4 *B. savilei* (Table 2): the collection included 46 *Rictularia tani* (all females), a single female *Capillaria gastrica*, an unidentified female oxyurid and six unidentified female trichostrongylids.

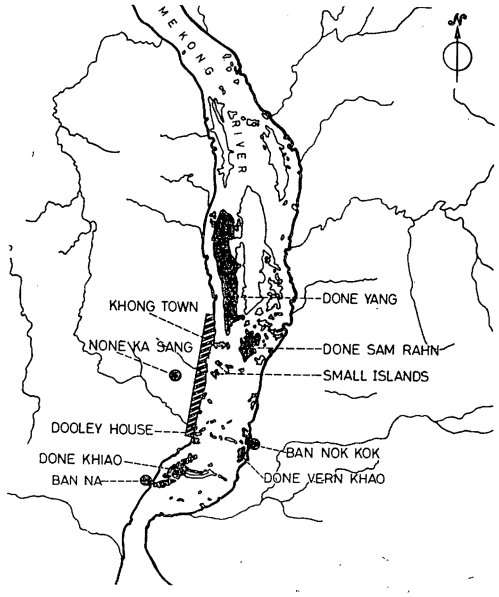

Fig. 1—Lower southeast edge of Khong Island (to the left of the Mekong River, which flows south) and immediate vicinity, showing named localities where rats were trapped and animal faeces collected. Scale: 7 mm = 1 km.

Table 2

Results of a parasitological examination of rodents on Khong Island, using perfusion technique and gut dissection.

Species	Site	No. exam.	Positive		
			Trematodes	Nematodes*	Cestodes**
Rattus exulans (in houses)	Dooley House	10	0	0	0
	Done Khiao	1	0	0	1
	Done Yang	1	0	0	0
Rattus rattus molliculus (edge of water)	Military camp	1	0	1	1
	Ban Xieng Wang	2	0	1	1
	Done Yang	8	0	2	3
	Done Sam Rahn	24	0	7	15
	Small islands	4	0	2	1
	Dooley House	8	0	5	3
	Ban Na	5	0	2	2
	Done Khiao	26	0	7	9
	Done Vern Khao	3	0	0	0
Bandicota savilei (edge of water)	Ban Nok Kok	5	0	4	0
	Done Vern Khao	5	0	0	0
Total		103	0	31 (27.9%)	36 (32.4%)

* Predominantly *Rictularia tani* but also a female *Capillaria gastrica*, an oxyurid and six trichostrongylids.
** *Hymenolepis diminuta* and *Raillietina* sp.

The cestodes that were recovered included *Hymenolepis diminuta* and an unidentified species of *Raillietina*. Thirty-six rodents (32.4%) harboured cestodes (Table 2).

No stages of the Mekong *Schistosoma* were recovered from any of the rodents included in the examination.

Pigs: The pigs were not heavily parasitized and more than half were negative for helminth eggs. *Ascaris suum* was encountered in 6 of 15 pigs (40%) and isolated infections with *Orientobilharzia harinasutai*, *Ascarops* sp., *Oesophagostomum* sp., and *Macracanthorhynchus hirudinaceus* were recorded (Table 3). Eggs of *Opisthorchis viverrini*, found in two pigs, were assumed to be the adventitious result of coprophagy (see Discussion). No eggs or miracidia of the Mekong *Schistosoma* were found in the pigs included in the survey, using either the M.I.F.C. or the hatching technique. Eggs of *O. harinasutai* were detected by M.I.F.C. only.

Cows: Approximately two-thirds of the faecal samples from cows examined by the M.I.F.C. and the hatching techniques were negative for helminth eggs. Nine of 43 cows were passing eggs of rumen flukes (Paramphistomatidae), the species of which could not be identified by egg morphology. Two cows had *Neoascaris vitulorum*, 5 had *Oesophagostomum* sp., one had *Thelazia* sp., and 2 had *Trichuris* sp. Neither eggs nor miracidia of the Mekong *Schistosoma* were demonstrated (Table 4).

