日本熱帯医学会雑誌

第20巻 第1号　　平成4年3月15日

内　　容

（裏頁に続く）

日熱医会誌.　日本熱帯医学会

第33回日本熱帯医学会総会講演英文抄録

会報・記録

日本熱帯医学会雑誌　　発行所　日本熱帯医学会
第20巻　第1号　　編集者　板倉英吾
1992年3月15日　印刷　　印刷所　昭和堂印刷
1992年3月15日　発行　　諫早市長野町1007-2（〒854）
☎0957-22-6000

本雑誌の刊行にあたりその費用の一部を文部省科学研究費補助金
（研究成果公開促進費）によった。

Jpn. J. Trop. Med. Hyg., Vol. 20, No. 1, 1992, pp. 1-9

NATURAL INFECTIONS OF BLACKFLIES WITH LARVAE OF ZOONOTIC *ONCHOCERCA* SPP. IN NORTHEAST JAPAN

HIROYUKI TAKAOKA[1], CHIHARU AOKI[1] AND HIROFUMI HAYAKAWA[2]

Received November 29 1991/Accepted January 16 1992

Abstract: Dissections of female blackflies collected while attacking a cow at the Omyojin Farm, Iwate, northern Japan showed that 4.4% of 1,104 *Simulium daisense,* 0.8% of 913 *S. aokii,* 0.5% of 741 *S. iwatense* and 0.9% of 340 *S. nikkoense* examined were naturally infected with filarial larvae. There were at least two types of third-stage larvae assignable to *Onchocerca,* of which type A found in *S. daisense* and *S. aokii* measured 914-1,212 μm long by 26-29 μm wide while type B found in *S. daisense* had its body size of 369-477 μm long by 14.9-17.9 μm wide. Morphometric observations suggest that types A and B each correspond to types I and III previously recorded in Kyushu, southwest Japan as the third-stage larvae of bovine *Onchocerca* (i.e., *O.* sp. and *O. lienalis*). This is the first record for natural infection of blackflies in Honshu (main island of Japan) with zoonotic *Onchocerca* larvae.

INTRODUCTION

In relation to the transmission of bovine and equine onchocerciasis in Japan, Hosoya *et al.* (1956) dissected several species of *Simulium* and *Culicoides* collected in the endemic areas, such as Tottori in western Honshu, Fukushima and Aomori in northern Honshu, but failed to detect any larvae of *Onchocerca,* though they found some flies infected with larvae of non-filarial nematodes bearing a sharply pointed tail. Ueno *et al.* (1956) also examined about 16,000 females of four *Culicoides* species caught by light traps in Tokyo and found no *Onchocerca* larvae. There was since then no investigation on the vectors of these zoonotic onchocerciasis in Japan. Recently, we found that several blackfly species collected at cattle sheds in Kyushu, southwest Japan were naturally infected with three types of *Onchocerca* larvae, of which two (types II and III) were each suspected as *O. gutturosa* Neumann and *O. lienalis* Stiles parasitizing cattle while the last (type I) remained unidentified (Takaoka and Bain, 1990). Subsequent infection experiments showed that microfilariae from the skins of slaughtered cattle or from the midgut of freshly blood fed blackflies successfully developed in blackflies to the third-stage larvae (L_3) almost identical to those of types I and III, (Takaoka, 1990). From the results of these and other works, it is now clarified that *S. bidentatum* (Shiraki) and *S. arakawae* Matsumura are the natural vectors of type I (*O.* sp.),

1 Division of Medical Zoology, Oita Medical University, Hasama, Oita 879-55, Japan
2 Department of Animal Production, Tohoku National Agricultural Experimental Station, Morioka 020-01, Japan

the former also serves as a vector of type II (probably *O. gutturosa*) and the latter as well as *S. kyushuense* Takaoka is also a natural vector of type III (=*O. lienalis*) in Kyushu (Takaoka, in preparation).

The present work was done in Iwate, northeast Japan to determine whether some other blackfly species are also involved in the transmission of zoonotic onchocerciasis. In this paper, two more blackfly species are added as a natural vector of zoonotic *Onchocerca* spp. (probably *O.* sp. and *O. lienalis*).

Material and Methods

Collections of adult blackflies were carried out at the Omyojin Farm (at about 40° N lat. and 141° E long.; 230-270 m in altitude), Faculty of Agriculture, Iwate University, at Shizukuishi-machi, Iwate Prefecture, in northeast Japan, from April to November in 1989. About 80 cattle were bred in the pasture (ca. 45 ha) of this farm. An hourly 10-minute collection of flies attacking a cow tethered to the wooden fence was made once a month using an insect net by one man, for 24 hrs starting from 11:00 hr. Air temperatures were hourly measured at the end of each collection. This regular collection was attempted to determine the seasonal pattern in biting activity of blackflies. Females captured were preserved in small vials with 80% ethanol solution, and sent to the laboratory in Oita. After being identified to species, these specimens were dissected in 5% Giemsa solution on glass slide under dissecting microscope and examined for filarial larvae. In addition to samples from regular collections, portions of female blackflies captured by a cow-baited trap and/or a CO_2 trap which were set at the same place and time were, if necessary, also dissected in the similar manner. Morphometric observations were microscopically made for all filarial larvae detected. Generic diagnosis of L_3 larvae followed that of Bain and Chabaud (1986).

Results

Table 1 shows the results of regular collections. At least 12 blackfly species belonging to *Prosimulium, Cnephia* and *Simulium* were collected. The biting activity of *Pro. yezoense* Shiraki was high in May but very low or nil in the following months. On the other hand, biting activity of most *Simulium* species became marked after May, continued to be relatively high until August with their peak in June or July and decreased in September. Thereafter, biting activity was still observed in several species but in small numbers except *S. aokii* (Takahasi). Among *Simulium* species, the most abundant were *S. aokii* and *S. daisense* (Takahasi), followed by *S. iwatense* (Shiraki) during the summer months from June to August.

In view of the critical air temperature of ca. 17°C below which larval development of *O. volvulus* (Leuckart) in the intermediate blackflies would cease (Takaoka *et al.*, 1982), dissections for natural infections with any filarial larvae were made only for samples of four *Simulium* species which were relatively abundant during a period from June to September when air temperatures were for the most part beyond 17°C (Fig. 1). The results of dissections are presented in Table 2.

All the four species dissected harboured filarial larvae in their thoracic and/or cephalic regions. The overall infection rates varied from 0.5% to 4.4% depending on the species.

Table 1 Number of female blackflies captured by a sweeping net on cattle at the Omyojin Farm, Iwate, northeast Japan, in 1989*

Blackfly species	Months of collection							
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.
Prosimulium yezoense	0	398	9	1	0	0	0	0
Other *Prosimulim* spp.	26	49	0	0	0	0	0	0
Cnephia sp.	2	19	0	0	0	0	0	0
Simulium uchidai	0	0	4	1	0	0	0	0
S. aokii	9	10	146	281	207	12	55	189
S. arakawae	0	3	0	11	16	0	2	1
S. daisense	0	0	722	201	108	9	5	0
S. iwatense	43	2	132	91	28	0	6	2
S. japonicum/kawamurae	4	50	2	8	0	2	34	0
S. nacojapi	0	0	0	1	0	0	0	0
S. nikkoense	0	1	16	71	15	0	0	1
S. rufibasis	0	0	3	3	1	0	0	0
Total	84	532	1,034	669	375	23	102	193

*: An hourly 10-minute collection was made for consecutive 24 hrs by one man, once a month.

Table 2 Natural filarial infections of blackfies collected on cattle at the Omyojin Farm, Iwate, northeast Japan

Blackfly species	Months of collection				Total
	Jun.	Jul.	Aug.	Sep.	
S. aokii					
No. infected/no. dissected	3/210	1/247	2/310	1/146	7/913(0.8%)
No. and stage[a] of larvae	$4L_1$	$1L_3$	$2L_1$	$4L_2$	
No. larvae/infected fly					
Median (Range)	1(1-2)	1(1)	1(1)	4(4)	
S. daisense					
No. infected/no. dissected	17/569[b]	29/202	1/281	1/52	48/1,104(4.4%)
No. and stage of larvae	$54L_1+1L_2$	$6Mf+56L_1+29L_2+39L_3$	$2L_1$	$3L_2$	
No. larvae/infected fly					
Median (Range)	2(1-13)	4(1-27)	2(2)	3(3)	
S. iwatense					
No. infected/no. dissected	0/176	3/499[b]	1/66	—/—	4/741(0.5%)
No. and stage of larvae	—	$2Mf+2L_1+2L_2$	$2L_1$		
No. larvae/infected fly					
Median (Range)		2(1-3)	2(2)		
S. nikkoense					
No. infected/no. dissected	—/—	2/312	1/28	—/—	3/340(0.9%)
No. and stage of larvae		$8L_1+2L_2$	$23L_1$		
No. larvae/infected fly					
Median (Range)		4(1-9)	23(23)		

a. Mf, microfilaria; L_1, L_2 and L_3 represent first-, second- and third-stage larvae, respectively

b. A larva of non-filaria nematode was found in the thorax of one *S. daisense* and one *S. iwatense*

Table 3 Measurements of third-stage larvae (L_3) of *Onchocerca* spp. found in *Simulium aokii* and *S. daisense* collected at the Omyojin Farm, Iwate, northeast Japan

L_3 no.	Type of L_3	Host *Simulium* spp. and body parts		Landmarks*				
				BL	BW	OL/BL	TL	TL/TW
1	A	*S. aokii*	thorax	1,212.7	26.6	0.49	40.0	1.79
2	A	*S. daisense*	thorax	914.2	28.0	—	—	—
3	A	〃	〃	947.8	29.1	0.46	33.5	1.29
4	A	〃	〃	1,059.7	26.9	0.49	43.7	1.83
5	A	〃	〃	1,179.1	26.1	0.43	—	—
6	B	〃	head	369.4	14.9	0.66	—	—
7	B	〃	〃	373.1	17.9	0.67	—	—
8	B	〃	〃	403.0	16.8	0.62	33.5	2.25
9	B	〃	〃	410.5	16.8	0.61	—	—
10	B	〃	〃	440.3	16.8	—	33.5	2.01
11	B	〃	〃	447.8	17.9	0.62	—	—
12	B	〃	〃	447.8	16.8	0.65	29.1	2.05
13	B	〃	〃	447.8	15.7	0.58	—	—
14	B	〃	〃	451.5	15.7	0.69	29.8	1.95
15	B	〃	〃	462.7	16.8	0.66	—	—
16	B	〃	〃	462.7	—	0.68	—	—
17	B	〃	〃	466.4	16.4	0.68	—	—
18	B	〃	〃	477.6	16.4	0.63	33.5	2.25

* : BL, body length; BW, maximum body width; OL/BL, length ratio of oesophagus/whole body; TL, tail length; TL/TW, ratio of tail length/tail width.
— : not measured.

Filarial larvae found were at various developmental stages (Fig. 2a-h), of which L_3 larvae were found only in 1 or 0.4% of 247 *S. aokii* and 7 or 3.5% of 202 *S. daisense* examined in July.

There are apparently two types of L_3 larvae judging from the body size and relative length of oesophagus to whole body, as shown in Table 3. The longer type (designated as type A; Fig. 2h) was found in both of *S. aokii* and *S. daisense* whereas the shorter one (type B; Fig. 2f) was detected only from the latter blackfly species. Of the seven *S. daisense* infected with L_3 larvae, three had type A, while the others harboured type B. One of the four females with L_3 of type B was also infected with two preinfective larvae assignable to type A (Fig. 2g) due to long body size (630.6 μm and 570.9 μm) and short oesophagus (0.36× and 0.34× body length).

The L_3 larvae of type A were all found in the thorax and their number per infected fly was one or two, whereas those of type B were mostly recovered from the cephalic region and their number varied from 1 to 27. In one female infected with 27 L_3 larvae, all but one were found in the head, in particular, clustering within labrum of the mouthpart (Fig. 3).

Several large second-stage larvae (L_2) (e.g. Fig. 2g) measuring ca. 400-780 μm in body length (i.e., advanced L_2 or preinfective) found in *S. daisense* were evidently the same species as the L_3 designated type A. However, classification was impossible for most of other smaller L_2 (body length ca. 200-290 μm and oesophagus about 1/2× the body length) including four L_2 larvae found in *S. iwatense* and *S. nikkoense* Shiraki (Figs. 2d and 2e) as well as first-stage

larvae (L_1) (ca. 90-170 μm long) (Figs. 2b and 2c).

Microfilariae (Fig. 2a) were found in the thorax of one *S. iwatense* and three *S. daisense*. In the latter three females, L_1 and/or L_2 larvae were also found. All these microfilariae were similar in size (i.e., 186.6-223.9 μm long by 5.0-5.9 μm wide), were unsheathed and had a slender pointed tail.

