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Studies on Schistosomiasis in the Mekong Basin
I. Morphological Observation of the Schistosomes and Detection of Their Reservoir Hosts

TOSHIHIKO IIJIMA
Department of Parasitology, School of Medicine, Kyorin University, Tokyo
CHIN-TSONG LO
Museum of Zoology, The University of Michigan, Michigan
AND
YOICHI ITO
Department of Parasitology, National Institute of Health, Tokyo
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Introduction

The first case of human schistosomiasis in the Mekong Basin was reported by vic Dupond in 1957. Chaiyaporn *et al.* (1959) reported that the schistosomiasis was found on a farm laborer who spent his whole life in the village in Nakornsrithamaraj Province, Thailand. Harinasuta *et al.* (1960), on the basis of the intradermal skin tests and stool examination to the inhabitants of several villages in Nakornsrithamaraj Province, where Chaiyaporn *et al.* found the Schistosomiasis, recognized this Province as the endemic foci of schistosomiasis japonica in Thailand. In the years of 1963-1966, Barbier diagnosed 5 cases of schistosomiasis japonica on the persons coming from Laos. In 1967 Iijima and Garcia examined the feces of 547 inhabitants on Khong Island in Sithandone Province, South Laos. In this study, they found 8.5% of inhabitants infected with schistosomiasis japonica and recognized this area as an endemic focus of schistosomiasis japonica. In Cambodia also, an endemic focus was discovered in Kratie District by staff members of Pasteur Institute (Yen, 1968).

Thus a number of cases of schistosomiasis are known to occur over a considerably wide area in the Mekong Basin and they are tentatively recognized as cases of schistosomiasis japonica. However, the adult worms of the schistosomes, intermediate hosts and reservoir hosts had not been identified. Such being the case, several matters had to be cleared up before it could be decided whether or not the above-reported cases were actually those of schistososmiasis japonica.

With a view to elucidate these matters, the present authors and their colleagues, in the capacity of the Short-Term Consultants of World Health Organization, made an epidemiological survey of schistosomiasis in the Mekong Basin from 1968 through 1969; special emphasis being placed on the survey of the range of the infected area. As a result of the morphological examination of worms obtained from the dogs captured in the Mekong Basin, it was discovered that adult worms of the genus *Schistosoma* were parasitizing their bodies. The following is a detailed report on the examination and the observation.

Materials and Methods

The research was prosecuted in Khong Island (Fig. 1); where 24 ownerless dogs were captured and killed by the injection of Minebital and Heparine, and the worms were subjected to morphological examination.

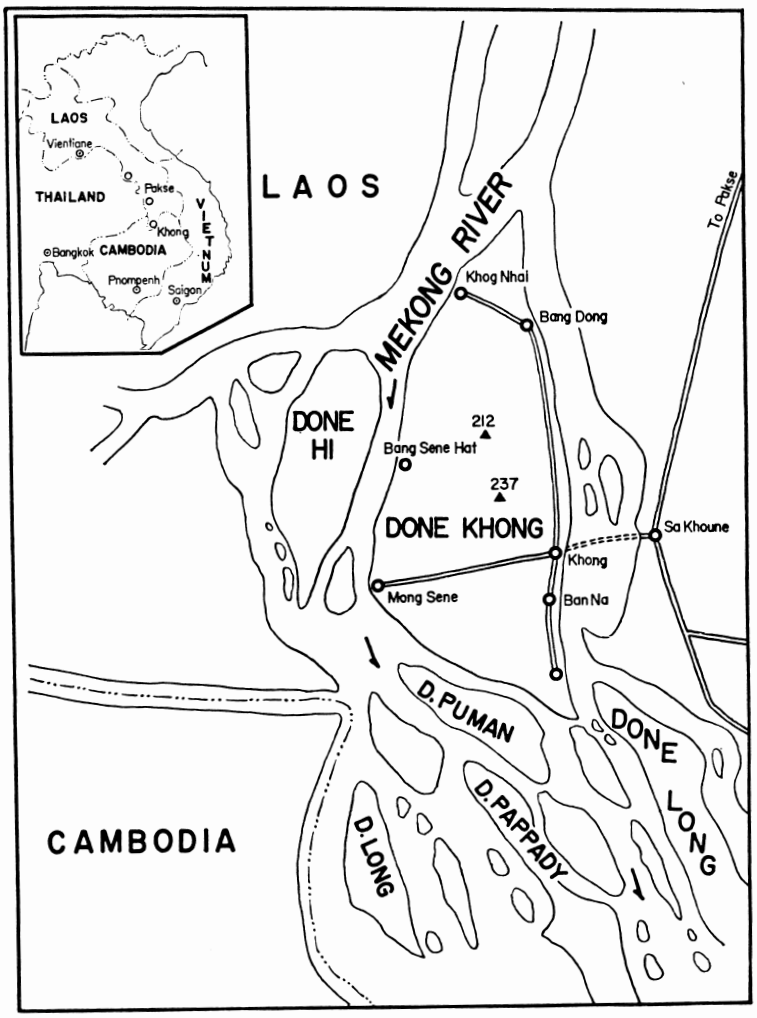

Fig. 1 The map of the Khong District, Sithandone Province, Laos.

In search for the worms, close examination was made for the lungs, hearts, spleens, the walls of small and large intestines, abdominal membranes, and portal veins; the worms thus discovered being picked out. From the livers, worms were gathered by perfusion. The collected worms were put in tepid water (37°C) and extended before the measurements were taken of their bodies. Then, morphological observation was made on the internal structure of the worms after fixing their bodies in a Faag's solution or a 5 per cent formalin solution, and dyeiny them with haematoxyline prescribed by Mayer.

For the purpose of comparison, the rabbits were expeimentally infected with the Japan strain of *Schistosoma japonicum*; and the same type of measurement and examination were made on the adult worms and eggs gathered from the bodies of these rabbits.

Results

1. *Infection Rate.*

Out of 24 dogs (male 17, female 7; ages 2 to 8 years) which were examined, seven were found to be parasitized by schistosomes.

2. *Location and Number of Worms.*

Table 1 Localization of the adult worms of Schistosome in the dogs

Hosts No.	Age	Sex	Worms	Localization: Portal vein liver	Mesentery vein small intestine	Mesentery vein large intestine	Mesentery vein rectum	Intestinal vein small intestine	Intestinal vein large intestine	Intestinal vein rectum	Toal (Number of worms)
			Pair	0	1	1	0	0	0	0	2
1	4-5	F	Male	2	0	0	0	0	0	0	2 (6)
			Female	0	0	0	0	0	0	0	0
			Pair	0	0	3	1	0	0	0	4
2	3	M	Male	0	0	0	1	0	0	0	1 (9)
			Female	0	0	0	0	0	0	0	0
			Pair	0	5	3	1	2	0	0	11
3	4	F	Male	8	0	0	0	0	0	0	8 (31)
			Female	1	0	0	0	0	0	0	1
			Pair	4	3	2	1	1	0	0	11
4	4	F	Male	7	1	0	1	0	0	0	9 (32)
			Female	0	0	0	1	0	0	0	1
			Pair	3	0	6	2	0	1	1	13
5	2	F	Male	15	2	2	0	0	0	0	19 (49)
			Female	2	1	1	0	0	0	0	4
			Pair	0	0	1	0	0	0	0	1
6	8	F	Male	0	0	0	0	0	0	0	0 (2)
			Female	0	0	0	0	0	0	0	0
			Pair	0	0	1	0	0	0	0	1
7	5	M	Male	0	0	0	0	0	0	0	0 (2)
			Female	0	0	0	0	0	0	0	0
			Pair	7	9	17	5	3	1	1	43
Total			Male	32	3	2	2	0	0	0	39
			Female	3	1	1	1	0	0	0	6
(Number of worms found)				(49)	(22)	(37)	(13)	(6)	(2)	(2)	(131)

As indicated in Table 1, many of the adult worms were discovered in the mesenteric veins of large intestine, and the portal veins of the liver. A small number of worms were discovered in the mesenteric veins of small intestines, but no worms were found in other organs. The smallest number of the worms discovered per host was two (1 pair), and the largest number was 46 (13 pairs plus 20 males).

3. *Morphological Structure of the Worms.*

Male : The body is greyish white coloured and has thick flesh. The body surface is smooth, and the body strongly bends just behind the ventral sucker. The anterior part of the body is slender and proboscis-shaped. The gynecopholus extends from the ventral sucker to the posterior part of the body. Tne length of the bodies varies : 9.9–18.0 mm (average : 15.2 mm) ; the maximum width of the body also differs : 0.38–0.63 mm (average : 0.58 mm.) The oral and

ventral suckers are both conspicuous, protruding from the body. The oral sucker, located at the anterior end of the body, is funnel-shaped and 0.26–0.30 mm in diameter. The ventral sucker, located 1.5 mm from the anterior end of the body, is oval (major axis : 0.3–0.4 mm) and funnel-shaped, and is slightly larger than the oral sucker. The esophagus is short, and there is only a trace of the pharynx. The intestine tube divides itself into two branches just before the ventral sucker and each of the branches stretches along the side of the body. The male had 3 to 8 testes ; and the testes (size : 0.17–0.20 mm) are located just behind the ventral sucker. The vas defference is short and the genital pore is open immediately at the behind of the ventral sucker.

Female : The body is slender and its posterior part is dark browm or blackish brown coloured. As in the case of the male, the body strongly bends immediately after the ventral sucker. The length of the bodies varies : 14.5–20.1 mm (average : 17.8 mm). The maximum width of the bodies is 0.28 mm. The oral and ventral suckers protrude from the body ; both are 0.08 mm in diameter. The intestine tube forks into two branches at a quarter from the anterior end of the body, but these separate branches join into one at a quarter from the posterior end. The ovary, whose shape is of an elongated ellipse or square, is located about the middle of the body ; its average size being 0.73×0.15 mm. The oviduct bends several times before it reaches the oötype. The uterus contains 120–130 ova.

4. *Eggs.*

The eggs picked out from the feces of inhabitants in the Khong area are of the shape of a compressed ellipse (major axis : 61.7±4.78 μ, minor axis : 51.2±4.30 μ) and contain mature miracidium.

5. *Comparison with the Japan Strain of Schistosoma japonicum.*

The adult worms and eggs of the schistosomes collected from the dogs in the Khong area, Laos were compared morphologically those from the rabbits infected with Japan strain of *S. japonicum*. The results of the comparison are shown in Table 2 and Figs. 2 and 3.

The Size of the Adult Worms : The size of the males collected in the Mekong Basin averages 15.2×0.58 mm and that of the females 17.8×0.23 mm. The size of the males and females of Japan strain were 10.4 ×0.53 mm and 11.6×0.23 mm respectively. On this data, the size of the worms in the Khong area in Laos is larger than that of the Japan strain of *S. japonicum*.

The Size of the Ovaries : As for the female worms gathered in Khong, the size of the ovaries averages 0.73×0.15 mm, whereas the size of the ovaries of the Japan strain of *S. japonicum* averages 0.52×0.14 mm ; on the basis of this obsevation, the size of the ovaries in the female worms gathered in Khong are larger than those of the Japan strain of *S. japonicum*.

The Number of the Testes : The male worms gathered in Khong were found to possess 3 to 8 testes, i. e., 6.5 on an average ; whereas the males of the Japan strain of *S. japonicum* have 5 to 7 testes (also 6.5 on an average) ; these two average numbers are the same. However, the worms is Khong show greater variations than those of the Japan strain of *S. japonicum* in the number of the testes.

The Sizs of the Eggs : The measurement of the eggs of schistosomes gathered from the feces of the inhabitsnts in the Khong area indicated that the smallest egg was 51.2×41.0 μ, the largest one 69.1×56.3 μ (average major axis : 61.69±4.78 μ, average minor axis : 51.2±4.30 μ), whereas the smallest egg of the Japan strain of *S. japonicum* was 64.0×56.3 μ, the largest one 92.2×56.3 μ (average major axis : 79.70±6.80 μ, average minor axis : 58.45± 51.6 μ). From these observations, eggs found in the Khong area are longer in major axis than those of Japan strain of *S. japonicum*. As to the shape of the eggs, those of schistosomes gathered in the Khong area were almost circular, whereas the eggs of the Japan strain of *S. japonicum* were oval (see Fig. 4).

Table 2 Comparison of the two sohistosomes, *Schistosoma japonicum* Japan strain and *Schistosoma* obtained form the dogs in Laos

		S. japonicum Japan strain		Schistosome obtained from the dog in Laos	
		Male	Female	Male	Female
Size of worms (mm)		10.0×0.53	10.8×0.20	12.8×0.53	14.5×0.28
		10.4×0.55	12.6×0.28	12.5×0.58	20.1×0.28
		9.5×0.55	9.0×0.23	9.9×0.38	17.9×0.30
		11.0×0.50	11.1×0.15	15.0×0.50	16.3×0.28
		10.4×0.53	14.5×0.30	18.0×0.63	20.0×0.28
				15.2×0.58	
	Average	10.4×0.53	11.6×0.23	15.2×0.58	17.8×0.28
Size of ovaries (mm)		0.62×0.21		0.77×0.15	
		0.57×0.18		0.72×0.14	
		0.57×0.15		0.72×0.15	
		0.46×0.15		0.72×0.15	
		0.14×0.12		0.72×0.15	
		0.52×0.15			
		0.52×0.15			
	Average	0.52×0.14		0.73×0.15	
Number of testes		7		7	
		6		6	
		7		3	
		7		7	
		5		6	
		7		8	
		6		8	
		7		7	
		7			
		6			
	Average	6.5		6.5	
Size of eggs (μ)	Length	Mean S.D.		Mean S.D.	
		79.70±6.80		61.69±4.78	
	Width	58.45±5.16		51.20±4.30	

Discussion

The worms (belonging to the genus *Schistosoma*) that were gathered from the dogs captured in Khong, Sithandone Province in South Laos bear a greater resemblance to *S. japonicum* than *S. mansoni* or *S. haematobium*, which are known to parasitize the human body.

In this connection, we must take into consideration of the reports by Ito (1955) and Hsü *et al.* (1957, 1958, 1962). They reported that worms belonging to *S. japonicum* may show morphological and biological differences according to the strain, the host, and the number of the worms parasitizing a host. As to the size of worms, Ito (1955) reported that the worms of the Japan of *S. japonicum*,

parasitizing rabbits, grew to a greater length (males : 10.8±3.24 mm, females 15.6±3.42 mm) than those parasitizing other mammalian hosts such as mice, guineapigs, and rats. Hsü *et al.* (1962) stated on the comparative measurement of the body-length of the Japan and the Formosa strains of *S. japonicum*, that the former grew to a greater length than the latter, though they did not provide the exact results of their mearurement. In view of these findings, it cannot be concluded that the schistosomes found in Khong belong to a distinct species from the Japan strain or the Formosa strain of *S. japonicum* merely on the basis of the fact that they grow to a greater length than the latter two strains.

As to the size of the ovaries, there has been no report provided as yet. But it seems quite probable that the schistosomes in the Khong area being greater in size, have larger ovaries.

Regarding the number of the testes per adult male worm, Hsü *et al.* (1957) reported, on the basis of their examinations of the four strains, i. e. Japan, Philippines, Formosa, and China, that the ratio of the individuals possessing 7 testes to the entire population of specimens differed from strain to strain (Japan strain : 95.4 %, Philippines strain : 77.4 %, Formosa strain : 88.4 %, and China strain : 91.9 %), and stated that these differences were not incidental but distinctive. Thus it has to be concidered that the average number of the testes may differ among the different strains and, therefore, the number of the testes can not be regarded as a criterion for identification of Schistosoms species.

As to the size of the eggs, Hsü *et al.* (1958) reported thet it varied according to the individual host, the kind of hosts, and the strain of the schistosomes. As to the variation of the size of the eggs according to the strain of the schistosomes, they reported that on an average the eggs of China strain were found to be the largest of all the strains examined, and that the eggs of Philippines and Formosa strains were, also on an average, smaller than those of China strain but larger than those of Japan strain, whose eggs were found to be the smallest of all the strains here concerned. Harinasuta *et al.* (1962) measured the size of the schistosomes' eggs gathered by means of rectal biopsy from the rectums of inhabitants in Nakornsithamarj Province, Thailand and found that the average size of the eggs to be 58.2 μ ; and they suggested that this small size may be due to the immaturity of the eggs gathered by the rectal biopsy or due to the difference of the schistosomes. Compared with the data provided by these reports, the average size of the eggs which were gathered in the Khong area proved to be the same as the average size of the eggs gathered by Harinasuta, being obviously smaller than the average size of the eggs of any strain already known to belong to *Schistosoma japonicum*, i. e., China, Formosa, Philippines, and Japan strains.

As staten earlier in this paper, the adult worms of the genus *Schistosoma* were gathered from the dogs and the eggs from the feces of human being in the Mekong Basin in Laos ; they were compared with the adult worms and eggs gathered from the rabbits that were experimentally infected with the Japan strain of *S. japonicum*. This comparative examination shows several notable differences between them. However, the morphological characteristics of individuals of *S. japonicum* vary a great deal according to their strain, the kind of their hosts, and the number of the worms parasitizing a host.

As the problem of intermediate host, all the efforts to determine the intermediate host of human schistosomes have not been succeeded in the Mekong Basin including Laos, Cambodia and Thailand. It is, at least, believed that the intermediate host of human schistosomes in this area may be different to *Oncomelania* snails known as the transmitted snails of *Schistosoma japonicum* in the Far East.

All of these facts suggest that schistosomes obtained from dogs in the Mekong Basin is different to any strains of *S. japonicum*

already known. The present authors and their colleagues are carrying on a further study to decide whether the schistosomes gathered in the Khong area belong to a new species of the genus *Schistosoma* or merely a new strain within *S. japonicum*.

Summary

The authors made a morphological observation of the adult worms and eggs of the genus *Schistosoma*, the eggs gathered from the feces of inhabitants in Muong Khong, Sithandone Provice in South Laos and the adult worms from the dogs captured in Khong and anatomized.

Both the worms and the eggs show remarkable morphological resemblsnces to those of *Schistosoma japonicum*. However, compared with the specimens gathered from the rabbits experimentally infected with the Japan strain of *S. japonicum*, they were proved to be different from this strain in several points: The ovaries are larger then those of Japan strain, the number of the testes has wider variation among the schistosomes discovered in the Khong area than among those of Japan strain, and the size of the eggs of the former is smaller than that of latter.

All of these facts suggest that schistosomes obtained from the dogs in the Mekong Basin is different to any strains of *S. japonicum* already known. The present authors and their colleagues are now carrying on a further study to decide whether the schistosomes gathered in the Mekong Basin belong to a new species or not.

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As mentioned earlier, our research is a part of the survey that we conducted of bilharziasis prevalent in the Mekong River Basin in the capacity of Short-Term Consultants, World Health Organization. We are particularly thankful to WHO and the Government of Laos for providing us with the opportunity to do this research as well as for extending their hearty support to us.

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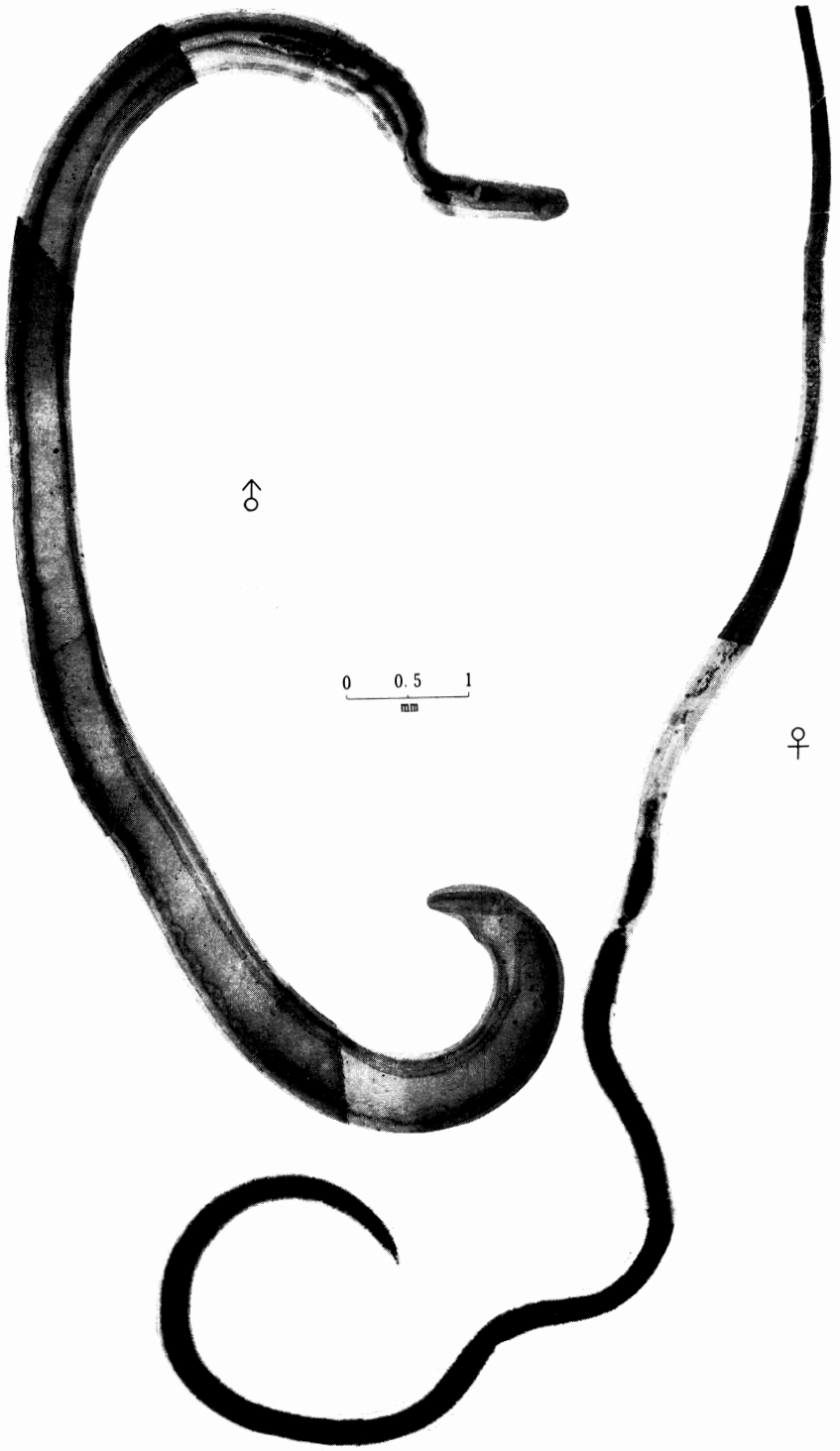
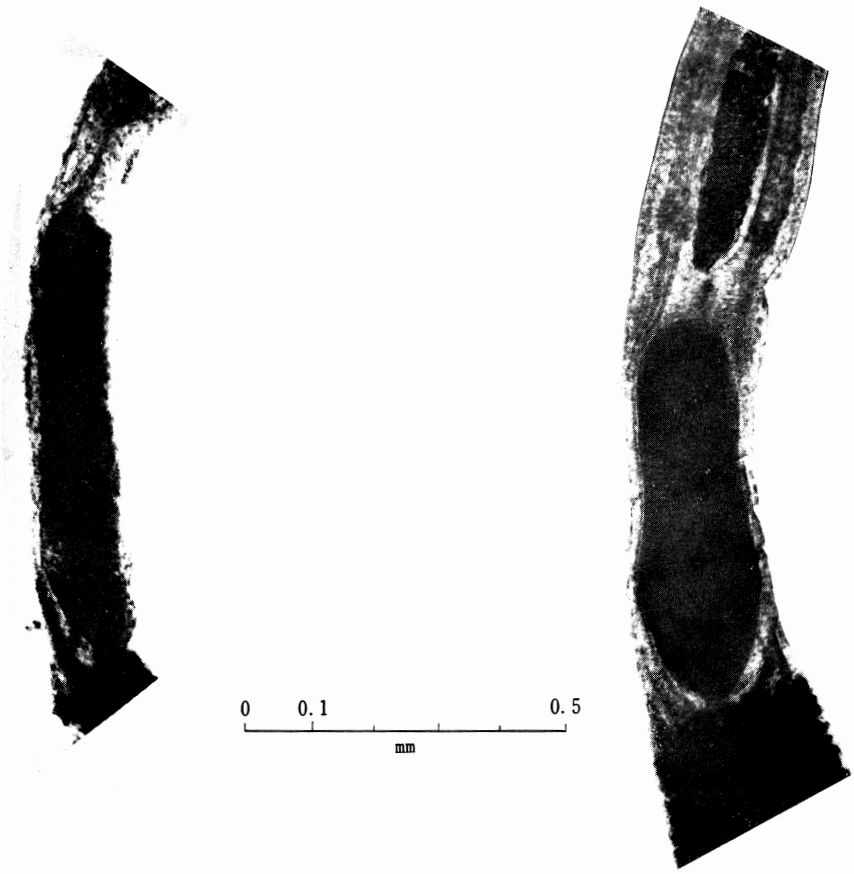


Fig. 2 Adult male and female worms of schistosome obtained from the dogs in the Khong District, Laos.



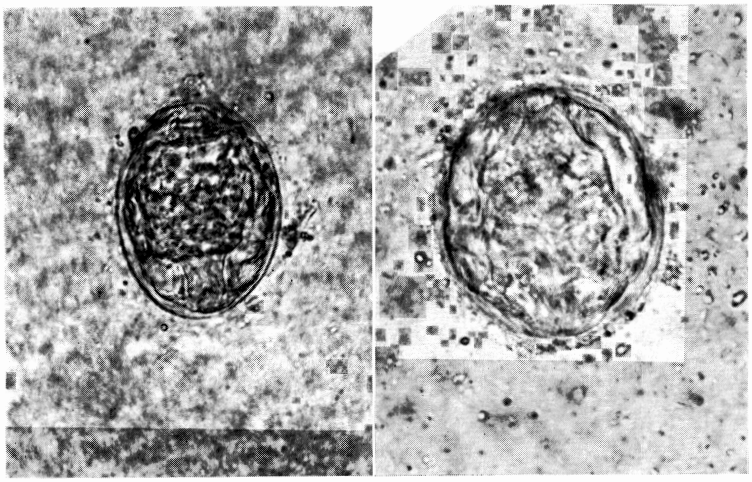


Fig. 3 Ovaries of *Schistosoma japonicum* Japan strain (right), and schistosome obtainted form the dogs in Khong District, Laos (left).

Fig. 4 Eggs of *Schistosoma japonicum* Japan strain (right) and schistosome obtainted from the dogs in Khong District, Laos (left).

Mekong 川流域における住血吸虫病の研究
I. 保虫宿主および虫体の発見，虫体の形態学的観察

飯島　利彦
（杏林大学医学部寄生虫学教室）
CHIN-TSONG LO
（ミシガン大学動物学教室）
伊藤　洋一
（国立予防衛生研究所寄生虫部）

Mekong 川流域における住血吸虫病は vic Dupond (1957) のラオス国住民の最初の症例報告以来各地から散発的に発見されたが，長い間，有病地と称すべき地域も発見されず，またその中間宿主ないしは保虫宿主も発見されなかつた．

しかるに，筆者らが WHO の Short-term Consultant として同地の住血吸虫病の調査をおこなつたところ，1967 年に Laos, Khong 地区に，1969 年に Cambodia, Kratie 地区にそれぞれ相当規模の同病有病地を発見し，同じく 1969 年に Khong 地区において犬から始めて住血吸虫虫体の採取に成功した．

これと，家兎に人工感染して得た日本住血吸虫（日本産）との形態の比較をおこなった．両者は虫体，虫卵共に酷似しているが，微細な相違点は，Laos, Khong で犬から得た虫体は日本産のそれに比し，体長がやや大であること，卵巣がやや大であること，睾丸数に変異が多いこと，虫卵がやや小であること等であった．

しかし以上の成績からは Khong 地区で犬から得られた住血吸虫は少なくとも既知の日本住血吸虫 Strain とは異なるが，別種であるとするにはなお慎重に検討する必要がある．