DISCUSSION

Wild Rodents: Experimentally, rats are considered to be poor hosts for "classical" *Schistosoma japonicum*. In Japan, Ito (1953) reported low experimental infection rates in white rats; most of the worms disappeared relatively early in the course of infection while growth of survivors was delayed, as compared with development of worms in rabbits, mice

Table 3

Results of a stool examination of domestic pigs *(Sus scrofa)* on Khong Island.

Site	No. exam.	No. neg.	Positive						
			Trematodes[a]			Nematodes[b]			Acantho-cephala[c]
			S.sp.	O.h.	O.v.[d]	A.s.	A.sp.	O.sp.	M.h.
Khong Town									
Market Square	1	1	0	0	0	0	0	0	0
Chom Tong	2	0	0	0	0	2	1	0	0
Pho Chai	2	0	0	0	2	1	0	1	1
Ban Na	9	7	0	1	0	2	0	0	0
Done Yang	1	0	0	0	0	1	0	0	0
Total	15	8	0	1	2	6	1	1	1

[a] S.sp. = Mekong *Schistosoma;* O.h. = *Orientobilharzia harinasutai;* O.v. = *Opisthorchis viverrini*.

[b] *A.s.* = *Ascaris suum;* A.sp. = *Ascarops* species; O.sp. = *Oesophagostomum* species.

[c] M.h. = *Macracanthorhynchus hirudinaceus.*

[d] Ova of *Opisthorchis viverrini,* which is not a natural parasite of swine, may have come from human faeces containing the eggs which were eaten by the swine. *O. viverrini* is not uncommon in humans on Khong Island.

Table 4

Results of a stool examination of domestic cattle in Khong Town, Khong Island, Laos.

Site	No. exam.	No. neg.	Positive					
			Trematodes[a]		Nematodes[b]			
			S.sp.	Para.	N.v.	O.sp.	Th.p.	Tr.sp.
Xieng Wang	11	5	0	3	0	3	0	1
None Ka Sang	32	22	0	6	2	2	1	1
Total	43	27	0	9	2	5	1	2

[a] S.sp. = Mekong *Schistosoma;* Para. = paramphistomatids (rumen flukes).

[b] N.v. = *Neoascaris vitulorum;* O.sp. = *Oesophagostomum* species; Th.sp. = *Thelazia* species; Tr.sp. = *Trichuris* species.

and guinea pigs; about a third of female worms failed to mature in rats and those that did mature produced a relatively small number of eggs.

In nature, Pesigan *et al.*, (1958) believed that naturally infected rats in the Philippines (Palo, Leyte) played a comparatively minor role in the transmission of *S. japonicum.* Although the rat population was high in their study (about 60 rats per hectare, or 10 rats per human being), the prevalence of infection was low (22.7%), the mean daily egg output was minute (21 eggs per rat), and the eclosion rate was only 10.6%, as compared with 42.4% from humans and 71.9% from cows.

Populations of rats and other rodents have not been estimated on Khong and its neighboring islands, but the impression gained

during the present work was of low numbers. *Rattus exulans* was found in and around houses and *R. r. molliculus* and *Bandicota savilei* were caught in fields, paddies, dry stream beds and along the shore of the Mekong River near the main transmission site. *B. savilei* was taken only on the mainland (left bank) and Vern Khao island which during dry season is connected to the mainland.

On Khong Island, Iijima and Garcia (1967) caught and dissected 45 "wild mice" and examined intestines and liver for signs of *Schistosoma* infection but without success. Sornmani *et al.*, (1971) trapped eight *R. rattus* at the river's edge within the boundaries of Khong Town; livers and intestines were examined microscopically after crushing between glass plates or slides, but no eggs or adults of any type of *Schistosoma* were reported.

In view of the resemblances between the Mekong *Schistosoma* and "classical" *S. japonicum*, we expected to find some evidence of the Mekong parasite in rats caught near the transmission site on Khong Island. However, the number of rats that were caught was relatively small. A total of 103 rodents, including 12 *Rattus exulans*, 81 *R. r. molliculus*, and 10 *Bandicota savilei*, were trapped in and near Khong Town and on neighboring islands in the Mekong River. About a third of these were infected with nematodes and cestodes but careful examination failed to reveal any *Schistosoma*. There are three possible explanations for this failure: (1) infected rodents may have been missed in the survey because of the relatively small size of the sample; (2) the rodents that were examined may not have come into contact with contaminated water long enough for cercariae to infect them; (3) the rodents may have been refractory to infection with the Mekong *Schistosoma*.

Pigs: In the Philippines, Pesigan *et al.*, (1958) examined 603 domestic pigs in the Palo area of Leyte and found 80 positive for *S. japonicum* (13.3%). However, mean daily egg output was small (481) and only about a third of the eggs hatched. These authors considered that pigs, like rats, played a minor role in maintaining the *S. japonicum* cycle in nature.

Iijima (1970) examined an unspecified number of pigs on Khong Island but found none infected with *Schistosoma*.

In the present work, faeces from 15 pigs that were usually kept penned in and near Khong Town were examined by the M.I.F.C. and hatching techniques; neither eggs nor miracidia of the Mekong *Schistosoma* were demonstrated although other helminth eggs were found including in one case those of another schistosome, *Orientobilharzia harinasutai*. Domestic pigs on Khong Island were not numerous and were seldom allowed to live more than 10 months before slaughter. In Khong Town and Ban Na, pigs were only rarely seen walking about outside the pen or drinking at the river. Thus, failure to demonstrate the Mekong *Schistosoma* in these pigs may reflect a relative lack of exposure to cercariae.

Two pigs in Ban Pho Chai (a section of Khong Town) were positive for eggs of *Opisthorchis viverrini*. Since this liver fluke is not a natural parasite of pigs, we believe that the eggs may have come from positive human faeces which had been eaten by the pigs. Liver fluke has been demonstrated in humans on Khong Island (Sornmani *et al.*, 1971).

With regard to *Orientobilharzia harinasutai*, we believe that this is the first report of the pig as a definitive host for this schistosome. *O. harinasutai* has previously been reported only from water buffalo in South Thailand (Kruatrachue *et al.*, 1965) and Khong Island,

Laos (Schneider *et al.*, 1975). It is of interest that miracidia of this schistosome were not recovered by the hatching technique. In the case of *S. japonicum*, low eclosion rates under suitable conditions have been associated with the presence of the adult worms in unsuitable hosts (Pesigan *et al.*, 1958). By analogy, it is possible that the pig is not a completely suitable host for *O. harinasutai*; this would have to be verified experimentally.

Cows: Elsewhere in Asia, cattle are considered (with dogs) as an important reservoir of classical *Schistosoma japonicum* (Wu, 1938; Pesigan *et al.*, 1958). In view of the morphological similarity between adults of *S. japonicum* and the Mekong *Schistosoma* (Iijima *et al.*, 1971; Sornmani *et al.*, 1973), we had expected to find some evidence of infection in cows on Khong Island. However, the number examined was relatively small and the animals were transients. The negative results reported here must be viewed conservatively and we cannot speculate on the possible role of cattle as hosts for the Mekong *Schistosoma* under conditions more suitable for transmission.

SUMMARY

No evidence of infection with the Mekong *Schistosoma* was found in 12 *Rattus exulans*, 81 *R. r. molliculus*, and 10 *Bandicota savilei* caught in the vicinity of and downstream from the schistosomiasis transmission focus on Khong Island, South Laos, and examined by dissection and portal perfusion. Likewise, no eggs of the Mekong *Schistosoma* were detected in faeces of 15 domestic pigs or 43 domestic cattle examined on Khong Island both by merthiolate-iodine-formalin concentration and by the hatching technique. These results suggested that the wild rodents listed above, as well as pigs and cattle in the vicinity of Khong Town, may not contribute significantly to the transmission of the Mekong *Schistosoma* under present conditions.

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