Two larvae of non-filarial nematode were each found in the thorax of *S. iwatense* and *S. daisense*. Both of these larvae had a sharply pointed tail (Fig. 2i) and measured 492.5 μm long by 14.9 μm wide and 856.0 μm long by 31.3 μm wide, respectively.

DISCUSSION

The present work shows that four blackfly species attacking cattle in northeast Japan were naturally infected with at least two types of filarial larvae developing in the fly's thorax, which are probably assignable to the genus *Onchocerca* by the very small, caudal lappets of L_3 larva.

Out of two types, type A found in *S. daisense* and *S. aokii* is characterized by the very long body (average 1,062.7 μm) and the short oesophagus {(0.43-0.49) × the body length}. With these characters type A does not fit with any known *Onchocerca* species (Bain and Chabaud, 1986), and reminds us of type I, L_3 of *Onchocerca* sp. (body length, 1,075-1,380 μm; ratio of oesophagus/body length, 0.45-0.50) which was reported from wild caught *S.*

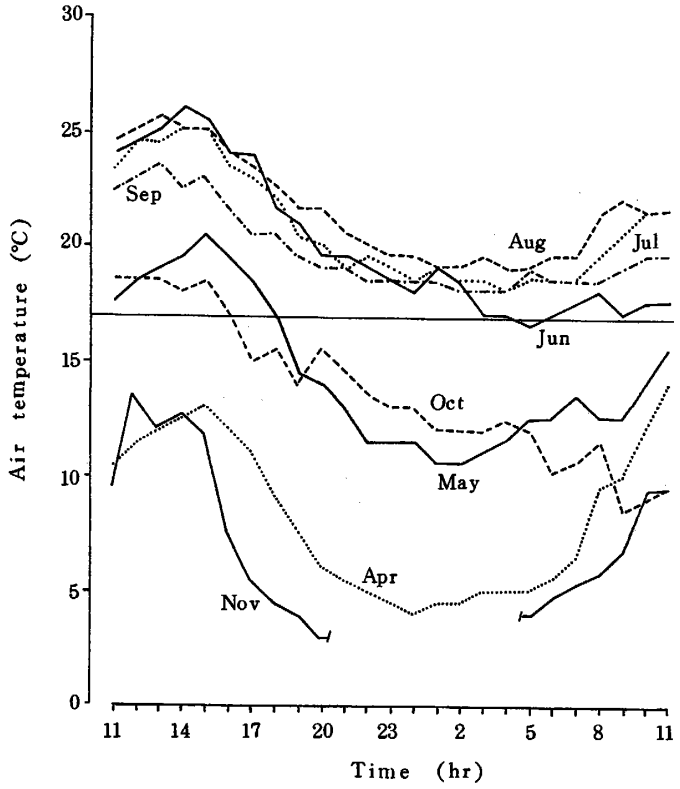

Figure 1 Daily fluctuation of air temperatures by months at the Omyojin Farm, Iwate, northeast Japan. Horizontal line shows a critical air temperature of ca. 17°C for development of *Onchocerca volvulus* larvae in the vector blackfly. Data on air temperatures from 21:00 to 04:00 hr in November were not available due to the cessation of the collection during the night.

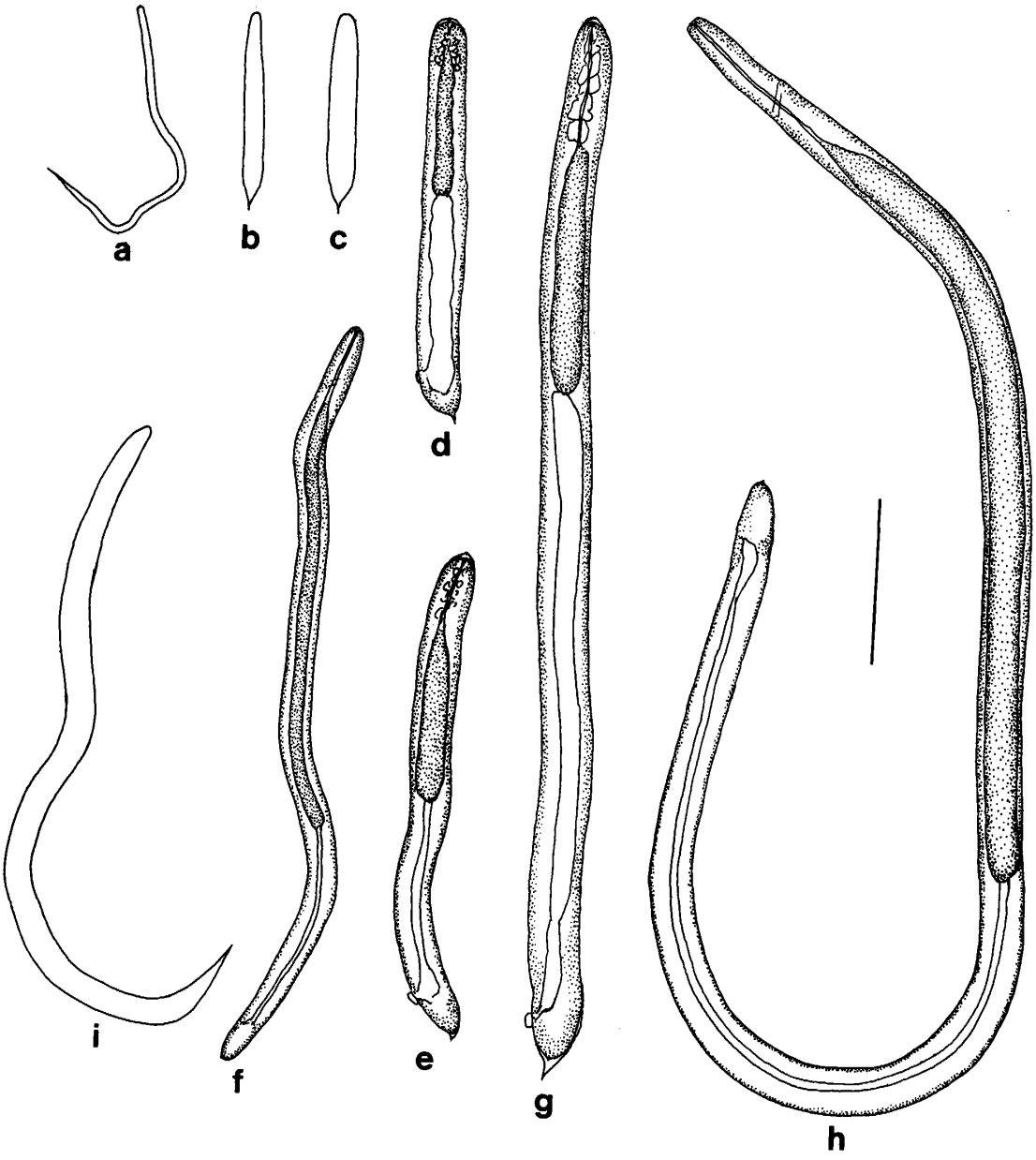

Figure 2 Filarial and non-filarial larvae found in *Simulium* spp. caught on a cow at the Omyojin Farm, Iwate, northeast Japan. a, microfilaria found in *S. iwatense*; b and c, first-stage larvae found in *S. daisense*; d, second-stage larva found in *S. iwatense*; e, second-stage larva found in *S. nikkoense*; f, third-stage larva of *Onchocerca* sp. (type B) found in *S. daisense*; g, preinfective larva of *Onchocerca* sp. (type A) found in *S. daisense*; h, third-stage larva of *Onchocerca* sp. (type A) found in *S. aokii*; i, non-filaria larva found in *S. iwatense*. All larvae were found in the thorax except type B larva (f) which was found in the head. Scale=100 μm.

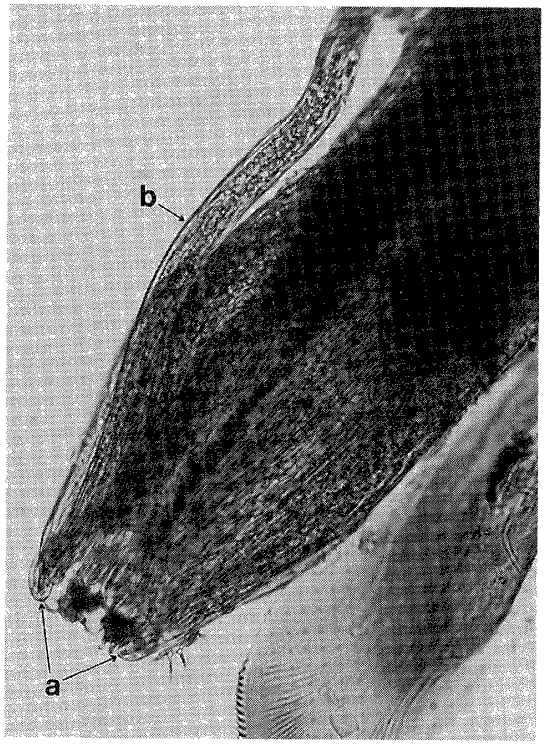

Figure 3 The labrum of the mouthpart of *Simulium daisense* clustered with many third-stage larvae of *Onchocerca* sp. (type B). a, anterior tip of two larvae; b, middle part of larval body.

bidentatum and *S. arakawae* (Takaoka and Bain, 1990) and was soon proved to be of bovine origin in Kyushu (Takaoka, 1990). In fact, it is difficult to separate type A from type I. The L_3 larvae of *Dirofilaria ursi* Yamaguti of bears and *D. immitis* (Leidy) of dogs are somewhat similar to those of type A in having a long body size and small caudal lappets but their development takes place in the Malpighian tubules of the blackflies (Addison, 1980; Takaoka and Baba, 1987).

Type B, found in *S. daisense,* seems to conform to type III, L_3 of *O. lienalis* parasitizing cattle, reported from *S. arakawae* and *S. kyushuense* in Kyushu too (Takaoka and Bain, 1990; Takaoka, in preparation). The body length of type B (369.4-477.6 μm, average 435.4 μm) is shorter than that of type III (510-530 μm) reported by Takaoka and Bain (1990). This gap is attributed mainly to the difference in the fixative solution used (ethanol vs. formalin).

The finding that L_3 larvae of type B were found together with preinfective larvae of type A in the same fly, though once only, strongly suggests that both of these types share the same animal as a definitive host.

From these results as well as high biting and infection rates of *S. daisense* caught on the cow, it is likely that both type A and B are a bovine parasite. However, further studies are needed to confirm this observation since no data has been available yet on the *Onchocerca* infection in cattle bred in and near the Omyojin Farm, and also on the biting preference of these blackflies to other animals. Whether or not the filarial larvae found in *S. iwatense* and *S. nikkoense* are a bovine *Onchocerca* remained to be studied.

Our results could add two more blackfly species, *S. daisense* and *S. aokii,* as a natural vector of zoonotic *Onchocerca* in Japan, though the latter species has been reported as an experimental vector of type I in Kyushu (Takaoka, 1990).

The biting activities of blackflies were observed throughout the surveyed period from April to November. And, four of these eight months (i.e., from June to September) were expected as the probable period for *Onchocerca* transmission in and near the Omyojin Farm from the air temperature conditions. However, actual transmission time of these zoonotic *Onchocerca* spp. would be more restricted, because female blackflies carrying L_3 larvae were collected only in July.

Acknowledgements

We would like to thank Prof. H. Itagaki, Azabu University, and Prof. I. Inoue, Nihon University, Kanagawa, for their kindness in providing valuable information and references on bovine onchocerciasis and its transmission in Japan. Our appreciation goes to Miss K. Ogata, Medical College of Oita, for her assistance in dissecting flies. This study was in part supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 01570216).

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東北地方のブユ成虫におけるオンコセルカ幼虫自然感染

高岡　宏行[1]・青木　千春[1]・早川　博文[2]

岩手県雫石町御明神牧場において，1989年4月から11月にかけて，牛囮法で合計12種のブユを得た。6月－8月に比較的捕獲数の多い *Simulium aokii, S. daisense, S. iwatense* および *S. nikkoense* の4種3,098個体を解剖し，それぞれ0.8% (7/913)，4.4% (48/1,104)，0.5% (4/741)，0.9% (3/340) にフィラリア幼虫の感染を認めた。*S. aokii* を除く他の3種のブユからは，今回初めてフィラリア幼虫が見付かった。第三期幼虫 (L_3) は，7月に採集された *S. aokii* および *S. daisense* から得られた。L_3の形態から2種が含まれていることが分かった。type Aは両種ブユから見いだされたが，type Bは *S. daisense* からのみ得られた。type AおよびBは，それぞれ九州のブユから得られている *Onchocerca* type I, II, III のうちのtype I (牛に寄生する *O.* sp.) とtype III (*O. lienalis*) と思われる。他の2種のブユからはL_3は得られなかったので，フィラリア種の同定は出来なかった。動物寄生性オンコセルカに感染された人体症例が，既に九州から報告されているが，今回の調査により，本州においてもブユの媒介による，同様の人体感染例が発生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　農林水産省東北農業試験場家畜虫害研究室

Jpn. J. Trop. Med. Hyg., Vol. 20, No. 1, 1992, pp. 11-21

ULTRASTRUCTURAL STUDIES ON CUTANEOUS LEISHMANIASIS IN ECUADOR

ABDUL MANAN BHUTTO[1], SHIGERU OKADA[1], SHIGEO NONAKA[1], EDUARDO A. GOMEZ L.[2] AND YOSHIHISA HASHIGUCHI[3]

Received December 4 1991/Accepted January 21 1992

Abstract: Ultrastructural observations were made of the lesions of three Ecuadorian patients with cutaneous leishmaniasis. Parasites were located both within the macrophages, either inside the intracytoplasmic vacuoles (parasitophorous vacuoles) or free in cytoplasm and outside host cells. Amastigotes were rounded or oval with a mean length of 2.62 μm ($\pm$0.17 S.D.) and mean width of 2.18 μm ($\pm$0.28 S.D.). Parasites showed degeneration intracellularly both within the vacuoles and in the cytoplasm of macrophages. Lymphocytes were seen in close contact with parasitized macrophages as well as directly attached to the parasites. Furthermore, spongiotic vesicle was observed in the epidermis where *Leishmania* parasites were found, surrounded by lymphocytes and other mononuclear cells. Amastigotes attached to mononuclear cells were also observed inside and between the keratinocytes. Mononuclear cells containing melanin granules showed amastigotes in their cytoplasm.

The parasite-macrophage relationship, the role of T-cells in combating the parasites and the fate of the parasites inside the host body are discussed.

INTRODUCTION

Cutaneous leishmaniasis is a tropical disease distributed widely in both the Old and New World. It is a serious dermatological problem, especially in the two continents of Africa and South America. The disease can be classified largely into two forms: an Old World form caused mainly by *Leishmania tropica* complex and a New World form caused by *Leishmania* (*Viannia*) *braziliensis* and *L.* (*Leishmania*) *mexicana* complexes (Lainson and Shaw, 1987; Pearson and Sousa 1985). In order to obtain information on the clinical, epidemiological and immunological features of the latter form of the disease in Ecuador we performed detailed investigations at different endemic sites. The results have already been reported by Nonaka *et al.* (1990a, b) and Hashiguchi *et al.* (1990, 1991).

Cutaneous leishmaniasis is prevalent throughout Ecuador and six *Leishmania* species (*L. braziliensis, L. panamensis, L. guyanensis, L. amazonensis, L. mexicana* and sp. near *L. major*) have been recorded from the country to date based on zymodeme, serodeme and schizodeme analysis of samples from humans and animals (Mimori *et al.*, 1989; Hashiguchi *et al.*, 1990,

1 Department of Dermatology, Nagasaki University, School of Medicine, Nagasaki 852, Japan.
2 Departamento de Medicina Tropical, Facultad de Medicina, Universidad Catolica Santiago de Guayaquil, Guayaquil, Ecuador.
3 Department of Parasitology, Kochi Medical School, Nankoku, Kochi 783, Japan.

1991; Armijos *et al.*, 1990). Morphology and ultrastructure are also useful in differentiation of *Leishmania* species, although less information is available on these methods.

This article reports the results of an ultrastructural study of the cutaneous lesions of Ecuadorian leishmaniasis patients, and is intended to give a better understanding of the morphology and nature of *Leishmania* parasites in the country.

Materials and Methods

Patients:

The Ecuadorian patients with cutaneous leishmaniasis, being of different ages and sex, were diagnosed on the basis of clinical features and history. The clinical and histopathological features of the three patients, i.e., I-02 (*L. guyanensis* infection), I-04 (*L. panamensis* infection) and I-25 (*Leishmania* sp., as yet characterized) were described in a previous articles (Nonaka *et al.*, 1990a, b). None of these patients had received treatment before biopsies were taken.

Processing of biopsy material:

Four-millimeter punch biopsies were taken under local anaesthesia from the edge of ulcers or nodules and fixed in different fixatives. The biopsy material was divided into two parts, one of which was fixed in 10% formalin and then embedded in paraffin. Five-micron sections were cut and stained with haematoxylin-eosin. The other part was cut into small pieces and fixed in cold 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The tissues were then washed with 0.1 M cacodylate buffer, and post fixed in 2% osmium tetraoxide for 2 hrs. After dehydration in different concentrations of alcohol, the specimens were embedded in Epon 812. One micron semi-thin sections were cut with a glass knife on an LKB ultrotome and stained with toluidine blue. Ultra-thin sections were cut with a diamond knife, stained with lead citrate and uranyl acetate, and examined under a JEM 1200 EX electron microscope (JEOL Japan).

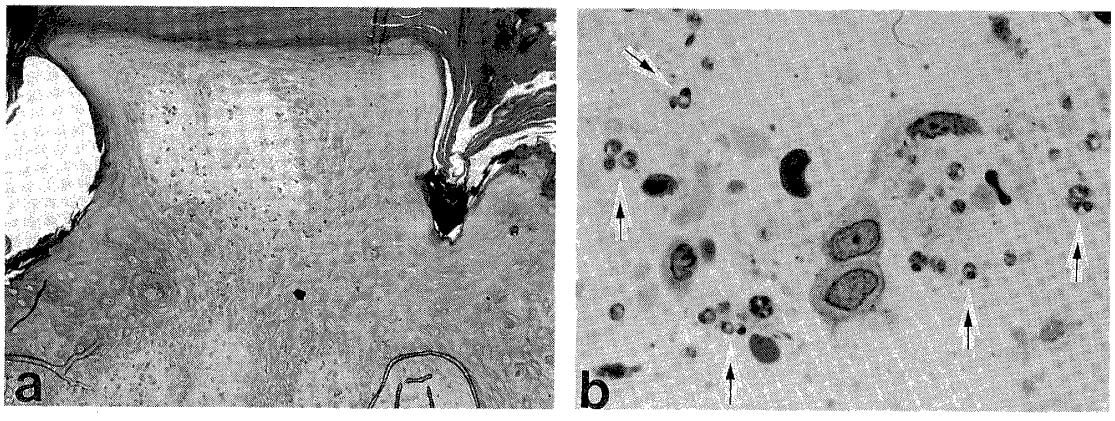

Figure 1 Light micrograph of an epon embedded semi-thin section of cutaneous leishmaniasis. a) spongiotic vesicle in the epidermis
b) arrows indicate the amastigotes inside the epidermal vesicle.
(Toluidine blue stain ×100, ×400)

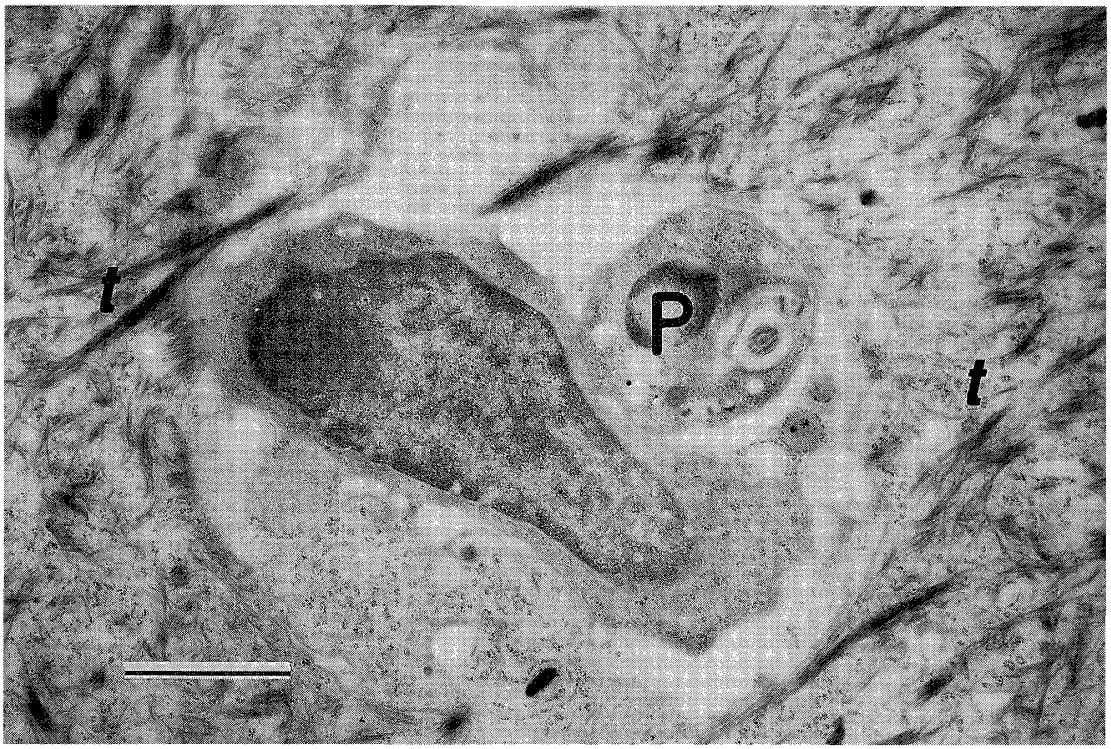

Figure 2 Electron micrograph of cutaneous leishmaniasis in Ecuador. *Leishmania* parasite (P) is in close contact with the mononuclear cell surrounded by tonofibrils (t) in the epidermis. bar=2 μm.

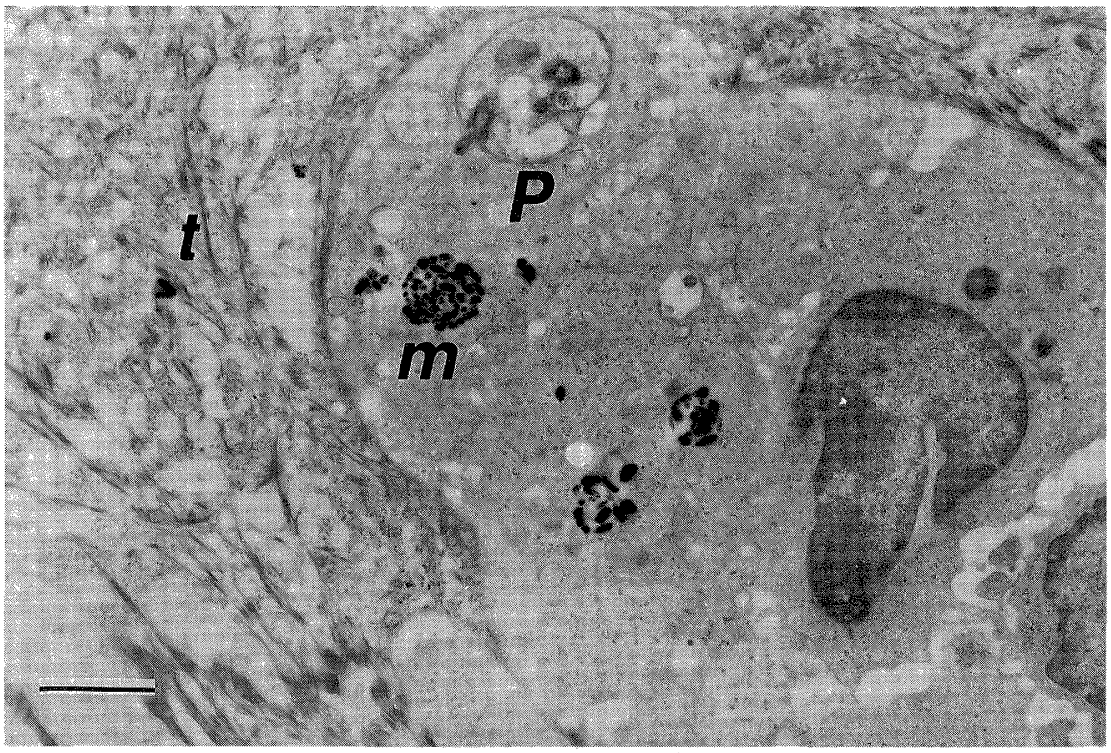

Figure 3 In the epidermis, a mononuclear cell with melanin granules (m) containing one *Leishmania* parasite (P) in its cytoplasm. (t) indicates the tonofibrils. bar=2 μm.

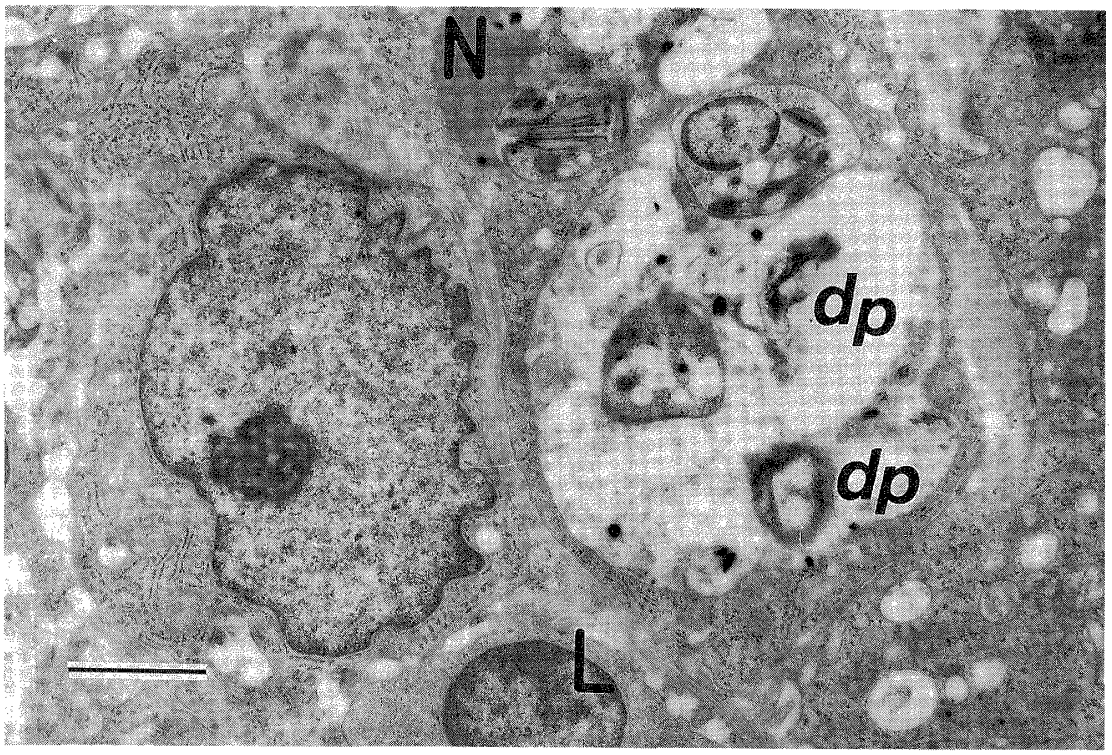

Figure 4 In the dermis, degeneration of some *Leishmania* parasites (dp) is visible inside the parasitophorous vacuole of macrophage. The host cell appears to be under parasitic attack and shows damage to the host cell nucleus (N). A lymphocyte (L) is also visible near the vacuole. bar=2 μm.

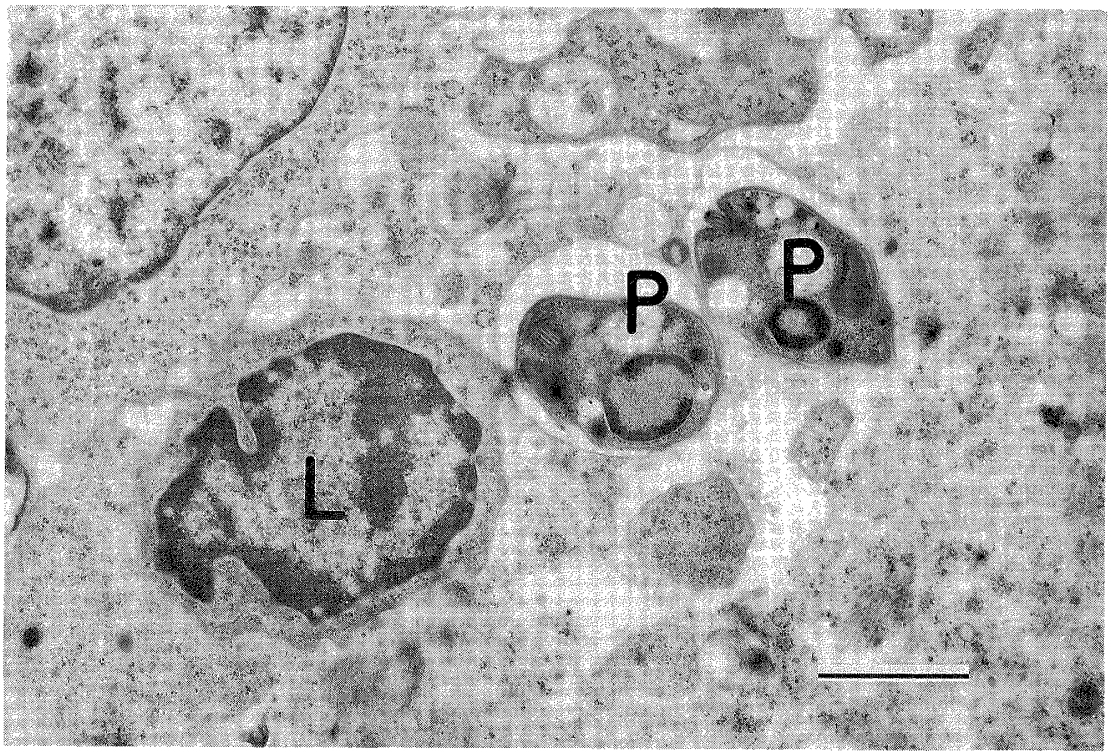

Figure 5 A lymphocyte (L) showing direct attachment to the parasites (P) in the dermis. bar=2 μm.

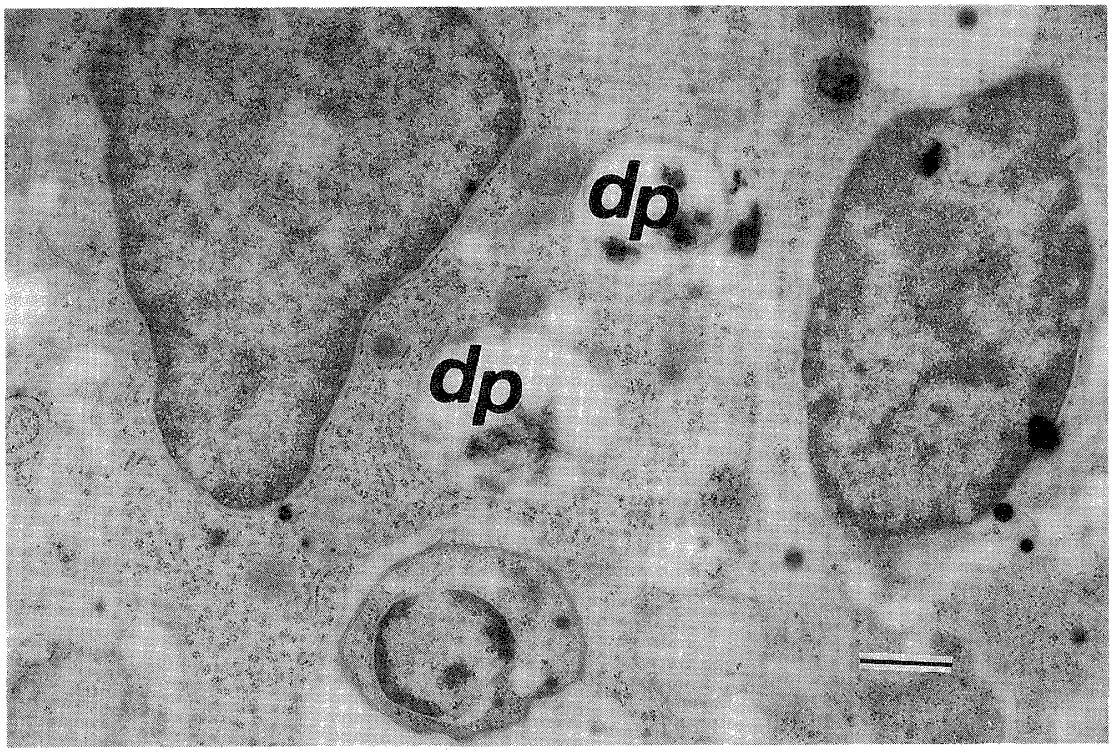

Figure 6 Degeneration of parasites (dp) inside the cytoplasm of host macrophage, with one parasite still intact. bar=1 μm.

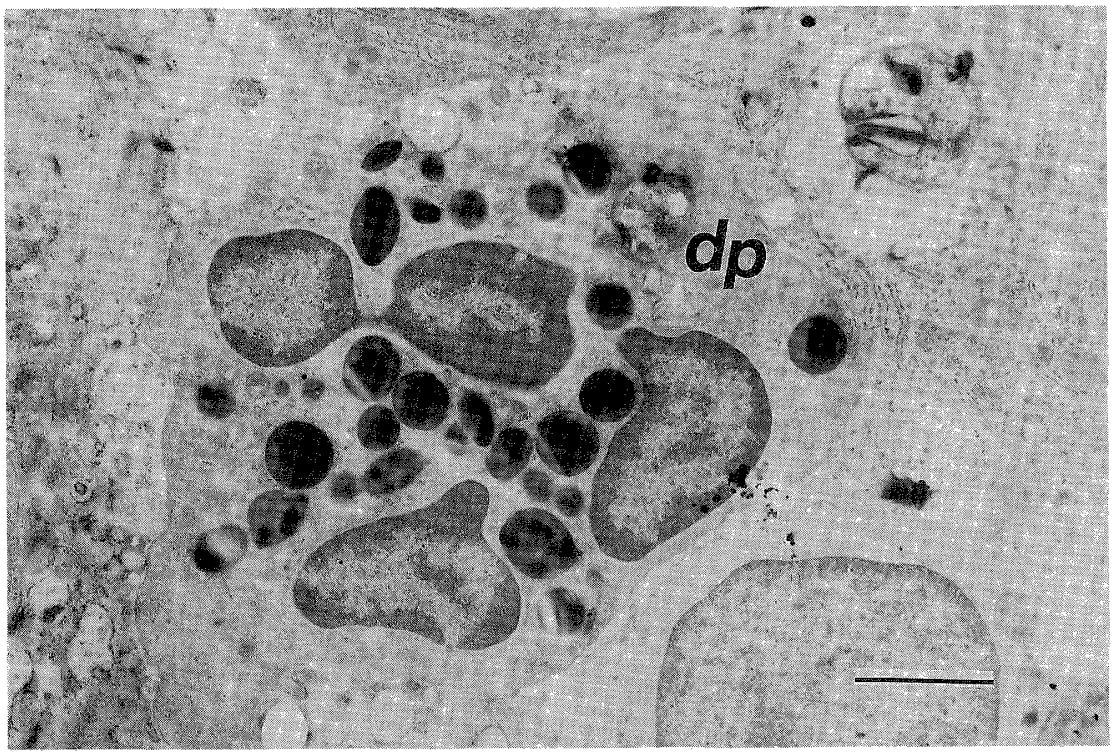

Figure 7 *Leishmania* parasite undergoing a degeneration process (dp) in the cytoplasm of eosinophil. One parasite seems to be outside the cell. bar=2 μm.

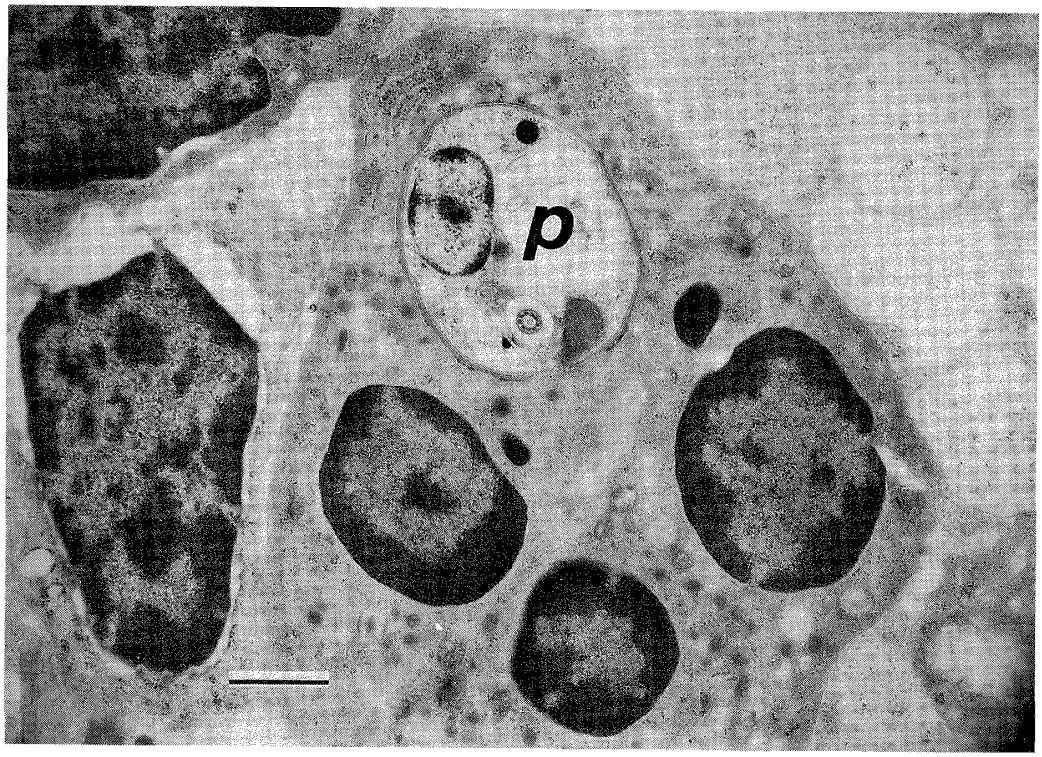

Figure 8 *Leishmania* parasite (P) is present in the cytoplasm of neutrophil. bar=1 μm.

RESULTS

Light microscopic observations:

Along with other minor alterations, an intraepidermal spongiotic vesicle was observed in the epidermis of one (I-25) of the three patients (Fig. 1). *Leishmania* parasites and mononuclear cells were observed inside the epidermal vesicle and between the keratinocytes. The dermis showed cellular infiltration with a great number of amastigotes.

Electron microscopic observations:

1. Epidermis:

Leishmania parasites inside the epidermal vesicle were found in ultra-thin sections, where lymphocytes and other mononuclear cells were present near the parasites. Parasites were also observed in and between the keratinocytes, either attached to mononuclear cells that may have been macrophages (Fig. 2) or free in the microvesicle abscesses. A mononuclear cell containing melanin granules had a *Leishmania* parasite in its cytoplasm (Fig. 3).

2. Dermis:

Parasites were found both intracellularly and extracellularly. Amastigotes located in the cytoplasm of macrophages either inside or outside the parasitophorous vacuoles. Parasites in the macrophages were varied from 1 to 14 in number either inside the vacuole or free in the cytoplasm, showed the multiplication of parasites inside the cells. Usually, one parasitized vacuole was seen in one macrophage. Many host macrophages were seen activated against the phagocytized parasites. Electron-dense granules were frequently observed in the

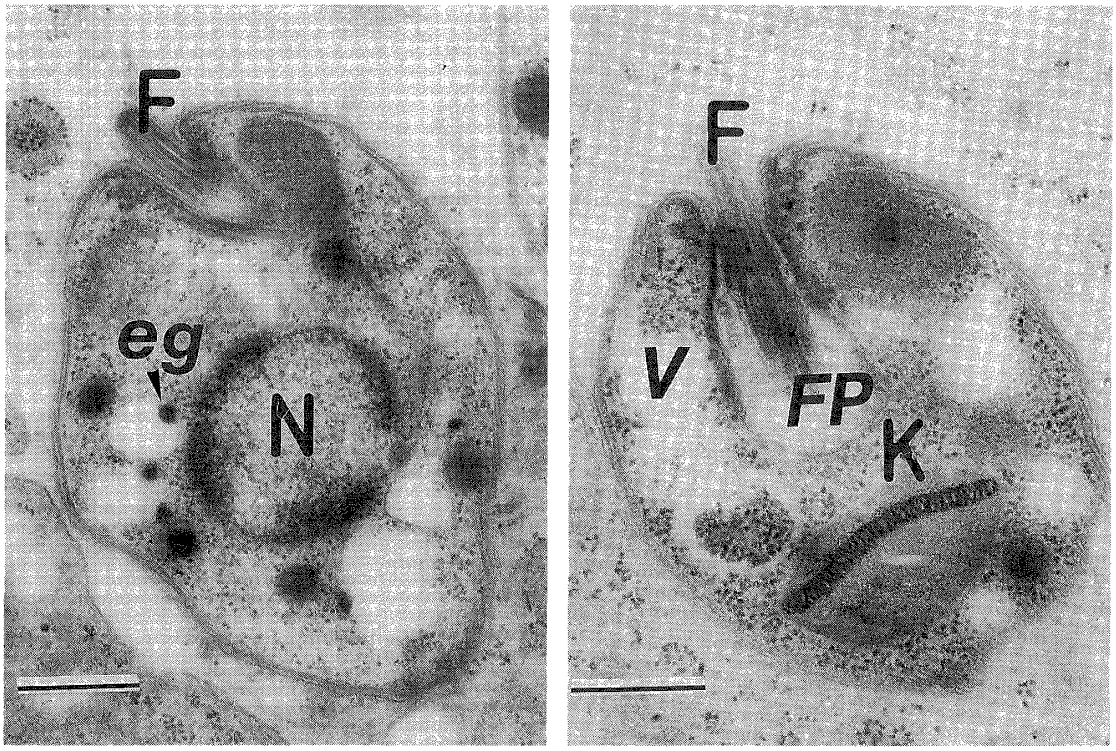

Figure 9 Ultrastructure of the *Leishmania* parasite. F=flagellum, N=nucleus, eg=electron dense granules inside the vacuole, FP=flagellar pocket, V=vacuole and K=kinetoplast. bar=500 nm.

parasitophorous vacuoles or outside the cell near the parasites. Specific lymphocytes were active in the specimens; some were in close contact with parasitized-macrophages (Fig. 4) and some were directly attached to the parasites (Fig. 5). Intracellular degeneration of parasites was observed inside the cytoplasm of the macrophages (Fig. 6). Degeneration of parasites was also noted inside the vacuole of macrophages that were almost destroyed by infections (Fig. 4). Parasites were also located in the cytoplasm of the eosinophils (Fig. 7) and neutrophils (Fig. 8) but no cellular vacuoles were seen in these cells. Almost similar changes were noted in the dermis of all three specimens.

3. Ultrastructure of amastigote:

The amastigotes seen were rounded or oval and of almost uniform size. The mean length was 2.62 μm ($\pm$0.17 S.D.) and the mean width was 2.18 μm ($\pm$0.28 S.D.). The amastigotes were surrounded by two layers of membranes and contained a rounded nucleus with a small nucleolus. The flagellum, flagellar pocket, kinetoplast, vacuoles and electron-dense granules could be distinguished (Fig. 9).

Discussion

Various microscopic and histologic changes have been reported in the epidermis of lesions of cutaneous leishmaniasis, whether infected with New World or Old World *Leish-*

mania species (Zaar *et al.*, 1982; Grimaldi *et al.*, 1980). We observed amastigotes associated with lymphocytes and other mononuclear cells in spongiotic vesicles within the mid-epidermis. Mononuclear cells that may be macrophages, were seen in close contact with amastigotes. The close contact of these antigen-presenting cells with *Leishmania* suggests their capacity to recognize the parasite and facilitate its destruction. We also noted melanin granules in macrophages infected with more than one amastigote, again within the epidermis. The purpose of these melanin granules is unknown but leukocytes carrying similar bodies have been observed in the blood of healthy humans, reptiles and amphibians (Wasserman, 1965), melanin-containing macrophages are present within the epithelium of certain groups of fish. The histological studies of Kurban *et al.* (1966) revealed the presence of amastigotes in the prickle cell layer of 3 of 27 human cases, associated with intra-epidermal abscesses. Our findings suggest that, as in Old World species, New World *Leishmania* can infect the epidermis, and that keratinocytes appear to play an active part in cellular defence against the parasites.

Amastigote morphology was similar to that described for other *Leishmania* species, and no marked differences were discerned between biopsy material from patients with *L. guyanensis* (I-02) and *L. panamensis* (I-04) infections. We observed small, electron-dense granules and rounded or rod-shaped structures both inside parasitized vacuoles within the host macrophages and outside the cells. A similar finding was reported by Schurr *et al.* (1987), who considered that the granules originated either from the macrophage or the amastigotes. We suggest that they arise from the latter, because they occur regularly in the intracellular parasitized areas; their function is however unknown. Other intracellular bodies of undetermined function seen in *Leishmania* parasites including the lysosomes seen in *L. tropica* by Lin *et al.* (1986), liposomes containing a wax-like material noted by Scorza *et al.* (1979) in the amastigotes of *L. garnhami,* and the virus-like particles in *L. hertigi* were described by Molyneux (1974). None of these bodies were seen during the present study.

Various theories have been proposed to explain the fate of the parasite within the host cell. It is generally agreed that *Leishmania* is able to multiply and grow within the macrophage (Berman *et al.*, 1979), but there have been conflicting opinions about the role of the macrophage in killing the parasite intracellularly. By electron microscopic examination, Bretana *et al.* (1983) showed the macrophages are incapable of digesting the parasites. Farah *et al.* (1975) reported that parasites can only be destroyed on the surface of macrophages but not the inside of the cell. Our studies found the degeneration of parasites within the macrophage both either inside the vacuoles or free in the cytoplasm. This indicates the active role of macrophages in the intracellular destruction of parasites. Similar results have also been presented by the Sandbank (1976), who showed the degeneration of parasites within the macrophage in human specimens. Moreover, we observed the degeneration of parasites in the macrophage where the host cell was also undergoing degeneration. The degeneration of the host cell may be due to the parasitic load or attack or to some unknown factors of the cell itself. The conditions and mechanism of the intracellular destruction of parasites were not elucidated in the present study.

Lymphocytes were seen to be in close contact with parasitized macrophages. This macrophage-lymphocyte combination represents the cooperation of two cells against the parasites. Here the function of lymphocytes can be assumed to be: (1) to recognize the parasitized macrophage that may finally result in the destruction of host macrophage and

thereby liberate the parasite (Schurr *et al.*, 1987); (2) to help the macrophage kill the invading *Leishmania* (Mauel *et al.*, 1978), as shown in our results. On the basis of immunocytochemical and electron microscopic studies in animals, it has been suggested that T-cells provide lymphokines that can activate the host macrophage to destroy the parasites intracellularly or that T-cells play a cytotoxic role, killing the infected macrophages and helping to destroy the liberated extracellular parasites (McElrath *et al.*, 1987). Interestingly, mononuclear cells which were morphologically determined as lymphocytes, were attached to parasites, showing their capacity to attack the parasites directly, although, exact function and mechanism remain unknown. Lymphocytes attached to parasitized macrophages may be cytotoxic T-cells. During the present study degeneration of amastigotes was seen within host cells subjected to attack by the immune system, and from this finding we conclude that the parasites may be destroyed by the macrophage they have invaded, although the destruction mechanisms are unknown. Observation of amastigotes inside the cytoplasm of eosinophils and neutrophils suggest that they are phagocytized by these cells. Amastigotes have also been reported in the eosinophils of laboratory-infected BALB/c mice (Barral-Netto *et al.*, 1987; McElrath *et al.*, 1987).

Based on our findings in the present study, we suggest that macrophage serves to destroy the parasite intracellularly, and that lymphocytes (probably cytotoxic T-cells) have a major role in the immune response to Leishmania infection.

Acknowledgements

The authors are grateful to Professor and Director Hikotaro Yoshida, Department of Dermatology, Nagasaki University, for his valuable discussions.

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エクアドル共和国における皮膚リーシュマニア症の電顕的検討

A.M. BHUTTO[1]・岡田　茂[1]・野中　薫雄[1]・
E.A. Gomez L.[2]・橋口　義久[3]

エクアドル共和国で，3例の皮膚リーシュマニア症患者皮膚病変について，電顕的検討を行った。リーシュマニア虫体は真皮および表皮内に存在し，細胞内外に見られた。細胞質内の虫体の存在する部位には，空胞（parasitophorus vacuoles）が見られたが，空胞がない状態でも虫体は観察された。Amastigotesは円形ないし卵円形で，その平均直径は，長径2.62 μm（±0.17 S.D.），短径2.18 μm（±0.28 S.D.）であった。虫体は貪食細胞の細胞質内あるいは空胞内で，変性像を示しているものが見られた。それらの貪食細胞の付近では，リンパ球が虫体と直接接触した像や，虫体を有する貪食細胞と密接に接したりする像が見られた。さらに表皮内に海綿状小水疱が観察されたが，その部では虫体が確認され，同時にリンパ球や単核球の浸潤が確認された。虫体は表皮細胞内外にも観察され，同様に単核球との接着が見られた。単核球内にはメラニン顆粒が観察され，それらの細胞質内にも虫体が見られた。その他好酸球や多核球の一部にも，細胞質内に虫体が見られた。

電顕的観察により，リーシュマニア虫体は，真皮のみならず表皮にも存在することが確認された。これらの虫体，あるいは貪食細胞に対して，リンパ球が密接に接着し，なんらかの免疫反応を起こしているものと示唆された。以上の点から，虫体と貪食細胞の関係，虫体に対するT細胞の役割，貪食細胞内の虫体の死滅について文献的検討を行った。

1　長崎大学医学部皮膚科学教室
2　Departamento de Medicina Tropical, Facultad de Medicina, Universidad Catolica, Santiago de Guayaquil, Ecuador
3　高知医科大学寄生虫学教室

Jpn. J. Trop. Med. Hyg., Vol. 20, No. 1, 1992, pp. 23-28

Case report

TWO SUSPECTED CASES OF *BORRELIA* INFECTION AFTER THE INFESTATION OF *HAEMAPHYSALIS FLAVA* AND *IXODES PERSULCATUS*

MINORU YAMADA[1], SHINJI MATSUDA[1], NAOKI ARIZONO[1], MAYA OHNISHI[2], KEIKO OKABAYASHI[2], HIROKAZU YASUNO[2], MOTOO MATSUBARA[3] AND EMIKO ISOGAI[4]

Received November 27 1991/Accepted January 16 1992

Abstract: We report here two cases of the infestation by ixodid ticks. In both cases the patients developed high serum titers to *B. burgdorferi,* but did not show typical migrating erythema. Case 1: A 5-year-old girl had tick bites on the left occipital region and developed fever (38°C) and multiple swollen lymph nodes on the left side of the neck. The tick was removed and identified as *Haemaphysalis flava.* After treatment with antibiotics, the fever subsided and the lymph nodes gradually decreased in size. The IgG antibody titers to *B. burgdorferi* examined by an ELISA using *B. burgdorferi* antigens, HO14 and HP3, were significantly high. Case 2: A 60-year-old woman had a sticking tick on the occipital region with local skin rash, indulation, and pain after travelling in Hokkaido. The tick was removed and identified as *Ixodes persulcatus.* After treatment with antibiotics, these symptoms disappeared. The IgG antibody titers to *B. burgdorferi* antigens were significantly high. In both cases, serological tests for syphilis and microscopic agglutination tests using different antigens obtained from seven serovars of *Leptospira interrogans* were negative. These findings suggest that both patients were infected with *B. burgdorferi,* but the symptoms were suppressed by the early treatment with antibiotics. *H. flava* found in one of the patients has not been reported as a vector for *B. burgdorferi.* Further investigations are necessary to examine the possibility.

Occurrence of Lyme disease, a disease caused by *Borrelia burgdorferi* (Burgdorfer *et al.,* 1982) and transmitted by Ixodid ticks, has been reported in North America, Europe, Australia, and Asia. In Japan, many cases have been reported especially in the residents of Hokkaido (Miyamoto *et al.,* 1990; Sato *et al.,* 1990).

In the reported cases in Japan, almost all patients manifested chronic migrating erythema and high serum titers to *B. burgdorferi.* In a few cases a pathogenic agent was isolated from both human skin and ixodid ticks (Miyamoto *et al.,* 1991). However, it is sometimes difficult to diagnose this disease without comprehensive assessment of clinical, epidemiologic, and laboratory features, because its signs and symptoms are often nonspecific (Rahn and

1 Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kyoto 602, Japan
2 Department of Dermatology, Kyoto Prefectural University of Medicine, Kyoto 602, Japan
3 Matsubara Skin Clinic, Omi-hachiman, Shiga 523, Japan
4 Department of Preventive Dentistry, Higashi Nippon Gakuen University, Hokkaido 061-02, Japan

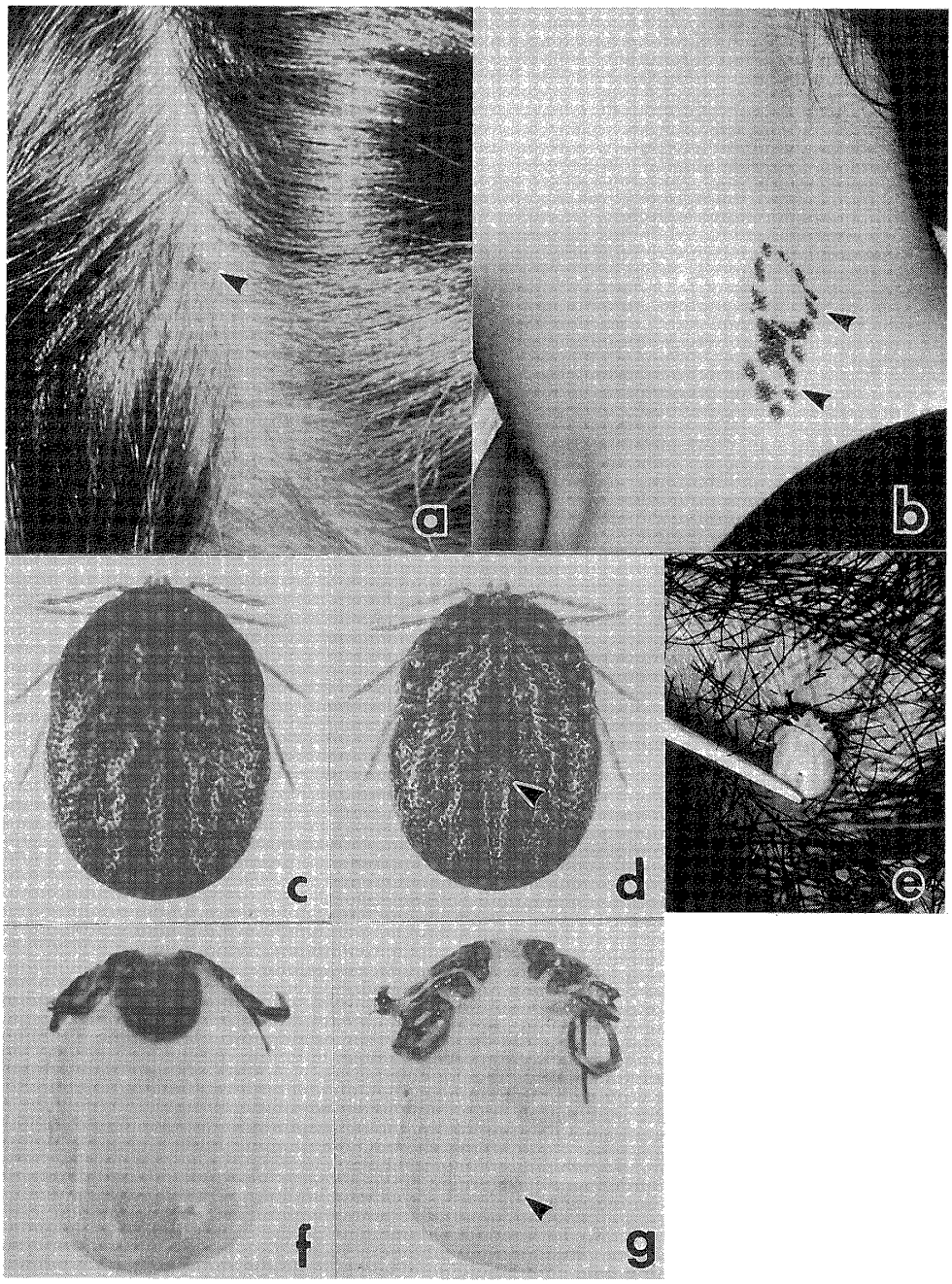

Figure 1a Tick bite (arrow head) on the occipital region of Case 1.
Figure 1b Swellings of lymph nodes of left neck in Case 1 (arrow heads).
Figure 1c and 1d Dorsal (1c) and ventral view (1d) of an adult female of *Haemaphysalis flava* removed from the skin in Case 1. arrow head: anus.
Figure 1e Tick bite (arrow head) on the occipital region of Case 2.
Figure 1f and 1g Dorsal (1f) and ventral view (1g) of an adult female of *Ixodes persulcatus* removed from the skin in Case 2. The hypostome of the tick is not observed, because it remained in the skin. arrow head: anus.

Malawista, 1991). In Europe, recently the cases with positive serological responses against *B. burgdorferi,* have been diagnosed as Lyme borreliosis irrespective of the presence of clinical symptoms (Bozsik *et al.,* 1986).

In the two cases of infestation of ixodid ticks reported here the patients developed high serum titers to *B. burgdorferi,* but did not show typical migrating erythema.

Case 1: Infestation of a tick was noticed in a 5-year-old female child on the left occipital region in 27 March 1991 (Fig. 1a), and she was admitted to Kyoto Prefectural University Hospital. Six to eight days after admission, the girl showed multiple swollen lymph nodes on the left side of the neck, and a body temperature of 38°C (Fig. 1b). The tick, which was removed from the skin, was identified as *Haemaphysalis flava* Neumann, 1897 (Figs. 1c and 1d). Vastocylin was administered orally for 12 days and gentamycin ointment was applied to the skin lesion. The fever subsided the day after the beginning of the treatment and the lymph nodes gradually decreased in size. The IgG antibody titers to *B. burgdorferi* were examined by an ELISA technique (Isogai *et al.,* 1991), using antigens, HO 14 and HP 3, both of which had been purified from *B. burgdorferi* isolated from the Ixodid ticks in Hokkaido in Japan. As shown in Figs. 2 and 3, the titer of the patient's serum obtained one month after the tick bite was significantly high. Serological tests for syphilis (Wassermann reaction, VDRL and TPHA) performed using the patient's serum 2 months after the tick bite were negative. Microscopic agglutination test using different antigens obtained from seven serovars of *Leptospira interrogans* were also negative (<1 : 20).

Case 2: A 60-year-old woman noticed a sticking tick on the occipital region with local

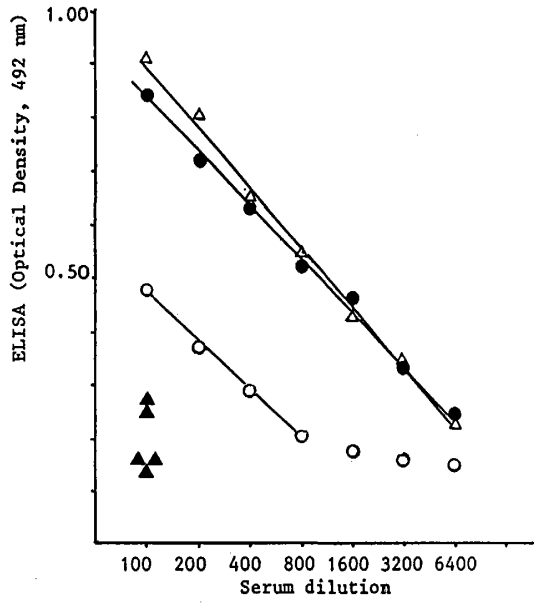

Figure 2 ELISA assay of IgG antibody titers to HO 14 antigen of *B. burgdorferi* (△: a definite case of Lyme disease, ▲: healthy persons (1:100), ●: Case 1, ○: Case 2)

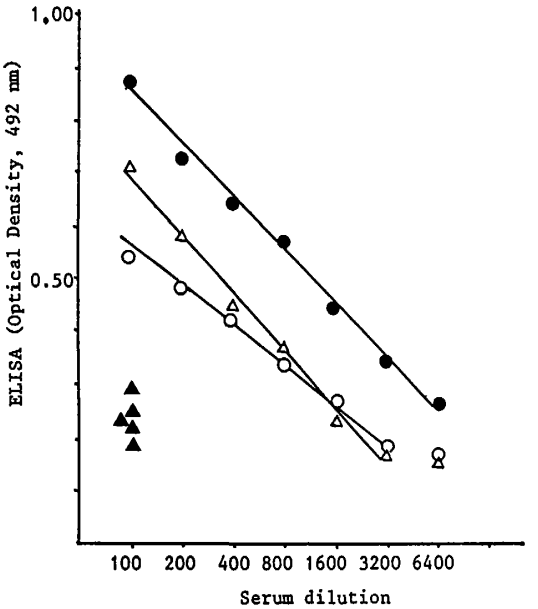

Figure 3 ELISA assay of IgG antibody titers to HP 3 antigen of *B. burgdorferi* (△: a definite case of Lyme disease, ▲: healthy persons (1:100), ●: Case 1, ○: Case 2)

skin rash, indulation and pain, after travelling in Hokkaido in Japan for four days in April 1991 (Fig. 1e). The tick was removed together with the surrounding skin after the injection of 10% formalin solution into the tick. The tick was identified as *Ixodes persulcatus* Schulze, 1930 (Figs. 1f and 1g). After removal of the tick, Minomycin was administered for 17 days. Neither fever nor migrating skin rashes developed. The IgG antibody titers against *B. burgdorferi* antigens, HO 14 and HP 3, were significantly high in the patient's serum obtained 2 weeks after the tick bite (Figs. 2 and 3). Serological tests for syphilis examined one month after the tick bite were negative and microscopic agglutination tests for *Leptospira interrogans* were also negative (<1 : 20).

Steere *et al.* (1977) reported that some cases of Lyme disease were not accompanied by the history of migrating skin rashes. According to Aeschlimann *et al.* (1986), local itches were only one clinical manifestation in 61 out of 350 cases with a positive serological test against *B. burgdorferi.* In the present cases, the serum antibody titers to *B. burgdorferi* were significantly high, though neither patient showed migrating skin rashes. Sera from patients with various spirochetal infections sometimes showed a cross reactivity to *B. burgdorferi* by indirect fluorescent antibody (IFA) test and ELISA (Magnarelli *et al.,* 1987). However, in the present cases, serological tests for syphilis and seven serovars of *Leptospira interrogans* were negative. Thus, the high serum titers to *B. burgdorferi* seem to be specific, though the possibility that some unknown pathogens may have exhibited an antigenic cross reactivity to *B. burgdorferi* cannot be excluded.

On the other hand, both patients received antibiotics as soon as the ticks were removed. As antibiotic treatment reduces skin rashes (Steere *et al.,* 1983), early treatment may suppress the manifestation of the disease. This suggests that both patients had developed an infection of *B. burgdorferi,* but the symptoms were suppressed by the early antibiotic treatment.

The species of ticks reported to transmitt *B. burgdorferi,* are *Ixodes dammini, I. scapularis,* and *Dermacentor variabilis* in the north-eastern region, *I. pacificus* in the western region in USA (Anderson *et al.,* 1985; Schmid, 1985), and *I. ricinus* in Europe. In Japan, ixodid ticks, *I. persulcatus* and *I. ovatus,* have been suggested to be the only possible vectors (Kawabata *et al.,* 1987; Sato *et al.,* 1990). In field surveys these ticks were predominantly found in northern and central Japan. These species can infest a variety of mammalian hosts, including dog, cattle, horse, wild rabbit, and deer (Yamaguti *et al.,* 1971) in these regions. Thus, the serologic survey of dogs has been recommended for identifying the possible endemic area of *B. burgdorferi* infection (Isogai *et al.,* 1990).

In the present cases, one patient was infested with *I. persulcatus,* but the other patient had infestation with *H. flava.* According to Yamaguti *et al.* (1971), *H. flava* has been collected almost everywhere in Japan. *H. flava* has been found from hares, dogs, cows, horses, wild boar, deer, bear, small rodents and birds. This species can transmit the pathogenic agent of tularemia in the northern area of Japan, but has not been reported to be a vector for *Borrelia burgdorferi.* Recently, in Spain, this spirochaete has been found in *Haemaphysalis punctata* by the method of indirect immunofluorescence with anti-*B. burgdorferi* monoclonal antibodies (Marquez and Constan, 1990). Further investigations are required to prove whether the transmission of *Borrelia* by *H. flava* was only accidental or not.

ACKNOWLEDGEMENTS

The authors are most grateful to Dr. Nobuhiro Takada, Department of Immunology and Parasitology, Fukui Medical School, for the identification of the ticks and valuable comment on Lyme disease and Dr. Kenji Miyamoto, Department of Parasitology, Asahikawa Medical College, and Dr. Noboru Yamaguti, Saitama Meidical School, for valuable information on Lyme disease.

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症例報告

Haemaphysalis flava および *Ixodes persulcatus* 刺咬により *Borrelia* 感染が疑われた2例

山田　稔[1]・松田　信治[1]・有薗　直樹[1]・
大西　真世[2]・岡林　啓子[2]・安野　洋一[2]・
松原　基夫[3]・磯貝恵美子[4]

ライム病は欧米に患者が多く，マダニ類によって媒介され，その病原体はスピロヘータの1種 *Borrelia burgdorferi* とされている。今回，同病原体の感染が疑われた2例のマダニ刺咬を経験したので報告する。両例とも患者は *B. burgdorferi* 抗原に対し高い抗体価を示したが，典型的な慢性遊走性紅斑を示さなかった。（症例1） 5歳女児，京都市在住。京都市内で後頭部に刺咬を受け，38°Cの発熱および左頚部のリンパ節の腫大を認めた。ダニはキチマダニ *Haemaphysalis flava* Neumann, 1897 の雌成虫と同定された。抗生物質投与後解熱し，リンパ節も縮少した。血清について北海道で分離された *B. burgdorferi* HO 14 および HP 3 株の抗原を用いて ELISA で *Borrelia* に対する IgG 抗体を調べたところ，非常に高い抗体価を示した。（症例2） 60歳女性，滋賀県在住。北海道旅行中にマダニの刺咬を後頭部に受けた。刺咬部位に皮膚炎，硬結，痛みが見られた。ダニはシュルツェマダニ *Ixodes persulcatus* Schulze, 1930 の雌成虫と同定された。症例1と同様，*B. burgdorferi* に対する抗体価の上昇を認めた。両例共梅毒血清反応，*Leptospira interrogans* に対する抗体は陰性であった。以上より，両例共 *B. burgdorferi* の感染を受けたと考えられるが，抗生物質の早期投与により，症状が軽度に抑えられたものと考えられた。今回の1例で見つかったキチマダニは現在のところ，*Borrelia* のベクターとしての報告がなく，今後調査が必要であると考えられる。

1　京都府立医科大学医動物学教室
2　京都府立医科大学皮膚科学教室
3　滋賀県近江八幡市開業（松原皮膚科医院）
4　東日本学園大学歯学部口腔衛生学教室

日熱医会誌，第20巻　第1号　1992年，29-35頁

症例報告

血小板減少と熱帯熱マラリア抗体価の上昇が見られた卵形マラリアの1症例

遠藤　匡亮[1]・内田　和彦[1]・大槻　眞[1]・堀尾　政博[2]・
嶋田　雅暁[2]・狩野　繁之[3]・鈴木　守[3]

平成3年12月19日受付／平成4年1月22日受理

はじめに

本邦では，1959年土着マラリアの発生が終息したが，経済成長と国際化の発展，さらには航空機の発達による海外渡航者数の増加により年々輸入マラリア症例が増えている。1980年から1989年までの10年間で，我が国でのマラリア発症数は781例になるが，そのうち卵形マラリアの発症数は14例（1.8%）（大友，1990）で比較的稀である。

今回筆者らは，形態学的に卵形マラリアと診断したにもかかわらず，間接蛍光抗体法で熱帯熱マラリアの抗体価が著増し，さらに感染赤血球の著明な増加と血小板の著しい減少を認めるなど，その診断ならびに経過において，特に注意を必要とした1例を経験したので報告する。

症　例

症例：25歳，日本人男性

現病歴：1988年3月技術指導員として，イギリス，ケニアを経由してマラウイに赴任した。赴任後よりマラリア予防のため，クロロキン300 mg base/week を内服していたが，約半年で内服を中止した。1988年12月，39℃前後の発熱があり現地でマラリアの診断を受け，クロロキンを内服したが軽快せず，スルファドキシン・ピリメタミン合剤(ファンシダール®)を内服して軽快した。1989年9月に再び熱発症状があり，この時もクロロキンは無効で，ファンシダール®を服用して軽快した。1990年3月にも同様の症状があったが，クロロキンは服用せずファンシダール®で軽快した。その後全く症状はなく，1990年9月中旬マラウイを発ち，ケニア，エチオピア，スーダン，エジプト，トルコ，イタリア，イギリスを経由し，1990年9月25日帰国した。帰国時の健康診断で軽度の肝機能障害を指摘され，また同時に採取された血清は，群馬大学医学部寄生虫学教室において，間接蛍光抗体法により，マラリアの抗体価が検査された。

1990年10月中旬，ふらつきを自覚し近医を受診，この時熱発はなかったが，肝機能障害と脱水の診断を受けたため入院し，安静加療により1週間で軽快した。その後自覚症状は消失していたが，1990年11月15日夕方より発熱，悪寒戦慄が出現し，翌16日には食欲も低下してきたため，産業医科大学第三内科を受診し，入院となった。

海外渡航歴：上記以外なし。

入院時現症：身長162 cm，体重57 kg，体温39.7℃，脈拍120/分整，血圧110/60 mmHg。眼瞼結膜に貧血を認めず，眼球結膜に黄疸を認めな

1　産業医科大学第三内科学教室（〒807 北九州市八幡西区医生ケ丘1-1）
2　産業医科大学医動物学教室（〒807 北九州市八幡西区医生ケ丘1-1）
3　群馬大学医学部寄生虫学教室（〒371 前橋市昭和町3-39-22）

かった。口腔，胸部に異常を認めず，腹部は平坦で圧痛無く，肝脾触知しなかった（腹部エコーでは脾腫を認めた)。

下腿に浮腫は無く，また意識は清明であり，神経学的にも異常は認められなかった。

入院時検査成績：一般血液検査では，軽度の貧血と肝機能障害，血清蛋白および血小板の低下と血清CRPの上昇を認めた（表1)。入院時に作製した末梢血薄層塗抹標本では，成熟栄養体の大きさは感染赤血球の約⅓で，感染赤血球は卵形に変形し，辺縁は鋸歯状でシェフナーの斑点を認めた(写真1)。成熟栄養体は著しいアメーバ状は呈さず，液胞をほとんど認めず(写真2)，ガメトサイトは円形であった（写真3)。赤血球10,000個につき，マラリア原虫体赤血球72個を認めた。

入院後経過（図1)：入院後直ちにクロロキン600 mg base/day，続いて300 mg base/dayを2日間投与した。第3病日には37℃以下に解熱し，全身状態も改善したが，第4病日には血小板が$4.1\times10^4/mm^3$にまで低下した。また血沈も6 mm/hrと遅延したためメシル酸カベキサート（FOY ®）投与を行った。第5病日には血小板は$13.4\times10^4/mm^3$，血沈13 mm/hrと回復した。

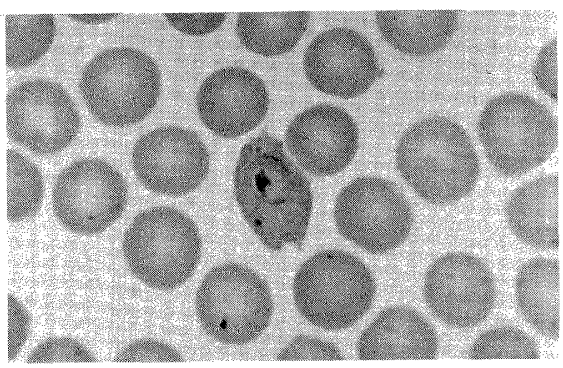
肝内型の原虫による再発を防ぐため，プリマキン15 mg/日を2週間投与したが，その間特に副作用を認めなかった。

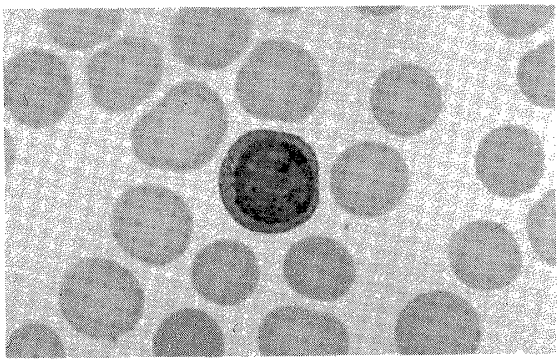
感染赤血球率の推移：末梢血薄層塗抹標本をギムザ染色して，検鏡下に感染赤血球率を計算した。入院時は0.7%であったが，クロロキン投与14時間後には0.07%と著明に低下し，19時間後には虫体を検出不能となった。

間接蛍光抗体法検査：帰国時（9月25日)，および入院時（11月18日）の検査では，熱帯熱マラリアの抗体価は1,024倍と高値であった。その後，12月

Table 1 Laboratory data on admission

WBC	5,500/mm³	TP	5.4 g/dl
Neutro	55%	Alb	3.6 g/dl
Eo	1%	T-bil	1.5 mg/dl
Ba	0%	D-bil	0.5 mg/dl
Ly	30%	GOT	40 IU/l
Mono	12%	GPT	58 IU/l
RBC	$413\times10^4/mm^3$	γ-GTP	117 IU/l
Hb	13.2 g/dl	ALP	3.0 K.A.
Ht	38.5%	ChE	461 IU/l
Plt	$5.9\times10^4/mm^3$	LDH	226 IU/l
Reti	45‰	T-Cho	125 mg/dl
		TG	63 mg/dl
Urinalysis		PL	138 mg/dl
pH	7.5	BUN	8 mg/dl
Ketone body	(−)	Cre	0.8 mg/dl
Prot	(Tr)	Na	138 mEq/l
Glu	(−)	K	3.4 mEq/l
Bil	(−)	Cl	101 mEq/l
Urob	2.0	FBS	90 mg/dl
S.G.	1.017	CRP	5+
Stool		ESR	16 mm/h
G method	(−)	PT	86.2 %
O method	(−)	APTT	28.5(30.5) s
		Fibrinogen	386 mg/dl
		FDPE	78.5 mg/dl





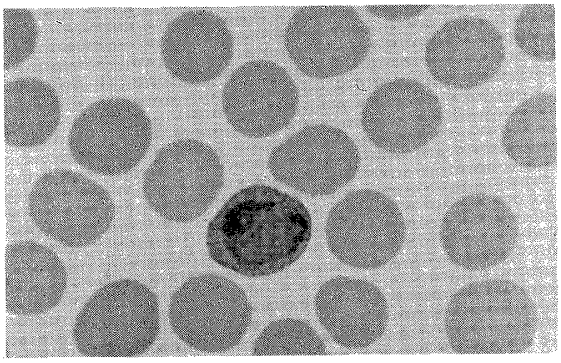


Photo. 1 | Photo. 3

Photo. 2

Photos. 1-3. Blood film (Giemsa stain) of the case.
Photo 1 A young trophozoite: The infected cell is egg-shaped and contains Schüffner's dots.
Photo 2 An old trophozoite: Compact, not amaeboid, vacuole inconspicuous.
Photo 3 A gametocyte: Spherical and compact.

21日には64倍となり1991年7月23日には陰性となった。一方，三日熱マラリアの抗体価は，帰国時，入院時とも64倍と軽度上昇で，1991年1月24日には16倍となり，1991年7月23日にいたるも16倍を維持した（図2）。

考　察

卵形マラリアの診断に際して，形態学的に類似点の多い三日熱マラリアとの鑑別がしばしば問題となる。本症例においては，検鏡下に以下の特徴を認めたため，卵形マラリアと診断した。（1）感染赤血球が卵形に変形しているものが多い。（2）成熟栄養体が著しいアメーバ状を呈していない。（3）成熟栄養体の入った赤血球の膨大化が著しくない。（4）栄養体の液胞が比較的小さい。

マラリア間接蛍光抗体法は，本邦では熱帯熱マラリア原虫と三日熱マラリア原虫の抗原を用いて抗体価を測定し，種の鑑別を行っている。熱帯熱，三日熱マラリア感染では両抗体価が共に上昇することが多いが，より高い抗体価を示した方を特異的と判断し，今までに例外はない。また，現在我が国で間接蛍光抗体用の抗原が得られない卵形マラリアの場合では，現在までに調べた少数例において熱帯熱よりも三日熱マラリア原虫抗原に対する抗体価が上昇している（Kano *et al.*, 1990）。この所見は，三日熱マラリア原虫と卵形マラリア原虫が近い関係にあること（Garnham, 1966）から考えても正当性がある。

本症例では卵形マラリアであるにもかかわらず，入院時熱帯熱マラリア原虫に対する抗体価が1,024倍と著しく高く，三日熱マラリアの抗体価は64倍であった。しかし，熱帯熱マラリア抗体価は治療後比較的早期に64倍まで低下し，およそ10カ月後には陰性となった。マラウイ滞在時の病歴並びに帰国時のマラリア抗体価とあわせて判断す

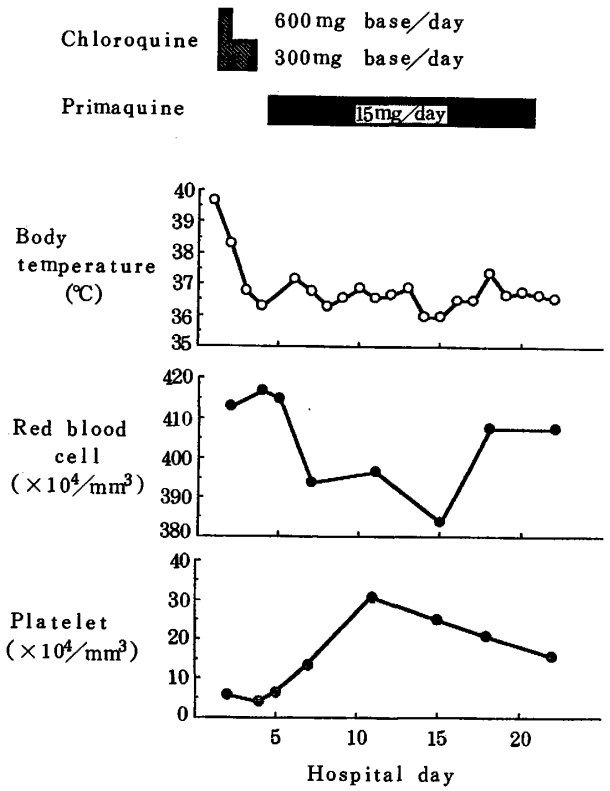


Figure 1 Clinical course. After administration of chloroquine, fever went down immediately. On the fourth hospital day platelet count reached the nadir of 41,000/mm³.

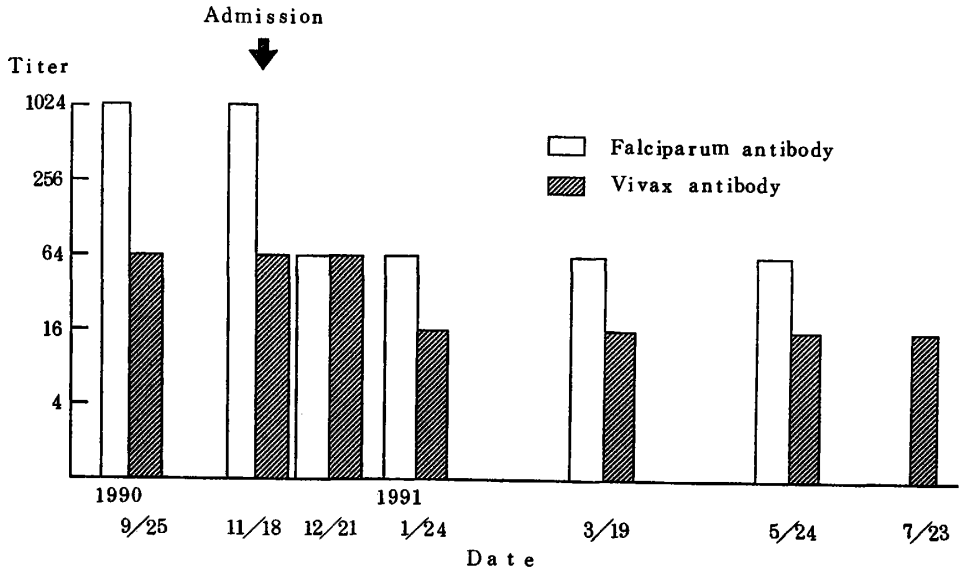


Figure 2 The change of malarial antibody to *Plasmodium falciparum* (Pf) and *P. vivax* (Pv). The antibody titer of Pf (1:1,024) was higher than that of Pv (1:64) at the begining of the course but decreased faster than the Pv after chloroquine therapy. Ten months later, the titer of Pf became negative (<1:4) but the titer of Pv still remained positive (1:16).

ると，本症例は，1988，1989，1990年に熱帯熱マラリアに感染したと推測される。従って，熱帯熱マラリア治癒後，卵形マラリア感染により熱帯熱マラリア抗体産生が非特異的に強く刺激され，卵形マラリアの治癒とともに，熱帯熱マラリア抗体価が早期に低下した可能性が考えられる。

帰国後51日目に発症していることや，形態学的に熱帯熱マラリアの所見が全く認められなかったことから，熱帯熱マラリアと卵形マラリアとの混合感染ではないと考える。本症例のように，異なる数種のマラリア感染を繰り返す可能性のある熱帯地長期滞在者の場合，熱帯熱，三日熱マラリア以外の抗原が入手できない現時点では，免疫学的な種の鑑別は危険で，感染したマラリアの種の鑑別は，あくまで形態学的診断によってなされなければならない。今後，卵形マラリア抗原，四日熱マラリア抗原が準備され，より種特異的な免疫学的診断法が確立されることが期待される。

本邦の輸入マラリア患者419例の血液所見に関する研究（海老沢ら，1990）では，感染原虫数は熱帯熱マラリアでは200×10^4/mm^3以上のこともあるが，卵形マラリアでは平均350/mm^3，最大でも3,800/mm^3である。また血小板数の減少は熱帯熱マラリアでは1×10^4/mm^3以下になることもあるが，卵形および四日熱マラリアの患者では最も低下したものでも最低6.4×10^4/mm^3と報告されている。

この血小板の減少の機序としては，脾機能亢進による血小板の破壊や，血小板中に侵入したマラリア原虫による血小板の破壊（Fajardo, 1973, 1979），抗血小板抗体の産生や免疫複合体の関与（Kelton *et al.*, 1983）が考えられている。また，稀ではあるがクロロキンによる副作用として血小板の減少が報告されている（Nagaratnam *et al.*, 1978）。血小板の減少はDICの準備状態であることから患者の管理において常に注目されている（Tani *et al.*, 1984）が，マラリアでDICを併発するか否かにおいては，否定する報告もある（Vreeken and Cremer-goote, 1978）。熱帯熱マラリアや三日熱マラリアで血小板が著しく減少する場合には，稀にDICを合併することが報告されているが（田辺，島田，1990）卵形マラリアでは極めて稀である。

本症例では，感染原虫数は約3.6×10^4/mm^3と上記報告に比較し，卵形マラリアのなかでは最も多い。臨床的な所見としては，DICを含め重篤な症状は認めなかったものの，血小板の減少は経過中4.1×10^4/mm^3にまで達し，重症マラリアの指標である5×10^4/mm^3以下にあてはまる（天野，1984）。血小板の減少は初診時より認められており，クロロキンの副作用であることは考えにくい。本症における著しい血小板の減少は，感染した原虫数が卵形マラリアでありながら比較的多かったことが，引き金になっている可能性がある。また，この血小板の減少が，熱帯熱マラリア抗体価の著しい上昇と同時に認められたことも興味深い。しかし，これらの関連については，今後の課題として残される。今後は，卵形マラリアであっても，原虫数，マラリア抗体価をよくモニターしながら，血小板の減少やそれにひき続くDIC合併など，臨床症状悪化の可能性を念頭に置き，治療にあたる必要があると考える。

おわりに

1）輸入マラリアの中で稀とされている，卵形マラリアの1例を報告した。

2）本症例では，本邦での輸入卵形マラリアの報告と比較して，著しく感染赤血球数が多く，血小板数の著明な減少があった。

3）本症例では，形態学的に卵形マラリアであるにもかかわらず，三日熱マラリア抗体価の上昇に比し，熱帯熱マラリア抗体価は著しく上昇していた。これはマラリアの流行地，長期滞在中におけるマラリア既往を，反映している結果と考えた。

本稿を終えるにあたり，本症例の診断に際しご指導ご教示いただいた，大分医科大学生物学教室，宮田　彬博士に深謝いたします。

なお，本論文の要旨は，第212回日本内科学会九州地方会（福岡市，1991年）において発表した。

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Case report

OVALE MALARIA ASSOCIATED WITH THROMBOCYTOPENIA INCREASED THE TITER OF ANTIBODY TO *PLASMODIUM FALCIPARUM*

MASAAKI ENDO[1], KAZUHIKO UCHIDA[1], MAKOTO OTSUKI[1],
MASAHIRO HORIO[2], MASAAKI SHIMADA[2],
SHIGEYUKI KANO[3] AND MAMORU SUZUKI[3]

Received December 17 1991/Accepted January 22 1992

We report a case of imported ovale malaria with a severe thrombocytopenia, that had high titer of malaria antibody to *Plasmodium falciparum* (Pf) and to *P. vivax* (Pv). The platelet count was lower than previously reported in imported malaria in Japan. The titer of Pf antibody was higher than the titer of Pv antibody.

A 25-year-old Japanese man was admitted to a hospital because of chill and fever. Thirty-two months earlier, he arrived at Malawi for technical assistance. He used chloroquine as malaria prophylaxis during the first 6 months of his stay. Nine months later, he had fever. A diagnosis of malaria was suspected but it was not confirmed. Chloroquine did not effect his fever but pyrimethamine-sulfadoxine did. He also got similar episodes nine and fifteen months later.

On admission the temperature was 39.7 °C, the pulse was 120/min. