日本熱帯医学会雑誌

第 18 巻 第 1 号　　平成 2 年 3 月15日

内　　容

（裏面に続く）

日熱医会誌　　日本熱帯医学会

Japan. J. Trop. Med. Hyg., Vol. 18, No. 1, 1990, pp. 1-10

INFECTIONS OF BLACKFLIES (DIPTERA: SIMULIIDAE) WITH THREE TYPES OF ZOONOTIC *ONCHOCERCA* LARVAE IN OITA, JAPAN

HIROYUKI TAKAOKA[1] AND ODILE BAIN[2]

Received August 21 1989/Accepted November 21 1989

Abstract: Wild female blackflies were collected at five cattle sheds in Oita, southern Japan, where a human zoonotic onchocerciasis had occurred. Among the eight *Simulium* species captured, *S. bidentatum* was predominant, followed by *S. arakawae*. Natural infections with filarial larvae were found in 10.5-20% of the parous *S. bidentatum* collected at three cattle sheds, and also in 6% of the parous *S. arakawae* captured at one cattle shed. Only one infected *S. arakawae* harbored two third-stage larvae in the thorax while the others had 1-4 either first- or second-stage larvae in the thorax. Third-stage larvae obtained from wild flies maintained alive for 6-9 days after being collected are distinguished into three types of *Onchocerca*: type I recovered from *S. bidentatum*, *S. arakawae* and *S. aokii* is probably new, having an elongated body length (1,075-1,380 μm), and types II and III, recovered from *S. bidentatum* and *S. arakawae* respectively, are each indistinguishable from *O. gutturosa* and *O. lienalis*. It is suggested that due to its anthropophily as well as zoophily *S. bidentatum* is the most probable vector of zoonotic *Onchocerca* (either type I or II) which may be acquired by humans in the western suburbs of Oita.

INTRODUCTION

In relation to the transmission of human zoonotic onchocerciasis found in Oita, Japan (Hashimoto *et al.*, 1990), we carried out collections of wild blackflies using human baits at the residential area of Tabaru, the suspected infection place, and suggested that *Simulium bidentatum* (Shiraki) might have been the vector of this zoonotic parasite to humans due to its high anthropophily, abundance and natural infections with larvae of *Onchocerca* spp. (Takaoka *et al.*, 1989). The identity of these zoonotic *Onchocerca* larvae and their natural animal hosts have remained to be studied yet.

We made further investigations on filarial infections of blackflies at several cattle sheds around Tabaru in the western part of Oita City. In this paper, we report that *S. bidentatum* is the predominant species attacking cattle and is naturally infected with larvae of two types of *Onchocerca* (one type, unknown and the other, resembling *O. gutturosa* Neumann, 1910). It

1 Division of Medical Zoology, Medical College of Oita, Hazama, Oita 879-56, Japan
2 Laboratoire de Zoologie Vers. Museum National d'Histoire Naturelle, 61, Rue de Buffon, 75231 Paris Cedex 05, France

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 01570216)

is further demonstrated that *S. arakawae* Matsumura is also infected with *Onchocerca* larvae indistinguishable from *O. lienalis* Stiles, 1908.

Materials and Methods

Collections of adult blackflies were carried out at five cattle sheds in the western part of Oita City. All of these cattle sheds are situated in lowland (ca. 20-30 m above sea level) and located within a 3-kilometer radius from the residential area of Tabaru (altitude ca. 30 m above sea level; 131°35'E and 33°10'N), where the zoonotic *Onchocerca* infection was presumed to have occurred.

The locality of each cattle shed surveyed, distance from Tabaru, race and number of cattle raised, structure of cattle shed and date of collections are as follows: 1) Kokubu, ca. 0.7 km north, ca. 50 Holstein, cattle shed well constructed, with windowed wall, May 1989; 2) Shimoonozuru, 2.6 km north-east, 18 Holstein, cattle shed well constructed, with windowed wall, November 1988; 3) Kamionozuru, ca. 2 km north-east, 20 Holstein, cattle shed roughly constructed, without wall, October 1988; 4) Takajou, 2.5 km east, 15 Japanese Black, cattle shed with roof but without wall, October 1988; 5) Uchiwasada, ca. 1.6 km south-east, 4 Japanese Black, cattle shed with roof but without windowed wall, October 1988. Cattle sheds 1, 2, and 3 are all situated in the rice fields along the banks of the Oita River, while cattle sheds 4 and 5, situated along the bank of the Nanase River, lie at the northern foothill of Mt. Ryouzen (596 m high). There is a low hilly area (50-100 m in altitude) with a shrubbery extending from west-south to east-north between the two river basins.

Collections were made between 16.00 and 18.00 hours, and flies landing on the inside surface of glass windows of cattle sheds 1 and 2 or flying around the body surface of the cows or collectors at cattle sheds 3-5 were captured by an aspiration tube or by an insect net, and held overnight in small plastic containers. On the following day, flies were dissected in saline on a glass slide, and parity was determined by the presence of follicular relics (Detinova, 1962). Parous females were microscopically searched for filarial larvae. The larvae found were preserved in formalin-glycerol (Wharton, 1959) for morphometric observations.

About 230 blood-fed blackflies were collected at cattle shed 2 and examined for the presence of microfilariae of bovine *Onchocerca* in the blood ingested. Microfilariae found were fixed in formalin solution, dried on slide and stained with Giemsa solution. In addition, around 1,000 unfed and blood-fed flies collected at cattle shed 2 were individually maintained alive with sucrose solution at 25°C for 6-9 days, and dissected for the presence of third-stage filarial larvae. Generic and specific diagnosis of third-stage larvae followed that of Bain and Chabaud (1986). However, it should be noted that the results given are as the most probable since the specific identification of third-stage larvae is generally difficult.

Results

Table 1 shows the results of dissections of blackflies collected at five cattle sheds. The majority of blackfly species at cattle sheds 1-4 were *S. bidentatum,* followed by *S. arakawae,* whereas the latter species predominated at cattle shed 5.

Natural infections of *S. bidentatum* with filarial larvae were found in three of five cattle sheds surveyed, with infection rates of parous flies being 10.6%, 10.5% and 20%. All the

infected *S. bidentatum* harbored a few first-stage larvae in their thorax except one fly collected at cattle shed 1 with two second-stage larvae (ca. 430 μm in body length). On the other hand, natural filarial infections were found in three of 50 parous flies (or 6%) of *S. arakawae* captured at cattle shed 5, and one of three infected flies had two early third-stage larvae in the thorax. The measurements of one of the two larvae were as follows: body length 943 μm, body width 26 μm, length of oesophagus 396 μm, length of tail 32 μm. The remaining two infected flies had one and three first-stage larvae, respectively.

In dissection of blood-fed flies two principal types of unsheathed microfilariae (X, Y) were found in three blackfly species (Table 2). All of the positive flies had no mixed infections. Type X (Fig. 1 A-D) measures 177-318 μm long and 5.0-6.5 μm wide, and is not coiled, while type Y (Fig. 1 E and F), which is 137-235 μm long and 5.0-7.5 μm wide, is coiled in the posterior region and has a thick cuticle. Type X shows variations in head morphology, as shown in Fig. 1 A-D.

Table 3 shows the results of dissections of unfed flies collected at cattle shed 2 and maintained alive for 6-9 days. The filarial infection rates of flies examined (rates of parous flies in parenthesis) were 0.9% (5.9%) for *S. aokii,* 3.0% (10%) for *S. arakawae* and 3.3% (8.6%) for *S. bidentatum.* Third-stage larvae were found in all three blackfly species examined. These third-stage larvae are classified into three types by their morphology (e. g., whole body length and length of oesophagus relative to body length) (Table 5). Type I, the longest in body length (Fig. 2 A), was found in the thorax and abdomen of three blackfly

Table 1 Species composition, parity and filarial infections of blackflies collected at five cattle sheds (sites 1-5) in western part of Oita City, southern Japan

	Collection sites														
	1			2			3			4			5		
Simulium spp.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.	No. col.	No. par.	No. inf.
aokii	10	3	0	26	14	0	2	1	0	0	—	—	0	—	—
arakawae	20	3	0	48	4	0	12	4	0	0	—	—	115	50	3
bidentatum	185	47	5	60	19	2	45	11	0	6	5	0	18	5	1
japonicum	0	—	—	0	—	—	0	—	—	0	—	—	2	0	0
nikkoense	1	0	0	0	—	—	0	—	—	2	0	0	0	—	—
quinquestriatum	1	0	0	6	3	0	3	0	0	2	2	0	9	0	0
rufibasis	0	—	—	0	—	—	0	—	—	1	0	0	0	—	—
takahasii	2	0	0	0	—	—	4	1	0	0	—	—	0	—	—

Table 2 Microfilariae (mf.) found in the blood of the stomach of three blackfly species collected at cattle shed 2

Simulium spp.	No. examined	No. with mf. (No. mf: median, range)	
		Type X	Type Y
aokii	24	4 (13, 1−71)	1 (1, 1)
arakawae	52	5 (1, 1−30)	2 (1, 1)
bidentatum	151	7 (1, 1−21)	19 (2, 1−5)

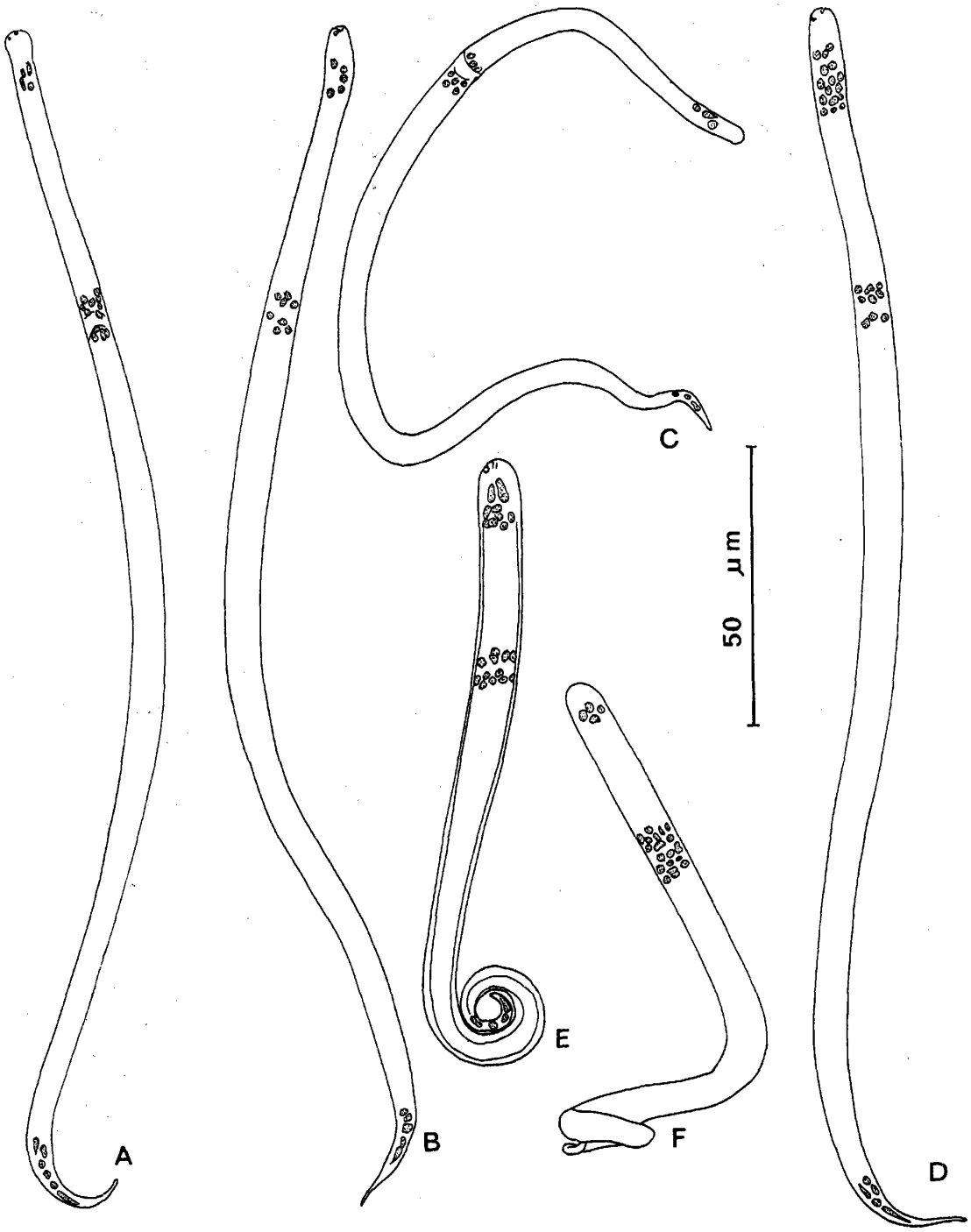

Figure 1 Microfilariae found in blood meals ingested by blackflies collected at cattle shed 2. A-D, type X assigned to *Onchocerca* spp.; A, C: ? *O. gutturosa*; B, D: ? *O. lienalis*; E, F, type Y, unknown species.

species. Type II with intermediate body length (Fig. 2 B) was found in abdomen of *S. bidentatum*. Type III, the shortest in body length (Fig. 2 C), was recovered from the head of *S. arakawae*.

These three types of third-stage larvae were also recovered from blood-fed flies dissected 6-9 days after collection (Table 4). Types II and III were found in *S. bidentatum* and *S. arakawae* respectively, whereas type I was found in both species. The overall infection rates of these blood-fed flies examined on days 6-9 after collection were 2.9% for *S. aokii*, 7.1% for *S. arakawae* and 2.4% for *S. bidentatum*.

The measurements of three types of third-stage larvae are shown in Table 5. The third-stage larvae found in the thorax of the flies were presumably young and then their measurements were not included in the table. Type I (Fig. 2 A and Fig. 3 A-E) is characterized by its large body size (1,075-1,380 μm long by 24-26 μm wide). The axial terminal caudal lappet is well distinct (Fig. 3 D and E). Tail is thick with a ratio of length/width 1.7-2.2. On the other hand, type II (Fig. 2 B and Fig. 3 F-J) measures 870-950 μm in body length and 20-21 μm in body width and has an elongated oesophagus (over half of body length). The tail is slender (Fig. 3 G and H), with ratio of tail length/width 2.5-2.7. Type III (Fig. 2 C and Fig. 3 K-P) is distinct from the former two types by its short body length (510-530 μm).

Table 3 Filarial infections in unfed females of three blackfly species collected at cattle shed 2, and maintained alive at 25°C for 6−9 days in the laboratory

Simulium spp.	No. dissected	No. parous	No. infected	No. & stages of larvae[1] Head	Thorax	Abdomen	Type of larvae[2]
aokii	107	17	1			1 L_3	1I
arakawae	99	30	2	4 L_3		1 L_3	1I, 4III
bidentatum	151	58	5		1 L_p, 2 L_3	2 L_3	3I, 1II

1. L_p and L_3 each mean preinfective and third-stage larvae.
2. See text for definition of types.

Table 4 Filarial infections in blood-fed females of three blackfly species collected at cattle shed 2 and maintained alive at 25°C for 6−9 days in the laboratory

Simulium spp.	Days post ingestion	No. dissected	No. infected	No. & stage of larvae[1] Head	Thorax	Abdomen	Type of larvae[2]
aokii	6, 7	24	1		1 L_2		
	8, 9	10	0				
arakawae	6, 7	36	3		1 L_1, 1 L_3	1 L_3	1I, 1III
	8, 9	20	1	3 L_3			3III
bidentatum	6, 7	270	4		2 L_2, 5 L_p	2 L_3	1I, 1II
	8, 9	232	8		1 L_2, 5 L_3	4 L_3	7I, 2II

1. L_1, L_2, L_p and L_3 each mean first, second, preinfective and third-stage larvae.
2. See text for definition of types.

Table 5 Measurements of third-stage larvae[1] of *Onchocerca* spp. found in three blackfly species collected at cattle shed 2 and maintained alive for 6-9 days in the laboratory

No.	Type	Characters[2] BL	BW	NR	OE	(GL)	OE/BL	TL	TW/TL	Host *Simulium*
1	I	1,220	26	70	600	(470)	0.50	40	1.8	*S. aokii*
2	I	1,220	26	75	550	(410)	0.45	40	2.0	*S. arakawae*
3	I	1,075	26	78	500	(365)	0.47	38	1.7	*S. bidentatum*
4	I	1,120	25	65	530	(460)	0.47	40	2.0	*S. bidentatum*
5	I	1,230	26	80	600	(470)	0.48	45	2.0	*S. bidentatum*
6	I	1,320	24	80	600	(460)	0.45	40	1.8	*S. bidentatum*
7	I	1,380	25	80	650	(510)	0.45	40	2.2	*S. bidentatum*
8	II	870	20	80	575	(440)	0.67	51	2.8	*S. bidentatum*
9	II	890	21	85	600	(475)	0.67	36	2.5	*S. bidentatum*
10	II	900	20	75	610	(490)	0.67	46	2.4	*S. bidentatum*
11	II	950	20	75	520	(390)	0.54	42	2.7	*S. bidentatum*
12	III	510	17	80	350	(240)	0.69	37	2.1	*S. arakawae*
13	III	510	17	70	330	(215)	0.59	36	2.1	*S. arakawae*
14	III	510	18	80	350	(220)	0.69	33	2.1	*S. arakawae*
15	III	530	18	85	330	(195)	0.62	32	2.1	*S. arakawae*

1. Only mature larvae recovered from head (no. 13-15) and abdomen (no. 1-12) were measured.
2. BL: total body length; BW: maximum body width; NR: distance from head tip to nerve ring; OE: length of oesophagus; GL: length of glandular part of oesophagus; OE/BL: ratio of length of oesophagus/total body length; TL: tail length; TW/TL: ratio of tail width/tail length.

Discussion

In the present study, three types of third-stage larvae were recovered from three blackfly species. These are all assigned to the genus *Onchocerca* by possessing the very small, almost indiscernible caudal lappets. Larvae of type I, chiefly infecting *S. bidentatum*, appear to be more frequently encountered than type II, as shown in Tables 3 and 4. The young third-stage larvae found in *S. arakawae* at cattle shed 5 probably are the same species as type I by its body length and ratio of oesophagus/body length. Type I does not correspond to any of the known third-stage larvae of *Onchocerca* (Bain and Chabaud, 1986). On the other hand, type II infecting only *S. bidentatum* is assumed to be *O. gutturosa*, a common *Onchocerca* of cattle, although this bovine filaria has been reported to develop in *Culicoides* in European region (Bain, 1979). The measurements of type III larvae recovered from *S. arakawae* agree well to that of *O. lienalis*, another bovine *Onchocerca* species, given by Bain and Chabaud (1986).

In order to know whether these three types of *Onchocerca* have originated from cattle bred in surveyed area, blood-fed blackflies were examined for the presence of microfilariae. Two principal types (X, Y) of microfilariae were found in three blackfly species (Table 2). A comparative analysis of these microfilariae and the slide-mounted microfilariae of determined *Onchocerca* from Museum National d'Histoire Naturelle, Paris, revealed that type X seems to be composed of at least two different species, i.e., one resembling *O. lienalis*, which

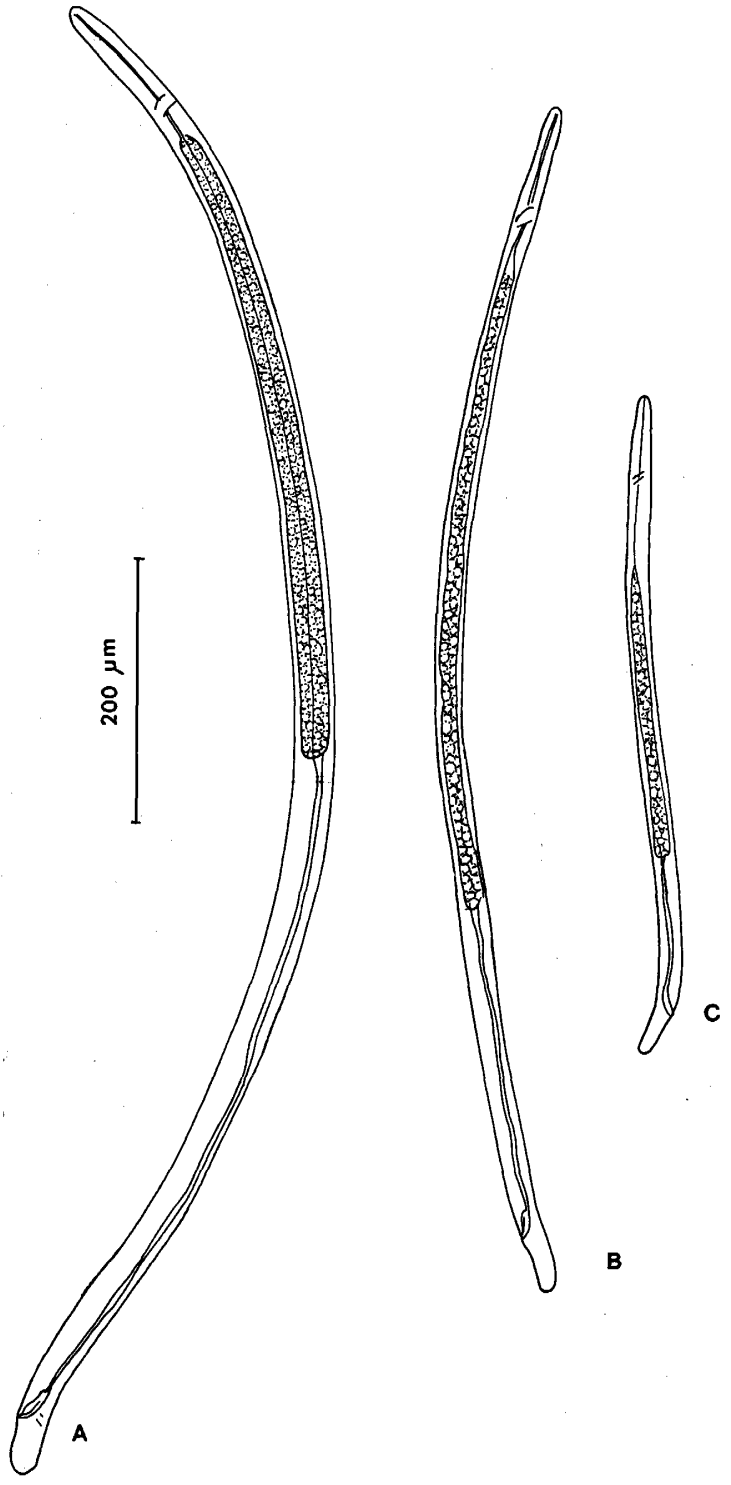

Figure 2 Third-stage larvae found in blackflies collected at cattle shed 2, and assigned to genus *Onchocerca*: general morphology. A, type I, unknown species; B, type II, ? *O. gutturosa*; C, type III, ? *O. lienalis*.

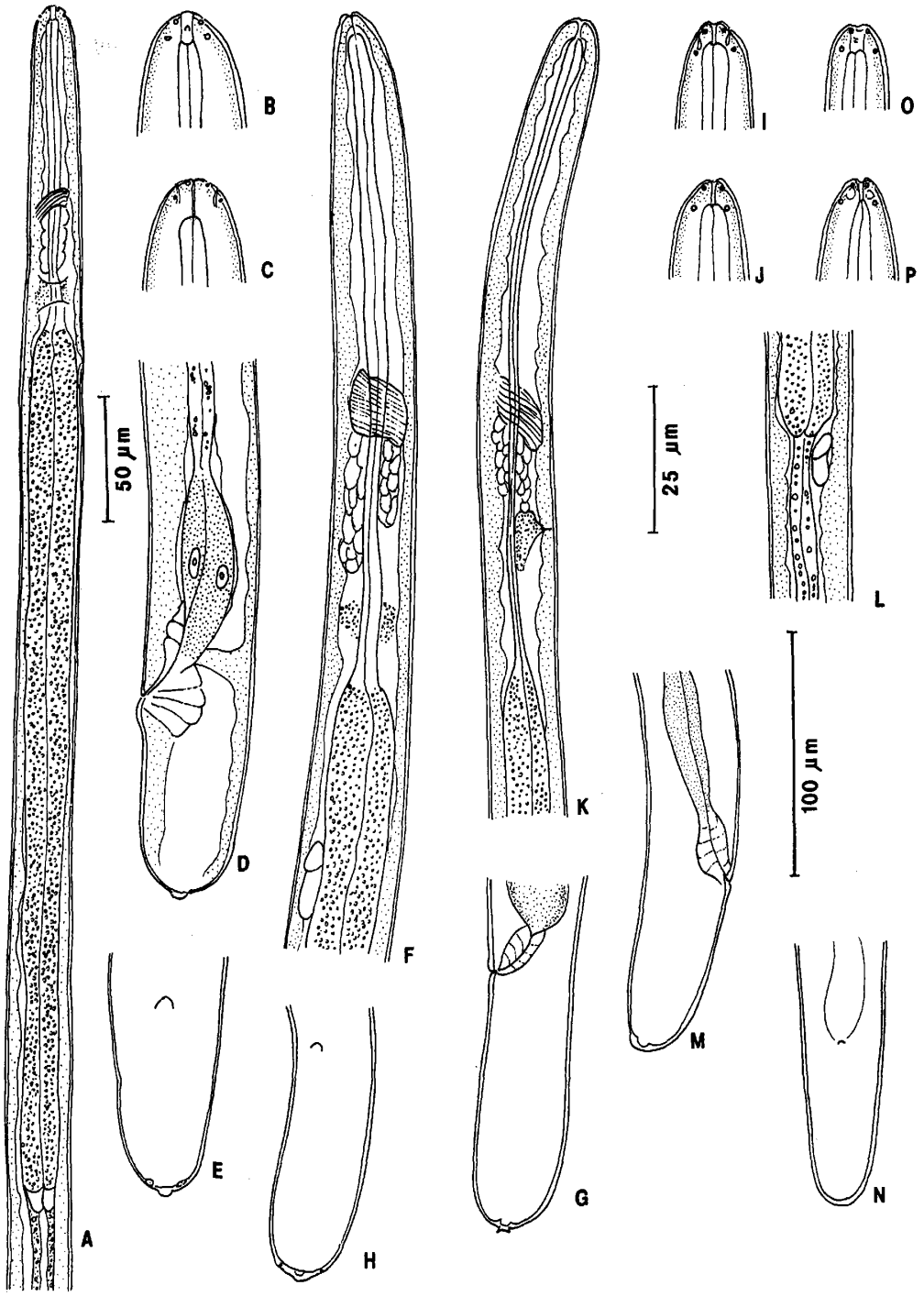

Figure 3 Three types of third-stage *Onchocerca* larvae: detailed morphology. A–E, type I, unknown species; F–J, type II, ? *O. gutturosa*; K–P, type III, ? *O. lienalis* (B, C, I, J, O, P: lateral view of heads; D, G, M: lateral view of tails; E, H, N: ventral view of tails). Scales 100 μm for A; 50 μm for F, K, L; 25 μm for the others.

is wider, especially in the anterior third of the body and presents a trapezoidal or rounded head according to the orientation, as shown in Fig. 1 B, D; the other resembling *O. gutturosa* with an inflated or slim head, as shown Fig. 1 A, C. On the other hand, type Y remains unidentified, although it resembles some species of *Cercopithifilaria* Eberhard, 1980 by its flattened body; microfilariae of this genus are dermic and vectors are ticks (Bain *et al.*, 1988).

Blood-fed flies were maintained alive for 6-9 days in an attempt to determine whether microfilariae ingested by blackflies at cattle shed 2 could develop to third-stage larvae. The rate of blood-fed flies harboring third-stage larvae of each type was compared to that of unfed flies (Tables 3 and 4). Our data show that the rate of blood-fed *S. arakawae* infected with third-stage larvae of type III (3.6%) was slightly higher than that of unfed flies (2.1%), suggesting the development to the third stage of a proportion of microfilariae ingested. However, no such an increased infection rate was observed in *S. bidentatum* for type I (1.2% for blood-fed flies vs. 2.0% for unfed ones) and type II (0.6% vs. 0.7%). Most of the third-stage larvae of types I and II found in blood-fed *S. bidentatum* might have originated from the previous infection; it should be noted that the rate of *S. bidentatum* ingesting microfilariae resembling *O. gutturosa* is suspected to be very low, since majority of type X microfilariae are likely to be *O. lienalis,* judging from the characteristic consistent pattern of acid phosphatase activity (unpublished data), as observed in the microfilariae of *O. lienalis* (Trees *et al.*, 1987). Further studies are needed to related type X microfilariae to types II and III third-stage larvae. The identity of the type I third-stage larvae is at present uncertain. It is possible that this also is a bovine *Onchocerca,* because of the absence of other ungulates as a reservoir host in and around the study area, as well as its frequent occurrence in the cattle-attacking blackfly species. The possible relationship of type I third-stage larvae with type Y microfilariae, both of which were found most frequently in *S. bidentatum,* is under investigation.

In the previous paper (Takaoka *et al.*, 1989), we reported that *S. bidentatum* was anthropophilic and was naturally infected with two types of zoonotic *Onchocerca* larvae, suggesting the possible involvement of this blackfly species in the transmission of human zoonotic onchocerciasis in Oita, southern Japan. Our results demonstrate that *S. bidentatum* is the predominant blackfly species attacking cattle in the same area, followed by *S. arakawae,* and that three types of *Onchocerca* larvae are infecting three blackfly species. *Simulium bidentatum* is infected with two (types I and II) of these three types, as mentioned above. It should be noted that two types of third-stage larvae reported from *S. bidentatum* collected on human baits at Tabaru correspond to these types I and II respectively. It is thus suggested that *S. bidentatum* is the most probable potential vector of zoonotic onchocerciasis which may be acquired by humans in the western part of Oita City, and types I and/or II will be responsible as its causative parasite. The possibility of type III being transmitted to humans should not be ruled out since *S. arakawae,* the probable vector of this parasite, has been reported to bite man in the same area (Takaoka *et al.*, 1989).

ACKNOWLEDGEMENTS

We are grateful to Dr. I. Tada, Kumamoto University Medical School, for his valuable comments. Thanks are also due to Dr. M. Baba, Miss C. Aoki, and K. Ogata, Medical College of Oita, for their assistance.

References

1) Bain, O. (1979): Transmission de l'Onchocerque bovine, *Onchocerca gutturosa,* par *Culicoides,* Ann. Parasitol. Hum. Comp., 54, 483-488
2) Bain, O. and Chabaud, A.G. (1986): Atlas des larves infestantes de filaires, Trop. Med. Parasit., 37, 301-340
3) Bain, O., Wamae, C.N. and Reid, G.D.F. (1988): Diversité des filaires du genre *Cercopithifilaria* chez les babouins, au Kenya, Ann. Parasitol. Hum. Comp., 63, 224-239
4) Detinova, T.S. (1962): Age-grouping methods in Diptera of medical importance, Monograph Ser. W.H.O. no. 47, pp. 217
5) Hashimoto, H., Murakami, I., Fujiwara, S., Takayasu, S., Takaoka, H., Uga, S., Akao, N., Kondo, K. and Yoshimura, H. (1990): A human case of zoonotic onchocerciasis in Japan, J. Dermatol., 17, (in press)
6) Takaoka, H., Baba, M. and Bain, O. (1989): Natural infections of *Simulium bidentatum* (Diptera: Simuliidae) with larvae of *Onchocerca* spp., in relation to a human zoonotic onchocerciasis in Oita, Japan, Japan. J. Trop. Med. Hyg., 17, 279-284
7) Trees, A.J., McCall, P.J. and Crozier, S.J. (1987): Onchocerciasis in British cattle: a study of *Onchocerca gutturosa* and *O. lienalis* in North Wales, J. Helminthol., 61, 103-113
8) Wharton, R.H. (1959): A simple method of mounting and preserving filarial larvae, Bull. Wld. Hlth Org., 20, 729-730

大分のブユ成虫における動物寄生性オンコセルカ3種幼虫の感染について

高岡　宏行[1]・Odile Bain[2]

大分市で見いだされた動物寄生性オンコセルカの人体寄生症例の起因種，および媒介ブユ種を追究する目的で，人体への感染が起きたと思われる地区周辺の牛舎においてブユの採集を行い，フィラリア幼虫の感染を調べた。採集されたブユ8種のうち，キアシツメトゲブユ *S. bidentatum* が優先種で，ヒメアシマダラブユ *S. arakawae* が次に多かった。フィラリアの自然感染は，調べた5牛舎のうち3牛舎で採集されたブユに認められた。フィラリアの種を確かめるため，1牛舎で採集したブユを6－9日間飼育した後，第3期幼虫を回収し，形態学的な検討を行った。その結果，牛のオンコセルカとして知られている *O. gutturosa* および *O. lienalis* と思われる幼虫が，それぞれキアシツメトゲブユおよびヒメアシマダラブユより見いだされた。さらに，未記録種と思われる第3の幼虫が，上記2ブユ種とアオキツメトゲブユ *S. aokii* に感染していることが分かった。既に報告した，人囮法によって採集したブユのフィラリア感染の結果と合わせて，*O. gutturosa* と思われる種と未記録種の2種のオンコセルカが，キアシツメトゲブユによって人へ感染する可能性が示唆された。

1　大分医科大学医動物学教室
2　Laboratoire de Zoologie Vers., Museum National d'Histoire Naturelle, Paris, France

Japan. J. Trop. Med. Hyg., Vol. 18, No. 1, 1990, pp. 11-16

Research note

POSSIBLE CAUSES LEADING TO AN EPIDEMIC OUTBREAK OF SLEEPING SICKNESS: FACTS AND HYPOTHESES

DAWSON B. MBULAMBERI

Received March 13 1989/Accepted December 1 1989

Abstract: Sleeping sickness epidemics have been noted to occur with some degree of periodicity and the question as to why this is so has been asked for quite a long time. These epidemics have been partially controlled in the past using the conventional methods of bush clearing, mass diagnostic surveys and treatment. Political, social and economic upheavals have been found to be very important factors in the recurrence of these epidemics. In addition, a number of facts and hypotheses have been advanced as possible causes of epidemic outbreaks of sleeping sickness.

This paper presents a brief account of factual epidemic outbreaks of sleeping sickness in south eastern Uganda (Busoga) and then proceeds to discuss, in general terms, a number of hypotheses that have been incriminated to date, as possible causes that might lead to an epidemic outbreak of the disease.

HISTORICAL PERSPECTIVE

The sleeping sickness epidemic which devastated the shores of Lake Victoria at the beginning of this century is a famous event in the annals of tropical medicine. It is famous because an estimated one quarter to one third of a million people lost their lives (Langlands, 1967). The same epidemic brought controversy as to whether Dr. Castellani or Colonel Bruce first identified the trypanosome as the cause of the epidemic (1962).

The cause of the epidemic was attributed to *Trypanosoma gambiense* introduced to this part of the country by a party accompanying the explorer Lugard from the Congo basin on relief of Emin Pasha expedition in 1894 (Christy, 1903). However, more properly the cause lay in the general increase in social, commercial and military mobility which developed throughout tropical Africa in the late nineteenth and early twentieth centuries.

Another outbreak involving about 2,500 persons occurred in the same area from Jinja eastwards to the border with Kenya between 1939 and 1945 (Machichan, 1944). The most striking feature of this epidemic was the virulence and rapid course of illness as contrasted with *T. gambiense* infection. An early and profuse appearance of posterior nucleate forms and a rapid and fatal course of the disease was observed on animal inoculation. Thus, it is believed, the epidemic was caused by *T. rhodesiense*. The first cases detected were among migrant workers employed on Kakira Sugar estates. Since these immigrants came from

Uganda National Sleeping Sickness Control Programme, P. O. Box 1241, Jinja, Uganda

areas of reasonable proximity to the infected areas of Tanganyika (now Tanzania), this epidemic was thought to have been introduced by them.

Since that outbreak, cases continued to be reported from within the infected area, though not in epidemic numbers. In 1971, infection spilled north of the usual focus and involved up to 169 persons. Through the combined efforts of EATRO (now UTRO) and the Ministry of Health, this outbreak was soon brought under control.

Present Situation

Following the control of the small epidemic of 1971, surveillance programmes were not instituted because of the prevailing political and economic atmosphere in the country at that time. There was indiscriminate and haphazard movement of people and livestock across the traditional trypanosomiasis barrier zone. Besides smuggling of commodities including cattle between Kenya and Uganda across the zone, became a means of livelihood. It was therefore difficult for the Ministry of Health teams to enforce surveillance measures. The Tsetse Control Department could not carry out control programmes due to lack of insecticides, transport and manpower. Thus, there was total breakdown of control measures and hence, by 1976, the stage was set for another epidemic outbreak of the disease in the area.

In August, 1976 a report was received at the then East African Trypanosomiasis Research Organization (EATRO) now Uganda Trypanosomiasis Research Organization (UTRO) of an outbreak of sleeping sickness in Luuka County of Iganga District. A preliminary survey by EATRO (now UTRO) medical team revealed 12 positive cases out of 812 persons examined (prevalence rate of 1.5%) (EATRO unpublished observations, 1976). Unfortunately, in June 1977, the East African Community collapsed and EATRO lost valuable logistics to a partner state. These included vehicles and laboratory equipment. In addition, several members of staff were forced to go into exile because of the harsh political and economic atmosphere prevailing in the country at the time of Amin's rule. Thus, UTRO, probably the department in the best position to help contain this epidemic during its outset, was rendered helpless.

The Ministry of Health, Uganda Government, posted microscopists to the area and later opened up treatment centres in the area. Since then, however, the incidence of the disease has continued to increase from 52 cases in 1976 to over 8,000 cases in 1980 (see Table 1).

Hypotheses of Epidemiological Importance

To date, a number of general hypotheses have been advanced to explain the occurrence of sleeping sickness epidemic outbreaks. Some of these hypotheses are discussed as follows.

Increased man-fly contact:

This phenomenon occurs most commonly during the hot, dry season with the result that transmission is enhanced. Indeed a period of drought almost invariably means an increase in the number of infections because few sources of water are shared by man, fly and game animals, in the case of *T. rhodesiense,* in close association; there is also more hunting and more searching for wild forest products at times when crops are bad. This phenomenon has been described by Willet (1965) and many other workers.

Changes in climate, vegetation and tsetse fly distribution:

Climate appears to be of more than ordinary importance. At higher temperatures, there is increased salivary gland infection rate in the tsetse flies, and in addition to this direct effect, there are many ways in which the climate influences a closer association between man and fly.

In Kenya a succession of heavy rains provided *G. f. fuscipes* with suitable conditions well outside its usual riverine habitat so that it was able to live and breed in the vegetation surrounding the homesteads-conditions which gave rise to the Alego outbreak of 1965. This same factor has had a role in the current epidemic in Uganda where the abundant growth of *Lantana camara* thickets near homesteads, has attracted *G. f. fuscipes* into peridomestic contact with man.

Table 1 Annual incidence of sleeping sickness in south eastern Uganda 1976-1987

Year	Number of cases
1976	52
1977	586
1978	2,076
1979	4,991
1980	8,465
1981	1,938
1982	1,309
1983	1,199
1984	1,956
1985	3,517
1986	4,446
1987 (up to end of July)	5,053
Total	35,588

There is too the influence of climate in determining where people choose to live, and the population density of both flies and human beings, as discussed by Ford (1965) and this is relevant to the proper use and planned full development of land which is the ideal at which to aim in the eradication of the tsetse fly and trypanosomiasis.

Infection rates in the tsetse flies and their infectivity:

Wijers (1958) observed that infection rates were highest in flies taking an infective blood meal on the day on which they emerged, somewhat lower on the second day after emergence, and did not occur thereafter. Thus the fact that flies emerging during the hot season are likely to feed early in their adult life means that infection rates in the fly are maximal during the hot, dry season. However, the number of trypanosomes inoculated by an infected tsetse fly varies greatly even among flies infected from the same host and in the same fly at different times.

It has been estimated that the minimum infective dose of *T. rhodesiense* for man is of the order of 350 parasites and it is probable that a similar number would be required to establish infection with *T. gambiense* (Fairbairn and Burtt, 1946)

Population density of the tsetse flies and their feeding behaviour:

A tsetse fly feeding on a number of animals and possibly also on man, may become infected with many different strains of trypanosome. Most of these strains will be non-pathogenic for man and even if a man-infective strain is acquired by the fly, the tendency will be for it to be so diluted by the non-pathogenic strains that it will not be passed on in a number sufficient to cause an infection in man.

Tsetse longevity:

Flies emerging at the end of the hot, dry season are particularly receptive to trypanosome infection since they will feed early in adult life. With the onset of the rainy season, the

expectation of life of the tsetse flies is maximal so that a combination of these factors produces a situation in which infected flies are liable to survive for protracted periods which enhances the potential for these flies to transmit the disease, of course depending on their infection rates.

Presence of domestic and wild animal reservoir hosts:

The pig in the case of *T. gambiense* and cattle in the case of *T. rhodensiense* have been incriminated as domestic animal reservoir hosts, while the kob and hartebeest in the case of *T. gambiense* and the bushbuck in the case of *T. rhodesiense* have been incriminated as wild game reservoir hosts. The bushbuck is particularly important because it tends to live in thickets near human habitation which puts it in close contact with man.

Appearance of different forms of the parasite:

The appearance of such parasites may be due to either the parasites being introduced from outside the area or genetic changes in the parasite.

There is at least a suspicion, based on field observations, that zymodemes of trypanosome introduced into fresh localities may exhibit an enhanced ability to spread through the community. Scott (1961) reported two instances in which the introduction of infected persons from an established epidemic area resulted in sharp outbreaks of the disease in endemic localities far removed from the original focus of infection. There are other similar observations suggesting that severe local outbreaks which quickly follow the introduction of infected persons to fresh localities are in some way connected with enhanced ability of the zymodeme to spread. Indeed, the possible existence of epidemic trypanosome zymodemes has been advanced by some workers.

Changes in population movements and population growth:

It is generally supposed that population movements are liable to precipitate epidemics. Refugees displaced as the result of war, famine, earthquakes and other similar occurrences are notoriously prone to disease in epidemic form as are also immigrant labourforces recruited for large scale construction work (tropical aggregation of labour) and pilgrims attending major religious festivals.

A new population in an area may spark off an epidemic outbreak of sleeping sickness in the area as a result of imported cases among them, which may be sufficiently large to augment the reservoir of infection available to the insect vector and so in a quantitative manner promote transmission. An imported strain may also show quantitative differences such as enhanced virulence or ability to spread or may be one to which the indigenous population has not been previously exposed and to which no resistance has been acquired. This phenomenon can also operate vice versa. Further, as with some other diseases, the periodicity of epidemics of sleeping sickness may be associated with growing up of a new generation of people with no previous experience of the disease.

Occurrence of subacute cases of the disease:

Another factor which might conceivably influence the spread of sleeping sickness is the presence of an undetected and perhaps unsuspected reservoir of infection in the form of human 'healthy carriers' of the disease which has been reported by several workers (Buyst, 1977; Rickman, 1974; Woodruff, 1982).

Under conditions in which man-biting tsetse are common where people congregate, the ambulant human carrier assumes a powerful potential for the onward transmission and spread of sleeping sickness. The exact extent of asymptomatic carriers is certainly low.

However, sleeping sickness cases with non-specific symptoms (fever, headache) who remain ambulant for several weeks are common, and they too may be important reservoirs of infection where man-fly contact is intense (Wurapa, 1984). This threat is also present among many early cases of the Gambian disease in which the initial stages are generally relatively mild and the victim may continue at work for many months or even for years, before he is eventually driven by increasing illness to seek treatment or to retire to his home. During this time, he is a constant source of infection to tsetse so that the very nature of the illness provides great opportunities for its spread. In the Ugandan situation, however, the question of delayed diagnosis and treatment is a big factor.

Human behaviour and activities in the fly habitat:

Man becomes infected during travel, hunting, fishing, collection of honey, or when working in the fly bush. Fishing and 'honey-hunting' are particularly hazardous occupations. While fishing in the riverine pools surrounded by thickets, man may be in close contact with tsetse fly for many days at a time, a situation in which the association between man, bushbuck and the tsetse fly is likely to be significant and therefore conducive to transmission and spread of the disease. Wyatt *et al.* (1985) working in north east Zambia found fishing to be more common among cases of sleeping sickness than controls and that it represented a hazard either while walking to the stream or while engaged in the activity of fishing itself.

Large regional and national development projects:

Projects like the on-going Onchocerciasis Control Programme in the Volta River Basin, construction of dams for power generation or irrigation, and agricultural or agro-industrial projects bring about changes in the local ecology in general. Moreover, such projects attract migrants and, whether spontaneous or planned, the resulting settlements are often inadequately supervised from the health and sanitation point of view. Also, the risk of sleeping sickness is rarely specifically considered when such projects are planned.

Political and economic upheavals:

These cause extensive and often uncontrolled movements of people into areas that were previously abandoned because of epidemics thereby promoting circulation of the parasite in the population and the risk of contact between people and the tsetse flies. These upheavals lead, in the final analysis, to a breakdown in vital social services including systematic medical and vector surveillance programmes. This is obviously the most important factor in the case of the current epidemic in Uganda as is the case in other countries like Angola, Mozambique and the Sudan which are also bedevilled with civil strife.

Acknowledgements

My sentiments of acknowledgement are extended to Drs. G.B. Wyatt and D.H. Smith for their guidance on the preparation of this paper.

References

1) Buyst, H. (1977): The epidemiology of sleeping sickness in the historical Luanga Valley, Annales de la Societe Belge de Medicine Tropicale, 57, 349-359

2) Mackichan, I.W. (1944): Rhodesian sleeping sickness in eastern Uganda, Trans. Roy. Soc. Trop. Med. Hyg., 38, 49

3) Mbulamberi, D.B. (1982): A survey of the prevalence of human trypanosomiasis in Kigulu County in Iganga District, Uganda. A dissertation submitted for the Academic Postgraduate Diploma in Public Health, University of Makerere
4) Morris, K.K.S. (1959): The epidemiology of sleeping sickness in East Africa. A sleeping sickness outbreak in Uganda in 1957, Trans. Roy. Soc. Trop. Med. Hyg., 53, 384
5) Onyango, R.J., Van Hoeve, K. and De Raadt, P. (1966): The epidemiology of *T. rhodesiense* sleeping sickness in Alego location, Central Nyanza, Kenya with evidence that cattle may act as reservoirs to most of the trypanosomes infective to man, Trans. Roy. Soc. Trop. Med. Hyg., 60, 175
6) Rickman, K.R. (1974): Investigations into an outbreak of human trypanosomiasis in the lower Luangwa Valley, Eastern Province, Zambia, East African Med. J., 51, 467-487
7) WHO Expert Committee on the Epidemiology and Control of African Trypanosomiasis (1986): Wld. Hlth. Org. Tech. Report Series, No. 739
8) Wyatt, G.B., Boatin, B.A. and Wurapa, F.K. (1985): Risk factors associated with the acquisition of sleeping sickness in north-east Zambia: A case control study, Annls. Trop. Med. Parasit., 79, 385-392
9) Woodruff, A.W., Evans, D.A. and Owino, N.O. (1982): A 'healthy' carrier of African trypanosomiasis, Journal of Infection, 5, 89-92

症例報告

輸入動物の寄生虫
V. オオガラゴに見出された舌虫幼虫

影井 昇[1]・七里 茂美[2]

平成元年6月15日受付/平成元年12月6日受理

輸入動物の寄生虫類の中には，その動物が輸入されることによって，それらの動物から直接人体に感染し問題を提起するものと，輸入動物に寄生していた寄生虫が我が国に土着し，それでもって我が国に流行をもたらすものとがある（影井，1988)。この後者のような過程をとって流行する寄生虫病は，その流行した後の撲滅作業が極めて困難になることのあることは，第二次大戦後礼文島で発見され，現在は北海道をも侵襲し，蔓延を続けている多包条虫の感染例を見れば明らかであろう。

我が国における舌虫類に関する調査は，利岡（1968）やKeegan *et al.*（1969）による日本産動物についての報告以外に，輸入動物からの舌虫類の報告も決して少なくはなく（中川ら，1967; 小山ら，1974; Kugi, 1977; 山口，庄司，1978; 山本ら，1978; 町田，1983)，今後も輸入され，報告されるであろう。

本報告では，アフリカから輸入されたオオガラゴ *Galago crassicaudatus* に見出された舌虫幼虫の寄生虫学的検索と，今後の問題点について考察する。

症　例

アフリカ東部産のオオガラゴ4頭（成・幼獣雌雄各1頭ずつ）を，1987年11月17日に購入・輸入し，多摩動物園にて飼育中の翌月16日に雌幼獣の1頭（体重650g）が死亡したので，死因究明のため剖検を行ったところ，写真1に示すように各臓器表面，ならびに内部に多数の虫体寄生が見出されたので，それらについて寄生虫学的検索を行った。なおその他の寄生虫類については，各臓器ともその感染は証明されなかった。

死亡したオオガラゴは，外観上は可視粘膜が蒼白であったが，潰瘍などは認められず，皮下脂肪は少なかった。天然孔の汚れは見られなかったが，肛門に乾燥した硬固便の付着が見られた。

剖検により，表1のごとく各臓器より乳白色の虫体が計25匹見出された。表1の備考に，簡単な肉眼所見を記した。

生存している残りの3頭中，死亡幼若雌と同居していた幼若雄は，体重の増加はあまり見られていないが，X線ならびに糞便検査では認めるべき異常はなく，現在も元気に生活している。この幼若雌ガラゴの死亡原因については不明で，舌虫寄生が死亡に至らしめたか否かも不明である。

寄生虫学的検索

虫体は各組織（肝臓，肺臓，脾臓，横隔膜，腸間膜，腹壁）の表面近く，あるいは組織内に埋まったような形で，それぞれの部位でC字型渦巻き状に薄い透明な膜によって包まれた形で被嚢しており（写真1，直径4-6 mm)，25匹のうち剖検時に遊離した虫体2匹（写真1，2）の計測値は，それぞれ体長17.6 mm，16.7 mm，体幅2.48 mm，2.10 mmを示した。それぞれの虫体表面に

1 国立予防医学研究所寄生虫部
2 東京都多摩動物公園衛生第2係

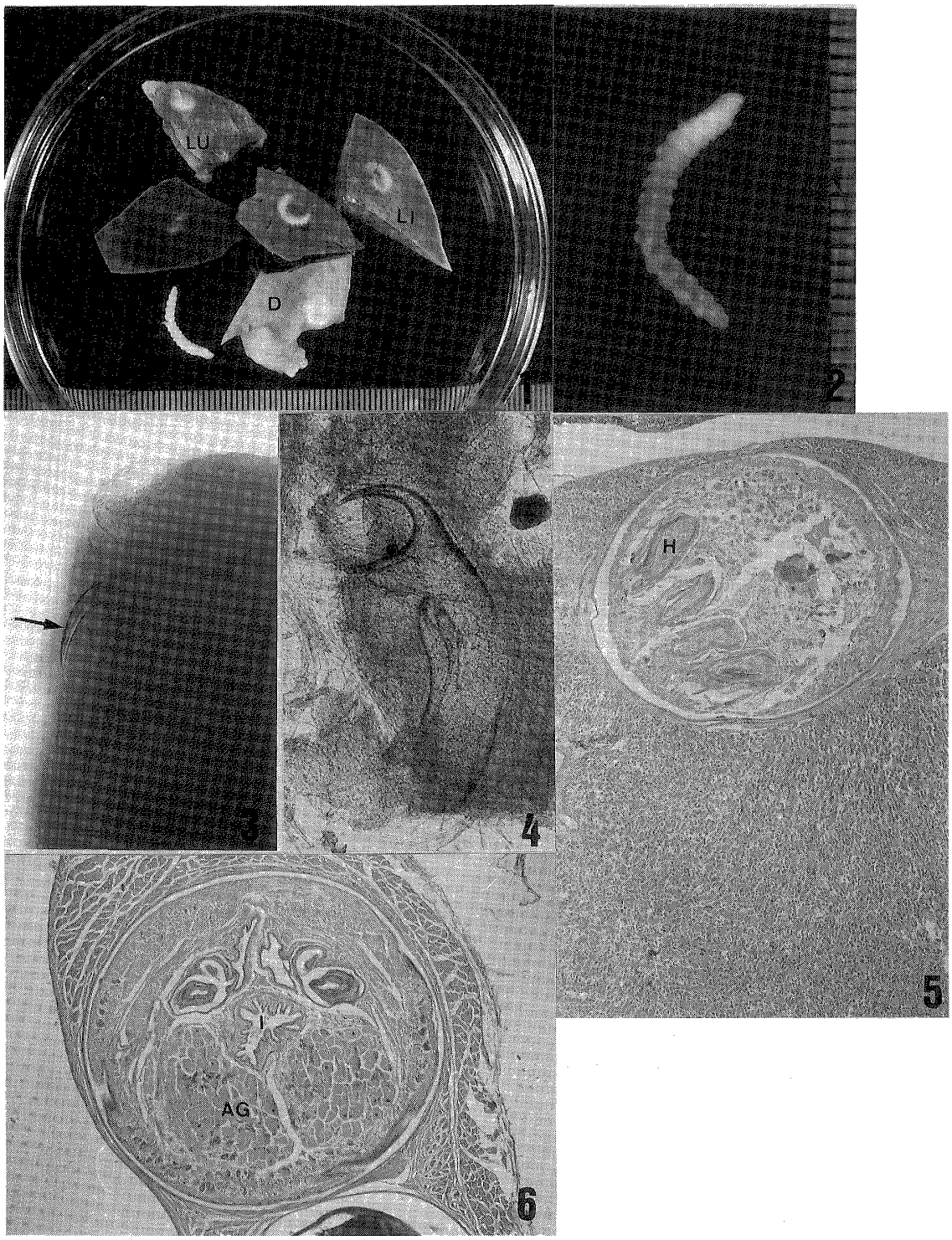

Photo. 1 Many small cystic nodules were found in the liver (LI), diaphragm (D) and lung (LU) of a died thick-tailed bush baby.
Photo. 2 *Armillifer armillatus* nymphs removed from the cyst showing typical annulations.
Photo. 3 Anterior part of *A. armillatus* nymph showing a hook (arrow).
Photo. 4 Hook piked up from anterior part of *A. armillatus* nymph.
Photo. 5 Cross section of *A. armillatus* nymph encysted on the liver. Hematoxylin-eosin stain. H: hook.
Photo. 6 Cross section of *A. armillatus* nymph encysted on the diaphragm. Hematoxylin-eosin stain. AG: acidophilic glands. I: intestinal tract.

Table 1 Number of cysts in each organs and its views with the necked eye

Organs	No. of cysts	Appearances
Right abdominal-wall	4	
Left lung (upper)	1	Mucous membrane of bronchus is brown color
(under)	1	
Right lung (upper and middle)	1	Larvae parasite between upper and middle lung with kitting, and there are abscess
Diaphragm	1	
Liver (diaphragm-side)	5	
(internal organ-side)	5	
Spleen	3	
Mesenterium	4	
Total	25	

は体環（annulations）が見られ，その数は16ならびに20個であった。

虫体の頭部には，2対の鎌状に湾曲した鉤（写真3の矢印）があり，その大きさは978 μm×208 μm，その先端は写真4に示すように2分して，互いに向きあった状態で爪状に湾曲し，その先端はそれぞれ尖っている。この2本の爪は一方が長さ308 μmと長く，他は192 μmと短かった。

以上の虫体長，ならびに外皮に極めて明瞭で特徴のある体環を備えていることと，その体環数から本虫が *Armillifer armillatus* の nymphs であると同定された。

肉眼的には肺の一部に癒着が見られ，また肺と肝臓において嚢瘍が見られた以外は，大きな所見はなかった（表1）。

肝臓（写真5），ならびに横隔膜（写真6）内における虫体寄生部位の病理組織学的検査で，薄い膜に包まれた虫嚢中には被嚢した虫体が見られ，虫体は6.4 μmの厚さの角皮層に包まれ，その角皮層の一部に sclerotized openings が見られるが，ほぼ表面は滑らかで，体表には棘などは見られなかった。角皮には腺と筋線維が見られ，体内には好酸性の特徴のある腺（AG）と，腸管（I）が縦走するのが認められた。肝臓においても横隔膜においても，虫体周囲における組織の炎症性反応は見られなかった。

考　察

節足動物と環形動物の両門に類似性を持った舌虫類は，現在13属43種が知られており（Sambon, 1922），我が国でも日本産動物における報告，ならびに輸入例についてはいくつかの報告が見られるが，それらの中に *Armillifer armillatus* の報告はない。

今回オオガラゴから見出された舌虫幼虫は，その外皮に棘が無いこと，特徴のある一定数の体環を有することから *Armillifer* 属舌虫類と考えられたが，*Armillifer* 属には4種類（*A. armillatus*, *A. grandis*, *A. angkistrontis* および *A. moniliformis*）が報告されている。うちアフリカ産動物には，*A. armillatus* ならびに *A. grandis* が報告されているが，近年は東南アジア産 *A. moniliformis* もアフリカに進出して来ているといわれる（Riley and Self, 1981）。ただ現在までに示されている *A. armillatus* の体環の数は，15-19（雄虫），18-22（雌虫）であるのに比べて，*A. moniliformis* は23以上であるという報告からするならば，今回見出された虫体は明らかに後者とは異なると考えられた。また *A. grandis* は *A. armillatus* より小型である（9-13 mm, Bygbjerg and Rask, 1978）とされているので，本虫は *A. armillatus* と同定さ

れた。もっともSelf (1969) は体環の数には変異があり，最終の脱皮が行われなければ確実な数にはならないと述べているが，Riley *et al.* (1985) は確かにその数に変異はあるが，少なくとも *Armillifer* 属においてはnymphの時期にその体環数は確立し，同定が可能であると報告している。

A. armillatus のnymphsは霊長類，食虫類，食肉類，ゲッ歯類，有蹄類などに属する約80種類の中間宿主に寄生することが報告されているが，ガラゴ（*Galago senagalensis*）における寄生はFain (1961) の報告のみでオオガラゴからの報告はなく，当然 *A. armillatus* の我が国からの報告も本例が初めてである。

A. angistrodontis を除く *Armillifer* 属4種は，すべて人体感染例が報告され，特に *A. armillatus* の人への感染に関する問題は，既に1847年にアフリカ人から報告されており（Fain, 1966），その後も感染者は決して少なくなく（Smith *et al.*, 1975），その寄生部位や寄生虫体数いかんでは急性症状を呈したり（Herzog *et al.*, 1985），中には腹痛と水様性下痢便排出後死亡した例も見出されており（Cannon, 1942），人畜共通の寄生虫としては，軽視することの出来ない問題を有している（Fain, 1966）。

A. armillatus は熱帯アフリカにのみ見られ，その終宿主は *Bitis* 属（*B. arictans* および *B. nasicornis*），あるいは *Python* 属（*P. sebae* および *P. regius*），*Cerates* 属（*C. cornutus*）などの大型の蛇類である。成虫はそれら蛇類の肺に寄生し，そこで産出され，糞便あるいは唾液と共に排出された虫卵により汚染された水や，野菜などから前述の中間宿主となる動物が感染するが，人もその例外ではない。これらの動物においては，幼虫（nymph）の形で腹腔内各臓器に寄生する。自然界における各動物でも寄生虫体が多数見出されているが，本症例でも25匹の虫体が見出された。しかしGraham (1960) も述べているように，虫体寄生部位における組織反応は殆ど見られず，通常高率寄生でも殆ど発病につながるような問題は認められないとされているが，稀にWhitney and Kruckenberg (1967) に見るように，24頭の実験用の尾長猿の7頭が本虫寄生により腹膜炎を起こして死亡したという報告があるので，実験用動物の使用上の問題がないとは言えない。特に虫体感染後の細菌などの二次感染で肺炎を起こしたり，ある器官への多数の集中寄生が，強い炎症を起こす場合などが考えられる（Riley, 1986）。

予防法としては，先ず虫体の発見にあるが，nymphの形で寄生している中間宿主動物の輸入例では，剖検を行わないと発見には至らないし，終宿主蛇類における糞便検査などは殆ど行われないので，未発見のものも多いと考えられ，終宿主は人をも含めた他動物への感染という観点からは，十分な注意が必要である。

輸入動物の死亡に伴って，それらの動物の肉や内臓を蛇類へ投与することなどはまず考えられないので，中間宿主動物の輸入でもって日本での流行が見られるとは余り考えられないが，大型蛇類の食品としての輸入，あるいはペットとしての輸入は，不完全な蛇料理によって（Azinege *et al.*, 1978），あるいはその飼育いかんによっては，蛇類の排出する便，ならびに唾液から直接，あるいは種々の食物・器物が虫卵で汚染され，飼育者本人はもちろん，家族その他における感染が懸念されるので，輸入後の検疫によって虫卵排出の有無を確かめ，予防対策を立てることが望ましいと考えられる。

人体感染者の輸入症例，あるいは我が国でのペットなどからの感染者に対しての腫瘍との鑑別診断が重要であり，Bygbjerg and Rask (1978) の報告に見られるX線による診断は効を奏すると考えられるし，免疫血清学的診断法についても，今後次第に検討がなされるものと考えられる。

ま と め

アフリカ産オオガラゴが輸入され，その飼育中に死亡し，剖検したところ，25匹の舌虫 *Armillifer armillatus* の幼虫が見出されたので，その日本における最初の輸入例として報告した。

本幼虫による日本での流行は考えられないが，今後成虫の輸入に伴っての日本の動物間での流行はもちろん，人体への感染症例も懸念されるので注意を喚起した。

参 考 文 献

1) Azinge, N.O., Ogidi-Gbegbaje, E.G., Oscunde, J.A. and Oduah, D. (1978): *Armillifer armillatus* in Bendel State (midwest) Nigeria (a village study in Ayogwiri Village, near Auchi), J. Trop. Med. Hyg., 81, 76-79
2) Bygbjerg, I.C. and Rask, M.P. (1978): Pentastomiasis and cancer of the colon, Trans. Roy. Soc. Trop. Med. Hyg., 72, 54-55
3) Cannon, D.A. (1942): Linguatulid infestation of man, Ann. Trop. Med. Parasit., 36, 160-166
4) Fain, A. (1961): Les pentastomids de l'Afrique centrale. Musee Royal de l'Afrique Centrale Anales, Serie in 8, Sci. Zool., 92, 1-115
5) Fain, A. (1966): Pentastomida of snakes—Their parasitological role in man and animals, Mem. Inst. Butantan Simp. Internac., 33, 167-174
6) Flynn, R.J. (1973): Parasites of Laboratory Animals, The Iowa State Universsity Press
7) Graham, G.L. (1960): Parasitism in monkeys, Annals N.Y. Acad. Sci., 85, 842-860
8) Herzog, U., Marty, P. and Zak, F. (1985): Pentastomiasis: Case report of an acute abdominal emergency, Acta Tropica, 42, 261-271
9) 影井　昇 (1988)：最近話題の寄生虫病(1)(2)，月刊薬事，30，1691-1698；2389-2402
10) Keegan, H.L., Toshioka, S., Matsui, T. and Suzuki, H. (1969): On a collection of pentastomids from East and Southeast Asia, Sanitary Zool., 20, 147-157
11) Kishida, K. (1928): A new linguatulid, *Armillifer yoshidai* with notes on the Porocephalidae, Ann. Zool. Japon., 11, 396-405
12) 小山　力，熊田三由，町田昌昭(1974)：輸入サル類の寄生虫について，昭和49年度人畜共通伝染病調査報告，44-70
13) Kugi, G. (1977): *Armillifer monilifromis heymonsi* (Pentastomoidea: Porocephalidae) from the reticulate python, *Python reticulatus*, J. Japan. Vet. Med. Ass., 30, 152-153
14) 町田昌昭 (1983)：マカク属サルの蠕虫類，霊長類と疾病 (昭和58年度)，33-35
15) 中川志郎，町田昌昭，市原醸郎，亀谷　了 (1967)：上野動物園飼育動物の寄生虫(2)，ヘビ類の寄生虫，寄生虫誌.，16，229-230
16) Riley, J. and Self, J.T. (1981): Some observations on the taxonomy and systematics of the pentastomid genus *Armillifer* (Sambo, 1922) in South East Asian and Australian snakes, Systematic Parasitology, 2, 171-179
17) Riley, J., Spratt, D.M. and Banaja, A.A. (1985): Pentastomids (Arthropoda) parasitic in Australian reptiles and mammals, Australian J. Zool., 33, 39-53
18) Riley, J. (1986): The biology of pentastomids, Advances in Parasitology, Vol. 25, 45-128
19) Sambon, L.W. (1922): A synopsis of the family Linguatulidae, J. Trop. Med. Hyg., 25, 188-206 (June), 391-428 (Dec.)
20) Self, J.T. (1969): Biological relationship of the pentastomida; A bibliography on the pentastomida, Exper. Parasitology, 24, 63-119
21) Smith, J.A., Oladiran, B., Lagundoye, S.B., Lawson, E.A.L. and Francis, T.I. (1975): Pentastomiasis and malignancy, Ann. Trop. Med. Parasit., 69, 503-512
22) 利岡静一，松井孝爾 (1964)：台湾産蛇類より採集した Pentastomid の 3 種について，爬虫類学雑誌，11，7-8
23) 利岡静一 (1968)．奄美大島産蛇類の舌虫について，爬虫両棲類学雑誌，2，47
24) Whitney, R.A. and Kruckenberg, S.M. (1967): Pentastomid infection associated with peritonitis in mangabey monkeys, J. Am. Vet. Assoc., 151, 907-908
25) 山口　昇，庄司直嗣 (1978)：マレーオオトカゲより得た舌虫の一種について，寄生虫誌.，27(増)，82
26) 山本　久，利岡静一，三島章義，小林睦生，小倉信夫 (1978)：本邦で採集された舌虫類について，

Snake, 10, 143-150
27) 山下次郎, 中俣充志(1952): 錦蛇の肺臓より得た一舌虫について, 北大農学部邦文紀要, 1, 309-311

Case report

PARASITES OF IMPORTED ANIMALS TO JAPAN V. *ARMILLIFER ARMILLATUS* NYMPHS IN THICK-TAILED BUSH BABY, *GALAGO CRASSICAUDATUS*, FROM AFRICA

NOBORU KAGEI[1] AND SHIGEMI SHICHIRI[2]

Received June 15 1989/Accepted December 6 1989

We had the opportunity to examine the carcass of a female thicktailed bush baby (*Galago crassicaudatus*) bred in Tama-Zoo, Tokyo. This carcass was one young galago of 4 head (2 males and 2 females) which were caught at the east part of Africa and died at one month after the importation to Japan at November 1987.

As the results of necropsy, 25 encapsulated nymphs of pentastomes were found in or on the parenchyma of the abdominal-wall, diaphragm, mesenteries, liver, lung and spleen, recognized as conspicuous annular worms and coiled within a transparent thin-walled cyst. These measured 4-6 mm in diameter. No other gross lesions were found. The cause of the *Galago*'s general illness was not ascertained, nor was the source of the pentastomes.

The cylindrical nymphs were identified as *Armillifer armillatus* based on the characteristic pronounced annulations of the integument and by the number of these annulations (16 to 20).

At microscopical examination of sections of encysted nymphs in liver and diaphragm, the nymph was enclosed in a fibrous cyst wall and there were a encysted nymph showing prominent acidophilic glands and intestinal tract; the chitinous cuticle was smooth without spins, 6.4 μm thick; subcuticlar glands and muscle fibres were apparent beneath the cuticle. Neither degenerative nor granulomatous inflammatory reactions noted in the adjacent tissue.

Nymphal pentastomes are capable of developing in a wide of intermediate hosts. *Armillifer armillatus* nymphs have been reported from various terrestrial animals, including insectivora, carnivora (lion, dog, leopard, mongoose), rodentia (rat, squirrel), ungulata (antelope), primates (monkey, macaques, galagos, guenons, mangabey, baboons, chimpanzee, man) (Sambon, 1922; Flynn, 1973). Although this parasite occurs naturally only in tropical Africa, when these worms were imported to Japan with these animals, this parasite will be possible to occures the problem as a zoonotic parasites.

1 Department of Parasitology, National Institute of Health, Tokyo 141
2 2nd Laboratory of Hygiene, Tama-Zoo, Tokyo 191

Japan. J. Trop. Med. Hyg., Vol. 18, No. 1, 1990, pp. 23-30

Case report

COMMUNITY OUTBREAKS OF FOOD BORNE INFECTION WITH SO-CALLED *VIBRIO FURNISSII* IN JAPAN AND THE PHILIPPINES

ETSUSHI OKUMURA[1], TOSHIHIRO NAKAJIMA[2],
MITSUTAKA HATA[3] AND EDY L. MYPA[4]

Received September 22 1989/Accepted December 8 1989

Abstract: Laboratory examinations on fecal specimens collected from 34 subjects who complained of diarrhea on an occasion of group checkup in January 1987 at Bacolod City, Negros Island, the Republic of the Philippines, revealed *Vibrio furnissii* in 2 of these cases (5.9%). Meanwhile, in June 1987, three siblings who had eaten a luncheon packed in a chip box, especially fishes and shellfishes such as sliced raw turbo, ark shell and tuna meat, deep fried prawns and mackerel sushi served at a Buddhist service for the death, developed diarrhea in a Osaka City. Bacteriological examination of fecal specimens from them demonstrated *Vibrio furnissii.* These three cases were diagnosed as food poisoning due to contamination with this pathogen. Diarrhea, gripes, nausea, vomiting and fever were the principal manifestations with which all these patients presented. The symptoms appeared 10-14 hours after the luncheon and diarrhea persisted for 12 to 30 hours. One patient had mixed infection with *Vibrio parahaemolyticus* and was eventually in a serious condition with dehydration. These three cases of food poisoning due to *Vibrio furnissii* infection are the first to be reported in Japan, to our knowledge. It is generally recognized that pathogenic strains of *Vibrio furnissii* and *Vibrio parahaemolyticus* cause infection in man chiefly by way of contaminated fishes and shellfishes, hence important as causative agents for food poisoning.

INTRODUCTION

It has been suggested that the new species of organism designated *Vibrio furnissii* by Brenner *et al.* in 1983 may cause diarrhea, but cases of overt infection by the organism reported are few as yet (Brenner *et al.*, 1983; Hickman-Brenner *et al.*, 1984).

In January 1987, however, we obtained *V. furnissii* isolates from rectal swabs in 2 of 34 children with diarrhea examined during medical cooperative checkups in Negros Island, the Republic of the Philippines. In June 1987, again, we experienced cases of siblings with enteric *V. furnissii* infection that occurred after luncheon served at Buddhist service for the death in Japan. The cases are the first to be reported in this country to our knowledge, and such cases

1 Department of Medical Technology, Kohchi Gakuen Junior College, Kohchi
2 Department of Pediatrics, Minami-Osaka General Hospital, Osaka
3 Department of Medical Technology, Minami-Osaka General Hospital, Osaka
4 Department of Gynecology, Bacolod Sanitarium Hospital, Bacolod, the Philippines

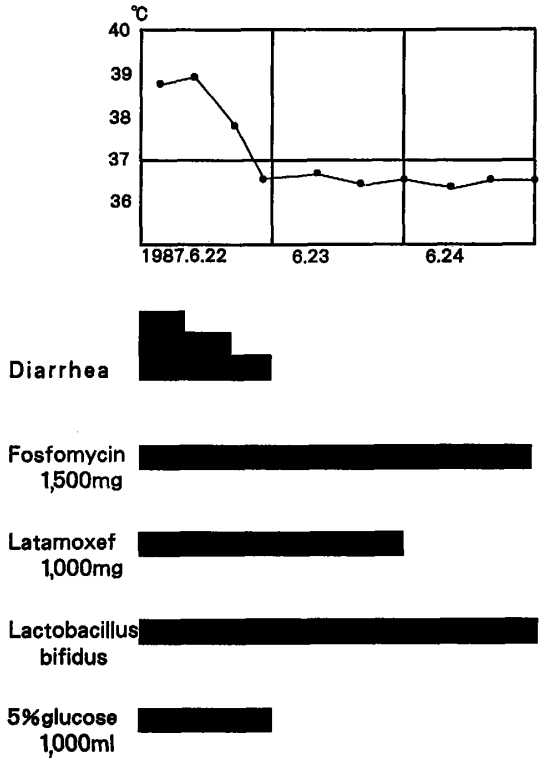

Figure 1 Clinical course K.H. 13 y.o. female.

Table 1 Laboratory findings
K.H. 13 y.o. female

WBC	14,700/mm³	TP	7.2 g/dl
Stab	10%	Alb	69.6%
Seg	66%	α_1—gl	3.3%
Eosin	1%	α_2—gl	8.8%
Mono	4%	β —gl	6.4%
Lymph	19%	γ —gl	11.9%
RBC	477×10⁴/mm³		
Hb	14.1 g/dl	ASLO	80
Ht	42.4%	ASK	160
Plt	22.3×10⁴/mm³	RA	(−)
		CRP	(−)
GOT	10 IU/l		
GPT	7 IU/l	Urine	
Alk-P	19.4 KA-U	pH	7.0
FBS	94 mg/dl	Protein	(−)
BUN	12.1 mg/dl	Keton	(−)
U-A	4.9 mg/dl	Urobilinogen	(±)
Creatinin	0.5 mg/dl		
Na	139 mEq/l	Cultivate bacteria of stool	
K	3.7 mEq/l	*V. furnissii*: positive	
Cl	102 mEq/l		
Ca	5.1 mEq/l		

will probably increase in our communities as well. This paper describes clinical features of the infection with primary reference to the cases we have experienced.

Brief Report of Cases

Case 1: A 13-year-old girl with a body weighing 50 kg.
Chief complaint: Hypogastric pain and watery diarrhea.

On June 21, 1987, she had supper after 5 o'clock which consisted of a luncheon in a chip box her parents had brought home from a Buddhist service, particularly of raw tuna and turbo slices, omlette, etc. She awoke early in the following morning, at about 5 o'clock, and suffered from nausea, vomiting and frequent watery diarrhea. The patient was examined at the Pediatric Clinic of Minami-Osaka General Hospital at 9 a.m. that morning and was immediately hospitalized.

Hospital course: At admission the patient had fever at 38.8°C with hypogastric pain, vomiting and frequent severe watery diarrhea. Hematologic examination revealed leukocytosis and *V. furnissii* was isolated from feces.

She was treated with intravenous drip of 5% glucose solution with 1,500 mg of fosfomycin and 1,000 mg of latamoxef. By the evening of that day she became afebrile and other symptoms including diarrhea markedly subsided. The patient was discharged much improved on 3rd hospital day, June 24 (Table 1 and Figure 1).

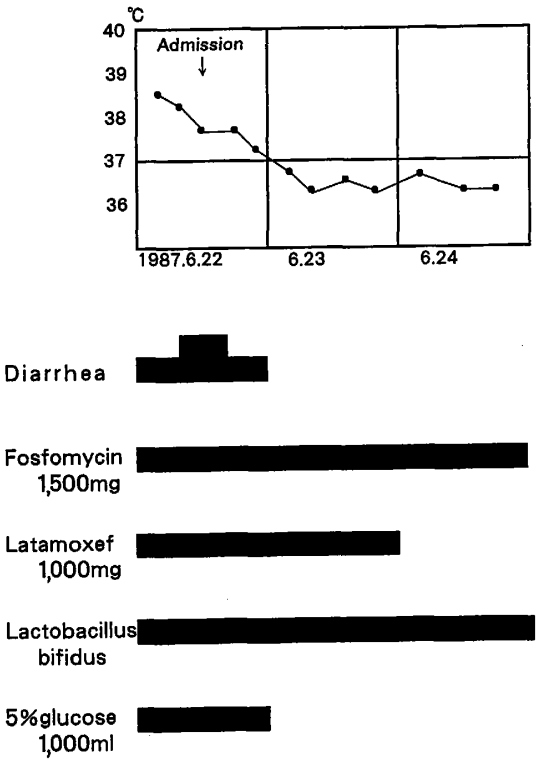

Figure 2 Clinical course N.H. 11 y.o. female.

Table 2 Laboratory findings
N.H. 11 y.o. female

WBC	10,600/mm³	TP	7.3 g/dl
Stab	21%	Alb	69.7%
Seg	63%	α_1—gl	3.2%
Mono	7%	α_2—gl	7.6%
Lymph	9%	β —gl	7.0%
RBC	470×10⁴/mm³	γ —gl	12.5%
Hb	13.7 g/dl		
Ht	40.4%	ASLO	60
Plt	20.0×10⁴/mm³	ASK	80
		RA	(−)
GOT	15 IU/l	CRP	(−)
GPT	7 IU/l		
Alk-P	22.6 KA-U	Urine	
FBS	118 mg/dl	pH	5.5
BUN	16.3 mg/dl	Protein	(−)
U-A	4.7 mg/dl	Keton	(−)
Creatinin	0.6 mg/dl	Urobilinogen	(±)
Na	140 mEq/l		
Cl	105 mEq/l	Cultivate bacteria of stool	
K	3.8 mEq/l	*V. furnissii*: positive	
Ca	5.4 mEq/l		

Case 2: An 11-year-old girl with a body weighing 37 kg.
Chief complaint: Hypogastric pain and watery diarrhea.

The patient, a younger sister of the patient of Case 1, ate particularly ark shells, deep-fried prawns and boiled fish paste of same luncheon after 7 o'clock that evening. As she had no discomfort the following morning, she went to school. Just at the time the morning gathering began, about 8:30 a.m., she suddenly began to suffer from griping with vomiting. Laid on bed in public health nurse room of the elementary school, she was given an antispasmodic but as high fever (38.5°C) and watery diarrhea persisted, she was taken home. Her parents were gone to the hospital where her sister and brother were already admitted, and she stayed home alone bearing the pain. But, as the symptoms became increasingly severe, she at last called the neighbor for help and was brought to the hospital after 12 o'clock. She also was admitted to the pediatric ward.

Hospital course: Examination of blood revealed leukocytosis, a shift of the polymorphonuclear leukocytes to the left, neutrophilia and lymphocytopenia, and *V. furnissii* was isolated from feces.

The patient was treated with the same therapy regimens as Case 1. Towards evening the fever began to recede with gradual improvement of other symptoms. She was dismissed much improved on the third hospital day (Table 2 and Figure 2).

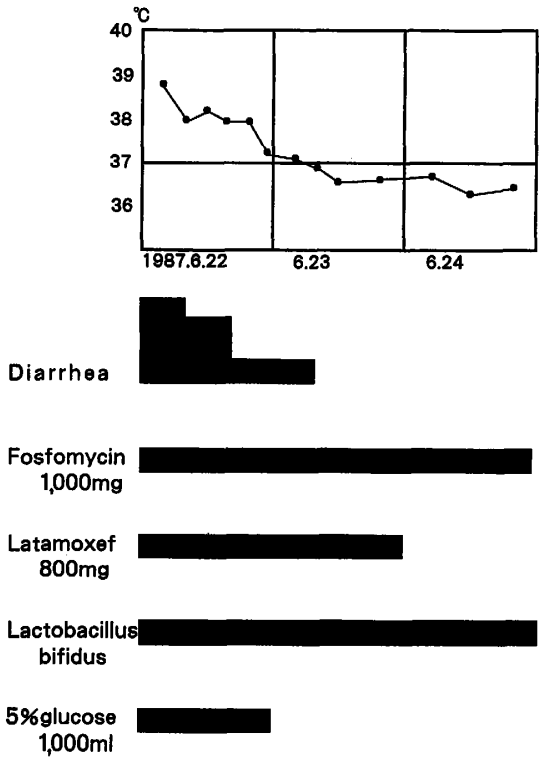

Figure 3 Clinical course Y.H. 9 y.o. male.

Table 3 Laboratory findings
Y.H. 9 y.o. male

WBC	11,700/mm³	TP	6.9 g/dl
Stab	19%	Alb	71.2%
Seg	64%	α_1—gl	3.0%
Eosin	1%	α_2—gl	8.6%
Baso	1%	β—gl	7.4%
Mono	3%	γ—gl	10.8%
Lymph	12%		
RBC	477×10⁴/mm³	ASLO	160
Hb	13.2 g/dl	ASK	160
Ht	38.7%	RA	(−)
Plt	24.7×10⁴/mm³	CRP	(−)
GOT	12IU/l		
GPT	10IU/l	Urine	
Alk-P	27.5 KA-U	pH	5.0
FBS	94 mg/dl	Protein	(−)
BUN	21.9 mg/dl	Keton	(±)
U-A	4.7 mg/dl	Urobilinogen	(±)
Creatinin	0.6 mg/dl		
Na	139 mEq/l	Cultivate bacteria of stool	
Cl	104 mEq/l	*V. furnissii*: positive	
K	3.7 mEq/l	*V. parahaemolyticus* K10 positive	
Ca	4.9 mEq/l		

Case 3: A 9-year-old boy with a body weighing 32 kg.
Chief complaint: Hypogastric pain and watery diarrhea.

The patient, a younger brother of the patients of Cases 1 and 2, ate a bellyful of the same luncheon, particularly, raw ark shell, tuna and turbo slices, deep-fried prawns and mackerel sushi after 4 o'clock on the evening of same day. He awoke with griping at about 2 o'clock the following morning but stayed in bed without any remedies. Around 4 o'clock the same morning he began to suffer from nausea and vomiting and to have watery diarrhea 5 times an hour, with increasingly severe hypogastric pain. He was examined at this hospital together with his sister (Case 1) and admitted to the pediatric ward.

Hospital course: On admission, laboratory examinations disclosed leukocytosis, a shift of the polymorphonuclear leukocytes to the left, neutrophilia, lymphocytopenia and elevation of BUN. *V. furnissii* and *V. parahaemolyticus* were isolated from stool specimen. He had a fever at 38.9°C with vomiting, griping and frequent watery diarrhea. In addition, symptoms of dehydration developed. The patient thus was in a serious clinical condition, compared to his sisters.

The patient was given 5% glucose solution by intravenous drip with 1,000 mg of fosfomycin, 800 mg/day of latomoxef and a *Lactobacillus bifidus* preparation. He became afebrile the following morning and other symptoms gradually subsided. The patient was discharged improved together with the sisters (Table 3 and Figure 3).

Two cases in Negros Island: During medical cooperative checkups in Negros Island of

Philippines in January 1987, we had collected the rectal swabs of 34 children with diarrhea, griping, fever and vomiting. And, we demonstrated *V. furnissii* infection in 2 (5.9%) of 34 stool cultures (Table 4).

Discussion

Vibrio fluvialis, described as a new species by Lee *et al.* in 1981, is detected as a contaminant of sea water, coastal mud, fishes and shellfishes. Case of *V. fluvialis* infection via them reported have been progressively increasing in recent years (Lee *et al.*, 1981; Kobayashi *et al.*, 1983; Miyata *et al.*, 1986; Tokoro *et al.*, 1984). Meanwhile, the organism with the same

Table 4 Clinical symptoms and examination of rectal swab

	age	sex	diarrhea	abdominal pain	vomiting	fever	results
1.	2	m	+	+	−	−	normal flora
2.	3	m	+	−	−	−	normal flora
3.	?	f	‡ (w)	+	−	+	EPEC OK4(+)
4.	6	f	±	−	−	−	normal flora
5.	?	f	+	−	−	−	normal flora
6.	4	m	+	−	−	−	normal flora
7.	10	m	+	−	−	−	normal flora
8.	7	f	+	+	−	−	normal flora
9.	5	m	+ (w)	+	−	−	normal flora
10.	1	f	‡ (w)(m)	+	+	+	ETEC OK4(+) LT(+)
11.	4	f	+	−	−	−	normal flora
12.	1	m	+	−	−	−	normal flora
13.	2	f	+ (w)	−	−	−	normal flora
14.	4	f	+	−	−	−	normal flora
15.	1	m	±	−	−	−	normal flora
16.	1	f	+	−	−	−	normal flora
17.	6	f	+	−	−	−	normal flora
18.	1	?	+	+	−	−	normal flora
19.	1	f	‡ (w)	+	−	−	normal flora
20.	3	f	+ (w)	−	−	+	normal flora
21.	2	m	+	−	−	−	normal flora
22.	1	f	+	−	−	−	normal flora
23.	2	f	+	−	−	−	normal flora
24.	1	f	+	−	−	−	normal flora
25.	5	m	+	−	−	−	normal flora
26.	5	m	‡ (b)	+	−	+	*Aeromonas hydrophila*
27.	5	m	+	−	−	−	normal flora
28.	1	f	+	−	−	−	normal flora
29.	7	m	+	−	−	−	normal flora
30.	3	m	+	−	−	−	normal flora
31.	1	m	+	−	−	−	normal flora
32.	4	m	‡ (w)	+	+	+	*Vibrio furnissii*
33.	1	m	‡ (w)	+	−	+	EPEC OK4(+)
34.	12	f	‡ (w)	+	+	+	*Vibrio furnissii*

(diarrhea‡ : more 6 times/day ‡ : 4~5 times/day + : 2~3 times/day ± : 1 time/day)
(w : waterly m : mucous b : bloody)

morphologic and biologic characteristics as *V. fluvialis* but with the ability to ferment glucose with the production of gas has been classified under a separate, independent species with a taxonomic nomenclature *V. furnissii* by Brenner *et al.* (1983). Their differential characteristics have been further clarified (Brenner *et al.*, 1983; Ohta, 1988; Chikahira and Hamada, 1988). However, cases of infection by the organism reported have been few. They include a case of a one-year old infant with mixed infection by *V. fluvialis* and *V. furnissii* and cases of food poisoning that occurred due to contaminated in-flight meal on Tokyo-Seattle route, with retrospective isolation of *V. furnissii,* cited by Morris *et al.* in their article entitled (Hickman-Brenner *et al.*, 1984; Morris and Black, 1985). We have continually been attentive possibility of infection by this organism in cases of food poisoning which occur among those whose favorite dishes are of fishes and shellfishes, in as much as *V. furnissii* infect humans via these foods.

We also demonstrated *V. furnissii* infection (not *V. fluvialis*) in 2 (5.9%) of 34 children with a chief complaint of diarrhea examined, with stool cultures, in Negros Island of Philippines, in January 1987. The clinical manifestations in these cases included gripes, vomiting and fever besides diarrhea.

To our knowledge, there has been no case of *V. furnissii* infection reported in Japan and probably the three cases described herein are the first to be reported in this country. To sum up the clinical manifestations seen in the present series, hypogastric pain procedes, followed by vomiting and watery diarrhea with remittent fever over 38°C, as in the pediatrics cases experienced in Negros Island. In the above described 23 cases of in-flight food poisoning, diarrhea occurred in 91% of cases, abdominal cramps in 79%, nausea in 65% and vomiting in 39% (Morris and Black, 1985). It has been described that clinical manifestations in *V. fluvialis* infection were diarrhea in 100% of cases, nausea and vomiting in 97%, abdominal pain in 90%, and fever; hence those in *V. furnissii* infection are remarkably similar to these (Kobayashi *et al.*, 1983; Ohta, 1988).

In the present cases, diarrhea persisted for 12 to 30 hours and the incubation period was 10 to 14 hours (mean: 12 hours) although it would vary with the amount of infecting organisms and also depend on individual variations and other factors. It appeared likely that the greater the amount of organisms ingested, the shorter the incubation period, producing a serious condition as in Case 3, as a general trend. In fact, Case 3 was of mixed infection with *V. parahaemolyticus* which, too, was isolated.

Laboratory examinations revealed leukocytosis, with neutrophilia and corresponding lymphocytopenia, in all cases. Marked dehydration resulting from frequent diarrhea as in Case 3 would lead to a serious clinical condition as indicated by BUN elevation. Therefore, it is undeniable that *V. parahaemolyticus* hold a dominant position in Case 3.

V. furnissii infection has been generally attributed to ingestion of marine products, especially to contaminated raw fishes and shellfishes (Tokoro *et al.*, 1984; Miyata *et al.*, 1986; Ohta, 1988). Although no attempt could be made to establish causal relation of the suspected foods to the infection by detection of organism in them in the cases reported herein, it should be noted that those to whom fishes and shellfishes are to favorite dishes in the Philippines, Japan and, of course, other areas may have ample opportunities for infection by this organism. It would be of importance to pay much attention to possible occurrence of this organism in cases of food poisoning particularly during the rainy and hot summer seasons which are referred to as seasons of food poisoning in this country.

REFERENCES

1) Brener, D.J., Hickman-Brenner, F.W., Lee, J.V., Steigerwalt, A.G., Hollis, D.G., Farmer, J.J., Weaver, R.E., Joseph, S.W. and Seidler, R.J. (1983): *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and environment, J. Clin. Microbiol., 18, 816-826

2) Chikahira, M. and Hamada, K. (1988): Enterotoxigenic substance and other toxin produced by *V. fluvialis* and *Vibrio furnissii,* Jap. J. Vet. Sci., 50, 865-873

3) Hickman-Brenner, F.W., Brenner, D.J., Steigerwalt, A.G., Schreiber, M., Hormberg, S.D., Baldy, L.M., Lewis, C.S., Pickens, N.M. and Farmer, J.J. (1984): *Vibrio fluvialis* and *Vibrio furnissii* isolated from a stool sample of one patient, J. Clin. Microbiol., 20, 125-127

4) Kobayashi, K., Taguchi, M., Shimada, T. and Sakazaki, R. (1983): Ten cases of gastroenteritis possibly caused by *Vibrio fluvialis* and its enterotoxigenicity, J. Jap. Assoc. Inf. Dis., 57, 375-378

5) Lee, J.V., Shread, P., Furniss, A.L. and Bryant, T.N. (1981): Taxonomy and discription of *Vibrio fluvialis sp. nov* (synonym group F Vibrios, group E F 6), J. Appl. Bacteriol., 50, 73-94

6) Miyata, Y., Taguchi, M., Harada, K., Tsukamoto, T., Ishibashi, M., Kinoshita, Y., Abe, H., Hashimoto, S., Goto, I., Ichiki, S., Asano, N., Arita, M., Honda, T., Miwatani, T. and Takeda, Y. (1986): Bacteriological study of traveller's diarrhea at Osaka Airport Quarantine Station during 1980-1983, J. Jap. Assoc. Inf. Dis., 62, 108-122

7) Morris, J.G. and Black, R.E. (1985): Cholera and other vibrios in the United States, New. Eng. J. Med., 312, 343-350

8) Ohta, K. (1988): *Vibrio fluvialis* and *Vibrio furnissii,* Clin. Microbiol., 15, 577-582

9) Tokoro, M., Kato, M., Goto, K., Watanabe, M., Yamada, F., Sako, T., Otsuka, K., Sugiyama, O., Furukawa, M., Niwa, S. and Nagayama, C. (1984): Community outbreak of mixed food borne infection with *Vibrio parahaemolyticus* and *Vibrio fluvialis,* J. Jap. Assoc. Inf. Dis., 58, 1038-1045

Vibrio furnissii による食中毒症例
—本邦とフィリピンにおける自験例の臨床像について—

奥村　悦之[1]・中嶋　敏宏[2]・
秦　　光孝[3]・Edy L. Mypa[4]

1987年1月，フィリピン・ネグロス島，バコロド市における住民検診時，下痢を訴えた34名の糞便を直腸スワブにて採取し，起因菌を検索し，2例（5.9%）に *Vibrio furnissii* が分離同定された。また一方1987年6月，大阪市において，法事の際の折詰弁当，特に赤貝，サザエ，マグロ刺身，海老の天ぷら，鯖寿司などの魚介類を食べた同胞3児が下痢を訴え，それぞれの糞便を検索したところ，同様に *Vibrio furnissii* を検出し，これら3例は本菌による食中毒症例と診断された。主要症状は下痢，腹痛，悪心嘔吐，発熱の4主徴が必発した。潜伏期間は10～14時間，また下痢持続時間は12～30時間であった。3症例のうち1例は *Vibrio parahaemolyticus* との混合感染例であり，当然のことながら脱水症などを合併して重篤となった。これら3症例の *Vibrio furnissii* 感染による食中毒症例は本邦最初の報告例と思われる。

また *Vibrio furnissii* は *Vibrio parahaemolyticus* や *Vibrio fluvialis* と同様，病原性を有する株が，主として魚介類を介して感染するとされており，食中毒起因菌として今後も充分注目されるべきであろう。

1　高知学園短期大学衛生技術科
2　総合病院南大阪病院小児科
3　総合病院南大阪病院臨床検査科
4　Department of Gynechology, Bacolod Sanitarium Hospital, the Philippines

日熱医会誌.，第18巻　第1号　1990年　31-38頁

症例報告

陳旧性日本住血吸虫症に発生した胃癌の一症例

神田　享勉[1]・小山　幸男[1]・加藤　達也[1]・前田　光久[2]・
篠原　宏康[2]・伊藤　秀明[3]・狩野　繁之[4]・鈴木　　守[4]

平成元年12月20日受付/平成2年1月25日受理

はじめに

日本住血吸虫症（以下日虫症と略す）は，我が国において減少しており，ここ数年新罹病患者は認められていない。しかし各種の住血吸虫症の慢性罹患者は，アジア，アフリカなどで2億人以上いると言われており，地球規模でみると稀な疾患ではない。交通の発達により人的物質交流が盛んになった今日では，日虫症非浸淫地域でも日虫症患者に遭遇することも，少なくないと思われる。しかも本症は消化器系腫瘍との因果関係や，慢性肝疾患の発症原因としても問題となっている。我々は今般，胃癌に日虫症を合併した，宮入貝生棲地である山梨県南巨摩郡出身の症例を経験したので報告する。

症　例

患　者：戸○志○ゑ，79歳女性
主　訴：腹痛
出身地：山梨県南巨摩郡増穂町
既往歴：気管支喘息，心房細動，高血圧
現病歴：上記疾患にて外来通院。昭和63年3月3日赤飯を食べた後，腹痛出現。数日後タール便を認め，当科で受診となった。来院時に，眼瞼結膜貧血性で，上腹部の圧痛と下肢の浮腫を認めた。検査所見でRBC 191×10⁴/mm³，Ht 18%，Hb 6.0 g/dl，Plt 13.4×10⁴，WBC 3,500と高度の貧血を呈し，T-P 5.0 g/dl，GOT 48 u，GPT 30 u，ALP 21.9 KE，ZTT 12.3 u，TTT 12.3 u，LDH 372 uと慢性肝疾患の合併を示唆していた。またCEA 4.9 IU，CA 19-9 34 IUと高く，消化器系癌が疑われた。そこでMDLを施行し，CardiaからEC-Junction部にかかる硬化像を認め生検，病理組織診断にて，高分化型腺癌と報告された。摘出標本より，日虫症の虫卵（日虫卵）が見出された。そのための精査を行い，肝・脳への日虫卵残存が考慮された。手術後も頭痛，微熱が続いた。
手術所見：正中切開にて開腹，腹水認めず，肝転移なし。腫瘍は噴門部小湾に径2 cm触知し，Borrmann III型を示し，No. 3の脾腫大とリンパ節腫大が認められた。腫瘍を含め胃全摘と脾摘を施行し，Roen-Y吻合し手術を終了した。
摘出標本所見：腫瘍は高分化型腺癌で（図1），リンパ節No. 3に転移があった。また日虫症の虫卵が胃傍リンパ節No. 3，4，5，8，9，12と脾門部リンパ節に多数認められた（図2，3）。虫卵は楕円形で，大多数は変性していたが，日本住血吸虫卵特有の突起を有していた。また虫卵周辺には，軽い線維化が認められたが，細胞浸潤はなかった。
糞便検査：虫卵は検出されなかった。
免疫学的検査：東京大学医科学研究所寄生虫研究部・田中　寛教授のもとで，虫卵周囲沈降反応（COP test），補体結合反応を施行したが両者共陰性であった。
腹部エコーおよびCT所見：腹部エコー像は，帯

1　篠原病院内科
2　篠原病院外科
3　群馬大学医学部中央検査部
4　群馬大学医学部寄生虫学教室

状の比較的太い線状エコーが亀甲状パターンを呈しており，典型的日虫症の所見であった（図4）。肝CT像は左葉外側区，尾状葉の腫大と右葉の萎縮，そして門脈走行に一致したと考えられるseptal calcificationを示した（図5）。また脾は腫大していた。

脳CTおよびMRI所見：脳CTで右側頭葉の皮質下領域に，石灰化病変と周囲の萎縮を認めた（図6A）。所見より日虫症による，calcified parenchymal granulomaの可能性が考えられた。またMRIにて，同部に脳梗塞病変（図6B）を認めた。

肝生検所見：グリソン鞘の線維化と，肝細胞の空胞変性を認めた。また組織の一部に虫卵が認められ，日虫症の合併があった（図7）。日虫症性肝線維症の所見であった。

考　察

本例は胃癌患者の胃壁，および脾門部に及ぶリンパ節と，肝臓に虫卵を認めた症例である。日本住血吸虫症は，山梨県甲府盆地，広島の片山地方，福岡・佐賀の筑後川流域，利根川下流などの宮入貝生棲地に見られる。本例は山梨県南巨摩郡で生まれ，20歳頃まで当地での生活経験があり，日虫症感染以後60年間経過したことになる。

日虫症における消化器，肝臓病変については多くの報告がある。内藤ら（1978）によると，剖検4,050例，肝生検1,679例中，虫卵陽性はそれぞれ215例（5.3%），84例（5%）であり，久留米という特殊地域では高頻度であった。天野（1981）によると，日本での胃十二指腸疾患手術材料中，日虫症を合併した報告は284例で，この中に胃癌が179例（63%）と報告している。また病巣との関係では，胃癌病巣内に日虫卵を見たもの57.0%，癌病巣内に日虫を認めなくても，病巣辺縁より5 mm以内に日虫卵を認めた例を含めると86.8%となり，癌病巣より離れた場合のみに日虫卵を認める例は，わずか13.2%であったという。また肝癌との関連は，Nakashimaらの報告があり，1929-1977年の4,611例中hepatocellular carcinomaは227例で，その24例（10.6%）に日虫症との関連を認めている。ただしHBsAgは，27%と高陽性率を示している。また本例の脳CT所見から，虫卵の脳組織への移行も推定されたが，臨床症状としてのてんかん発作は認めなかった。しかし頭痛は，現在までも続いている。

日本住血吸虫卵と発癌との関連について，発癌作用有りとする説では，(イ)長期にわたる虫卵の重複感染の結果，粘膜のびらん・再生が繰り返され，粘膜の悪性変化を起こすという考えがある（内藤ら，1978）。例えば，天野（1981）は，日虫卵の見られる胃隆起性病変では，粘膜筋板の断裂や肥厚，粘膜下組織の血管増生が認められたという。また猪口ら（1978）も，日虫卵の介在する層は粘膜，および粘膜下層である症例が殆どで，粘膜面での再生上皮形成に影響を与えることは，充分に推測し得るとした。(ロ)汚染地と非汚染地との大腸癌（Xu and Su, 1984）や，肝癌（Inaba, 1988）発生の頻度が統計的に有意な差がある。特に，Inaba (1988)の山梨県における肝癌発生では，日虫症の相対危険度の推定（odds ratio）が9.5と高く，肝癌のco-factorであるとした。逆に発癌作用なしとする説では，(イ)癌腫に見られる虫卵は古いもので，粘膜下層に多く粘膜上皮の癌化に関与しない。(ロ)虫卵の分布と癌腫とは，一致しないという所見を主張している。Dimmette *et al.*,（1956）は，住血吸虫症に合併した大腸良性ポリープを検討し，半数に腺腫変化を認めず，ポリープ変化と，癌化との関係はないとした。

我々の症例は，胃癌で原発巣に虫卵は検出されず，リンパ節と脾臓で虫卵が認められた。その経緯としては，日虫卵は大腸から門脈系に入り，日虫症肝線維症進展と共に左胃，右胃静脈，および脾静脈に到達したか，またはリンパ行性に直接到達したものと考えられる。本例の日虫症感染は50年以上前と考えられ，しかも現在検便で虫卵が見出せず，免疫反応も陰性であることから，自然発癌としても矛盾はない。しかし陳旧化した虫卵が，消化管（特に胃）に長期間沈着してきたことは疑いようもなく，その刺激と発癌との関連は，否定しきれないと考えられる。特にマンソン住血吸虫症と比較して，日虫症と発癌との相互関係は強いと考えられる。なおマンソン住血吸虫症の汚染地

域である南米やアフリカにおいては，日虫症に見られるような消化器癌との関連は認められていない（Cheever, 1978）。今回は，胃癌切除標本中に，日本住血吸虫症が認められた山梨県出身の一女性を報告し，文献的考察を加え報告した。

文　　献

1）天野皓昭（1981）：山梨県の消化器手術材料に見られた日本住血吸虫症の研究，寄生虫誌.，30，135-149

2）猪口嘉三，足立　剛，山内　胖，磯本浩晴，高森邦明，篠原　誠（1978）：癌と日本住血吸虫症との関係，医学研究，48(2)，93-100

3）内藤寿則，神代正道，坂本和義，猪狩民生，中島敏郎，中山和道（1978）：日本住血吸虫症における肝臓，消化器病変，胃と腸，13(12)，1717-1726

4）Cheever A.W. (1978): *Schistosomiasis* and neoplasia (Editorial), J. Natl. Cancer Inst., 61(1), 13-18

5）Chen, M., Hu J., Chang P., Chuang C., Ts'ao P., Chang S., Wang F., Ch'en T. and Chou S. (1965): Pathogenesis of carcinoma of the colon and rectum in schistosomiasis japonica, Chinese. Med. Journal, 84, 513-525

6）Dimmette, R.M., Elwi A.M. and Sproat H.F. (1956): Relationship of schistosomiasis to polyposis and adenocarcinoma of large intestine, Am. J. Clin. Pathol., 26, 266-276

7）Inaba, Y (1988): Liver cancer in an endemic area of schistosomiasis japonica in Yamanashi prefecture, Japan, pp. 211-218, Japan Sci. Soc. Press, Tokyo/Taylor & Francis LTD., Tokyo

8）Xu, Z. and Su, D.L. (1984): *Schistosoma japanicum* and colorectal cancer, An epidemiological study in the people's Republic of China, Int. J. Cancer, 34, 315-318

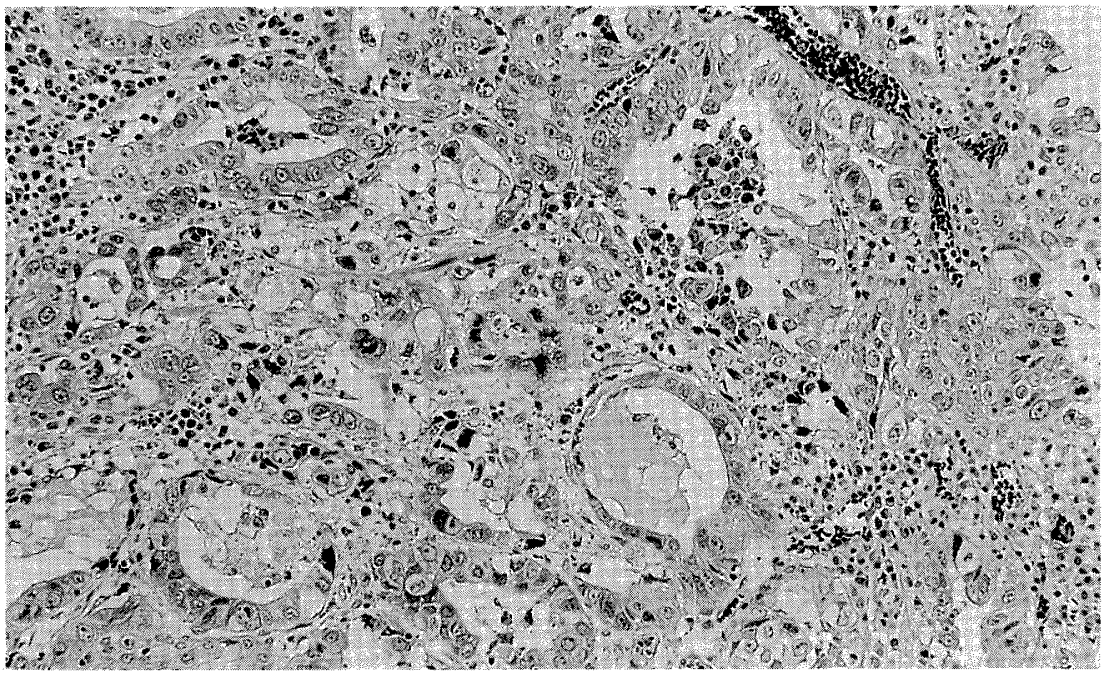

Figure 1 A histological finding on the surgical specimen of the stomach. A well differentiated adenocarcinoma with nodular formation is seen (H.E. staining ×150).

Figure 2 *Schistosoma japonicum* ova detected in the lymphnodes located at the hilus of the spleen. Fibrosis surrounding the eggs is remarked, however, focal cell infiltration is not seen. (HE staining ×300)

Figure 3 A heavy egg deposition is noted in a left gastric lymphnode., Fibrosis without cell infiltration is remarked around the eggs. (H.E. staining ×300)

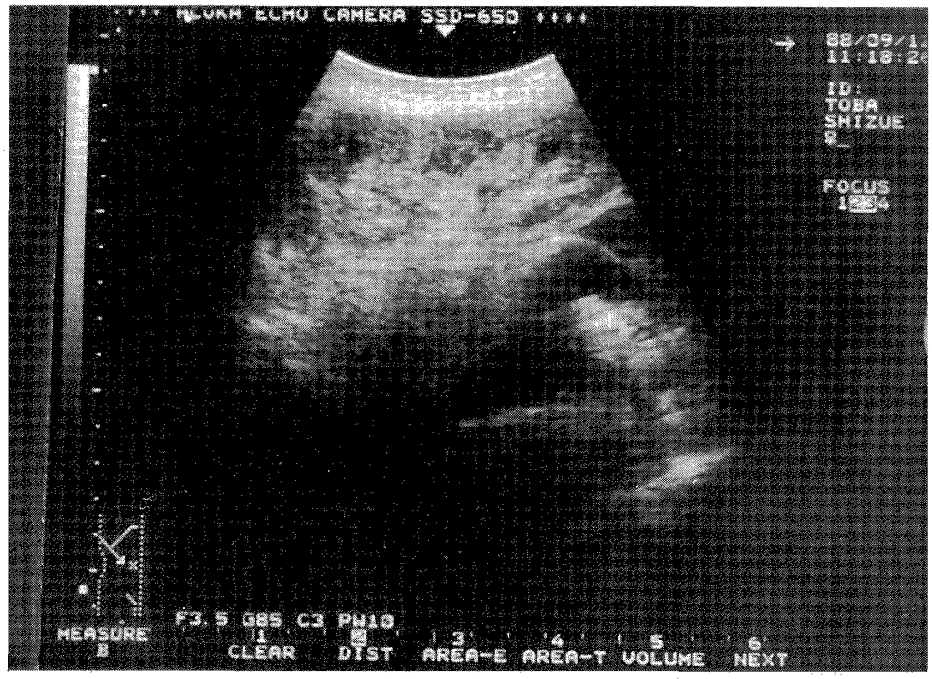

Figure 4 Ultrasonic echo pattern of the patient's liver. A network formation of fibrosis is observed in the liver.

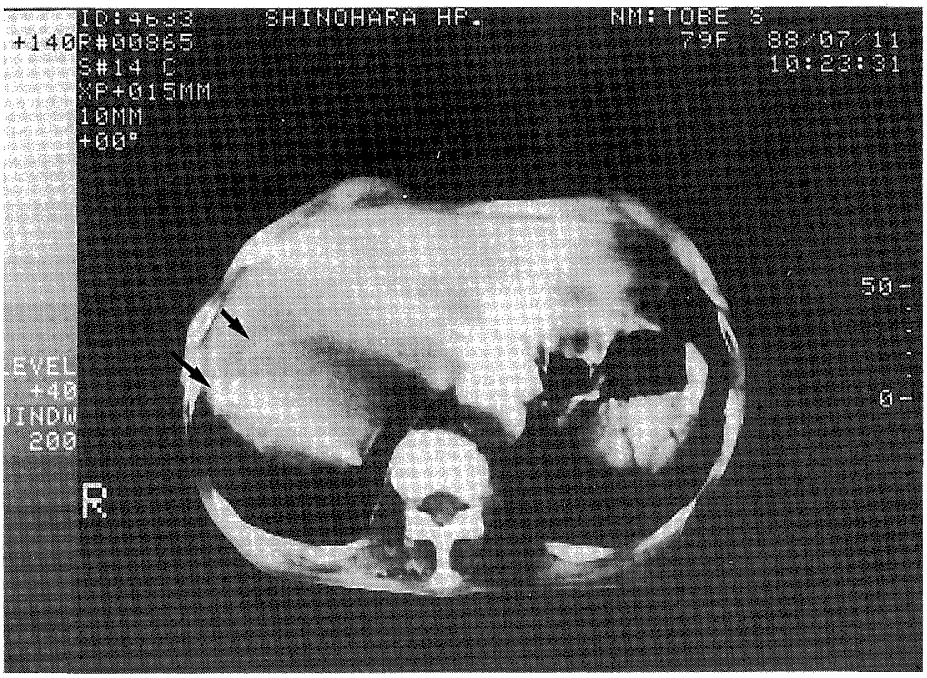

Figure 5 High density funicular patterns by CT examination is shown in the photograph. Septal calcification (indicated by arrows) in the edge of the liver is suspected.

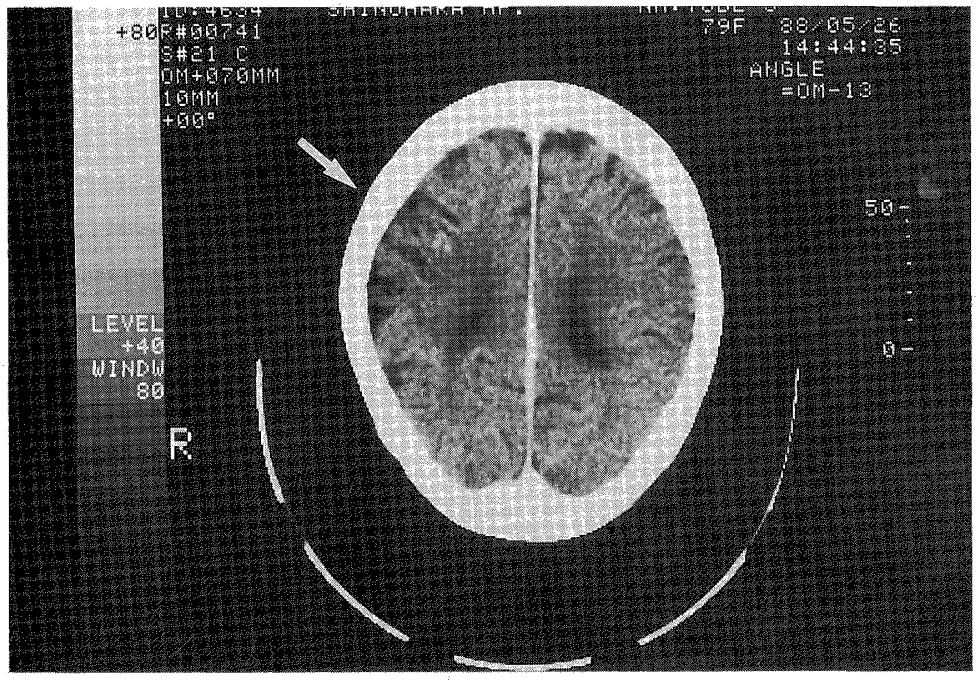

Figure 6(A) Plain CT picture of the brain of the patient. Arrow indicates localized atrophied area with calcification at the right temporal lobe. The calcified parenchymal granuloma is suspected from this figure.

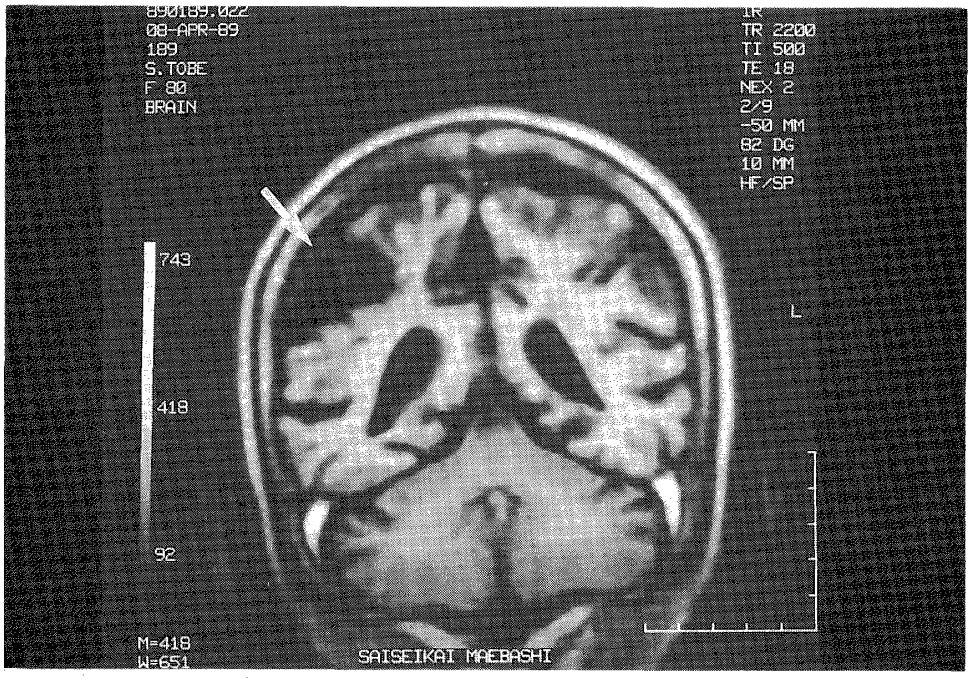

Figure 6(B) MRI image of the brain shows cerebral infarction (indicated by arrow) at the same place of the focal calcification in the CT.

Figure 7 Microphotograph of a part of the biopsied liver specimen from the patient. Two schistosomal ova are seen. Development of the fiber around the egg is poor. Cells are not infiltrated in the area. (H.E. staining ×300)

Case report

A GASTRIC ADENOCARCINOMA CASE WITH DEAD *SCHISTOSOMA JAPONICUM* EGG DEPOSITION IN THE REGIONAL LYMPHNODES

TSUGIYASU KANDA[1], YUKIO KOYAMA[1], TATSUYA KATO[1],
MITSUHISA MAEDA[2], HIROYASU SHINOHARA[2], HIDEAKI ITO[3],
SHIGEYUKI KANO[4] AND MAMORU SUZUKI[4]

Received December 20 1989/Accepted January 25 1990

A 79 years old woman with past history living in an endemic area of *Schistosoma japonicum* developed gastric adenocarcinoma. She lived in Yamanashi, Masuno until 19 years old, then left the place and never returned back there. The patient was subjected to a total gastrectomy with splenectomy. By histological examination, *Schistosoma japonicum* eggs were detected in a considerable number of lymphnodes along the lesser curvature. The eggs were also found in the splenic lymphnodes. Fibrosis with the egg deposition in the Glisson's sheath of biopsied specimen of the liver was partly observed but not very extensive. By stool examination no egg was demonstrated and the serological tests by COP and CF were negative, which supported the view that the patient did not carry alive parasite. Although any definite evidence was not raised in the present case, an etiological role of the *Schistosoma japonicum* eggs in the development of stomach cancer was suggested even 50 years after the infection. This kind of cases should be further studied for the clarification of carcinogenic stimulus by dead *Schistosoma japonicum* eggs.

1 Department of Internal Medicine, Shinohara Hospital
2 Department of Surgery, Shinohara Hospital
3 Department of Clinical Pathology, Gunma University School of Medicine
4 Department of Parasitology, Gunma University School of Medicine, Maebashi 371, Japan

Japan. J. Trop. Med. Hyg., Vol. 18, No. 1, 1990, pp. 39-41

Short communication

IN VITRO BROMODEOXYURIDINE INCORPORATION ASSAY FOR DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN THE FIELD

HIROYUKI DOI[1], SYAFEI[2] AND AKIRA ISHII

Received August 24 1989/Accepted October 22 1989

Drug susceptibility test using anti-bromodeoxyuridine (Doi *et al.*, 1988) was carried out with *P. falciparum* cases in coastal villages of North Sumatra, Indonesia between December 1988 and February 1989. The peripheral blood specimens were withdrawn from inhabitants with consent from vein and were put into culture in test tubes. The initial parasitemia were ranged 960-6,400/mm^3. Blood samples containing parasites were transported in test tubes to Regional Health Laboratory of Medan. Experiments were carried out with 4 h culture and 16 h culture; 4 h culture was conducted in test tube at ambient temperature during transportation time, 16 h culture was done for 4 h in test tube at ambient temperature and for 12 h in candle jar at 37°C (Trager and Jensen, 1976). Experiments were conducted with 2 μM of BrdU (Sigma, USA) in the presence of 0.5 μM and 1.0 μM of chloroquine concentrations (Aralen, Winthrop Products, USA). After BrdU flash, the ELISA was done according to our method described previously (Doi *et al.*, 1988). Chloroquine effect on DNA synthesis was monitored by measuring BrdU incorporation at 4 h and 16 h of incubation using ELISA.

Four hours culture of *P. falciparum* did not show any differences of BrdU incorporation under different concentration of chloroquine in 6 cases. The ring forms of 4 h culture could not be distinguished morphologically from pre-culture ones under microscopic observation. It may be mainly due to the shortage of time for the parasite to adjust to *in vitro* culture.

Results of 8 cases of *P. falciparum* demonstrated that drug susceptibility was distinguished by BrdU uptake when cultured for 16 h in this assay, which implied incorporation of BrdU into parasite nucleic acids (Fig. 1). At the chloroquine concentration of 0.5 μM, BrdU uptake was inhibited in the range of 21-83%, and the mean of inhibition of BrdU uptake was 39%. *P. falciparum* free fresh blood was used as a negative control, and its ELISA value was 0.16 after BrdU flash. The value was the same as that of BrdU-free culture. Therefore, human leucocytes in whole blood did not incorporate BrdU in this assay.

The concentration of 0.2-0.3 μM of chloroquine is the same level of patients blood administered that of 10 mg/kg. It is, thus, reasonable and feasible to monitor and judge chloroquine resistance at the concentration of 0.5 μM. If only slight inhibition of BrdU uptake was observed at the concentration of 0.5 μM chloroquine in isolated *P. falciparum*, chloroquine is not an effective drug against that strain *in vivo*.

1 Department of Parasitology, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan

2 Medan Health Laboratory, Jl. Laboratorium No. 5 Medan, North Sumatara, Indonesia

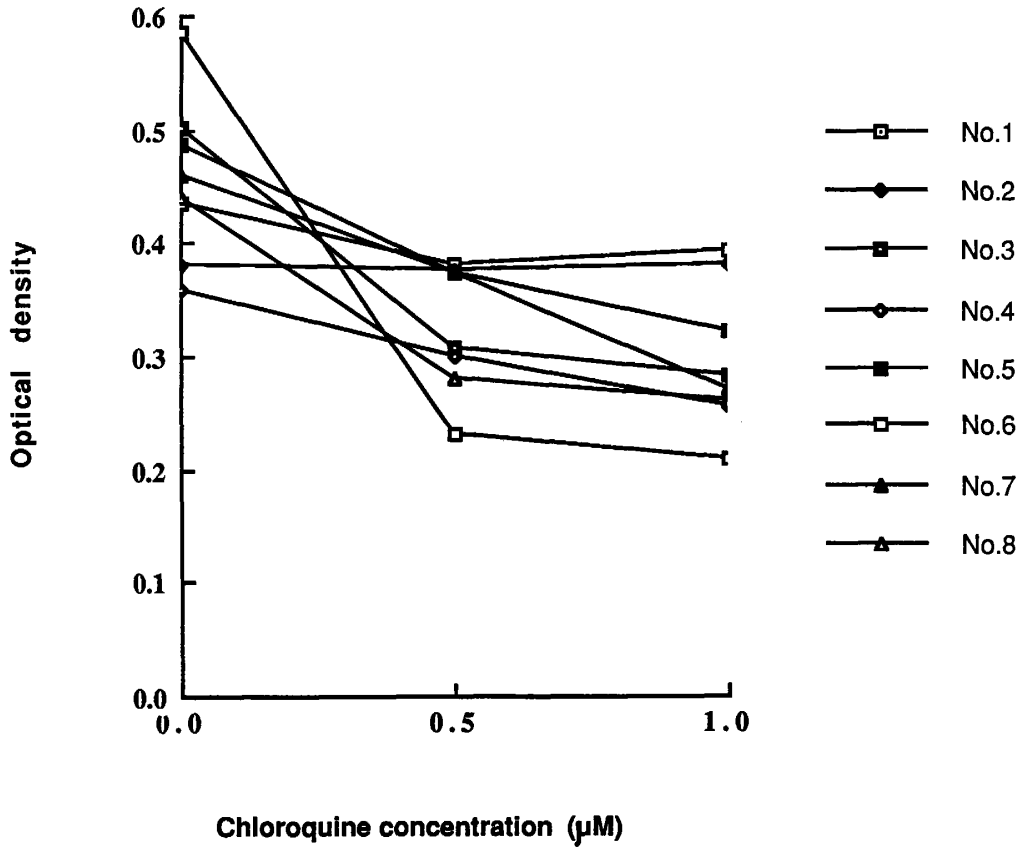

Figure 1 Inhibitory concentration of chloroquine for *P. falciparum* isolates from North Sumatra

In the present study, the drug effect on the proliferation of *P. falciparum* was monitored using BrdU incorporation in the field. This system does not need special equipments (Geary and Jensen, 1983; Waki *et al.*, 1989), and only portable spectophotometor is enough. It would overcome the disadvantages of visually counting parasites (Desjardins *et al.*, 1979; Jensen *et al.*, 1981; Geary *et al.*, 1983). Small amount of blood (200 μl) from malaria patients is enough to test drug susceptibility in this assay. We comfirmed the feasibility of our method in the field. It would offer a possibility of an accurate method of monitoring the effects of a variety of antimalarial drugs, and large-scale screening of drug resistance *P. falciparum* could be performed.

ACKNOWLEDGEMENTS

This investigation was supported by JICA (Japan International Cooperation Agency) and Ministry of Health, Republic of Indonesia. The authors are most grateful to Dr. Munthe, Dr. W. Panjaitan and Dr. T. Suzuki. Excellent technical assistance was given by the field workers especially, Mr. R. Siagian. We owe them all our best thanks.

REFERENCES

1) Desjardins, R.E., Canfield, C.J., Haynes, J.D. and Chulay, J.D. (1979): Quantative assesment of antimalarial activity *in vitro* by a semiautomated microdilution technique, Antimicrob. Agents Chemotherapy, 16, 710-718
2) Doi, H., Ishii, A. and Shimono, K. (1988): A rapid *in vitro* assay system using anti-bromodeoxyuridine for drug susceptibility of *P. falciparum,* Trans. Roy. Soc. Trop. Med. Hyg., 82, 190-193
3) Geary, T.G., Divo, A.A. and Jensen, J.B. (1983): An *in vitro* assay system for the identification of potential antimalaria drugs, J. Parasitol., 69, 577-583
4) Geary, T.G. and Jensen, J.B. (1983): Effect of antibiotics on *Plasmodium falcipurum in vitro*, Am. J. Trop. Med. Hyg., 32, 221-225
5) Jensen, J.B., Capps, T.C. and Carline, J.M. (1981): Clinical drug-resistant falciparum malaria acquired from cultured parasites, Am. J. Trop. Med. Hyg., 70, 523-525
6) Trager, W. and Jensen, J.B. (1976): Human malaria parasites in continuous culture, Science, 193, 673-675
7) Waki, S., Jun, L., Meiyin, Z., Yongle, Q., Takagi, T., Lin, C., Haoming, G. and Suzuki, M. (1989): A field trial of fluorometric *in vitro* drug sensitivity test for *Plasmodium falciparum* in Hainan Island, Trans. Roy. Soc. Trop. Med. Hyg., 83, 165-166

フィールドにおけるブロモデオキシウリジンの取り込みによる熱帯熱マラリアの薬剤感受性試験

土居　弘幸[1]・Syafei[2]・石井　明[1]

我々は，先に発表したブロモデオキシウリジンの取り込みを測定する方法で，熱帯熱マラリアの薬剤感受性試験を，北スマトラ州東海岸のマラリア流行地にて行った。種々の薬剤濃度下で，普通の滅菌済み試験管を用い，患者の血液を16時間室温，室内で培養した。8例の患者から分離した熱帯熱マラリア原虫では，0.5×10^{-6}Mのクロロキンの濃度で，ブロモデオキシウリジンの取り込みが21-83%抑制された。この方法は，特殊な培養器具を必要とせず，分光光度計があれば実施できる簡便な薬剤感受性試験であり，マラリア流行地で広範な調査が可能であることを示した。

1　岡山大学医学部寄生虫学教室
2　メダン国立衛生試験所

Japan. J. Trop. Med. Hyg., Vol. 18, No. 1, 1990, pp. 43-106

PROCEEDINGS OF XXXI ANNUAL MEETINGS OF JAPANESE SOCIETY OF TROPICAL MEDICINE

18-19 November 1989, Nagoya

Emeritus president
Tetsuzo Totani
(Emeritus Professor, Fujita-Gakuen Health University)
President
Toshio Nakabayashi
(Professor; Department of Parasitology, Fujita-Gakuen Health University School of Medicine)

CONTENTS

2 Schizodeme characterization of kinetoplast DNA minicircles of *Trypanosoma cruzi* and *Leishmania* isolates from Paraguayan Chagas' disease and leishmaniasis patients H. Yamasaki *et al.*

3 Improvement of enzyme immunoassay for *Trypanosoma cruzi* epimastigote L. Zhao *et al.*

4 *In vitro* induction of monomorphic bloodstream forms from pleomorphic bloodstream forms of *Trypanosoma brucei brucei* T. Yanagi *et al.*

5 Toxoplasma infection and soil-transmitted helmintheases in Brazilian children A. Hamada *et al.*

6 Biological activity of flavivirus nonstructual protein NS3 in virus reproduction Z. Edward *et al.*

7 Development of high sensitive enzyme immunoassay for the quantification of *Vibrio cholerae* 85P3 in several foods H. Tanimori *et al.*

8 Bacterial respiratory infections in Chiang Mai, Thailand N. Rikitomi *et al.*

9 A developing pattern of *Dirofilaria immitis* larvae in mosquitoes E.M. Zytoon *et al.*

10 Phylogenetic relationships among species of *Dirofilaria* Railet and Henry, 1911 (Nematoda: Filarioidea) B.B. Omar *et al.*

11 Studies on *in vitro* maintenance of adult *Dipetalonema viteae* for a long period J. Maki

12 Basic studies on Mongolian gerbils as the susceptible host to filarial infection (3) Thy-1 antigen M. Shimizu *et al.*

13 Basic studies on Mongolian gerbils as the susceptible host to filarial infection (4) complement K. Shichinohe *et al.*

14 Distribution pattern of output eggs from schistosoma haematobia patients and evaluation of mass treatment based on the egg counts K. Makiya *et al.*

15 Ultrasonographic diagnosis of schistosomiasis japonicum in Leyte H. Ohmae *et al.*

16 Application of multi spot enzyme immuno-assay to serodiagnosis of trematodiasis M. Itoh and S. Sato

17 Change of cytokine and natural killer activities in *Ascaris suum* infected mice D. Tao *et al.*

18 Monitoring of malaria vectors after application of some control methods V. Kerdpibule *et al.*

19 Response and monitoring of the vectors of Japanese encephalitis by sound trap after application of two control methods S. Leemingsawat *et al.*

20 Insecticides for use with wide-mesh net curtain against malaria vectors T. Kurihara

21 Susceptibility of chemosterilized *Mansonia uniformis* to *Brugia malay* infection V. Kerdpibule *et al.*

22 Effect of repeated infection to maintain immunity to *Plasmodium yoeli* W-J. Ai and K. Yano

23 Relationship between clinical severity and the adherence of *Plasmodium falciparum* infected erythrocytes to C32 melanoma cells S. Nakazawa *et al.*

24 Hematological and biochemical findings in malaria patients I. Ebisawa *et al.*

25 DNA diagnosis of falciparum malaria K. Watanabe *et al.*

26 A study on serological malaria monitoring in a hypoendemic Amazonian settlement M. Suzuki *et al.*

27 A seroepidemiological study in the estimation of potential danger of malaria outbreak in hypoendemic areas in Sudan S. Kano *et al.*

28 A survey on Guinea worm disease in Anambra State, Nigeria H. Ohara

29 Study of microfilariae from human blood in Jaboatão, Perunambuco, Brazil S. Miura *et al.*

30 Control of subperiodic bancroftian filariasis in Samoa by single-dose mass treatment with diethylcarbamazine E. Kimura

31 Prevalences of serum antibodies to spotted fever Rickettiae among inhabitants in southeast Asia N. Takada *et al.*

32 Yellow fever in Ghana W.K. Ampofo *et al.*

33 Prevalence of IgG-ELISA antibodies against Japanese encephalitis, West Nile and type 2 dengue viruses in human population in Nepal C.M. Sharma *et al.*

34 An efficient method for isolation of dengue virus from dengue hemorrhagic fever patients and analyses of data (Jakarta, Indonesia, 1988) N. Fujita *et al.*

35 Immunoelectron microscopic detection of dengue haemorrhagic fever viruses from *Toxorhynchites* mosquitoes in Indonesia T. Matsumura *et al.*

36 Present situation of rickettsiosis in Awaji Island, Hyogo Prefecture T. Awata *et al.*

37 Incidence of *Giardia lambria* infection in travellers to India and Nepal A. Kimura *et al.*

38 Pathogenicity of ameba strains in Brazil T. Takeuchi *et al.*

39 A modification of agar plate method for detection of *Strongyloides* S. Kasuya *et al.*

40 A case of strongyloidiasis where accompaning gastro-intestinal disorders remarkably improved Y. Otsuji *et al.*

41 A case of myiasis caused by *Dermatobia hominis* R. Maeda *et al.*

42 Study on the immunogenicity of purified Siamese cobra toxoid Y. Kawamura and Y. Sawai

43 Myonecrotic factors with phospholipase A_2 activity in *Trimeresurus flavoviridis* (Habu snake) venom H. Kihara *et al.*

44 Investigation of schistosomiasis in Sulawesi, Indonesia

Special lecture

THE EPIDEMIOLOGY AND CHEMOTHERAPY OF SCHISTOSOMIASIS AND OTHER SNAIL-BORNE TREMATODE INFECTIONS

A. DAVIS, MD, FRCPE, FFCM, DTM & H.
Lately Director, Parasitic Diseases Programme,
World Health Organization, Geneva, Switzerland

The different life cycles of the human digenetic parasitic trematodes *Schistosoma japonicum, S. mansoni, S. haematobium, Clonorchis sinensis,* various species of the genera *Opisthorcis* and *Paragonimus,* and those of lesser pathogenic importance, *Fasciolopsis buski, Heterophyes heterophyes* and *Metagonimus yokogawai,* illustrate the complexity of the clinico-epidemiological profiles of these syndromes.

The one factor common to all of these infections is the necessity of a primary snail intermediate host to complete the biological life cycles: some additionally require a secondary intermediate host which may be several species of fresh-water fish, crustaceans or aquatic plants. Conversely, the mode of transmission to man varies from dermal penetration of infective cercariae in the schistosome infections, to the consumption of infective metacercariae in raw or undercooked fresh-water cyprinoid, fresh-water fish in clonorchiasis and opisthorciasis and the eating of fresh-water crabs or crayfish in paragonimiasis or the ingestion of water caltrop, water chestnut and bamboo in fasciolopsiasis.

Yet, despite these variations, several general common features characterise the different epidemiological cycles:—

1. the prevalences of the infections are highest in rural populations, frequently higher in males, commonly occurring in the younger age-groups and the distributions all follow the inverted binomial pattern, itself characteristic of those infections in which there is no replication of a parasite population within the human host;
2. an important feature of their propagation is the indiscriminate disposal of human faeces in areas deficient in sanitary provisions or in the use of "night soil" where this practise remains part of the prevailing agricultural pattern;
3. transmission in all of these infections is via human behavioural variables—either obligatory water-contact in the case of the schistosomes, or age-old eating habits in the other trematodes—human attitudes and customs which are extremely difficult to alter and which are often resistant to conventional health education;
4. animal reservoirs of infection, are, with the exception of *S. haematobium,* extremely common.

It thus appears inherently improbable that complete eradication of these infections by the permanent interuption of transmission will be achieved on a global scale—at least in the immediate future and certainly not before the year 2000.

We should appreciate, in a terminological sense, exactly what is implied by the word "control". The customary practical definition, in both medical and epidemiological terms, is that "control" means the reduction of the prevalence and intensity of these infections to levels

which do not constitute major public health hazards and this achievement will imply the parallel reduction of human morbidity and mortality. It follows too, that because of the complex interactions of the numerous groups of variables characteristic of the snail-transmitted trematode infections, control strategies, tactics and projections will tend to be area-specific, country-specific and intermediate-host specific. There is no substitute for locally aquired expertise in the investigation and analysis of the problems which the infections produce.

Since schistosomiasis is the major globally-distributed example of the human trematode infections, it will be taken as an example of the linkage between epidemiological analysis and control strategy and will be dealt with in greater detail than the other trematode infections which, nowadays, are of greater regional than global significance.

The control of schistosomiasis will be covered under the headings of objectives, minimal requirements for the planning of control, epidemiological, parasitological and biological techniques in current use, the concept of an "integrated" approach with emphasis on population-based chemotherapy to reduce human morbidity, the importance of the chemotherapeutic "delivery system", the principles of health care and participation in and by the community, the analytical framework necessary for decisions on follow-up action, the importance of primary health care in control and the contraints experienced in the management of operations.

Illustractive examples will be given from on-going schistosomiasis control projects.

The discussion on the drugs available for chemotherapy of trematode infections will be incorporated into the epidemiological section of the control approach.

Symposium

I Report of the North Sumatra Health Promotion Project

1 GENERAL ASPECT

TAKESHI SUZUKI
Azabu University

The North Sumatra Health Promotion Project was started in April 1978 as one of the technical cooperation projects of JICA to Indonesia, which was terminated in March 1989 after 11 years activities. At the initial stage, the Project was aimed at backing up the Asahan Project, a gigantic project of alminium smelter which had been constructed under cooperative support of Indonesian and Japanese Governments. As the construction works came to an end, objective of the Project turned to community health, in accordance with the national policy of Indonesia.

The sectoral programmes involved in the Project were malaria control, intestinal communicable disease control, rural water supply, tuberculosis control and maternal and child health. These programmes were later tried to be integrated into primary health care of community, which showed success at least to some extent.

During the entire period, a total of 67 Japanese experts were assigned to the Project, with the breakdown of 25 for malaria control, 19 for water supply, 6 for intestinal communicable disease control, 4 for TB control, 4 for maternal and child health, etc. On the other hand, 32 Indonesian counterparts were accepted in Japan as fellows. Equipments and materials donated to Indonesian side consisted of vehicles, microscopes, computers, laboratory equipments etc., which were amounted to more than 700 million yen.

The results of the cooperation were summarized in a Final Report published in March 1989, which was divided into two parts: Part I PROJECT REPORT (214 pp.) dealing with administrative matters and Part 2 TECHNICAL REPORT (260 pp.) dealing with technical matters. Summary of these activities will be reported briefly by each expert in the following sessions.

2 VECTOR CONTROL TRIALS OF COASTAL MALARIA IN NORTH SUMATRA, INDONESIA

EIJI TOKUHISA[1] AND MASAYUKI YASUNO[2]
Teiso Kasei Co., Ltd.[1] and National Institute for Environmental Studies[2]

The project activities are divided into two phases.

In the first phase (1978-1984), the following facts were elucidated. *Anopheles sundaicus* would be as a principal vector of coastal malaria in the project area. This species is

exophilic. Due to this nature, DDT indoor residual spraying was not effective at all. Therefore, larval control measure was considered instead of adult control. In the second phase (1984-1989), larval control operations were carried out.

After a two year preparatory period, a preliminary operation was started in the coastal area of 32.5 km² including a part of three villages: Perupuk, Guntung and Pematang Panjang in September 1986. Three measures were employed as components of the integrated control: environmental management, biological control by guppies, and chemical control by temephos. The environmental management is a method to reduce or eliminate mosquito emergence through drainage or circulation of stagnant water. This management should be the first task prior to application of other methods.

The preliminary operation was terminated in December 1986. However, man-hour density (MHD) of *An. sundaicus* at mosquito collection point (St. Cl) in Perupuk rose up to ca. 70 in October 1986. This was due to the vast swamps created by the blockade of the mouth of Piyai River, one of the creeks running through the operation area (later the mouth was cut open). In addition, a large swamp which had been overlooked before was heavily infested by *An. sundaicus*. Chemical application was difficult because of its extent. Guppies were less effective in the area in which carnivorous fish lived or water decayed, because fish density could not be maintained to be sufficiently high for the suppression of mosquito larvae under such conditions.

Comprehensive operation was carried out for 15 months from April 1987 to June 1988 based on the preliminary operation.

Environmental management made as the first step had worked satisfactorily. They were channels to drain water from swamps or to prevent flooding. A low meadow was converted to fish-farming ponds connecting a river with a channel.

Temephos was applied to breeding places fortnightly as routine but applied occasionally if larvae were detected. Unfortunately, some breeding places had remained without larviciding because of refusal by some villagers.

Guppies were released into non-decayed water bodies with the average density of ca. 200/m² which was extremely high considering the reduction by predation of carnivorous fishes. The high density could be maintained for about two months, but was reduced by heavy rainfall in August 1988.

As far as vector density is concerned, MHD of *An. sundaicus* at St. Cl had maintained a low level: 15.8 in average, which was obviously far lower than that in the preceding period.

However, it was considered from epidemiological studies that the supression of MHD of *An. sundaicus* to the level of around 20 for 15 months was not enough to stop the transmission of malaria.

The larval control operation was switched to an adult control operation using insecticide-impregnated bed-nets. This method is considered to be effecive against exophilic species.

More than 700 coarse mesh (2.5 mm) nylon bed-nets were impregnated with permethrin (77 mg/m²) using custom-made apparatus by six unexperienced villagers in 14 hours in July 1988. A total of 726 bed-nets were distributed to villagers. Of 409 target houses, 404 houses accepted the nets, the coverage being 98.8%. A questionnaire survey showed that 92.7% (265/286) of them actually used the nets.

The bed-nets distributed were re-impregnated again with permethrin in November 1988, four months after the first impregnation (dose 78-196 mg/m²).

The high coverage suggests that insecticide-impregnated bed-nets are the applicable control method.

MHD of *An. sundaicus* at St. Cl gradually decreased and became below 3 at least for 6 months since late September 1988, which was never observed in the past 8 years.

The parasite rate significantly dropped from 6.9% in July to 2.7% in December 1988 ($p < 0.001$). This may be due to combined effect of bed-netting and chemotherapy which was done concurrently.

Thus, impregnated bed-nets seems to be promising in controlling coastal malaria. Further studies are needed to conclude if concurrent chemotherapy treatment is necessary.

3 SEROEPIDEMIOLOGICAL APPLICATION OF MALARIA ELISA AND CHEMOTHERAPEUTIC MALARIA CONTROL IN NORTH SUMATRA, INDONESIA

HIROYUKI DOI[1], AKIRA KANEKO[2] AND AKIRA ISHII[1]
Department of Parasitology, Okayama University Medical School[1] and WHO, Vanuatu[2]

An enzyme-linked immunosorbent assay for malaria was used in North Sumatra, Indonesia as an epidemiological survey tool but not as a diagnostic purpose. Malaria-ELISA using crude *Plasmodium falciparum* antigen cross-reacted on *P. vivax* cases. A total of 878 blood samples were examined by ELISA and microscopy. Among these samples, 85 *P. falciparum* cases and 126 *P. vivax* cases were detected by microscopy. Malaria-antibody reading figure expressed as antibody unit (ABU) in ELISA was compared with parasitological diagnosis in malariometric survey and passive case detection. The mean value of ELISA (ABU) was 0.340 (SD: 0.121) for *P. falciparum* cases, and 0.283 (SD: 0.121) for *P. vivax* cases, the difference being statistically significant ($p < 0.05$). The mean ELISA value of control sera was 0.041 (SD: 0.008). The seroepidemiological survey was concentrated on students of junior high school to which students attend from surrounding villages. They showed enough antibody production to malaria as well as adults. Serum samples obtained from them were classified according to their native villages. The mean of ELISA values of each village group was compared with the parasite rate in primary schools in their native villages. A good correlation was observed between them ($r = 0.96$). The results indicated that survey of malaria in junior high school by ELISA could give useful information on malaria prevalence of several peripheral villages. Province-wide information on malaria prevalence can easily be obtained by malaria-ELISA in junior high school in tropical rural area.

Malaria is prevalent throughout east-coastal villages in North Sumatra, Indonesia. The appearance of chloroquine-resistant *P. falciparum* and ineffectiveness of DDT residual spraying have been observed. Mass drug administration, especially combination therapy of schizonticidal plus gametocyticidal drug has been repeatedly advocated, but its substantial value in the field has not been well documented. Prior to chemotherapeutic malaria control, the gametocyticidal effect of primaquine was investigated on 72 *P. falciparum* gametocyte-carriers after treatment with Fansidar plus primaquine and on 12 carriers with Fansidar

alone in Tanjung Tiram District. The gametocyte-positive rate (GPR) after treatment with Fansidar plus primaquine became 2.7% (2/72) on Day 7 and 0% on Day 14, but the GPR after treatment with Fansidar alone was 47% (5/12) on Day 7 and 33% (4/12) on Day 14.

Based on the results of active case detection which covered a total of 690 cases in Guntung Village in August 1988 and in Bagan Dalam Village in December 1988, age group (pre-school children, school children and others) specific pattern was analyzed. Parasite positive rate of pre-school children, school children and others were 7.5%, 26.6% and 8.9%, respectively. Malaria prevalence in school children was significantly higher ($p<0.05$) than other age groups. Gametocyte positive rate was the highest in school children ($p<0.05$).

Selective age group treatment and village scale chemotherapeutic malaria control operation were carried out in east-coast villages in North Sumatra, Indonesia in 1987/1988. A single dose of Fansidar plus primaquine was adopted as the drug regimen to cut the transmission of malaria at gametocyte stage. The significant reduction of *P. falciparum* prevalence in the community was obtained by successive selective age group treatment in primary school, however, *P. vivax* prevalence still lasted.

Village scale active case detection was carried out by one health center staff and two village health volunteers who were key persons of the community, and almost of the households were surveyed. After eight months, *P. falciparum* prevalence was reduced from 14% to 1%, however, in the control study area malaria prevalence was still high of 27%. Therefore, the effectiveness of chemotherapeutic malaria control will last more than one year. Treatment once a year would prevent a possibility to give rise of drug resistant strain of *P. falciparum*. Once malaria prevalence was suppressed, the frequent activity in the following period would not be necessary. As the results of the chemotherapeutic control activities covering high-prevalent villages in the coastal area, malaria prevalence in 1988 became very low, as compared with the status in 1985/1986. The intensive active case detection and treatment with Fansidar plus primaquine, especially with good community participation is suggested as a possible successful measure of malaria control.

4 A CHOLERA EPIDEMIC IN NORTH SUMATRA

NORICHIKA H. KUMAZAWA
Department of Veterinary Public Health, Faculty of Agriculture, Tottori University

A big epidemic of cholera in North Sumatra Province started at Nias Island on the 19th of March in 1978 and spread to the main area of the province. A total of clinically suspected cases reached to 23,900 in which 1,110 died by the 30th week of 1980. I collected data on the epidemic at Provincial Health Service of North Sumatra, Regional Health Services of Medan and Asahan, health centers in Asahan and Tapanuli Utara regencies and Regional Health Laboratory in Medan to analyze the spread of the epidemic.

Number of the clinical cases incleased from the beginning of 1978 to reach a peak at the beginning of 1979, decreased to the end of the year and made another small peak in 1980. From the day of onset of the outbreak in each regency, the epidemic was found to start at Nias Island on March 1978 and, after establishing at Sibolga City in Tapanuli Tengah

regency, spread along a national highway to Medan and afterward to Asahan and Labuhan Batu regencies along Malacca Strait in the main area of the province. The epidemic wave branched at Sibolga entered to Tapanuli Selatan regency. The spread was rather slow and took about 20 weeks from Nias to Deli Serdang and about 25 weeks from Deli Serdang to Labuhan Batu. The epidemic was not entered to Karo and Dairi regencies.

From the figure of the epidemic in each regency, second epidemic was found to start at an area including Deli Serdang and Medan in the beginning of 1979 to spread reversely along the same highway to Sibolga and reached to Nias Island where the first epidemic in 1978 started. The second epidemic was also entered from Medan and Deli Serdang to Asahan and Labuhan Batu. The second epidemic was also slow and took about 6 weeks from Deli Serdang to Nias.

Vibrio cholerae eltor Ogawa strains were isolated from specimens submitted to Regional Health Laboratory in Medan including feces and rectal swabs taken from about 7,500 suspected cases along the first spread from Nias to Deli Serdang until the first detection of an eltor Inaba strain at Deli Serdang on the 26th of December 1978. Four days later, second Inaba strain was detected in the same regency. Since the beginning of 1979, Inaba strains were detected consecutively with Ogawa strains at Deli Serdang, Medan, Tapanuli Utara and Asahan regencies followed by Tanjung Balai, Simalungun, Pematang Siantar and Labuhan Batu regencies and municipalities. In Nias, only Inaba strains were isolated at the second epidemic. Conclusively, the second epidemic was caused by eltor Ogawa and Inaba strains in which Inaba strains overwelmed the preceding Ogawa strains in Nias on February 1979.

Source of the first epidemic in Nias Island was traced to an ice-producing factory in Gunung Sitori, a capital of Nias regency, where the water was heavily contaminated with human excreta. As a cholera epidemic was reported at Simeulue Island in Aceh province, about 150 km northwest from Nias Island, from January till March 1978 with 480 cases and 82 deaths, the organism caused the first outbreak in Nias was supposed to have been imported from Simeulue.

Since September 1981, the system has changed for all diarrheal diseases to be reported collectively. This change brought some merit as that trends of diarrheal diseases became easily grasped together with the demerit as that the acute diarrhea suspecting cholera became hidden in whole diarrheal diseases.

5 WATER SUPPLY IN RURAL AREA

HIROMI AMANO
Sapporo Waterworks Bureau

Enough supply of safe water is the essential prerequisite to protect inhabitants residing in rural area from infection of intestinal communicable diseases such as cholera and other bacterial diseases.

The preliminary survey on water supply system was done in the initial stage of the Project to improve water supply facilities in the Project area.

In order to obtain basic data for planning of future water supply program in rural area

extensive surveys were carried out. The various data were collected to know the present condition of environmental sanitation and water utilization, etc.

Based on the result of the surveys, many activities for water supply, mainly construction of deep wells, were conducted, through cooperation with Indonesian counterparts.

The outlines are mentioned as follows.

(1) Construction of deep wells and water supply facilities

Based on the preliminary survey construction of the deep wells were decided at five sites in Asahan regency in 1981. The construction work was completed in 1981. Furthermore new six deep wells were constructed in 1987. The facilities consist of a deep well with a submergible pump, a steel-made elevated tank, polyvinyl chloride pipelines, stand pipes and bathing rooms for public use. The sites for construction of the deep wells, where it is difficult to get clean water and the typical water-borne diseases had broken out, were selected by Indonesian government.

The operation and maintenance of these water supply facilities by community people was organized in each village. In general rural people do not notice necessity of the cost for water. But after discussion with villagers to solve the problem, their consent was obtained on charging for water. After a training course was held for maintenance and operation of the deep well, the facilities are maintained by people in villages. One of them is already broken down, but the others are in good condition, which have been used by many villagers.

(2) Surveys for collecting the basic data and other water supply activities

According to the result of surveys, it was found that the rate of damaged hand pumps in Project area was about 80%, almost all samples of water from dug wells were not good in quality and were contaminated by coliform and bacteria through contact with human excrement or the medium of animals, in general dug wells were used by many villagers, etc.

Therefore it was necessary to propose the following countermeasures for the problems.

a. Establishment of workshops and training courses; Preparation of tools and spare parts and improvement of knowledge for the maintenance and repairing of water supply facilities.

b. Dissemination of simple sand filters; Improvement of water quality

c. Construction of dug wells with sanitary facilities (washing and bathing room, toilet, septic tank, etc.); Promotion of sanitary awareness and improvement of environmental condition, etc.

Thus the water supply activities have achieved the purpose of safe water supply through the construction of deep wells and other activities in Project area, in spite of facing various problems. Participation of the villagers was promoted, as community in the villages was organized to maintain the water supply facilities. Water supply is their own essential problem, but it is difficult for villagers to accept our water supply activities because of the difference in culture or economical condition between Indonesia and Japan.

It is very important to promote sanitary awareness of villagers, and appropriate technology for water supply in rural area should be developed to improve environmental condition.

Health education by local government is necessary to promote sanitary awareness of villagers. At the same time it is desirable to construct facilities such as small-scale plants for water supply using water from the deep well or river, sewage and garbage diaposal.

The policy for improvement of environment and sanitation including water supply in

rural area should be advanced as Indonesian government policy in the near future.

6 TUBERCULOSIS PROGRAMME IN THE "NORTH SUMATRA HEALTH PROMOTION PROJECT"

AKIKO FUJIKI[1] AND RYOJI TAKAI[2]
International Cooperation Department, The Research Institute of Tuberculosis, JATA[1] and Team Leader: 1980-1984, JICA[2]

Tuberculosis control programme in the Project concentrated its activities on the clarification of TB epidemiological status in the district and on improvement of TB laboratory techniques. The main achievements were as follows:

TB Status Survey in North Sumatra:

To estimate annual risk of infection, tuberculin skin tests and BCG-scar surveys were carried out among school children in North Sumatra. Tuberculin positive rate and BCG vaccination coverage rate for new born infants were 13.14% and 31.9% respectively. From these results, the annual risk of infection during childhood was calculated to be 1.69%.

Expansion of TB Centers:

The TB control programme has been implemented through TB centers attached to Health Centers. In North Sumatra, 5 TB centers were first established in 1971 and thereafter the number increased to 61, corresponding to 25% of the total number of Health Centers in 1980. Pagurawan and Lima Puluh health centers have participated in the TB control programme ever since the project started in 1980. In the Asahan regency progress rate of participation in the TB control programme was 38.9%, with the highest rate reported among regencies in North Sumatra.

Improvement of TB Centers Functions:

In 1980, TB centers in North Sumatra carried out 19,026 sputum-examinations and detected 2,653 new tuberculosis cases. These figures showed significant increases compared with those of previous years, and such increases were most remarkable at the Indra Pura and Pagurawan health centers which participated in the TB Control Programme.

Manpower development:

Health workers and microscopists in the Health Centers were trained to improve TB Control Programme Management. Key personnel for the TB Control Programme consisting of three doctors and one laboratory technician were sent to Tokyo, Japan to participate in the International TB Training Course held at the Research Institute of Tuberculosis.

TBB examination technology transfer:

Technology concerning sputum culture examination for TB bacilli was transferred to the Medan Health Laboratory. Sputum smear positive specimens and sputum specimens from suspected TB cases were collected at 6 institutions: BP_4 (Chest Disease Center), 3 health centers in Medan, the Medan Health Laboratory and the Indra Pula Health Center. A total of 566 specimens were sent to the Medan Health Laboratory: all specimens were cultured by the modified Kudoh swab method using 2% Kudoh medium except the specimens from Indra Pula Health Center, where the cultures were done on the spot, because this health center is

located about 100 km from Medan. Out of 566 cultures, 451 (79.7%) were free from contamination but 115 (20.3%) cultures were either partially or totally contaminated. At BP_4 an attempt was made to reduce this high contamination rate by collecting sputum directly into a clean, sterilized and disposable container and by preserving it in a refrigerator at a temperature of 4°C–5°C. At the Medan Health Laboratory, contaminant rate was reduced by increasing the sputum pretreatment time with 4% NaOH from 2 minutes to 5 minutes. Once these procedures were implemented, heavy contamination was no longer observed.

Conclusion:

TB control programme in the project area has been greatly improved by implementing recommendations based on epidemiological indices and information obtained through field research. The National Standard Method of TBB detection by direct smear examination of sputum was adopted. Techniques for culture examination, TB identification test and drug sensitivity test were successfully transferred to the Medan Health Laboratory and as a result the Medan Health Laboratory has been functioning as a first class TB laboratory in carrying out these examinations in Indonesia.

7 MATERNAL AND CHILD HEALTH

YASUHIDE NAKAMURA
Mitaka Health Center, Tokyo Metropolitan Health Department

The Government of Indonesia has implemented a new strategy on health care delivery to reduce infant mortality rate, under-fives mortality rate and fertility rate. This approach is called Integrated Family Health Package (IFHP) and its activities are being carried out through Posyandu (Integrated Service Post) in all over Indonesia.

The IFHP activities of the Project started in Sep. 1986. At first, the survey for collecting health information was needed to decide the practical target of the IFHP of the Project. In the second stage, health promotion development was done in a model village, Tinggi Raja Village in North Sumatra Province. Finally, integrated primary health care was implemented as community health training courses.

IFHP in Tinggi Raja Village: The area of Tinggi Raja Village is 21.4 km^2 and its total population as of January 1988 amounted to 5,779. Number of households was 1,039.

The household health survey was conducted to reduce under-fives mortality rate and to strengthen intersectoral cooperation. Planning and implementation of the survey was done by Kader (village health volunteers). About 60 Kader visited all households and got information on vital statistics, environmental factors and health situation of women and children. This community-based survey made villagers active and self-reliant.

Tinggi Raja Seminar was held every month to strengthen practical knowledge on health in the community by means of information, education and communication to Kader. Date, place and content of the Seminar were decided by Kader. Lecturers were decision makers at Provincial level and chairmans were Kader from the community. This seminar encouraged villagers; dropout rate of Kader in this village became very low and new candidates for Kader increased markedly.

Under-fives mortality rate of Tinggi Raja Village decreased markedly from 111.9/1,000 newborns (1986) to 21.9 (1988). Leaders of Kader made a booklet on health report of their own village with handwriting. They were able to calculate infant mortality rate, under-five mortality rate and fertility rate. This means that villagers can evaluate their health activities by themselves.

Community Health Training Courses: In the final year of the Project, comprehensive and integrated training courses were implemented for the reinforcement of the health care delivery system, in consideration of community needs and community self-care system. All the courses were done under the cooperation of the Indonesian officials and Japanese experts. Its specific objectives were to cultivate trainers at regency and health centers and to mobilize community resources and community participation. Contents were as follows; training on health management for regency and municipality health workers in North Sumatra, training on medicine, nutrition, family planning, malaria and water supply for health center staffs, health education on community health development for village health volunteers. Actual number of attendants amounted to 396.

Cooperation project for community health development should be planned under the basic principles of primary health care, including community participation, community needs orientation, community resource mobilization, appropriate technology and intersectoral cooperation. From this viewpoint, all activities of our Project was integrated into community health training courses.

II Diarrhea in Tropical Countries: Its Problems Related with Changing Socioeconomic Situation

1 DIARRHEAL DISEASES IN THE PHILIPPINES

MEDIADORA C. SANIEL
Research Institute for Tropical Medicine, Department of Health, the Philippines

Diarrheal disease is a major cause of death and illness among Filipino infants and young children. Over 25 million episodes occur annually among children aged five and below resulting in as many as 80,000 deaths in this age group. Furthermore, diarrhea cases occupy 30-50% of hospital beds in pediatric wards creating a heavy demand on health facilities and the limited national health budget.

In response to this problem, the Department of Health launched a national program to control diarrheal diseases (CDD) in 1980. The program aims to reduce mortality through effective diarrhea case management utilizing oral rehydration therapy (ORT). For reduction of morbidity and its associated ill effects, particularly malnutrition in infants and young children, interventions are focused on promotion of breastfeeding and improved weaning practices, use of safe and abundant water, good personal and domestic hygiene, and immunization against measles.

Major efforts have been exerted and significant gains achieved in the institutionalization of effective case management through training activities targetting different levels of health care providers, establishment of ORT corners, oral rehydration salts (ORS) production and supply distribution, and public and professional promotion. Diarrhea preventive strategies are being strengthened.

Despite all these efforts by our government in collaboration with the private sector, much more needs to be done. ORS and ORT use rates among children with diarrhea have improved but remain low. Diarrhea case management in health facilities leaves much to be desired. Intravenous fluids, anti-diarrheals and antibiotics continue to be prescribed inappropriately. Availability of ORS supply in adequate quantities at different levels and at all times remains elusive. The national CDD program is currently addressing these issues and re-evaluating its strategies to achieve its targets.

2 OVERSEA TRAVELLERS' DIARRHEA IN TOKYO

MAKOTO OHASHI
Tokyo Metropolitan Research Laboratory of Public Health

Since the 7th cholera pandemic due to *Vibrio cholerae* O-1, biovar eltor began in the early 1960's, it affected Japan in various ways during the past three decades. In 1977, a domestic epidemic of cholera occurred in Arida City, involving about 100 cases, and its infectious source was presumed to be body waste from a person who returned from a cholera-infected

country to Japan and sub-sequently manifested the disease but remained at home, unaware that the illness was cholera. In the next year, 1978, there was a cholera outbreak due to mishandling of frozen rock lobsters imported from a cholera-endemic country at catering service of a wedding hall in Tokyo. The outbreak involved a total of 49 cases inhabited in 10 different Prefectures.

In the same year, 1978, the Tokyo Metropolitan authorities established the service to carry out fecal examinations of oversea travellers returning or coming from cholera-infected area. The examination has been carried out by our laboratory. It has come from the idea that early detection and proper treatment of the imported cases of food- and water-born communicable diseases, such as cholera, typhoid fever, and shigellosis are of the utmost importance in the prevention of domestic spread of these diseases. As the result of the service, we found about 50% of all the cholera cases reported in Tokyo, and we also found patients or carriers due to a variety of enteropathogenic bacterias other than cholera vibrio. These findings made us possible to grasp the general outline of international transmission of diarrheal diseases from tropical area to Japan.

According to the results obtained in the past 7 years, the frequency appearance of known and possible enteric pathogens was about 60% in the group of acute diarrheal cases and 25% in the group of convalescent cases and normal subjects. Diarrheagenic *Escherichia coli,* including enterotoxigenic, enteroinvasive, enterohaemorrhagic and of enteropathogenic serotypes, had the highest frequency (29.4%). It was followed by *Salmonella* (21.5%), *Vibrio parahaemolyticus* (12.3%), *Campylobacter jejuni/coli* (10.7%) and *Shigella* (6.7%). Detection rate of *V. cholerae* O-1 was 0.4%. Interestingly, multiple kinds of pathogens were detected concomitantly from single individuals in approximately 20% of pathogen-positive cases.

Serotypes of *Shigella* and *Salmonella* have shown different distributions in isolates obtained from domestic cases and imported cases.

In a shrinking world like today with fast and frequent international travells and transportations of food, the transmission of diarrheal diseases across the boundaries is inevitable. In our attempts to cope with such a situation, we have to share internationally the responsiblility and the work of seeking a way to intelligently solve our common problem, control of these diseases. In order to achieve this goal, establishment of a surveillance system supported by relevant information of laboratory origin in each country and its international organization should be most effective.

3 CURRENT TREND OF AMEBIASIS AND GIARDIASIS IN JAPAN

HIROSHI OHTOMO
Department of Parasitology, Gifu University School of Medicine

Amebiasis and giardiasis resulting from the infections of *Entamoeba histolytica* and *Giardia lamblia* are the protozoan diseases distributed widely in the world, centering on the tropical and subtropical zones. They have been found prevalent in the areas with inferior sanitary environment since cysts excreted into the asymptomatic carrier's feces become the source of the infection. However, in recent years, attention has been drawn to an increase

in domestic occurrence, in advanced countries, of the amebiasis and giardiasis with the mode of transmission different from the prevalence in developing countries. In Japan, too, re-advant of amebiasis and giardiasis with a new mode associated with the transition in the social conditions is posing a grave issue of medical measures.

Regarding amebiasis, Walsh (1988) estimates that in 1984 there are about 500 million persons with the infection all over the world. Of them, 40,000 developed severe colitis or amebic abscess, of whom at least more than 40,000 or largest 110,000 died. Also in our country, emergence of a considerable number of patients was seen in the period of confusion after the Second World War. Incidence, however, decreased sharply along with an improvement in the living environment with a decrease in the number of cases reported to the Ministry of Health and Welfare as legal epidemic to about 10 cases per year in the 1960s. However, the cases reported have shown a sharp increase since 1979, and furthermore, many of the cases were found to be males who had no experience of travelling overseas. As a result of conducting a survey, it was found that oral anal sex among male homosexuals was the main cause of transmission. It attracts our attention that the domestically infected amebiasis has become a disease belonging to the category of sexually-transmitted disease, more than half the number of the patients proving positive to serologic test for syphilis. Further, there are many cases who develop liver abscess among homosexuals with amebiasis in our country, and it is an interesting finding that the group of Prof. Takeuchi of Keio University clarified that the isozyme pattern of the infective protozoan is of pathogenic strain different from that of the protozoan isolated from homosexual patients in Europe and U.S.A. In this situation, lately the epidemiological phase of amebiasis in Japan has undergone a remarkable change. It is necessary for us to take precautious measures for this disease transmitted by travelers overseas and imported monkeys.

Giardiasis is also a disease distributed widely in the world, and its patients are estimated to be 200-300 millions. Occurrence of the infection in Japan today is extremely rare. However, the incidence in patients who returned from a trip to developing countries is not so low as we can ignore and the infection is one of the important causes of diarrhea in travelers. Particularly, there are many male patients in their 20-30s who contracted the disease in subcontinental India, and the authors' experiences reveal that about half the number of the cases had mixed infections with *Shigella, Salmonella* and *Campylobacter.* This disease also shows the aspect of sexually-transmitted disease in Europe and U.S.A. In Japan, however, most of the cases are those whose infections were carried into Japan from abroad, showing no such trend.

4 RECENT PROBLEMS OF STRONGYLOIDES INFECTION IN OKINAWA

ATSUSHI SAITO
First Department of Internal Medicine, School of Medicine, University of the Ryukyus

The infection due to *Strongyloides stercolaris* is widely ditributed in tropical or subtropical areas in the world.

Okinawa Islands are located southern most Japan and well known as the endemic area of the infection.

I report here several topics and new results for strongyloides infections.

1. Analysis of the cases of desseminated strongyloidiasis.
2. New agar plate method (SA plate #1) for detection of *Strongyloides stercoralis.*
3. Prevalence of strongyloides carrier in Okinawa related to anti-HTLV-1 antibody.
4. Failure of the trial of the new treatment.

1. Among the 7 cases of desseminated strongyloidiasis, 6 (86%) were positive anti-HTLV-1 antibody and the remaining was under treating with corticosterioid at the onset of the disease. Causative organisms other than *Strongyloides* were *Klebsiella pneumoniae, Enterococcus faecalis, Eikenella corrodens, Pneumocystis carinii* and *Staphylococcus aureus.* Leading clinical manifestations were meningitis, septicemia, pneumonia or liver abscess.

2. It had been reported until the new method was devised that the positive rate of the healthy carrier were around 1.0 to 1.5% among the residents in Okinawa. *Strongyloides* was detected in 18.0% out of 1,150 samples, whereas 3.0% and 4.3% by filter papar and MGL methods, respectively.

3. Average positive rate was now estimated as 7.2%, and the residents with positive anti-HTLV-1 antibody were revealed to have high infection rate with 47.0%.

4. We tried 4 regimens for the eradication of the organism. R-1: thiabendazole single use (1,500 mg/day for 5 days and discontinued 9 days, repeated 3 times). R-2: same as above, but repeated 4 times. R-3: combination of thiabendazole and mebendazole (thiabendazole 1,500 mg/day for 5 days and continued use of mebendazole 200 mg/day for 9 days, repeated twice). R-4: mebendazole single use (200 mg/day for 28 days).

Side effects such as nausea, vomitting, vertigo occured in high incidence in R-1 and R-2 groups with drop out rate of 42.7% and 55.7%, respectively. Mebendazole were well tolerated to be administrated, but lever disfunction were observed in 50.0% and 72.4% in R-3 and R-4 groups, respectively.

Eradication effect in short oeriods observation were satisfactory but finally this regimens were not recommended for the treatment for the strongyloides infection by the high incidence of the side effects.

General presentation

1 ENHANCE BY LOW pH OF TRANSFORMATION FROM TRYPOMASTIGOTES TO AMASTIGOTES IN *TRYPANOSOMA CRUZI*

HIROJI KANBARA, HARUKI UEMURA, SHUSUKE NAKAZAWA AND TOSHIHIDE FUKUMA
Department of Protozoology, Institute of Tropical Medicine, Nagasaki University

Transformation of trypomastigotes to amastigotes in *Trypanosoma cruzi* is assumed to be the spontaneous process since it occurs in various culture media. However, it was proved that low pH remarkably promoted the transformation, which corresponded with the prompt intracellular transformation. The transformation involved sepalation of lytic components to themselves, which were more active in high-virulent trypomastigotes. These components were slowly separated even in the culture medium of neutral pH because high-virulent trypomastigotes would completely disintegrated by longer incubation unless they were transformed to amastigotes. The lytic activity was neutralized with bovine albumin, therefore the rapid and intact transformation was obtained in the low pH medium with bovine albumin. The delayed transformation was in the neutral medium containing bovine albumin suggests that trypomastigotes probably have form-maintaining factors which will be removed were rapidly at low pH and slowly at neutral pH, but are different from lytic components.

2 SCHIZODEME CHARACTERIZATION OF KINETOPLAST DNA MINICIRCLES OF *TRYPANOSOMA CRUZI* AND *LEISHMANIA* ISOLATES FROM PARAGUAYAN CHAGAS' DISEASE AND LEISHMANIASIS PATIENTS

HIROSHI YAMASAKI[1], KIYOSHI KITA[1], TAKASHI AOKI[1], HIROSHI OYA[1],
BALBINA PAVÓN[2], OFERIA ARIAS[2], MYRIAM MORÁN[2] AND MARTA VERA[2]
Department of Parasitology, Juntendo University School of Medicine[1]
and Laboratorio de Investigación, Laboratorio Central e Instituto de
Medicina Tropical, Ministerio de Salud, Asunción, Paraguay[2]

Kinetoplast DNA (kDNA) minicircles, the constituents of mitochondrial DNA unique to trypanosomatid flagellates, possess genetic diversities. On the basis of these heterogeneities, electrophoretic patterns (schizodemes) of restriction endonuclease-generated fragments of kDNAs have been utilized to identify and classify the species, strains, isolates, and clones of trypanosomes and *Leishmania* species (Morel *et al.*, 1980; Lopes *et al.*, 1981; etc.). We applied this technique for the 3 isolates (CD, SF and VM) of the Paraguayan *T. cruzi* that we isolated and established as cell lines from 3 patients with acute phase infections of Chagas' disease; these patients were from areas that were geographically distant. Two Paraguayan *Leishmania* sp. (JE and BN) isolated from cutaneous leishmaniasis patients were also subjected to schizodeme-pattern analysis.

The isolates used in this study were cultivated at 28°C for *T. cruzi* and at 25°C for *Leishmania* sp. in a serum-free medium GIT which was supplemented with hemin and tryptose. Culture forms of these organisms, epimastigotes and promastigotes, were harvested at the stationary phase of growth and kDNAs were prepared according to the method of Ozaki and Czeko (1984). kDNA fragments produced by restriction endonucleases were electrophoretically separated on 2% agarose gels or 4-15% gradient polyacrylamide gels. Five *T. cruzi* reference strains were generous gifts from Dr. G. Grimaldi, Fundacao Oswaldo Cruz (Rio de Janeiro, Brazil). *L. mexicana, L. braziliensis panamensis* and *L. donovani chagasi* were purchased from ATCC. *L. mexicana amazonensis* was from the Department of Parasitology, Tokai University School of Medicine.

(I) *T. cruzi* isolates: Digestion by EcoR I of kDNAs of the 3 Paraguayan isolates resulted in almost the same schizodeme patterns. In comparison with the 6 reference strains, the patterns of these isolates were similar to that of the Fl strain isolated from *Triatoma infestans* in Rio Grande do Sul, a southern Brazilian state located near Paraguay. *T. cruzi,* the pathogen of Chagas' disease and *T. rangeli,* a non-pathogenic agent, often distribute in the same area and are difficult to distinguish morphologically. Interestingly, however, the schizodeme patterns with any of Hae III, EcoR I, or Msp I differed in these 2 species, possibly providing a useful tool to diagnosis of the infections with these trypanosomes.

(II) *Leishmania* isolates: Three different restriction enzymes (Msp I, Hae III and Taq I) were used for schizodeme analyses for the 2 Paraguayan pathogens which were expected to be *L. mexicana* or *L. braziliensis* complexes from the clinical symptoms of the patients. These two isolates yielded essentially the same patterns, showing major fragments of 1.0 and 0.8 kbp, 210 bp, and 350-360 bp with Msp I, Hae III and Taq I digestions, respectively. The patterns with Msp I were similar to that observed in *L. mexicana,* while those with Hae III and Taq I were not detected in any *Leishmania* reference strains.

3 IMPROVEMENT OF ENZYME IMMUNOASSAY FOR *TRYPANOSOMA CRUZI* EPIMASTIGOTE

LIPING ZHAO[1], MASAYUKI IWAMOTO[1], TSUNEHIRO KITAGAWA[1] AND HIROJI KANBARA[2]
Department of Microbiological Chemistry, Faculty of Pharmaceutical Sciences[1] and
Department of Protozoology, Institute of Tropical Medicine[2], Nagasaki University

This study was undertaken to improve a previous immunoassay method for epimastigotes of *T. cruzi,* the proliferative form in vector triatomine bugs. The original method which Tulahuen strain was used as the solid-phase antigen was the specific assay to the epimastigote of *T. cruzi,* Tulahuen strain. A novel improved method named selected antibody enzyme immunoassay (SAEIA) which is a general assay method for five different strains of epimastigotes was developed applying a new assay principle. Three immunological reagents used for the assay were rabbit antiserum specific to epimastigote of *T. cruzi,* Tulahuen strain, β-D-galactosidase labeled anti-rabbit IgG as the secondary antibody, and cell fragments of epimastigote absorbed on the surface of microtiter-plate as the solid-phase antigen. The SAEIA, the general assay method for five different strains of epimastigotes with

the same working range between 10^4 and 10^6 cells/m*l*, was developed by using cell fragments of epimastigote of Y strain instead of that of Tulahuen strain as the solid-phase antigen. The scope of the SAEIA was limited to epimastigotes of various strains. A basic study to apply the SAEIA for detection of pollution area of Chagas' disease was study: the proof that the contamination of an aliquot of the body homogenate of kissing bugs in the assay media did not disturb the SAEIA is also reported.

4 *IN VITRO* INDUCTION OF MONOMORPHIC BLOODSTREAM FORMS FROM PLEOMORPHIC BLOODSTREAM FORMS OF *TRYPANOSOMA BRUCEI BRUCEI*

TETSUO YANAGI[1], HIROYUKI HIRUMI[2] AND S.K. MOLOO[2]
Department of Protozoology, Institute of Tropical Medicine, Nagasaki University[1] and International Laboratory for Research on Animal Diseases, Nairobi, Kenya[2]

Monomorphic bloodstream form (mBSF) populations could be induced from clonal pleomorphic (p) BSF populations of *Trypanosoma brucei brucei* GUTat3.1 by treating the pBSFs *in vitro* with bovine haemoglogin (BHb), or murine erythrocyte lysates (MEL). The primary cultures of the BHb-treated BSFs were originated from infected tsetse salivary glands and those of the MEL-treated BSFs were initiated from infected Balb/c mouse blood. Not-treated BSFs, which were long slender (L-) BSFs *in vitro,* were subcultured in T-25 flasks containing feeder-layers derived from a foetal bovine spleen and maintained at 37°C using RPMI 1640 medium supplement 20% foetal bovine serum. Under these conditions the BSFs continued to grow in the medium without penetrating intracellular spaces of the feeder-layers. An addition of BHb or MEL to the medium resulted in the differentiation of L-BSFs to short stumpy forms (SSFs). All the SSFs rapidly disappeared by day five without changing the medium. However, a very small number of the surviving L-BSFs gradually increased in number and formed a pure L-BSF population by day ten when the medium containing BHb or MEL was changed every two days after the five-day-treatment. The treated L-BSFs showed only monomorphism when inoculated into rodent hosts and killed the hosts within a short time without developing parasitaemic waves. Tsetse flies, *Glossina morsitans centralis,* fed either on the treated L-BSFs by means of membrane feeding or on the infected mice, didn't become infected. On day seven, no L-BSFs were seen in any of the cultures when the medium hadn't be changed. However, a small number of procyclic forms (PCFs) were observed, which continued to proliferated at 37°C for long periods. The results suggested that (1) BHb as well as MEL regulates the pleomorphism of *T. b. brucei* BSFs, (2) L-BSFs in the original pleomorphic population vary in their sensitivity to BHb and MEL, (3) most, but not at all, the L-BSFs differentiate to SSFs at 37°C when exposed to BHb or MEL, depending on their sensitivity, amounts of BHb and MEL, and the duration of the exposure, (4) the nature of resistance (low sensitivity) to BHb and MEL is most likely irreversible for a long period of time, (5) the BHb- and MEL- treatment may eliminate BSFs, which are able to transform to SSFs, as well as to PCFs, from the pBSF populations and (6) the *in vitro* system developed in this study may facilitate further studies on the mechanism underlying this stage-specific differentiation of *T. brucei.*

5 TOXOPLASMA INFECTION AND SOIL-TRANSMITTED HELMINTHEASES IN BRAZILIAN CHILDREN

ATSUO HAMADA[1], MASASHI KOBAYASHI[2], EIICHI OKUSAWA[3], TOMOYOSHI NOZAKI[3], IVETE BARBOSA[4], IRAN DUARTE[4], AMARO COSME[4], SEIKI TATENO[4] AND AKIO KOBAYASHI[1]
Department of Parasitology, Jikei University School of Medicine[1],
Department of Parasitology, Chiba University School of Medicine[2],
Department of Parasitology, Keio University School of Medicine[3] and
Laboratorio de Imunopatologia Prof. Keizo Asami,
Universidade Federal de Pernambuco, Brazil[4]

Human toxoplasma infection is highly prevalent in tropics and it was supposed that the people in some of these areas may be infected through oocysts contaminated soil. We examined the prevalence rate of toxoplasma infection in relation to soil-transmitted helminthiases among school children in Recife, Brazil.

School in Varzea (VAR) is located in suburbs of Recife where sanitary condition is poor. School in Recant (REC) is in center of this city with well established sanitation. Stool and serum samples were collected from 110 students in VAR and 18 students in REC. Fecal samples were examined by Kato-Katz method. Seventy three (68%) of students in VAR and only one student (6%) in REC were found to be positive forsoil-transmitted helminth eggs. Sera were examined for anti-toxoplasma antibody (toxo-Ab) by indirect latex agglutination test. Toxo-Ab was positive (tiler>1:32) in 91 (84%) of students in VAR and 7 (39%) of students in REC. Thus, VAR students were significantly higher ($p<0.001$) than REC students in regard to the prevalence ratio of both helminthic infections and toxo-Ab. In addition, it was shown that as a high percentage as 64% of VAR students had toxo-Ab titers of 1:1,024 or higher, suggesting that most of those infected children were recently infected.

These facts may strongly suggest that VAR students frequently contact with the soil and other sources, which were highly contaminated with toxoplasma oocysts as well as soil-transmitted helminth eggs.

6 BIOLOGICAL ACTIVITY OF FLAVIVIRUS NONSTRUCTURAL PROTEIN NS3 IN VIRUS REPRODUCTION

ZULKARNAIN EDWARD, MASAKO SAHARA, SUSUMU HOTTA AND TSUTOMU TAKEGAMI
Division of Tropical Medicine, Medical Research Institute, Kanazawa Medical University

Recently, entire nucleotide sequence of flavivirus genomic RNA has been determined. Sequence data gave us a lot of informations about functions of virus specific proteins. However, the functions of nonstructural proteins (NS) are still unknown. It is suggested that NS3 (70 kDa) seems to be related to viral RNA synthesis and cellular immunity. To examine the biological activity of NS3, we prepared monospecific antibody against NS3.

Japanese encephalitis virus (JEV) (JaGAr-01 strain) and dengue virus type 1 (D1) (Mochizuki strain) were used as virus materials. JEV-infected C6/36 cells were homogenized in Tris buffer (pH 7.5) and fractionated by the centrifugation. Crude membrane fractions were subjected to SDS-PAGE and NS3 bands were cut. NS3 proteins eluted from the gel slices were injected into rabbit. Anti-NS3 rabbit sera recognized not only JEV-NS3 but also D1-NS3 in virus infected Vero cells. Western blot (WB) analysis using anti-NS3 showed that NS3 mainly existed in nuclear and membrane fractions in the cells. In addition, indirect fluorescence antibody experiment confirmed the localization of NS3 in the cells and showed that anti-NS3 could detect NS3 at the early stage after virus infection in JEV-infected (6 hr, p.i.) and D1-infected (1 day p.i.) Vero cells. In *in vitro* RNA synthesis, anti-NS3 inhibited the activity of RNA synthesis. These results suggest that NS3 has a role in viral RNA synthesis which associated with membrane fractions in the infected cells.

7 DEVELOPMENT OF HIGH SENSITIVE ENZYME IMMUNOASSAY FOR THE QUANTIFICATION OF *VIBRIO CHOLERAE* 85P3 IN SEVERAL FOODS

HIDEAKI TANIMORI[1], RITSUKO MURAKAMI[1], AKITO NAKAGAWA[1], MIHOKO SAKAE[1], TSUNEHIRO KITAGAWA[1], AKIYOSHI UTSUNOMIYA[2] AND TATSURO NAITO[2]
Department of Microbiological Chemistry, Faculty of Pharmaceutical Science[1]
and Department of Bacteriology, Institute of Tropical Medicine[2],
Nagasaki University

We have been developed a competitive enzyme-linked immunosorbent assay (CELISA) method for a strain named 85P3 of *Vibrio cholerae* using the antiserum specific to *V. cholerae* 85P3 and cell fragment of 85P3 loaded solid-phase antigen as the immunological reagents, and β-D-galactosidase labeled anti-rabbit IgG as a tracer. The lowest detection limit of a highly sensitive CELISA for *V. cholerae* 85P3 was 10^6 cells/m*l*. It was demonstrated that the application of this CELISA method was successfully applied to quantitative analyses of a growing state of *V. cholerae* 85P3 in various solid foods, such as banana, tomato, porridge and so on. *V. cholerae* 85P3 was inoculated and cultured in 1 g of banana at 28°C for a suitable time. The samples were collected at 1.5, 3, 6 and 12 h after the inoculation. The time course of the growth of *V. cholerae* 85P3 was able to trace by using the CELISA for *V. cholerae* 85P3. After 12 h of incubation period, the concentration of *V. cholerae* 85P3 increased up to 10^9 cells in 1 g of banana.

The similar growth curves were also obtained with other solid foods.

8 BACTERIAL RESPIRATORY INFECTIONS IN CHIANG MAI, THAILAND

NAOTO RIKITOMI[1], ATSUSHI TAKAHASHI[1], TSUYOSHI NAGATAKE[1],
HIDEHIKO HIROSE[1], KEIZO MATSUMOTO[1] AND PRASIT THARAVICHITKUL[2]
Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University[1]
and Maharajanakorn Chiang Mai Hospital, Thailand[2]

Antimicrobial sensitivity of 12 strains of *S. pneumoniae,* 58 strains of *S. aureus,* 5 strains of *B. catarrhalis,* 38 strains of *H. influenzae,* and 111 strains of *P. aeruginosa* isolated from sputa at Chiang Mai Hospital in 1988 were tested and compared to those in Japan.

All the *S. pneumoniae* strains at Chiang Mai showed high sensitivity (MIC≦0.05 μg/ml) for ampicillin, while 8 (16%) of them ranged from 0.1 to 0.78 μg/ml in Japan. The rates of methicillin resistant *S. aureus* were 59% at Chiang Mai and 68% in Japan. Both of them had low sensitivity for other β-lactam antibiotics. However rifampicin (95% were less than 1.56 μg/ml) and vancomycin (98% were less than 3.13 μg/ml) demonstrated high activity for the Chiang Mai strains and it also exhibited high activity for the Japanese strains. Frequency of minocycline resistant *S. aureus* (MIC≧6.25 μg/ml) were 57% and 31% at Chiang Mai and in Japan respectively. On the other hand resistance rate of *S. aureus* to ofloxacin were higher in Japan (66%) than Chiang Mai (5%). Activity of β-lactam antibiotics for 5 *B. catarrhalis* strains of Chiang Mai Hospital seemed not to be so different from Japan. Their MICs ranged from 0.39 to 12.5 μg/ml for ampicillin, more than 6.25 μg/ml for cefazolin, from 0.78 to 6.25 μg/ml and from 0.025 to 3.13 μg/ml for cefmenoxime. Ampicillin resistance (MIC≧1.56 μg/ml) of *H. infiuenzae* were seen both Chiang Mai and Japan and the rate was about 20%. High resistance was also seen in the *P. aeruginosa* strains of Chiang Mai for gentamicin and tobramycin. They showed resistance to β-lactam antibiotics. Imipenem, however, were more potent to the Chiang Mai strains than the Japanese strains.

In conclusion, the clinical isolates from Chiang Mai Hospital have similar antibiotic resistance to those of Japan. Further investigation is needed to make clear the factors which affects the emergence of antimicrobial resistance in Chiang Mai area.

9 A DEVELOPING PATTERN OF *DIROFILARIA IMMITIS* LARVAE IN MOSQUTIOES

EIMAN MOHAMED ZYTOON, EIJI KONISHI AND TAKEO MATSUMURA
Department of Medical Zoology, Kobe University School of Medicine

Microfilaria of dog heartworm nematode, *Dirofilaria immitis* (Leidy), resides in the blood stream of the definitive host with no potentiality for further development until ingested by a blood-feeding mosquito. In susceptible mosquitoes, prelarvae initiate the developmental stages. Microfilariae (mf) migrate from the midgut to the Malpighian tubules, and initiate further developments after this period, to attain the infective stage.

The purpose of the present study was to determine the developing larvae of *D. immitis*

in *Aedes albopictus* (Miki and Oahu strains) and to explain the developing changes taking place within these mosquitoes. A dog, naturally infected with a heavy microfilarimic level of 55,000-60,000 mf/m*l* in peripheral blood was used as a donor in these experiments.

Parallel feeding experiments were carried out in adult female mosquitoes (Miki and Oahu strains) for 4-6 hrs and infected by allowing them to engorge through an artificial feeding technique for 5 min (in collaboration with Dr. H. Yamanishi).

The survival rate, measurement and distribution of developing larvae in 10 mosquitoes were recorded every day, in which there were some differences in the distribution and measurement in some developing larvae, compared with the previous studies (Ohishi, 1986).

The infection rate in Malpighian tubules of the first stage larvae on day 1 ranged between 97-100%. The rate decreased over time. The mean number on day 1 ranged between 9.3-10 and decreased gradually up to day 5.

It is of considerable interest, therefore, that the second stage larvae were observed from day 9-12, which is later than the normal appearance of this stage noted in previous studies. Not all the second stage larvae were detected in Malpighian tubules which is believed to be the normal place of the larvae development but mainly observed in the crushed thorax with the maximum infection rate of about 20% and with the mean number ranging between 1.8-2, which decreased gradually up to day 12.

The third stage filarial larvae were observed from day 9 in 10% of mosquitoes, the mean number being 1.1. The larvae were observed in the labium from day 10 in 10% of mosquitoes. The rate increased to 40% up to day 14, and then decreased again to 10% on day 21.

On the other hand, the mortality rate of infected mosquitoes was 5% until day 4, followed by no death up to day 14. The results so far obtained suggest that the internal organs of the infected mosquitoes may be damaged by the first stage larvae.

Further studies are undertaken to clarify the developing pattern of mf in mosquitoes in more detail, including morphological studies stimulated by these results.

10 PHYLOGENETIC RELATIONSHIPS AMONG SPECIES OF *DIROFILARIA* RAILLIET AND HENRY, 1911 (NEMATODA: FILARIOIDEA)

BAHARUDIN B. OMAR[1], THOMAS C. ORIHEL[2] AND TOZO KANDA[3]
Department of Parasitology and Medical Entmology, University Kebangsaan Malaysia[1],
Department of Tropical Medicine, Tulane Medical Center, U.S.A.[2] and
Department of Medical Zoology, St. Marianna University School of Medicine[3]

Relationships among the filarioids of the genus *Dirofilaria* are examined based on cladistic analysis of morphological data of the adult worms and the microfilariae. Primitive and derived states for fourteen characters of subfamily Dirofilariinae and eleven characters of *Dirofilaria* are proposed and the data analysed using the computer program PAUP (Phylogenetic Analysis Using Parsimony). Consensus cladograms are constructed and proposed as phylogenetic hypothesis. Three nested groups consisting of 1) *D. panamensis* and *D. macrodemos*; 2) *D. acutiuscula, D. subdermata, D. ursi, D. repens* and *D. lutrae*; 3) *D. magnilarvatum, D. immitis, D. striata* and *D. tenuis*; and two lineages with a single taxon

consisting of *D. cancrivori* and *D. corynodes* respectively emerge among the 13 species of *Dirofilaria*. Synapomorphic (shared-derived) features of the number of preanal papillae, esophagus, cuticular ornamentation, and shape of left spicule are identified among the species to lead to the above groupings. *D. macrodemos* and *D. panamensis* infecting the sloths are the sister groups for the other eleven species and are hypothesised as the most primitive. They have the pleisomorphic (primitive) features of less than three preanal papillae, a divided esophagus, the lamina of the left spicule being long and filamentous, and the presence of longitudinal ridges that are long, almost regularly spaced and seldom branched. This study also proposes that the division of *Dirofilaria* into subgenera *Dirofilaria* and *Nochtiella* is not valid. The absence of cuticular longitudinal ridges and the large size of the worm are homoplasies, and arrangements of caudal papillae are too variable even between specimens of the same species. Analysis of the Dirofilariinae show that *Foleyella, Pelecitus* and *Loaina* as distinct monophyletic lineage and most derived among the subfamily and the genera of Dirofilariinae as polyphyletic. The current hypothesis of relationships can be used to speculate on the patterns of dispersals and centers of origin among *Dirofilaria*.

11 STUDIES ON *IN VITRO* MAINTENANCE OF ADULT *DIPETALONEMA VITEAE* FOR A LONG PERIOD

JUN MAKI
Department of Parasitology, Kitasato University School of Medicine

Adult female *Dipetalonema viteae* from jirds were maintained in NI medium (a 1:1 mixture of NCTC 135 and Iscove's modified Dulbecco's Medium) with a gas phase of 5% CO_2-95% N_2 for several weeks (the gas and medium were renewed every 3-4 days) to assess objectively the physiological integrity and viability of the adult worms and microfilariae released in the medium. When the culture period of adult worms was less than three weeks, the following results were obtained. Adult females, which had ceased releasing microfilariae in culture, could resume this activity when transplanted into jirds, indicating that this reproductive capacity was not irretrievably lost during culture. Microfilariae released and then injected into ticks could develop to third-stage larvae, which were then inoculated into jirds and became adult worms producing microfilariae, indicative of the physiological normalcy of the microfilariae released in the medium (Weinstein, Paul P., Department of Biology, University of Notre Dame is a coworker and superviser of this research, financially supported with a grant from WHO).

12 BASIC STUDIES ON MONGOLIAN GERBILS AS THE SUSCEPTIBLE HOST TO FILARIAL INFECTION (3) Thy-1 ANTIGEN

MASUMI SHIMIZU[1], KAZUHIRO SHICHINOHE[1], MASAMICHI ISHIZAKI[2],
SETSUKO TSUKIDATE[3] AND KOICHIRO FUJITA[3]
Department of Experimental Animal Science[1], Department of Pathology[2],
Nippon Medical School and Department of Medical Zoology,
Faculty of Medicine, Tokyo Medical and Dental University[3]

The mongolian gerbil has been used in the field of parasitology, especially as the susceptible host to filarial infection. The gerbil is a very useful model of experimental filariasis, but there has been almost no reports about immunological characteristics of the mongolian gerbil, and it is nessesary clarify it. We carried out experiments to know that the Thy-1 antigen exists or not and it becomes a specific marker of T-lymphocyte or not, how about its distribution pattern in the tissue in the mongolian gerbil, in order to analyse immunological kinetics of the mongolian gerbil.

Materials and Methods:

1. Antibodies

Anti-rat thymocyte serum (ARTS) preparing from rabbits immunized by thymocytes of Wistar-Imamichi (WI) rats was used as polyclonal antibodies. Monoclonal Thy-1.1 (Thy-1.1m. Ab) or Thy-1.2 antibody was obtained from commercial base.

2. Absorption and Cytotoxic test

ARTS was absorbed with thymocytes or with cerebral homogenate of either the Wistar rat or the gerbil. Monoclonal Thy-1.1 antibody was absorbed with thymocyte or spleen cells obtained from the Wistar rat, the AKR mouse or the gerbil. The cytotoxic potency of absorbed ARTS and absorbed Thy-1.1m. Ab were determined by a complement dependent cytolytic system.

3. Immunohistological examination

Cryostat sections made from the thymus, lymph nodes, the spleen and the kidney were stained by the indirect immunofluorescence technique.

Result:

1. Cytotoxic test

The cytotoxic potency of ARTS was absorbed by the rat thymocytes and the brain, as well as the gerbil thymocytes and the brain. Thy-1.1m. Ab was absorbed by the thymocytes of the gerbil. But no absorption effect was observed in the spleen cells of the animal.

2. Immunohistological examination

Thy-1.1m. Ab showed strongly positive reaction in the gerbil thymus, but the response to Thy-1.2m.Ab was completely negative.

In the spleen and lymph nodes of the gerbil, a few ARTS positive cells were observed. But the other cells in the T-cell region were negative. Further, Thy-1.1m. Ab and ARTS showed the same immunofluorescence in the glomerular mesangial region of the gerbil kidney.

Summary:

The Thy-1.1 antigen existed in the thymus, the brain and the glomerular mesangium of the gerbil. Its distribution pattern in the tissue was similar to that of the rat.

13 BASIC STUDIES ON MONGOLIAN GERBILS AS THE SUSCEPTIBLE HOST TO FILARIAL INFECTION (4) COMPLEMENT

KAZUHIRO SHICHINOHE[1], MASUMI SHIMIZU[1], MASAMICHI ISHIZAKI[2],
SETSUKO TSUKIDATE[3] AND KOICHIRO FUJITA[3]
Department of Experimental Animal Science[1], Department of Pathology[2],
Nippon Medical School and Department of Medical Zoology,
Faculty of Medicine, Tokyo Medical and Dental University[3]

The mongolian gerbil is very useful as the susceptible host to filarial infection in the studies of parasitology. Recently, immune response of the gerbil as a host to filarial infection has been studied. But, it is unknown the detail immunological or serological character of the gerbil.

We examined distribution pattern in the tissue of Thy-1 antigen in gerbil. It appeared that Thy-1.1 antigen existed in glomerular mesangium of this animal. Then, Thy-1.1 antibody was injected into gerbil and we attempted to induce experimental gulomerular nephritis.

We prepared anti-rat thymocyte serum (ARTS) from rabbit immunized with rat thymocyte as polyclonal antibodies. Gerbils were injected intravenously with only ARTS as group 1. As group 2, guinea pig sera as complement was given after ARTS injection to girbils. Some girbils of 2 groups each were killed at various time intervals after the injection. At the time of sacrifice, the kidneys were taken from gerbils and examined pathological changes using light or electron microscopy.

In group 1, the kidneys showed almost no pathological change. But in group 2, they were induced mesangiolytic changes caused by ARTS. After 12 hours of ARTS administration, mesangiolytic changes appeared with a picture of infiltration by plasma components, fibrin exudation in their lumen and degeneration or necrosis of the mesangial cells. After 2 days, migration of the monocytes and neutrophils was observed in mesangial region. After 8 days, mitotic figures of the mesangial cells were seen.

Then, we examined complement activity of the gerbil by complement dependent hemolytic reaction. Sheep red blood cell (SRBC) was immunized to rabbit or gerbil and prepared SRBC antibody of each animal. SRBC was sensitized with each anti-sera and added sera of guinea pig, rat and gerbil respectively as complement. As a result of this experiment, gerbil sera showed no hemolytic activity to SRBC.

As complement of gerbil showed no cytotoxic activity, it is suggested that gerbil has deficiency of the factor of complements or that its activity is very low.

14 DISTRIBUTION PATTERN OF OUTPUT EGGS FROM SCHISTOSOMA HAEMATOBIA PATIENTS AND EVALUATION OF MASS TREATMENT BASED ON THE EGG COUNTS

KIYOSHI MAKIYA[1], YOSHIKI AOKI[2], MASAAKI SHIMADA[2] AND NGETHE MUHOHO[3]
Department of Medical Zoology, University of Occupational and Environmental Health[1],
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[2]
and Kenya Medical Research Institute[3]

Schistosomiasis control project has been continued since 1982 in Kwale district, Kenya. In order to evaluate the control measure more effectively, analysis was made on the distribution pattern of output eggs from the patients. The variance/mean ratio of output eggs was revealed significantly bigger than unity using Morisita's $I\delta$ index, meaning that the eggs were distributed not randomly but unevenly among the inhabitants.

The negative binomial fitted well not only the total population but also each age group, the reciprocal of parameter k (index of overdispersion) being far lower in age group of 10-19 and becoming higher with age. Considering together with the high egg density in 10-19 age group, this means that the majority of this generation is excreting a large number of eggs in the population.

It is said that, for a high degree of parasite aggregation (low k), the mean worm burden decreases much more rapidly than the prevalence. Comparison was made between the infection rate and the mean egg count in the detection of significant successive change. Egg count was transformed by log $(x+1)$ to stabilize the variance before processing data. As a result, significant change was detected far more effectively by the mean egg count than by the infection rate.

15 ULTRASONOGRAPHIC DIAGNOSIS OF SCHISTOSOMIASIS JAPONICUM IN LEYTE

HIROSHI OHMAE, YUJI IRIE AND KAZUO YASURAOKA
Department of Medical Biology, Tsukuba University School of Medicine

Ultrasonographic examination is very useful for the diagnosis of hepatosplenic schistosomiasis and has been used in Sudan to evaluate the therapeutic efficacy of praziquantel in schistosomiasis mansoni infection.

Ultrasonographic (US) examination was performed on 17 admitted patients diagnosed as hepatosplenic schistosomiasis japonica and 15 new infected outpatients, in Schistosomiasis Hospital, Palo, Leyte. Correlation between US pattern and liver function was studied.

Splenomegaly (Spleen index: $20\leqq$) was found in the US studies of all hepatosplenic patients. Concerning portal hypertention, enlargement in diameter of the splenic vein ($8\leqq$) was found in 13 cases. Collateral circulation was seen in 3 cases. The net work pattern or fish scale pattern was seen in 3 cases. The most frequent US pattern was hyperechoic bands

or brilliant echogenicity. Liver cirrhosis was found in 3 cases. Hypertrophy of the left lobe was seen in 6 cases and hypertrophy of the right lobe was seen in 3 cases. In the new outpatients with egg-positives by KATO-KATZ method, splenomegaly which could not be diagnosed by palpitation was found in 4 cases.

No corelation between US pattern and GOT, GPT and ALP was found. Increase of TBA (total biliary acid) and decrease of Ch-E were found in the patients with net work pattern and LC pattern. The patients were treated with praziquantel, after which, ultrasonographic examination and liver function tests are being performed every three months.

16 APPLICATION OF MULTI SPOT ENZYME IMMUNO-ASSAY TO SERODIAGNOSIS OF TREMATODIASIS

MAKOTO ITOH AND SHIGEFUSA SATO
Department of Medical Zoology, Nagoya City University Medical School

Serodiagnosis utilizing enzyme conjugated antibodies has been widely used for diagnosis of parasitosis. Enzyme linked immunosorbent assay (ELISA) is useful for a mass-survey of parasitic diseases, since many samples can be examined at a time with high sensitivity. Immunoblotting has also been used for serodiagnosis with much more specificity. Both methods, however, are not so convenient to a examination of a few samples as ordinarily requested.

In this study, we applied 'Multi Spot Enzyme Immuno-Assay (MSEIA)' for serodiagnosis of trematodiasis. A nitrocellulose strip was spotted with antigens of trematodes such as *P. miyazakii, P. westermani, Fasciola* sp., *Clonorchis sinensis* and *Schistosoma japonicum.* After blocking the strip with 1% casein solution, it was dried up and kept at 4°C until use. For a examination, the strip was incubated in patient sera (×200) at 37°C for 1 hour and washed three times. Then it was incubated in peroxidase conjugated anti-human IgG (×500) at 37°C for 1 hour. 4-chloro-1-naphthol was used as a substrate.

Positive case was easily observed as a bluish purple dot. In all trematodiasis cases examined, antibodies to antigens of infecting parasite were dominantly detected as a spot with the naked eyes. Although cross-reactions with other trematode antigens were observed, it did not disturb the diagnosis.

Densities of the spots were measured as the absorbance at 560 nm with a densitometer and values were compared with those obtained with ELISA. Similar sensitivity and specificity were observed.

From these observations and an advantage that the antigens bound to a nitrocellulose strip are stable up to 6 months at 4°C, it was concluded that this method is useful for serodiagnosis of the trematodiasis as a ordinary examination.

17 CHANGE OF CYTOKINE AND NATURAL KILLAR ACTIVITIES IN *ASCARIS SUUM* INFECTED MICE

TAO DAO RYUZO SANO, MINORU HIGASA, HIKOSHIRO MIYAMOTO,
KAZUHIKO YAMADA, TOSHINORI KOMATSU AND SOHEI SHINKA
Department of Immunology and Medical Zoology, Hyogo College of Medicine

In order to analyze the cellular mechanism of immunosuppression during acute infection by *Ascaris suum,* cytokine and natural killer activities in *A. suum* infected mice were investigated.

Ten days after infection with 10^3 or 10^4 embryonated *A. suum* eggs, Concanavalin A (ConA)-induced interleukin 2 (IL-2) production by splenocytes were reduced. At the same time, NK activity of splenocytes and the levels of serum interferon induced by Poly I•Poly C were also reduced. On the other hand, culture supernatants of peritoneal adherent cells from *A. suum* infected mice showed significantly high interleukin 1 activity which reached peak levels on day 15 following lipopolysaccharide stimulation. Increase in the number of macrophages in the peritoneal cavity were also observed in this period. Furthermore, the culture supernatants of peritoneal adherent cells from 10-days *A. suum* infected mice exhibited a suppressive effect on both IL-2 production by normal splenocytes and IL-2 activity assayed using CTLL-2 cells. Dialysis and molecular filtration studies indicated that the factor with molecular weight below 10,000 related to the suppressive effect. There was, however, no significant increase of prostaglandin E_2 in the culture supernatants.

These results suggest, activated macrophages may play an important part in the immunosuppression by producing a soluble suppressive factor during acute infection by *A. suum.*

18 MONITORING OF MALARIA VECTORS AFTER APPLICATION OF SOME CONTROL METHODS

VANIDA KERDPIBULE, TOZO KANDA, THONGCHAI DEESIN AND SOMJAI LEEMINGSAWAT
Department of Medical Zoology, St. Marianna University School of Medicine

There are various methods to assess the effectivity of control methods of malaria. The present study adopted the investigation on nulliparity of newly emerged adult vectors collected by cattle bait. A control method of malaria by using an insect growth regulator (IGR), 2-[1-methyl-2(4-phenoxyphenoxy)ethoxy] pyridine was applied into two running streams and assessed its effect in this study. In order to examine the activity of the compound, the investigation on nulliparity was performed on the two principal vectors following to the three applications, being the first in February 1987, the second in November 1987 and the third in March 1988. The decreasing of nulliparity was evidently proved in this surveillance and maximum rate of nulliparous to be around 50 to 60%.

The effect of the compound with the concentration of 5 ppb applied in stream was

compared with the effect of predator, *Poecilia reticulata.* Study sites were selected at three hamlets, Wat Sapanhin with population of 200 by applying IGR, Wat Sabon with population of about 500 by predator and Wat Thamprapothisat with population of 200 for the control site. These hamlets are along different streams respectively.

Though the study is still going on, the number of malaria case was still remaining without decreasing at seven to six, whereas the decreasing of nulliparous rates of both *Anopheles minimus* and *An. maculatus* was evident after application of the compound in Wat Sapanhin as well as Wat Sabon, applied the predator, in comparison with the control.

19 RESPONSE AND MONITORING OF THE VECTORS OF JAPANESE ENCEPHALITIS BY SOUND TRAP AFTER APPLICATION OF TWO CONTROL METHODS

SOMJAI LEEMINGSAWAT, TOZO KANDA, VANIDA KERDPIBULE AND SUPATRA THONGRUNGKIAT
Department of Medical Zoology, St. Marianna University School of Medicine

Relating to the endemicity of Japanese encephalitis (JE) in the Metropolitic area around Bangkok, the present study was performed to monitor the prevalences of the vector species of mosquitoes. Four study sites were selected at Bang Na at southeast of Bangkok, Bang Phli located at east of Bangkok in Samut Prakarn Province; Bang Len located at 100 km west from Bangkok in Nakorn Pathom Province, and 2 km north of Supannburi in Supannburi Province. All of these are surrounded by swampy rice field, breeding site of the vectors. Sound traps emitting modified wing-sound of various frequencies combined with dry ice and one hamster or chicken in cage were variously adopted for monitoring of mosquito prevalence and for examining their response to various frequencies. Though the study is still on going, the host preference of the vectors was tested. Pig was the highest, but hamster was the lowest in the number of mosquitoes trapped. Responses of the vectors against modified wing-sound with various frequencies were different by study sites within same species and prevalence of *Culex tritaeniorhynchus* were different by the localities, being 530 Hz was dominant in Supannburi whereas three of different frequencies, being 350, 550 and 800 Hz were dominant at Bang Len, and *Cx. gelidus* was more prevalent than *Cx. tritaeniorhynchus* at two study sites in Samut Prakan. A comparative study was performed between two different control methods, such as sound trap in Supannburi and application of insect growth regulator (IGR), 2-[1-methyl-2(4-phenoxyphenoxy)ethoxy] pyridine in Bang Len. Application of consecutive trapping by sound traps for 21 days proved significant decrease of parous rate, but no decrease in whole number. Oppositely the application of the IGR proved decrease of both population number and nulliparous rate. So that these two control methods should be combined for the vector control for their earlier effective needs in the endemic area of JE.

20 INSECTICIDES FOR USE WITH WIDE-MESH NET CURTAIN AGAINST MALARIA VECTORS

TAKESHI KURIHARA
Department of Medical Zoology, Teikyo University School of Medicine

An insecticide-treated curtain of wide-mesh net has been developed for vector mosquito control as an alternative to sprying of residual insecticide. An earlier study found that impregnation of a wide-mesh net with certain insecticides influences mosquito behaviour, both by inhibiting the insect's contact with the net and, in other instances, by killing it. Contact bioassay is insufficient to determine the efficacy of an adulticide, so a cage-test method was developed to evaluate the net curtain treated with adulticides. The apparatus was composed of two wire-cages ($30 \times 30 \times 30$ cm each), placed side by side, one serving as a cage from which avid mosquitoes were released and the other as a mouse-baited cage. Each cage had two round holes and passageways between the cages were interposed with a wide mesh netting; mesh size was 10×10 mm and the thickness of the nylon fibers was 0.4 mm. Mosquitoes could go through this net passage to feed on the bait. The net was impregnated with one of three insecticides; phenothrin, cyphenothrin or permethrin. The dosage applied was 0.16 or 0.64 g/m^2. Female *An. stephensi,* 3 to 5 days after emergence, were introduced into the release cage and recovered the following morning. Observations were carried out 4, 10 and 20 weeks after impregnation of the net. Mosquito mortality at 20th week was highest in the experiment with permethrin and phenothrin was second most effective. All three insecticides inhibited entrance to the bait cage during the four weeks following impregnation but this was not prominent thereafter.

21 SUSCEPTIBILITY OF CHEMOSTERILIZED *MANSONIA UNIFORMIS* TO *BRUGIA MALAYI* INFECTION

VANIDA KERDPIBULE, THONGCHAI DEESIN AND SOMJAI LEEMINGSAWAT
Faculty of Tropical Medicine, Mahidol University, Thailand

Development of insecticide resistance is common among mosquito vectors, several alternative control methods are introduced in the mosquito control programme including sterile male release. A laboratory experiment on chemosterilization of *Mansonia uniformis* was performed to study the possibility of sterile male release technique for filariasis vector control. In particular, this technique required a large number of males, in which some females could be contaminated. These females when released could contribute the transmission of disease. Hence, their susceptibility to *Brugia malayi* infection was determined in laboratory. *Ma. uniformis* were sterilized by a chemosterilant, p,p,-bis(1-aziridinyl)-N-methylphosphinothioic amide (Bisazir) at pupal stage. The pupae were immersed in 0.25-2.0% solution of Bisazir for one hour, and studied the sterity of the emerged adults. Approximately 91-92% sterility was induced in males when mated with normal, females and it was 61.9% when

treated females mated with normal. The complete sterilization was evident in mosquitoes treated with 0.5% or higher concentrations of Bisazir. The sterile females infected with *Brugia malayi* by feeding on cat were examined for filarial infection 10 days later. It revealed that the sterile mosquitoes were less susceptible to *B. malayi* infection than those of the control group ($p<0.05$). The infection rate became less in the mosquitoes which had been exposed to higher concentrations of Bisazir, i.e. 18.2% in the mosquitoes treated with 2.0% while it was 69.6% in those treated with 0.25%. This suggested that it might be possible to control of *Ma. uniformis* by releasing sterile males which had been exposed to 0.5% Bisazir since it could induce complete sterility in nature. If some females contaminated in releasing, less than half of them (47.7%) could transmit the disease. However, if they were exposed to higher concentration of chemosterilant more reduction in transmission would occur.

22 EFFECT OF REPEATED INFECTION TO MAINTAIN IMMUNITY TO *PLASMODIUM YOELII*

WEN-JING AI AND KENYICHI YANO
Department of Protozoology, Institute for Microbial Diseases, Osaka University

Plasmodium yoelii (strain 17XL) and ddY mice were used for experiments. Five week-old mice were intraperitoneally (ip.) inoculated with 1×10^4 *P. yoelii*-infected RBC (I-RBC) and, from 4 days later, treated with sulfadimethoxine (0.5 mg/m*l*) in drinking water for 7 days (I-Immune mice). Some of them were ip. infected with 1×10^6 I-RBC once (II-Immune mice) and twice (III-Immune mice) at 4 weeks intervals. These 3 groups and control mice were ip. infected with 1×10^6, 1×10^7 and 1×10^8 I-RBC and examined their parasitemia in thin smears of tail bloods on 2, 4, 6, 8 and 10 days thereafter. Inapparent infection rates were caliculated as complete immunity to *P. yoelii* malaria, that any infected-RBC were not recongnized in thin smears.

With the challenge infection of 1×10^6 I-RBC, the inapparent infection rates were 7%, 44% and 18% in 3, 4 and 8 weeks of I-Immune mice, 100%, 100% and 7% in 2, 4 and 8 weeks of II-Immune mice and 100%, 100% and 83% in 2, 4 and 8 weeks of III-Immune mice, respectively. With the challenge infection of 1×10^7 I-RBC, the inapparent infection rates were 0% and 100% in 3 and 4 weeks of I-Immune mice, 38% and 56% in 4 and 8 weeks of II-Immune mice and 91% and 73% in 2 and 4 weeks of III-Immune mice, respectively. With the challenge infection of 1×10^8 I-RBC, the inapparent infection rates were 0% in both 3 and 8 weeks of I-Immune mice, 13% and 8% in 4 and 8 weeks of II-Immune mice and 94%, 67% and 30% in 2, 4 and 8 weeks of III-Immune mice, respectively. All immune mice were survived except each one in the challenge of 1×10^6 and 1×10^8 I-RBC in 3 weeks of I-Immune mice against all death of control mice.

Repeated infection increased immunity to *P. yoelii* malaria and III-Immune mice showed the most consistent inapparent infections to high dose challenge infection such as 1×10^8 I-RBC. The immunity of III-Immune mice became weaker in 8 weeks.

23 RELATIONSHIP BETWEEN CLINICAL SEVERITY AND THE ADHERENCE OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES TO C32 MELANOMA CELLS

SHUSUKE NAKAZAWA[1], HIROJI KANBARA[1], TOSHIHIDE FUKUMA[1], HARUKI UEMURA[1], CHIRASAK KHAMBOONRUANG[2] AND CHARIN CHESDAPAN[2]
Department of Protozoology, Institute of Tropical Medicine, Nagasaki University[1] and Department of Parasitology, Faculty of Medicine, Chiang Mai University[2]

Binding assays of infected erythrocytes with *Plasmodium falciparum* (P.f.) to amelanotic melanoma cells C32 were conducted in the Northern Thailand. Bloods were taken in heparin solution from the patients with various clinical manifestations at the hospital and malaria clinic in Mae Sod and Mae Hong Son. Bloods samples were transported to the Faculty of Medicine, Chiang Mai University within 24 hours after bleeding. They were washed with RPMI 1640, suspended in RPMI 1640 supplemented with 10% human AB type serum and incubated in a candle jar for 28 hours. Then the erythrocytes parasitized with mature form of P.f. were concentrated by gelatin floatation methods, and suspended in RPMI 1640 supplemented with 20% human AB type serum. The suspensions were inoculated into wells in the culture plate each well of which melanoma cells had been previously cultured on Thermanox cover slip. The plate was incubated in a 5% CO_2 incubator for 90 minutes. At the end of incubation cover slips were washed to remove non-adherent erythrocytes. After being dried fixed and stained, adherent erythrocytes to melanoma cell were counted under oil immersion. On the other hand, the effect of patient plasma on the adherence of his own infected erythrocytes to melanoma cells was investigated. The same method that was used to obtained numbers of adherent erythrocytes was employed except patient plasma was used in place of human AB type serum. IFA was also done to investigate whether the titer of IFA had correlation with the effect of patient plasma on the adherence of infected erythrocytes. Following results were obtained.

1. There was no relationship between the capacity of adherence of parasite and clinical severity.
2. Most patients plasma showed inhibitory effect on the adherence and there was no relationship between the level of inhibitory effect of patients plasma and clinical severity.
3. There were no relationship between the numbers of adherent erythrocyte and parasitemia.
4. There were not clear correlation between the level of inhibitory effect of patients plasma and IFA titers.

These results suggest that the in vitro binding assay using melanoma cell may not be proper to study the pathophysiology of cerebral malaria. The *in vitro* model which reflect clinial severty remains to be developed.

24 HEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN MALARIA PATIENTS

ISAO EBISAWA[1], HIROSHI OHARA[2] AND KIYOKATSU TANABE[3]
Keihin Railway Kawasaki Clinic[1], Overseas Cooperation Department,
National Medical Center[2] and Department of Medical Zoology,
Faculty of Medicine, Kagoshima University[3]

The extent of laboratory abnormalities caused by infection with three different species of malaria parasites, *P. falciparum* (Pf), *P. vivax* (Pv) and *P. ovale* (Po) was investigated. Laboratory data of 413 (170 Pf, 220 Pv and 23 Po malaria) patients, most of whom were aged between 21 to 40 years, were analyzed. The mean values, variances and the incidence of abnormal laboratory data were analyzed for 3 groups of patients infected by different malaria parsites. The significant difference of values between two groups was assessed at 1% level. Only the mean values of laboratory data are mentioned here. 1) The maximum parasite count per μl of blood before treatment was high in the order of Pf>Pv>Po. 2) The minimum red blood cell count and hemoglobin concentration was small in the order of the patients infected by Pf<Pv<Po. 3) The maximum leucocyte count before treatment was almost equal in three groups of patients. Leucopenia was seen in 17.3% of the patients. 4) The maximum lactic dehydrogenase LDH, total bilirubin, blood urea nitrogen BUN, creatinine, alanine aminotransferase SGPT and asparate aminotransferase SGOT values were high in the order of the patients infected by Pf>Pv≒Po. 5) The minimum values of serum total protein was low in the order of patients infected by Pf<Pv≒Po. 6) The minimum thrombocyte counts and serum cholesterol values were not different in patients infected by Pf, Pv and Po.

25 DNA DIAGNOSIS OF FALCIPARUM MALARIA

KAZUYO WATANABE[1], SATORU NAKAGAMI[2], TATSURO KAWASOE[2], AKIO YAMANE[2],
J.Y. KIMURA[3], AKIRA ISHII[3] AND YUSUKE WATAYA[1]
Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Okayama University[1], Central Research Laboratories, Wakunaga Pharmaceutical Co., Ltd.[2]
and Department of Parasitology, Okayama University Medical School[3]

Techniques utilizing DNA hybridization probes for the rapid detection of *Plasmodium falciparum* had been reported. However, these methods had many problems about the sensitivity and the stability of the probes. We have developed two types of plasmid-born DNA probes: One represents the species-specific 21-base long repetitive DNA sequence (5'-AGGTCTTAACTTGACTAACAT-3') chemically synthesized and ligated to generate five repeats. The other represents the junction DNA sequence of the DHFR (dihydrofolate reductase)-TS (thymidylate synthase) gene, which may be more conserved among various strains of *P. falciparum* than the repetitive sequence. When the junction probe was used, the target sequence was amplified by PCR (polymerase chain reaction) to increase the sensitiv-

ity. We used the Universal Probe Sysjem {Yamane *et al.*(1988): Nucleic Acids Res., Symposium series, 19, 93-95}. This system consists of two probes; a primary probe prepared from a chimeric phage-plasmid vector (pUCf1) containing a sequence complementary to a target, and a biotin-labeled secondary probe complementary to a portion of the primary probe, which is detected by the BCIP/NBT method. This system has a high sensitivity to the target DNA because the secondary probe is labeled with many biotin molecules.

With the 21-bp repetitive probe we can detect 100 pg DNA (1,000 parasites/50 μl blood) of *P. falciparum,* and with the DHFR-TS junction probe 10 pg (100 parasites/50 μl blood) if PCR is combined. These two probes have the sensitivity equal to that of the microscopical examination (250-1,000 parasites/50 μl blood).

26 A STUDY ON SEROLOGICAL MALARIA MONITORING IN A HYPOENDEMIC AMAZONIAN SETTLEMENT

MAMORU SUZUKI[1], MILTON M. HIDA[2], SHIGEYUKI KANO[1], KUMIKO SATO[3], HIROSHI YANAGISAWA[4], EIICHI NAKANO[4], LYNDON THOMAS[5], EIICHI SHIBATA[6], TAKESHI HONDA[6] AND YUJI IKUTA[6]
Department of Parasitology, Gunma University School of Medicine[1], UNESP Botucatu, São Paulo[2], College of Medical Care and Technology, Gunma University[3], Department of Public Health, Kyorin University School of Medicine[4], NEWI, Wales[5] and Beneficia Nipo-Brasileira da Amazônia[6]

Tomé-Açu is an equatorial Amazonian village developed in 1929 by Japanese settlers. Aroung 1935 and early half of 1960's malaria epidemics occurred there involving majority of the inhabitants. However, after the second episode, malaria has been well controlled with occasional incidental small number of cases. The recent gold rush occurred along Amazon basin has caused sharp increase of malaria cases in the communities around Tomé-Açu, and this endangers the controlled situation. Parasitological and serological surveys were undertaken in 1976, 1988 and also in 1989 August. In 1986 and 1987, both in August, small sized preliminary studies were conducted. The inhabitants in Tomé-Açu consisted of two distinct populations. Japanese settlers were permanent residents while majority of Brazilians were migrant labourers and their families hired by the farmers in the harvesting time. Both people did not manifest parasitemia by microscopic examination in any year studied until 1988. In 1989, one Japanese and one Brazilian developed malaria when our survey was undertaken. Generally, in such a hypoendemic area, features of malaria epidemiology is not available by means of parasite examination, and this was true in Tomé-Açu. However, when collected sera were studied by means of malaria indirect fluorescent antibody test, epidemiological pictures of both groups of inhabitants were closed up. In the study worked in 1988, both frequency distribution curve of titers and age positivity rate gave endemic patterns in the migrant labourers, while the pattern was not seen in Japanese settlers. In the studies worked in 1989, pattern suggesting the endemic malaria was not shown in both groups of inhabitants. Thus it was suggested that endemic malaria did not occur among Japanese settlers but the endemicity was seen among migrant labourers in some year. In 1989, labourers hired in a

pasture not very far from Tomé-Açu was studied. Again, no parasite was recorded, however, serological curve clearly showed endemic pattern of the studied people. These results were displayed in the map on a lap-top type computer screen using a soft system prepared by us. The seroepidemiological study and application of computer system will provide a new method for detailed malaria monitoring particularly suitable to hypoendemic area.

27 A SEROEPIDEMIOLOGICAL STUDY IN THE ESTIMATION OF POTENTIAL DANGER OF MALARIA OUTBREAK IN HYPOENDEMIC AREAS IN SUDAN

SHIGEYUKI KANO[1], SALAH HASSAN El SAFI[2], FAKHR ELDIN M. OMER[2],
KUMIKO SATO[3], AHMED AYOUB El GADDAL[2] AND MAMORU SUZUKI[1]
Department of Parasitology, Gunma University School of Medicine[1],
Ministry of Health, Blue Nile Health Project[2] and
College of Medical Care and Technology, Gunma University[3]

When a malaria control campaign comes to an advanced stage, the treated community becomes hypoendemic. On such occasions, the latent foci of parasites could endanger the population whose immunity tends to decrease. Microscopic observation of blood smears can miss lower parasitemia than the microscopic thresholds. A suitable method to define latent foci is required. Through the studies conducted in the Sudan Gezira in December 1987 and in January 1989, it was affirmed that the serological survey was useful to foresee future malaria outbreaks which might occur triggered by some events. In the present study, two low endemic villages, Sennar and Mobi were compared. Population movement was minor if any in both villages. In 1987, 80 school children were examined in Sennar and 5 children with parasitemia were detected. While in Mobi, none out of 46 examinees manifested parasitemia. Thus, by microscopic observation, the clear answer for the risk was not given by the parasite rate. However, by a comparative serological study between the two groups, the risk score was higher in Sennar than in Mobi (Sennar; 42 manifested titer at 1:64 and 14 showed 1:256, Mobi; 16 manifested titer at 64 and only 2 showed 1:256). The latent parasites or recent past infections were thought to be reflected in the high titers. In August, 1988, a heavy rainfall in the Sudan Gezira caused a flood covering both places, and this was thought to work as the trigger of the malaria outbreak. Therefore, in January 1989, re-examination was done in Sennar and in Mobi. Boosting of malaria cases was particularly seen in Sennar. Thirty four examinees out of 72 manifested parasitemia; 85% of the examined people were studied also in the survey in 1987. While in Mobi, only 7 out of 90 examinees showed parasitemia. In the study conducted in 1987, both Sennar and Mobi were designated as hypoendemic area by the microscopic examination. Malaria ABC-ELISA revealed higher malaria titers in Sennar than in Mobi, which was the indicator for the higher risk of future malaria in Sennar than in Mobi. This was proved after the flood by the survey in 1989.

28 INVESTIGATIONS ON GUINEA WORM DISEASE IN ANAMBRA STATE, NIGERIA

HIROSHI OHARA
Department of International Cooperation, National Medical Center Hospital

Investigations on guinea worm disease and treatment of the cases were conducted at Abakariki in Anambra State, Nigeria. The occupational items of 63 patients who were treated were agriculture (76%), students (19%) and others, and the average age was 21 years old. Each case was treated with oral admnistration of albendazole (400 mg) and observed until the worm was excreted. The average period between administration of the drug and complete excretion was 5 days followed by recovery of ulcerative skin lesion.

Investigation on quality of drinking water was carried out on 5 groups consisting of pond, shallow well, deep borehole, river and tap waters. The results revealed that contamination was severe in pond, shallow well and river waters, and many cyclops which were intermediate host of *Dracunculus medinensis* were found. This fact suggested that the inhabitants were easily infected with guinea worm. Investigation on primary school revealed that many students were absent from school due to guinea worm disease (16% of total absence), suggesting that this disease were exerting much influence both socially and economically, for instance, on school attendance and agricultural production.

Guinea worm disease is related closely to water sanitation and effective control of this disease is extremely important in this country. At present construction of deep borehole is proceeded. However, it is also important to promote, at the same time, health education among the inhabitants.

29 STUDY OF MICROFILARIAE FROM HUMAN BLOOD IN JABOATÃO, PERUNAMBUCO, BRAZIL

SACHIO MIURA[1, 2], SEIKI TATENO[1], ELIZABETH MALAGUEÑO[1] AND DIVANE A. ALBUQUERZUE[3]
Laboratorio de Imunopatologia Prof. Keizo Asami[1], Department of Parasitology,
School of Medicine Keio University[2] and Centro de Pesquisas Aggeu Magalhães FIOCRUZ[3]

Identification and description of the filarial species infecting man have usually been based on morphological features of the microfilariae. They have been studied with different taxonomic criteria, such as (1) the presence or absence of a sheath; (2) body length and width; (3) the distance of various organs-systems bihind the head expressed as a percentage of body length; (4) the somatic nucleation characteristics (discrete or smudgy and overlapping); (5) the appearance of the tail; (6) the presence or absence of the inner-body. Our studies have been done in Jaboatão Recife from Nobember 1987 to October 1988. The initial purpose of the present study was the collection of antigen for preparation of monoclonal antibody. Unfortunately most of our samples were air-dried thick blood films and stained with Giemsa; thus, these specimens were subject to morphological studies. They were obtained from people of the Jaboatão in Recife an endemic area of filariasis. In 1955 G.

Carvalho has described three morphological variants 'typica', 'type Y' and 'type Z' of *Wuchereria bancrofti* microfilariae in blood films from Recife in Brazil. Recently, J.F. Scharcher and Geddawi (1969) have rediscovered and comfirmed of Carvalho's type y and reported a new brazilian species *W. lewisi.*

Our morphological studies indicated that microfilariae showed various types. Most of the microfilariae were seathed but they have five variants. Group I of these microfilariae are very similar to the Carvalho's type Z concerning the somatic nucleation, intermediate width, average of total length, average distance between head and nerve ring, striated cuticula and an evident innerbody. Group II to IV of microfilariae have smudgy somatic nucleations; accordingly they seemed similar to the Carvalho's Y type but details about ratio of cephalic space length/width, intermediated width, average of total length were quite different and we have not confirmed the typical Carvalho's Y. Especially group II showed a very peculiar stumpy form. Group V appeared similar to the typical form of microfilariae of *W. bancrofti.* But we have not confirmed *W. lewisi* which has been described by J.F. Schacher (1969).

On the other hand, unseathed group had three variants and these were named Group VI, VII and VIII. Group VI of the microfilariae had fine transverse striations on the cuticle. The ratio of CSL:CSW was 1.23. Concerning this stumpy form, somatic nucleation is discrete. An innerbody was not evident in any of the microfilariae examined. Some of them might be similar to *Microfilaria bolivarensis* which has been described by G. Godoy, T.C. Orihel and G. S. Volcan (1980). But the location of key landmarks expressed in micrometer were a little different. Other two types of group VII and group VIII were slender and shorter than other groups. The microfilariae of group VII showed the nuclear column which dose not extend into the tip of the delicately tapered thin pointed tail; therefore they seemed to be *Mansonella ozzardi.* Group VIII showed nuclear column extending into the tip of the thick blunt tail which seemed compatible with *Mansonella perstans.*

In this endemic area, the possibility was shown that there are many kind of species of microfilariae involved in human infection. Thus identification of microfilariae are urgently needed for improvements of general public health of this area.

30 CONTROL OF SUBPERIODIC BANCROFTIAN FILARIASIS IN SAMOA BY SINGLE-DOSE MASS TREATMENT WITH DIETHYLCARBAMAZINE

EISAKU KIMURA
Department of Parasitology, Aichi Medical University

The filariasis control programme in Samoa, where subperiodic bancroftian filariasis is endemic, was inaugurated in 1975 when the first mass drug administration (MDA1) was carried out with 18 doses of diethylcarbamazine (DEC) at 5-6 mg/kg of body weight. The MDA1 succeeded in lowering the prevalence rate of microfilaraemia from about 20% to 2%. The second mass treatment (MDA2), which was conducted in 1971 with 12 doses at 6 mg/kg, further reduced the prevalence to 0.2%. However, filariasis had never been eliminated from this small island country, but increased gradually and steadily to about 5% level by 1979. Since 1981, a mass treatment with DEC at a single dose of 6 mg/kg has been adopted in

Samoa as a national strategy to control filariasis. Mass drug administrations with the new treatment scheme were carried out in 1982 (MDA3), 1983 (MDA4) and 1986 (MDA5). Before each treatment, more than 90% of the national total population (156,000-160,000) were registered individually by the Samoan Filariais Control Project, and 90-92% of those registered were treated. The evaluation blood surveys were conducted in 1981/82 (pre-MDA3), 1982/83 (post-MDA3), 1983/84 (post-MDA4) and 1987/88 (post-MDA5), each time on about 10,000 subjects using 60 mm^3 of finger-prick blood.

Before MDAs, the prevalence rate of microfilaraemia was 5.3% (male: 7.4%, female: 3.1%), which was reduced gradually to 4.2, 2.8 and to 2.4% by the three MDAs. The potential of transmission, which is the estimated mosquito infection rate in percentage when a population (including the microfilaria positives and negatives) was exposed evenly to mosquito bites, reduced from pre-treatment level of 2.18% to 0.67% after MDAs.

The results suggest that subperiodic bancroftian filariasis may be controlled by means of a single-dose mass treatment given at intervals of several years.

The study has been carried out in cooperation with the Government of Samoa, WHO, Japan Overseas Cooperation Volunteers and American Peace Corps.

31 PREVALENCES OF SERUM ANTIBODIES TO SPOTTED FEVER RICKETTIAE AMONG INHABITANTS IN SOUTHEAST ASIA

NOBUHIRO TAKADA[1], WEN-HSIUNG HUANG[2], YASUHIRO YANO[1] AND HIROMICHI IWASAKI[3]
Department of Immunology and Parasitology, Fukui Medical School[1],
Department of Parasitology, Cheng Kung University, Taiwan[2] and
First Department of Internal Medicine, Fukui Medical School[3]

Outbreaks of Japanese spotted fever which had been reported in 1984 first in Japan have increased over 60 cases for the past three years. According to our sero-survey by immunoperoxidase test (IP) using a spotted fever group rickettia isolated from a patient (named *R. japonica* by Dr. Uchida), the prevalences of antibody titer over 1:80 were noticeable (6-11%) among each of inhabitants in the endemic area or loggers as a high-risk group, while significantly low among inhabitants in the northern Japan.

In the present study, the prevalence in Southeast Asia was examined by IP using 3 antigens, *R. japonica* (Rj), *R. sibirica* (Rs) and TT-118 (TT) from Thai tick. Out of 113 outpatients in a hospital in Tainan City, Taiwan, high titers over 1:80 were shown in 3.5% for Rj, 4.4% for Rs and TT. Out of 122 rural inhabitants in Mae Chan City, North Thailand, the same titers were 9.0% for Rj, 10.7% for Rs and 21.3% for TT, respectively. In both groups examined, the positive titer was higher in male than in female, but not significantly different by ages. In Taiwan, 66.7% of all 18 cases titrated over 1:20 for any antigens was cross-reactive for 3 antigens. In Thailand, 31.1% of 45 cases titrated was reactive for TT alone, and each cross rate of TT with Rj and Rs was 40.0% and 37.8%. Additionally, reactivity for *R. typhi* was shown in about 30% of Taiwan and 3% of Thailand, but not significantly cross-reactive for spotted fever antigens.

In conclusion, it seems that spotted fever may be widely distributed in Southeast Asia and

associated with a certain difference between each of tick fauna in palearctic and oriental regions of zoogeography.

32 YELLOW FEVER IN GHANA

WILLIAM KWABENA AMPOFO[1, 3], TAKAO YOSHI[2], J.A.A. MINGLE[3] AND AKIRA IGARASHI[1]
Department of Virology, Institute of Tropical Medicine, Nagasaki University[1],
Department of Viral Diagnosis, National Institute of Health of Japan, Tokyo[2]
and Noguchi Memorial Institute, Chana[3]

Yellow fever (YF) is an endemo-epidemic acute infectious disease caused by a flavivirus (Bres, 1984). This arthropod-borne zoonotic and human viral disease is transmitted among monkeys, or between monkeys and humans, by certain species of mosquitoes, such as *Aedes africanus* and *Ae. simpsoni,* whilst *Ae. aegypti* is responsible for the transmission among humans in West Africa. In its most serious form, YF presents as hepato-renal dysfunction which proves fatal in the vast majority after 10 days. Ghana is located within the YF endemic zone of the African tropics and the period of 1969-1970 recorded 319 cases with 79 deaths (25% mortality); 1977-1979, 823 cases, 193 deaths (24%); and in 1983, there were 372 cases with 201 deaths (54%). Since then, no YF epidemics are known to have occurred, although an outbreak in a neighbor country Nigeria claimed over 1,000 lives in 1986.

Studies on YF in Ghana date back to the work by Noguchi, Stokes and Mahaffy, that led to the isolation of the Ashibi strain which was subsequently used in the production of the YF 17D vaccine. Various reports by Fabiyi (1969), Minami (1974), and Agadzi *et al.* (1984) confirm the continued circulation of the YF virus in Ghanaian population. Although entire nucleotide sequences of this Ashibi-17D parent-progeny paired strains of YF virus have recently been analyzed showing multiple amino acid replacements over the entire genome, especially in the envelope glycoprotein (E) region, many aspects of YF epidemiology and clinical virology still remain to be solved.

Due to the devastating potential of YF as shown by the past epidemics, there is the need for reliable rapid detection of the disease for appropriate containment measures. To support clinical diagnosis on YF in Ghana, especially to differentiate YF from viral hepatitis which are also common viral infections in Ghana, the Virology Unit of Noguchi Memorial Institute in Accra has been trying to establish rapid laboratory diagnostic tests on YF. So far, the sera from 2 YF-suspected cases in 1988 showed positive reaction of anti-IgM antibodies by the indirect immunofluorescence test using YF virus-infected Vero cells. Although this test is relatively easy to perform, it provides the answer after the antibody production and requires to be quantitated by introduction of other tests, such as IgM-capture ELISA.

Another future goal is to develop more rapid diagnostic tests to detect viral genome or antigen in the patient's specimens by the introduction of PCR (polymerase chain reaction) or antigen detection ELISA.

33 PREVALENCE OF IgG-ELISA ANTIBODIES AGAINST JAPANESE ENCEPHALITIS, WEST NILE AND TYPE 2 DENGUE VIRUSES IN HUMAN POPULATION IN NEPAL

CHINTA MANI SHARMA[1, 3], MATHURA SHRESTHA[2], BHOGENDRA SHARMA[2], ASHOK KUMAR SRIVASTAVA[3] AND AKIRA IGARASHI[3]

Department of Clinical Pathology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal[1], Department of Community Medicine, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal[2] and Department of Virology, Institute of Tropical Medicine, Nagasaki University[3]

Japanese encephalitis (JE) is an acute encephalitis with high mortality and grave sequelae caused by JE virus, a mosquito-borne flavivirus. Although the number of human JE significantly decreased in Japan after 1966 and in the Republic of Korea after 1982, JE has still been a major public health problem in several Asian countries. In Nepal, an epidemic of presumed JE occurred in 1978 in Southern Terai Plain along the Indian Border. The surveillance by Joshi *et al.* collected many mosquitoes including potential JE vectors of *Culex* species and detected anti-JE antibodies among 1/3 of human and animal sera by the hemagglutination-inhibition test. Since the test cannot often differentiate between JE and cross-reacting flaviviruses, such as West Nile (WN) and dengue viruses, both of which are prevalent in the Indian Subcontinent, we tried to see the IgG-ELISA antibody prevalence against these viruses in human sera collected in 1988 at 3 study areas in Nepal.

Sera were obtained from a total of 585 individuals of various age groups at 1) Kathmandu in Central Basin, 2) Biratnagar in Eastern Terai and 3) Bharatpur in Central Terai. Antibody titers were examined by the indirect micro IgG-ELISA using antigens of a) formalin-inactivated and purified JE vaccine concentrate Nakayama strain (Research Foundation for Microbial Diseases of Osaka University), b) JE virus JaOArS982 strain, c) WN virus Eg101 strain and d) dengue type 2 virus New Guinea B strain. The antigens b), c) and d) were purified from infected C6/36 cell culture fluids by similar procedures.

1) The sera from Kathmandu area showed low antibody positive rates (under 20%) to antigen a) in all age groups. While, the rates were higher (50-100%) to antigens b) and c) and intermediate (less than 50%) to antigen d).

2) The sera from Biratnagar area did not show significant difference of antibody positive rates by the assay antigens, although they showed age-dependent increasing trend. The rates were 40-60% to antigen c), and did not exceed 45% to antigen a), 70% to antigen b), and 52% to antigen d).

3) Sera from Bharatpur area generally showed similar trends as 1), although the results were not clear because of small sample size.

4) Geometrical mean ELISA titers showed similar trends as the antibody positive rates.

5) Correlation coefficients of antibody titers of individual specimen could not explain the results by the cross-reactions among these viruses.

The results suggested high endemicity of some virus(es) closely related to JE and WN viruses in Nepal, especially in Kathmandu area, and the common antigen could be lost by formalin.

34 AN EFFICIENT METHOD FOR ISOLATION OF DENGUE VIRUS FROM DENGUE HEMORRHAGIC FEVER PATIENTS AND ANALYSES OF DATA (JAKARTA, INDONESIA, 1988)

NOBUYA FUJITA[1], SUSUMU HOTTA[2], HISAO ESAKI[1], EIJI KONISHI[3], SUMARMO[4] AND SUJUDI[4]

Kyoto Prefectural Institute of Hygienic and Environmental Sciences[1], Division of Tropical Medicine, Medical Research Institute, Kanazawa Medical University[2], Department of Medical Zoology, Kobe University School of Medicine[3] and University of Indonesia[4]

In February 1988 at Jakarta we encountered an outbreak of dengue hemorrhagic fever (DHF) in which a number of severe cases were found. Investigating this epidemics, we attempted to isolate dengue viruses (DV) from DHF patients by an easy and efficient method we designated. The whole blood was drawn from patients, and immediately thereafter, 2 drops of specimen were introduced into *A. albopictus* (clone C6/36) cell cultures fed on a 24-well culture plate added with heparin of an appropriate concentration. After an incubation overnight at 28°C to complete virus-cell adsorption, the blood and heparin were washed off with PBS and fresh medium was introduced. The cultures were incubated at 28°C for further 6-7 days. The infected culture fluid was inoculated into BHK (clone 13) cell cultures and the infected cells were subjected to confirmation of positive DV isolation and typing of isolates by indirect immunofluorescent antibody tests using anti-DV mouse immune ascitic fluid and type-specific monoclonal antibodies, respectively.

Seventeen strains of DV were isolated from 100 blood specimens from 76 DHF patients. Fourteen strains thereof were type 3 and the remaining 3 were type 1. The data suggest that the principal etiology of dengue prevailing at Jakarta in early 1988 was type 3 virus. The successful isolation of virus had no apparent connection with anti-DV antibody titers of blood from which the virus was isolated nor with the severity of illness, i.e., WHO's grades. The possibility of virus isolation was significantly related to the time of blood drawing, i.e., periods after admission to hospital.

The investigation was financially supported by a Grant-in-Aid for Overseas Scientific Research from the Ministry of Education, Science and Culture, Government of Japan, No. 62041100 and No. 63043070.

35 IMMUNOELECTRON MICROSCOPIC DETECTION OF DENGUE HAEMORRHAGIC FEVER VIRUSES FROM *TOXORHYNCHITES* MOSQUITOES IN INDONESIA

TAKEO MATSUMURA[1], HIROSHI YAMANISHI[2], BERNARDUS E.F. DA SILVA[3], EIJI KONISHI[1], S. DJAKARIA[3], NOBUYA FUJITA[4], SOEKIMAN SOEDARTO[5] AND SUSUMU HOTTA[6]
Department of Medical Zoology, Kobe University School of Medicine[1], Kobe Gakuin Women's Junior College[2], University of Indonesia[3], Kyoto Prefectural Institute of Hygiene and Enviromental Science[4] and Airlangga University[5] and Division of Tropical Medicine, Medical Research Institute, Kanazawa Medical University[6]

Dengue haemorrhagic fever (DHF) has been one of the most important mosquito-borne, acute and febrile viral disease in Southeast Asian countries. The various methods used to study dengue fever virus interrelationships virologically and immunologically have been already reported.

It is necessary for the developing countries located in the Southeast Asia to establish the system to isolate and identify appropriately the etiological viruses according to the soft-and hard-ware situation in each country.

In the present paper have been reported preliminary results of immunoelectron microscopic detection of DHF viruses by using the protein A-colloidal gold method, based on the innoculation of C6/36 culture cells and/or intra-thoracical innoculation of genus *Toxorynchites* mosquitoes, in collaboration with the team of University of Indonesia, Indonesia.

36 PRESENT SITUATION OF RICKETTSIOSIS IN AWAJI ISLAND, HYOGO PREFECTURE

TETSUJI AWATA[1], KAZUYA KODAMA[1], HISASHI OHNISHI[1], TAKEFUMI MATSUO[1] AND TAKEO MATSUMURA[2]
Hyogo Prefectural Awaji Hospital[1] and Department of Medical Zoology, Kobe University School of Medicine[2]

We reported one case of tsutsugamushi disease and two cases of Japanese spotted fever (spotted fever) in Awaji Island where both diseases had not been recognized. The first case was tsutsugamushi disease found in 1986 and the second case was spotted fever found in 1988. The last case was a suspected spotted fever by the review of past patients who were admitted to our hospital complaining of high fever and skin rash.

The first case was a 52-year-old man. He was admitted to our hospital with high fever, headache and skin rash on September 12, 1986. Tache noir on the right lower abdomen suggested tsutsugamushi disease, administration of doxycycline rapidly imploved symptoms. The sera from the patient showed elevation of antibody titer to Gilliam strain of *R. tsutsugamushi* by indirect immunofluorescence (IF) method from less than 1:10 to 1:80. One year later, his sera remained 1:40 to Gilliam strain of *R. tsutsugamushi* by IF method and also showed 1:40 to spotted fever rickettsia (*R. japonica*) by IF method. We suspected that he

might have suffered from spotted fever before tsutsugamushi disease infection.

The second case was a 60-year-old man. He was admitted to our hospital with high fever, nausea, skin rash on August 11, 1988. Tache noir was found on the right lower abdomen. By IF method, his sera showed no elevation of antibody titer to *R. tsutsugamushi* but showed elevation of antibody titer to *R. japonica.*

The third case was a 54-year-old man. He was admitted to our hospital with high fever, headache, skin rash on June 24, 1977. Weil-Felix titer of the patient's serum reached 1:40. Serological diagnosis was not determined, but administration of chloramphenicol rapidly improved his symptoms.

After them new patients of both diseases were observed in Awaji Island. Now there are 7 cases of tsutsugamushi disease (5 cases of serologically confirmed, 2 cases of clinically suspected) and 4 cases of spotted fever (3 cases of serologically confirmed, 1 case of clinically suspected). Tsutsugamushi cases were distributed in the north area and spotted fever cases were distributed in the south area.

Irrespective of quite different vector both diseases are similar clinically. We compared and examined these cases. All cases of both diseases exibited mild liver disfunction and one case of tsutsugamushi disease showed DIC. Rise of *Proteus* OX2 titers were found in all cases of spotted fever, but those titers were low (1:20~1:80). Very interesting point was that patient of spotted fever (case 2) found a tick on the right lower abdomen, and preserved it for a while.

37 INCIDENCE OF *GIARDIA LAMBLIA* INFECTION IN TRAVELLERS TO INDIA AND NEPAL

AKIO KIMURA[1], YOSHIICHI MINEKAWA[1], NAGASHIGE IKEDA[2], KENJI SHIMOIRISA[3], YOSHIHISA KUSUI[2], TADASUKE ONO[4] AND TOSHIO NAKABAYASHI[5]

Osaka Prefectural Institute of Public Health[1], Osaka Airport Quarantine Station[2], Naha Qurantine Station[3], Department of Veterinary Pathology, University of Osaka Prefecture[4] and Department of Parasitology, Fujita-Gakuen Health University School of Medicine[5]

Stool examination for *Giardia lamblia* in patients of traveller's diarrhoea who had stayed in India and/or Nepal over 10 days were carried out in July, 1986 to October, 1989. Results obtained were as follows:

1) Stools from 566 patients were examined and in 49 cases with *Giardia lamblia* was detected (8.7%). The positivity rate in each year was: 13.6% (1986), 10.1% (1987), 2.7% (1988) and 9.0% (1989 till October), respectively.

2) The seasonal variation was recognized in the piositivity rate: Higher in April (15.1%), June (11.8%), September (10.0%), October (10.5%) and November (15.0%) than other months. There was no positive case in July and December.

3) More than 80% of the 49 positive cases were male and in the age of 20s, respectively.

4) 89.6% of the positive cases were the travellers over 15 days and 42.6% were the long term travellers over 30 days.

5) 98% of the positive cases had experience of stay in India. This suggested that India

might be an important country as infection source.

6) In 16 cases of the 49 positive cases, some species of pathogenic bacteria (e.g. *Shigella*) or other nonpathogenic intestinal protozoa (e.g. *Entamoeba coli*) were concomitantely detected.

7) Majour complaints of the positive cases were watery diarrhoea and lastig loose bowels.

38 PATHOGENICITY OF AMEBA STRAINS IN BRAZIL

TSUTOMU TAKEUCHI[1], SEIKI KOBAYASHI[1], YASUSHI MIYAHIRA[1], SEVERA MOTTA[1], EIICHI OKUZAWA[1, 2], TOMOYOSHI NOZAKI[1, 2], SEIKI TATENO[2] AND IVANIZE ACA[2]

Department of Parasitology, School of Medicine Keio University[1]

and Laboratório de Imunopatologia Prof. Keizo Asami, Federal University of Pernamco, Brazil[2]

We made a study on pathogenicity of *Entamoeba histolytica* in some areas in Brazil. Stool examination, serological examination, cultivation of amoeba and zymodeme analysis were carried out primary according to the method of Sargeaunt *et al.*

Nine hundred and sixty two school children in Northeast Brazil (Recife and Sao Luiz) and North Brazil (Belem and Manaus) were examined coprologically. Two hundred and ninety five cases (31%) were positive with amoeba cysts in stool, and one hundred and four (11%) with identified *E. histolytica* cyst. The same samples were inoculated into Robinson's medium. Of the 921 inoculation, 333 cases (36%) were positive for the culture. From 23 cases positive for *E. histolytica* in Recife, 20 strains were isolated (87% isolation). The 333 strains isolated were subject to isoenzyme analysis of hexokinase (HK), glucose phosphate isomerase (GPI), phosphglucomutase (PGI) and malate dehydrogenase (ME). Ninety six strains (10%) showed isoenzyme patterns compatible to those of *E. histolytica.*

Of the 96 *E. histolytica* strains isolated in Brazil, the majority had zymodeme I (36 strains). In addition, zymodeme VIII (23), XVII (15) and III (11) were common. A minor difference among these is the presence of gamma or delta bands of PGM and GPI. We encounterd only five pathogenic strains there, all of which were isolated in North Brazil and proved to be zymodeme XIX. In our study no zymodeme II, the most common pathogenic one, was found.

We got blood from 66 of the children from whom these zymodemes were isolated. Unfortunately we could receive only one serum from the children with zymodeme XIX, however all of the cases were negative by Ouchterlony's test. We also did micro-ELISA modified for detecting asymptomatic carriers. Even using this method, more than 70% of these carriers proved to be negative.

We also examined hospital patients with diarrhea in Recife. Three strains of *E. histolytica* were isolated from 126 patients with a chief complaint of diarrhea. Three were isolated from 130 patients without complaint of diarrhea (control group). All of the six strains showed zymodeme I.

We conclude from these data that non-pathogenic strains, chiefly zymodeme I, are prevalent in Northeast Brazil, and amebiasis seems to be a minor diarrheal disease. On the other hand in North Brazil pathogenic zymodeme XIX is distributed. It suggests that there would be clinical amebiasis in Belem and Manaus, although no seropositive cases were found.

39 A MODIFICATION OF AGAR PLATE METHOD FOR DETECTION OF *STRONGYLOIDES*

SHIRO KASUYA[1], KAORI KOGA[1], MASATOSHI IEDA[2], KENJI KITA[2],
NAOTAKA TAKATSUKA[2] AND HIROSHI OHTOMO[1]
Department of Parasitology[1] and Student[2], Gifu University School of Medicine

The agar plate method for detecting of *Strongyloides stercoralis* has been tried in Okinawa (Japan) and Chiang Mai (Thailand). This method has been proved that more *S. stercoralis* larvae are detected than in traditional methods. However, the larvae can crawl out of dish, therefore we must modify the agar method for safe. Our modification is to seal the dish with adhesive tape. A simulation experiment using *Rhabditis*-like worm isolated from *Rattus rattus* feces, we clearly demonstrated that mobility of the worm was not suppressed for long time by sealing of dish if drier dishes were used. Lighting through green filter facilitate to detect crawling traces on agar. Based on these data, this modification of the agar plate method has been proved to be useful.

40 A CASE OF STRONGYLOIDIASIS WHERE ACCOMPANING GASTRO-INTESTINAL DISORDERS REMARKABLY IMPROVED

YOSHIHITO OTSUJI[1], RYUJI HARADA[2] AND AKIRA NAKASHIMA[3]
Kagoshima Prefectural Comprehensive Health Center[1], The Second Department of Internal Medicine, Faculty of Medicine, Kagoshima University[2] and Imamura-Bunin Hospital, Kagoshima City[3]

Evidence has been presented that digestive system disorders in a case of strongyloidiasis strikingly improved as a result of Thiabendazole administration

Case: H.K., 60 year old, self-employed man.

Chief complaints: Sense of abdominal distention and loss in weight.

Social history: The patient was born in Amami-ōshima, Kagoshima Prefecture and had lived there until he was 20 year old. He from that time on moved to China, again back to Amami-ōshima, then to Okinawa. He has been living in Kagoshima City since 50 years old.

Present illness: In July, 1984 or thereabouts the patient started to have sense of abdominal distention, frequent flatulence and anorexia. He was diagnosed as having only mild pancreatic dysfunction based on examinations at a nearby hospital. Thereafter was weight loss of 4-5 kg in 3-4 months.

Physical findings and results of examination: On admission the patient was mesomorphic, height 156 cm and weight 43.5 kg. Neither anemia nor jaundice was noted. The spleen and liver were negative for enlargement and ascites was not noted. Routine blood studies showed that white blood cell count was 12,500/mm^3, with 7% eosinophils. Stool cultures and examinations for parasites revealed R-type mucous membrane and confirmed by biopsy procedure. A large number of the R-type larvae also found in gastric juice. Scout film

examination of the empty stomach revealed abnormal gas in the upper gastric region and findings suggestive of the edematous Kerckring's folds.

Treatment: Thiabendazole 50 mg/kg body weight/day in two divided doses, for 3 days. A week after initiating the treatment sense of abdominal distention subsided and an improved appetite ensued. There had been weight gain of 3 kg. Disappearance of the larvae from the stool and improvement of roentgenographic findings of the abdomen, as well as of endoscopic ones of the pars descending of the duodenum, came out.

The evidence shows that the antiparasitic effect of Thiabendazole has resulted in improvement of both subjective and objective signs and symptoms in the strongyloidiasis patient.

41 A CASE OF MYIASIS CAUSED BY *DERMATOBIA HOMINIS*

RYUICHIRO MAEDA[1], EMI MAKITA[1], MITSURU SEGAWA[2], TOSHIRO SHIBUYA[1] AND MIKIO OGINO[2]
Department of Parasitology, Teikyo University School of Medicine[1] and Department of Orthopedic Surgery, National Medical Center[2]

One case of human myiasis caused by *Dermatobia hominis* was reported.

The patient was a 22-year-old Japanese woman who had been in Brazil for 12 years. She lives in the suburbs of São Paulo City at present.

About the middle of December 1988, a slight bleeding was noticed between the first and the second toe of right foot. She came to Japan for sightseeing January 1 and visited National Medical Center January 23, 1989. When she consulted the doctor, the swelling with the oozing of blood was 10 mm in diameter. The affected part was not cured after 3 days of sterilization. January 27, a maggot was taken out from the lesion surgically. After the maggot was removed from the patient, the symptom disappeared rapidly. The maggot was 9.5 mm long and the color was yellowish white. The strong spines distributed evenly on the surface. The posterior spiracle was lost during the process of extirpation. It was identified as second inster larva of *Dermatobia hominis* after a detailed examination.

Occlusion of the punctum in the skin with vaseline causes the maggot to migrate out of the skin to breathe.

42 STUDY ON THE IMMUNOGENICITY OF PURIFIED SIAMESE COBRA TOXOID

YOSHIHARU KAWAMURA AND YOSHIO SAWAI
Japan Snake Institute

Detoxification of purified Siamese cobra (*Naja naja kaouthia*) venom by formalin and the immunogenicity of the toxoid have been investigated. Two per cent of the crude venom solution fractionated by ethanol was treated by formalin in increasing amount from 1 to 5%

at an interval of one week and the excess formalin was removed by dialysis. The toxoid was tested for complete detoxification in mice and then added with the same amount of aluminium hydrochloride as adjuvant.

Final concentration of protein was 1%. Immunogenicity of the toxoid was tested injecting the toxoid subcutaneously into mice and guinea pigs. Anti-lethal effect of sera taken from immunized mice and guinea pigs indicated that 0.5 m*l* of the sera neutralized 15 μg (2 mlds) and 6-9 μg (1-1.5 mlds) of the venom, respectively. The mice and guinea pigs also torelated and survived from the injections of 26 μg (2 mlds) and 300 to 600 μg (1-1.3 mlds) of the venom, respectively.

43 MYONECROTIC FACTORS WITH PHOSPHOLIPASE A_2 ACTIVITY IN *TRIMERESURUS FLAVOVIRIDIS* (HABU SNAKE) VENOM

HIROSHI KIHARA[1], RYUICHI UCHIKAWA[2], MOTONORI OHNO[3] AND SABURO HASHIMURA[1]
Department of Physiology[1] and Medical Zoology[2], Faculty of Medicine, Kagoshima University
and Department of Biochemistry, Faculty of Science, Kyushu University[3]

The envenomation of *Trimeresurus flavoviridis* (Habu snake) is characterized by a highly complex pathophysiological picture which includes a necrosis of skeletal muscle together with hemorrhage and edema. Several myotoxins which induce myonecrosis at the injection site have been isolated from snake venoms, and some of them are identified as phospholipase A_2.

We purified three isoenzymes of phospholipase A_2 (named PLA_2, BP-1 and BP-2) to homogeneity from *T. flavoviridis* venom by means of conventional chromatographic techniques, and then determined their amino acid sequences. They consist of 122 amino acid residues and are similar in sequence to each other. BP-2 is identical in sequence to BP-1 except for substitution of one amino acid residue. PLA_2 has high phospholipolytic and indirect hemolytic activities, but both activities of BP-1 and BP-2 were very low. The isoelectric point of PLA_2, BP-1 and BP-2 was estimated to be 7.9, 10.2 and 10.3 from the mobility on an isoelectric focusing electrophoresis, respectively.

Recently, we reported that PLA_2 led to an increase in plasma creatine kinase (CK) levels as well as to cause myonecrosis at the injection site in rabbits and rats (The SNAKE, 18, 84-91, 1986). When injected i.m. into mice, BP-1 and BP-2 induced a dose-dependent increase CK levels and a series of degenerative events in skeletal muscle which led to myonecrosis. The pattern of CK level increase is very similar after PLA_2, BP-1 and BP-2 injections. However, the levels are higher at each concentration in mice injected with BP-1 and BP-2 than those injected with PLA_2. PLA_2, BP-1 and BP-2 induced similar histological changes in mouse skeletal muscles 4 hr after injections. Coagulative necrosis of muscle fibers with stromal edema and cellular infiltrations was observed, but not hemorrhage. These changes were not caused by the injection of PBS.

The Ouchterlony double diffusion technique was performed to test antigenic differences among three toxins. Each toxin formed a single precipitation band in the gel plate against the *T. flavoviridis* antivenom. The precipitation band formed by BP-1 fused completely with

that by BP-2, but not with that by PLA_2. This suggests that BP-1 and BP-2 have a complete immunological identity, but differ from the antigenicity of PLA_2. The antivenom of 200 fold over in weight inhibited about 80% the increase of CK level by BP-1 and BP-2. The antivenom, however, was less effective in neutralization of CK increase by PLA_2.

44 INVESTIGATION OF SCHISTOSOMIASIS IN SULAWESI, INDONESIA (1) ENDEMIC AREA ON THE LINDU LAKE

KIYOSHI KAMIMURA[1], SYAFRUDDIN[1,6], MASATOSHI NAKAMURA[1,2], RYO ARAKAWA[1], KAORU KONDO[3], NOBUAKI AKAO[3], TAKAAKI OHYAMA[3], TAKAFUMI HAYASHI[4], NASIR BORMAN[5], HERMAN WIBOWO[5] AND KUNAR ABADI[6]

Department of Parasitology, Toyama Medical and Pharmaceutical University[1], Department of Parasitology, School of Veterinary Medicine, Azabu University[2], Department of Parasitology, School of Medicine, Kanazawa University[3], Toyama Yuai Hospital[4], Central Sulawesi Provincial Institute of Health[5] and Department of Parasitology, Faculty of Medicine, Hasanuddin University[6]

In August 1989, we visited the endemic areas of schistosomiasis japonica, along the west basin of Lindu Lake, in Central Sulawesi. The Lindu Valley is an area of approximately 100 km^2 in the Takolekaju Mountain range. At the north-east basin, the Gumbasa River, which is the only outlet, flows into the adjoining Palu Valley and so a dam being planned for irrigation downstream and simultaneuously to regulate waterlevel at the lake. Schistosomiasis controls were conducted throughout this area by National Institute of Health in the forms of: (1) Reduction of snail population by spraying chemical agents (Baylucide) into their nests, drainage and incineration of the foci, however, infection rates of *Schistosoma japonicum* in *Oncomelania hupensis lindoensis* varied considerably (av. 0.3%, own 2/10). (2) Mass treatment with praziquantel during 1981-1988. In the Lindu Lake area where the population numbers about 2,900, the prevalence of the disease has also been successfully brought down to a mere 1%, comparing to 58% at some 15 years ago. Transmission of the disease would not be cut successfully as long as there are reservoir hosts, particularly dogs and rodents. If so, eventually, schistosomiasis would return with a vengeance.

45 INVESTIGATION OF SCHISTOSOMIASIS IN SULAWESI, INDONESIA (2) ON THE RESULTS OF FECAL EXAMINATION AND SKIN TEST IN NAPU DISTRICT

KAORU KONDO[1], NOBUAKI AKAO[1], TAKAAKI OHYAMA[1], KIYOSHI KAMIMURA[2], SYAFRUDDIN[2], RYO ARAKAWA[2], MASATOSHI NAKAMURA[2] AND TAKAFUMI HAYASHI[3]

Department of Parasitology, School of Medicine, Kanazawa University[1], Department of Parasitology, Toyama Medical and Pharmaceutical University[2] and Toyama Yuai Hospital[3]

Schistosomiasis japonicum in Indonesia is localized at some villages surrounding of Lake Lindu in Central Sulawesi. In stool examination, Dogo *et al.* (1976) reported that positive rates of schistosoma eggs revealed 13-56% (1972) of the residents. After that, the control operations on schistosomiasis has been initiated by the Government and WHO. The mass treatment with praziquantel, the rate of positive cases decreased to about 0.5-4.7% (1988).

In August of 1989, we carried out the reassessment of schistosomiasis in Napu, one of endemic area of schistosomiasis near Lake Lindu. By stool examination, 2 out of 17 specimen were positive for schistosome eggs. Immuno-epidemiological survey, using the adults worm extracts antigen of *S. japonicum,* revealed that 16 out of 77 (20.8%) people showed the positive reaction by skin test; 17 out of 22 (77.3%) sera possessed a high antibody titer by ELISA, and the mean antibody titer was 1.79+0.16. In both the immunological tests, no significant differences were found between the group with a past history of the egg positive and the group without the history. We captured three wild rodents in the village Wuasa, and dissected and examined for the presence of schistosome worms. Two adults worms (one male and a female) were found in a rat.

46 STUDIES ON *SCHISTOSORA HAEMATOBIUM* INFECTION IN KENYA —RESPONCE OF PEOPLE TO THE INSTALLATION OF PIPED WATER SUPPLY—

MASAAKI SHIMADA[1], KATSUYUKI SATO[1], YOSHIKI AOKI[1], EISAKU KIMURA[2], SHINICHI NODA[3], ATSUO SATO[3] AND D.N. MUHOHO[4]

Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[1], Department of Parasitology, Aichi Medical University[2], Department of Medical Zoology, Faculty of Medicine, Kagoshima University[3] and Kenya Medical Research Institute, Kenya[4]

In a village of Kenya, Mwachinga, safe water supply was installed for a control measure of schistosomiasis. In order to determine the impact of safe water supply on the behavior of villagers, the relationship between the safe water consumption and the frequency of contact with river water was analyzed.

Two rivers flow through the village. They were the main water sources for the villagers before the installation of water supply. The water contact behavior was observed by local observers for 1 day per month at each of 16 observation sites. The observation was carried out over a period of 12 months before the provision of safe water supply and continued 12 months after the installation. At 5 safe water supply points, the quantity of water collected by each household was recorded for one year after the installation. The quantity of safe water used by a person was calculated by dividing the total quantity of safe water by the number of the household members.

The maximum quantity of safe water used by a person per day was only 16 liters and the geometric mean of the consumption of safe water per person per day was 2 liters. The per cent reduction of water contact with rivers after the installation of safe water was significantly higher in the households where each house member consumed more than 1,000 liters per year. If the distance to the nearest safe water point was more than 800 m, the

consumption of safe water was significantly low. The households more distant from safe water supply points used smaller quantity of safe water than the houses nearer to the points.

In conclusion, the provision of safe water had a great impact on the water contact behavior with rivers. The people who consumed much safe water tended to get rid of contacting with river water. One of the major factors which made the villagers to change their water contact behavior is the distance from their house to safe water supply points.

47 STUDIES ON *SCHISTOSOMA HAEMATOBIUM* INFECTION IN KENYA —COMPARISON OF SHORT AND LONG TERM EFFICACY OF METRIFONATE AND PRAZIQUANTEL—

MASAAKI SHIMADA[1], KATSUYUKI SATO[1], YOSHIKI AOKI[1], EISAKU KIMURA[2], SHINICHI NODA[3], ATSUO SATO[3], JUN-ICHI GYOTEN[4] AND D.N. MUHOHO[5]
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[1], Department of Parasitology, Aichi Medical University[2], Department of Medical Zoology, Faculty of Medicine, Kagoshima University[3], Department of Parasitology, Ehime University School of Medicine[4] and Kenya Medical Research Institute, Kenya[5]

In a village of Kenya, a control measure for schistosomiasis, mass chemotherapy with drugs was carried out accompanied with installment of safe water supply. The mass-treatments were carried out with 2 types of drug at different times; in March 1984 with metrifonate and in July 1986 with praziquantel. Metrifonate was given 3 times at a dose of 7.5 mg/kg body weight at an interval of 2 weeks and praziquantel was given at a single dose of 40 mg/kg. Three months, and 2 years after each treatment, urine examinations were done for the evaluation of short and long term efficacy of the drugs.

Three months after treatment, metrifonate reduced much less both overall prevalence (38.4%) and intensity (from 2.56 ± 0.99 to 1.18 ± 1.20 in logarithm) of infection, while praziquantel caused a marked reduction in prevalence (83.5%) and intensity (from 2.22 ± 0.93 to 0.20 ± 0.57 in logarithm). The efficacy of one drug on the patients who were not cured by the other drug was also examined. Out of 56 patients who had not been cured by metrifonate, 46 (82%) were cured by praziquantel, however metrifonate had cured only one patient who were not cured by praziquantel. By our treatment protocols, the efficacy of praziquantel was superior than that of metrifonate.

When we focus our attention on the increase of egg output level after treatment, the prevalence and intensity of infection increased slightly until one year after the treatment both with metrifonate and praziquantel. However, 2 years later, while the intensity of people treated with metrifonate remained stable, the people treated with praziquantel still continued getting reinfection and returned to the pretreatment level.

These results indicate that the installation of safe water reduced the infection pressure in the village, and the intensity of infection returned to a new equilibrium point of "loss and gain" of worms. Although the treatment with praziquantel reduced the intensity of infection much more than that with metrifonate, the intensity easily returned to the pretreatment level after 2 years.

48 UNTRASONOGRAPHIC EVIDENCE OF BILIARY TRACT STONES IN OPISTHORCHIASIS PATIENTS AFTER TREATMENT WITH PRAZIQUANTEL

SWANGJAI PUNGPAK[1], DANAI BUNNAG[1], TRANAKCHIT HARINASUTA[1],
SANTASIRI SORNMANI[1] AND TOZO KANDA[2]
Mahidol University, Thailand[1] and Department of Medical Zoology,
St. Marianna University School of Medicine[2]

Optitorchis viverini infection is common in Thailand. It was estimated that about 7 million people are suffered from this liver fluke infection. Praziquantel is very effective drug in treating opisthorchiasis. It was claimed that eversince praziquantel has been introduced for a large scale control programme in Thailand the incidence of biliary stones increased. The objective of this study is to find out the incidence of biliary stone in opisthorchiasis patients after treatment with praziquantel by using a portable ultrasound machine (Model 8 AL, 32B Toshiba).

The study was carried out at 3 villages in Khon Kaen Province, 450 km northeast of Bangkok. Six hundred and forty seven opisthorchiasis patients were recruited for the ultrasonographic examination. These patients had been treated with praziquantel during 1981 to 1986.

The treatments was repeated annually in those reinfected. The reinfection rate was 53.9% in the first year gradually declined. Ultrasonographic examinations were performed during 22nd to 29th December 1987. The findings were normal in 80.6% of subjects. The most common abnormal finding was liver enlargement (14.8%), followed by dilatation of the gallbladder (3.5%), sludge formation (2.1%) and thickening of the wall of gallbladder (1.0%). Gallstones were found in 7 cases (1.0%). Dilatation of intrahepatic bile ducts was detected in one subject (0.1%). The incidence of gallstones in treated patients was similar to that in a large necropsy of the general hospital population. The results of this study do not substantiate the claim that incidence of gallstone in opisthorchiasis patients increases after praziquantel treatment.

49 SECULAR CHANGES OF THE PERCENTAGE OF LBW INFANTS AT AN OBSTETRIC SERVICE IN NAHA CITY DURING THE LAST 33 YEARS (1955-1987)

SHINYA MATSUDA AND HIROAKI KAHYO
Department of Human Ecology, School of Medicine, University of Occupational and Environmental Health

Secular changes of the percentage of LBW infants at an obstetric service in Naha City during the last 33 years (1955-1987) were examined. The percentage of LBW infants was the highest at 1956 with 26.6%, then decreased into between 3 and 6% recently.

In order to investigate the cause of this phenomenon, we examined the relationship between 3 perinatal factors {maternal height, pre-pregnant maternal Body Mass Index (Quetelet's index), and parity} and the occurrence of LBW infant. Odds ratio of each risk

factor was calculated by the Logit model. Women with lower pre-pregnant BMI (<20) had increased LBW risk by 1.33 (95%CI: 1.13-1.56), compared to women with higher pre-pregnant BMI (≦20, 20<, ≦25). Lower maternal height (150 cm>) was associated with significant LBW risk (1.47; 95%CI: 1.00-1.47). Women of primiparae had higher LBW risk than women of multiparae (1.33; 95%CI: 1.14-1.56).

It is considered that maternal height reflect her long term nutritional status including the condition of her childhood, and that maternal BMI describes the short term nutritional condition.

Our result suggest that the remarkable decrease of LBW infants at this facility was due to the improvement of nutritional status of mothers, which was strongly connected with the socio-economical development of Okinawa after the second World War.

On the other hand, we concluded that an index describing birth weigh distribution could be available as a total health indicator of a particular community.

50 MALIGNANT TUMOR OF HEAD AND NECK IN CENTRAL AND SOUTH AREAS, THAILAND

KIICHI SATO AND SUSUMU HOTTA
Division of Tropical Medicine, Medical Research Institute, Kanazawa Medical University

We have participated the Rural Ear Surgery Camp in Thailand in collaboration with Thai otolaryngologists for five years. In these terms, we have visited seven Hospitals attached to Medical Universities and studied each Departments of Otolaryngology.

This time, since we have inquired into the Malignant tumor of head and neck from Siriraj Hospital, Mahidol University (M. Univ.) in Bangkok and Songkla University (S. Univ.) in Hadyai, the results have been reported. The compaired data of Japan was the Japanese Joint Committee on TMN classification, which had been published in National Cancer Center (1985-1986).

The patients of M. Univ. were mostly from Bangkok, Central East and West area, and those of S. Univ. were from South area.

The results are as follows: (1) The total number of the patients diagnosed as a malignant tumor by the pathohistological method of M. Univ. were 623 cases (1984), those of M. Univ. were 247 (1985). (2) according to the classification of organs, the nasopharygeal carcinoma occupied the largest number. 144 (23%) patients in M. Univ. had the nasopharyngeal carcinoma and 45 (18%) in S. Univ. Comparing with the prevalence in Japan (4%-5%), these were significantly higher. (3) the prevalences of the laryngeal carcinoma of both hospitals seemed to be high, but were lower than that of Japan. (4) About 90% of the patients were found to be a squamous cell carcinoma histologically. We suggested that the differences of the frequency may have involved in not only the differences of life styles but also those of foods and luxury goods. However, we consider that they are related to infection diseases exsiting constantly in tropical countries.

51 LYMPH NODE TYPE KAPOSI'S SARCOMA IN WESTERN KENYA

HIDEYO ITAKURA, KAN TORIYAMA, HIDEAKI ETO,
SATORU KOMURO AND KEN-ICHIRO MURATA
Department of Pathology, Institute of Tropical Medicine, Nagasaki University

African endemic type Kaposi's sarcoma (KS) is fundamentaly a soft tissue tumor and occurs usually on the extremities of adult male patients. Although KS in children is an uncommon disease, majority of the cases are found in lymph nodes and show oftenly generalized lymphadenopathy. We have observed 26 cases of lymph node type KS in western Kenya (Western, Nyanza and Rift Valley Province) during the period of 1979 to 1988. Eighteen out of 26 cases were taken from children. Five were from adults. Three were unknown. The male to female ratio was 3.3: 1.0. All children cases showed no associated skin lesions and 9 out of 26 cases showed generalized lymphadenopathy. Two out of 5 adult cases showed the aggressive form of KS associated with multiple skin lesions and 1 out of 5 cases showed human immunodeficiency virus (HIV) positive serologically. Ethnogeographically, the highest incidence of KS in children occurred in the Luo, an inhabitant of Nyanza Province, and that in adults occurred in the Luhya, an inhabitant of Western Province. All 6 cases of KS in children under 2 years old were found only in male and 4 of them occurred in the Luo. No adult cases of lymph node type KS were found in the Luo. These findings suggest that there are some differences of oncogenesity of lymph node type KS between children and adults. Although several reports in the U.S.A. said that KS is not a malignant tumor but a benign hyperplasia, some cases of lymph node type KS in western Kenya showed malignant features histologically, such as tumor emboli in lymphatic vessels, necrosis of tumor tissues and extranodal invasions.

52 A STUDY ON THE HEALTH CONDITION OF THE JAPAN OVERSEAS COOPERATION VOLUNTEERS (JOCV) MEMBERS IN THE THIRD WORLD

IKUKO SEKI[1], SUSUMU TAKAHASHI[1], HIROSHI NARUTO[1] AND HIROSHI OHARA[2]
Clinic of Japan Overseas Cooperation Volunteers, JICA[1] and Department of International Cooperation, National Medical Center Hospital[2]

The study was intended to clarify: (1) the type of the diseases and disorders which JOCV members, especially in younger generations, have tendencies to contract during their long term stay in developing countries, (2) if there were any characteristics in the structures of the diseases depending on the areas where the volunteers stayed.

The subjects on which the study was carried out consisted of 463 JOCV members (123 females and 340 males) whose average age was 25.8 at their assignment to each country in 1984. One hundred and sixty of them stayed in Asia, 180 in Africa, 103 in Latin America, and 20 in Oceania. The duration of their stay was 2 years and 2 months on average.

This study was carried out based on the reports concerning diseases and disorders which

were sent from each country to JOCV headquarters.

The following results were obtained: (a) Odontopathies, tropical parasitic diseases, and bone fractures and traumata caused by traffic accidents were most frequent among the subjects. (b) Diarrhea diseases and fever attacks of unidentified causes were also frequent, and tropical diseases were more common among males than females. (c) The subjects in Latin America contracted diseases with the highest frequency, followed by those in Asia and then in Africa. With respect to tropical diseases, they were more common in Asia and Africa than in other areas.

53 RECENT STATUS OF PARASITIC DISEASES IN PAKISTAN

YOUICHI ITO[1], KENJI SODA[2], SEIJI KIMURA[3],
SEIICHI ICHIKAWA[2] AND WATARU HIRANO[4]
Department of Parasitology, Kitasato University School of Medicine[1],
Department of Public Health, Yokohama City University School of Medicine[2],
Department of Pediatric Science, Yokohama City University School of Medicine[3]
and Department of Public Health, Teikyo University School of Medicine[4]

Japan International Cooperation Agency despatched the study team on infectious diseases composed of 6 members who cover public health, epidemiology, microbiology, parasitology and pediatric in Pakistan. The team has been in Pakistan for 24 days started from August 5 to 28, 1988 and studied available informations on the status and activities of various infectious diseases. This presentation is restricted only to the informations of parasitic diseases. (1) Malaria: The whole country is malarious with the exception of extremely cold areas and deserts. It is estimated that about 62 million people are exposed to the risk of infection. The recorded slide positive rate was 3.13% and it seems to be maintained at low level. The seasonal peak of malaria transmission may occur during rainy and post-rainy seasons (June-September). Two species of malaria parasite, *P. vivax* and *P. falciparum* have been observed and it appears that chloroquine resistance is quite widespread in the country now. Anti-malaria activities started from 1961 and the Third five years plan for malaria control has been carried out to maintain the low level of malaria prevalence. (2) Leishmaniasis: The visceral leishmaniasis was first reported from Baluchistan; the cases were 60 with the 80% of children cases. Following strict control measures it was controlled from that area. Nevertheless the disease reappeared not only the previously endemic areas but also from the neighbouring regions, parts of Northern Punjab and adjacent NWFP. The cutaneous leishmaniasis on the other hand is reported from all over the country. However, it has been mainly endemic in Baluchistan; the cases were 139 at vital with active cases of 70. (3) Dracunculiasis: The Pakistan Guinea Worm Eradication Programme was established in 1986 at aims of the elimination of dracunculiasis by 1990. During the first year the programme conducted a national search and validation survey to determine the prevalence of the disease in the country. A nationwide search of all villages (48,000) was carried out between April and June 1987 and a total of 401 villages were provisionally identified as having endemic guinea worm disease. (4) Hydatidosis: According to some sources of information the

incidence of hydatidosis in livestock is high; the rates were 12.3% for buffaloes, 9.6% for cattle, 8.3% for sheep and 7.5% for goat. Furthermore 26 human cases of hydatidosis were seen over a period of 2 years in Liaquat College Hospital, Hyderabad. Hydatidosis is one of most serious parasitic diseases in Pakistan but little study on the epidemiology of the disease has been undertaken. (5) Intestinal parasitic diseases: There are several reports which indicate that intestinal parasite infection is common in Pakistan. Among them giardiasis, amoebiasis, ascariasis and *Hymenolepis nana* infection are common having high prevalence rates.

54 SELF-TREATMENT OF MALARIA WITH SPECIAL REFERENCE TO AN IMPORTED *PLASMODIUM FALCIPARUM* MALARIA CASE FROM ZAIRE

TOSHIMASA NISHIYAMA[1], YUZO TAKAHASHI[1], TSUNEJI ARAKI[1] AND HIROYUKI AMAMO[2]
Department of Parasitology, Nara Medical University[1] and
Department of Overseas Medical Services, Tenri Hospital[2]

In recent years, the incidence of imported cases of malaria in Japan has increased exceeding 60 cases annually. Because of limitation of available medical facilities in endemic areas patients had to treat themselves for malaria. In this report, we pick up several points about self-treatment by showing one typical patient with imported malaria who failed to treat herself for *P. falciparum* with chloroquine.

Case: A.K., 33 y.o., female, housewife who stayed in Zaire from March 1986 through June 1989. In 1986, she used chloroquine (irregularly) for prophylactic purposes in Zaire, from the next year she stoped this drug because of side effect (nausea, epigastralgia). On June 11 in 1989, she came back to Japan from Zaire. On June 15, she suffered from sudden chilling and high fever. On the next day, she consulted a private practitioner in Japan and was diagnosed and treated as common cold. On 16, she took chloroquine sulfate because she self-diagnosed as malaria but no success in treatment. She was devoid of precise knowledge how to treat for malaria, and failed to follow company's instruction; she took the drug at the recommended dose but she divided it into 3 times in stead of one time administration, resulting in question that the drug concentration in the blood may have stayed under the effective one. As a consequence it remained unclear so far whether or not the strain is chloroquine resistant. On June 20, we began to treat using sulfadoxine/pyrimethamine and this patient successfully recoverd.

Other cases: among 8 patients who did self-treatment for malaria, 6 cases failed because of unsuitable doses of antimalarial drugs and 5 cases suffered from malaria in spite of correct and regural chemoprophylaxis.

Recently, increased travel to and from endemic areas have increased the likelihood that Japanese get infected with malarial parasites. Unfortunately Japanese laymen are devoid of precise knowledge and effective chemoprophylaxis. In conclusion we emphasize the importance of medical education from the point of tropical medicine; how to use prophylactic drugs and how to manage fever attack in endemic areas.

55 AN IMPORTED CASE OF SEVERE FALCIPARUM MALARIA INDICATIVE OF LOW SENSITIVITY TO FANSIDAR AND QUININE

YOSHIO KAMEDA[1], DAISUKE TAKAGI[1], TOSHIO NAKABAYASHI[2] AND KENICHI YANO[3]
Internal Medicine, Hyogo Prefectural Nishinomiya Hospital[1], Department of Parasitology, Fujita-Gakuen Health University School of Medicine[2] and Department of Protozoology, Research Institute for Microbial Diseases, Osaka University[3]

A 37 year-old Japanese man was admitted to this hospital on July 31, 1989, because of chill, high fever and anorexia for a week. He visited north Thailand from July 4 to 22, 1989 and after returning to Japan, he had the acute onset of high fever on July 26. The physical examination revealed hepatosplenomegaly, positive test for urinary protein, total bilirubin 1.8 mg/d*l* (normal 0-1.0 mg/d*l*), direct bilirubin 0.6 mg/d*l* (normal 0-0.3 mg/d*l*), alanine aminotransferase 76 u/d*l* (6-36 u/d*l*), total protein 5.9 g/d*l* (6.5-7.9 g/d*l*), LDH 912 u/d*l* (264-437 u/d*l*), urea nitrogen 58 mg/d*l* (9-20 mg/d*l*), creatinine 2.1 mg/d*l* (0.4-0.9 mg/d*l*), red blood cell count 535×10^4/mm^3, Hb 16.8 g/d*l*, Hct 49.6%, WBC count 10,200/mm^3 with 45% band form, platelet 2,000/mm^3, prothrombin time 11.1 sec., fibrinogen 453 mg/d*l* and FDP 80 g/m*l* (0-20 μg/m*l*). The blood smear stained with Giemsa showed 2.4% parasitemia of *Plasmodium falciparum,* ESR 4 mm/hour and blood culture negative. Then, the diagnosis was falciparum malaria associated with DIC. He was treated with 4 tablets of Fansidar for three days in combination with 1.5 g of quinine per day for two days followed by 400 mg Quinimax per day for two days. Although he had numerous petechiae on his legs, anti-DIC therapy with 2,000 mg of FOY and 1,500 U of anti-thrombin III per day suppressed the DIC. Parasitemia had been reduced from 6% to 0.5% but fever and parasitemia was still continuous until the 8th hospital day, so that the patient was received 200 mg of minocycline-HCl per day combined with 400 mg of Quinimax followed by 1.5-1.8 g of quinine per day for 8 days. Parasitemia and fever were repidly subsided, though a few gametocytes remained. From the 17th hospital day Primaquine 15 mg base/day was given for 5 days. The clinical course indicated that this falciparum malaria might be of low sensitivity to Fansidar and quinine.

56 A CLINICAL TRIAL OF FANSIMEF IN TWO PATIENTS INFECTED WITH FALCIPARUM MALARIA

HIROSHI OHTOMO[1], MASAYOSHI NEGISHI[2], GOHTA MASUDA[2],
YUKO SAGARA[3] AND HIROSHI TANAKA[4]
Department of Parasitology, Gifu University School of Medicine[1], Tokyo Metropolitan Komagome Hospital[2], Tokyo Metropolitan Toshima Hospital[3] and Public Health Laboratory of Chiba Prefecture[4]

The recent circumstances involving falciparum malaria are that there are not a few areas where the strains resistant to other schizontocide have emerged in addition to the various areas where the chloroquine-resistant strains of *Plasmodium falciparum* emerged

successively. For this reason, treatment of falciparum malaria has become more difficult than ever, and attention of the people concerned is attracted to the fact that mefloquine in 4-quinoline methanol group which was approved in Switzerland in November, 1984, exhibits an excellent therapeutic effect on the drug-resistant malaria. A report is made here on the summary of the successful results we have obtained in curing 2 cases of falciparum malaria who resisted the conventional treatments by administering Fansimef, a mefloquine preparation.

Case 1: A French male aged 29

The patient visited Japan on 12th of May from Thailand, developed high fever on 14th and was admitted to Komagome Hospital on 16th. A diagnosis of falciparum malaria was made. Fever recurred despite treatment with 2 tablets of Fansidar/day on 18th and 19th, for which intravenous drip infusion of quinimax was made for 4 days from 24th. However, a high fever at 39-40°C persisted, and parasitemia did not disappear, for which 3 tablets of Fansimef were administered on 30th. As a result, the fever dropped to 36.2°C at last on 31st, and the symptom improved with resolution of parasitemia on 1st of June.

Case 2: A Japanese male aged 34

The patient made a stay in a mountainous region in Thailand near the boundary with Myanmar from December, 1988, to 22nd of February, 1989. After his return to Japan, the patient developed high fever to 40°C, convulsion and disturbance of consciousness at the midnight of 4th of March. He was admitted to Toshima Hospital. The symptoms were diagnosed as falciparum malaria and treated with chloroquine. However, the fever recurred on 10th, and no disaapearance of parasitemia was observed though concurrent treatment with quinine and tetracycline was commenced. Then, the symptom was alleviated by a treatment with 3 tablets of Fansimef on 13th made accordingly.

The initial development and testing of mefloquine was persormed by the US Army, and then it has been developed by WHO, in collaboration with the Walter Reed Army Institute of Research and Roche (Switzerland). This drug has excellent antimalarial activity. But the half life of this mefloquine is longer than those of other schizontcide, for which it appears that careful consideration should be given to possible side effects in giving it to patients who have carried the infection with them into Japan. However, the drug exhibited excellent therapeutic effects on the present 2 cases without occurrence of side effects in either of them.

57 A CASE OF CEREBRAL MALARIA TREATED WITH FANSIMEF

KIYOKATSU TANABE[1], HIDETOKU SUGIYAMA[2], ATSUO SATO[1] AND KAORU SHIMADA[2]
Department of Medical Zoology, Faculty of Medicine, Kagoshima University[1] and
Department of Infectious Diseases, Institute of Medical Science, The University of Tokyo[2]

A 40 y.o. female (Japanese) was admitted to the Hospital of Tokyo University on Jan. 24, 1989, suffering from malaria with disturbance of her consciousness. She had been to Indonesia (the Lombok Island) from Dec. 23, 1988 to Jan. 16, 1989 for sight-seeing. She had no episode of malaria. No prophylaxis was done against malaria during the trip. Symptoms of remittent fever and diarrhea started on Jan. 16, the day of arrival to Japan.

Clinical signs on admission were as follows: BP 90/60, tachycardia (104/min, regular). Consciousness was drowsy. Skin and tongue were dehydrated. Bleeding from gum and genital organ were noticed. Ascites and pleural effusion were observed but splenomegaly and hepatomegaly were not detected. On laboratory examination parasitemia was remarkable ($10^6/mm^3$). Anemia, azotemia (BUN 56, Cr 2.4), hepatocellular injury (GOT 73 IU, GPT 88 IU) and DIC (Plt $1.5\times10^4/mm^3$, FDP 19.1, Fibrinogen 178).

Treatment for malaria was initiated by oral administration of chloroquine and sulfadoxine-pyrimethamine on the first day. Fever attacks ceased gradually after the treatment but, on 11th day of admission, high fever of 39°C started again. Parasitemia was still present (only small number of ring-form schizonts as well as gametocytes) therefore we suspected this case as a low sensitive one to malaria drugs (chloroquine or/and pyrimethamine). Fansimef (compounds of mefloquine and sulfadoxine-pyrimethamine) was given on 21th day and shown to be effective. Fever disappeared and other clinical symptoms were recoverd.

58 USE OF QUINIDINE IN TREATMENT OF IMPORTED *PLASMODIUM FALCIPARUM* MALARIA WITH SPECIAL EMPHASIS ON A CASE RESISTANT TO SULFADOXINE/PYRIMETHAMINE

HIROYUKI AMANO[1], TOMOKO NISHIO[2], TOSHIMASA NISHIYAMA[3], YUZO TAKAHASHI[3] AND TSUNEJI ARAKI[3]

Departments of Overseas Medical Services[1] and Internal Medicine[2], Tenri Hospital and Department of Parasitology, Nara Medical University[3]

The antimalarial efficacy of quinidine (diastereoisomer of quinine) was found over one century ago. But usually it has been used only as an antiarrhythmic drug because of its cardiotoxicity. Recently, clinical trials have been made to use quinidine for malaria mainly due to the appearance of drug resistant *Plasmodium* and difficult availability of conventional antimalarial drugs. Bunnnag *et al.* (1987) reported that the administration of quinidine sulfate (600 mg×3/day per oral) showed the cure rate of 100% to chloroquine resistant *Plasmodium falciparum* malaria, and that no cardiotoxicity was found although the diarrhea and vomiting was common during its treatment. Moreover, the MIC of quinidine in their study was about one half of that of quinine and the blood concentration of quinidine was exceeded the MIC.

We tried combination therapy of quinidine sulfate and doxycycline to a case resistant to sulfadoxine/pyrimethamine with imported *Plasmodium falciparum* malaria. The patient, a 32-year-old Japanese man had the febrile attack on his way home from lake-side area of Tanganyika, Zaire in April, 1989.

Self-treatment with chloroquine, sulfadoxine/pyrimethamine and quinine failed to eliminate his malarial symptoms. He was seen in our hospital 17 days after onset of the disease. On addmission, his temperature was 39.8°C by axilla, the patient had a clear consciousness although complained of severe fatigue, headache, muscular and joint pain. The diarrhea, anemia, jaundice, bleeding tendency and hepatosplenomegaly were not found. The important laboratory findings were: hemoglobin 12.9 g/dl, platelet $13.7\times10^4/\mu l$, CRP 8.2 mg/dl, BUN

13.0 mg/dl, creatinine 1.1 mg/dl, cholesterol 121 mg/dl, serum albumin 3.8 g/dl, total bilirubin 1.1 mg/dl, haptoglobin 4.2 mg/dl, LDH 932 IU, GOT 34 IU, GPT 45 IU and alkaline phosphatase 284 IU. The thin blood smear revieled the ring form of *Plasmodium falciparum* and the parasitemia was 5,084/μl.

The treatment with sulfadoxine/pyrimethamine (total 14 tablets used) was started on the second hospital day, however, parasitemia did not completely disappear. It re-increased from 8 days after stopping the drugs and 2 days later the patient had a high fever of 38.9°C (R2 resistant type). Then, oral quinidine sulfate (500 mg×3/day, 5 days) and parenteral DOXY (200 mg×2/day, 5 days) were added, resulting in the sufficient response to his malaria. The clearance of fever and parasitemia was 48 hours and 72 hours, respectively. No side effects except the diarrhea was found. ECG was recognized as almost same of the base-line and the dysrhythmia did not appeare. Additionally we had the experience of quinidine/DOXY therapy to 34-year-old man and 41-year-old woman with *Plasmodium falciparum* malaria. They were treated with sulfadoxine/pyrimethamine which resulted in successful response, and quinidine was added (1.8-1.5×5 days and 1.2×4 days, respectively). During the treatment, the former complained of diarrhea and fatigue, while the latter nausea and vomiting. Neither cardiotoxicity nor ECG change were seen.

It is worth noting that quinidine is effective even to drug resistant *Plasmodium falciparum* with minor side effects.

59 THREE CASES OF MALARIA WITH SERIOUS CLINICAL MANIFESTATIONS

KIYOJI ASANO[1], YOSHIMI ITO[1], YUKIHIRO HAYAKAWA[1], KUNIHIKO TSUCHIYA[1], MICHIO ARAKAWA[1], HIROYASU ITO[1], SENRI HIRAKAWA[1], KAORI KOGA[2] AND HIROSHI OHTOMO[2]
2nd Department of Internal Medicine[1] and Department of Parasitology[2], Gifu University School of Medicine

We have experienced 5 cases of "imported" malaria (2 of falciparum malaria and 3 of vivax malaria) from January to July in 1989 in the 2nd Department of Internal Medicine at the Gifu University School of Medicine. We report here 3 cases of those who showed serious clinical manifestations.

[Case 1] A 33 year-old female, housewife, who came back from Ghana was admitted to our hospital because of fever of unknown origin. On admission, she had high fever, depressed conscious level with delirium, hallucination, hepato-splenomegaly, ascites, bilateral leg edema, liver dysfunction, severe anemia and mild pericardial effusion. Examination of the blood revealed the ring form and gametocyte of *Plasmodium falciparum* and the diagnosis of falciparum malaria was made. Specific therapy was started with combined tablet of sulfadoxine and pyrimethamine, and tetracycline 2 hours after aimission. At the same time, supportive therapy was intensified. As a result, the number of malarial parasites the red blood cells decreased markedly and the patient became afebril and symptomes reflecting the encephalopathy disappeared on the hospital day 2. Renal and hepatic functions and general condition improved gradually, making a favorable progress.

[Case 2] A 39 year-old male, a staff of faculty of university, came back to Japan from Amazon. He was admitted to our hospital because of remittent fever and dark-colored oliguria. On admission, he had high fever, headache, sore eyes and brown-colored urine. Blood examination revealed ring form of *Plasmodium falciparum* and the diagnosis of the falciparum malaria was made. Therapy with chloroquine was started immediately but parasites remained unchanged. Since parasitemia and general condition did not improve, therapy was changed to that with intravenous quinimax and minocycline. When adequate diuresis occurred, oral quinine hydrochloride was substituted for quinimax and minocycline. As a result, renal function and general condition ameriolated gradually, making a favorable progress.

[Case 3] A 40 year-old male, a company employee, went to Indonesia for mountaineering. Two days after coming back to Japan, he started to have headache with fever. Because of possible malaria, blood examinations were repeated. However, all of the tests were negative, probably because he had been taking Fansidal and it took a week to detect *Plasmodium vivax.* During that period, hepatic and renal functions became deteriorated and coagulation abnormalities developed. The patient responded rapidly to suppressive treatment with chloroquine and tetracycline and there has been no relapse so far by radical therapy with primaquine in combination with the above therapy.

Three cases of malaria described above had serious clinical manifestations and severe complications, indicating that the "imported" malaria is dangerous disease. This suggests that the early diagnosis and pertinent management is very important.

Proceedings of XXXI Annual Meetings of Japanese Society of Tropical Medicine

ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

Vol. 18 No. 1 March 1990

CONTENTS

(Contents continued on reverse of this cover)

Japan. J. Trop. Med. Hyg.

JAPANESE SOCIETY OF TROPICAL MEDICINE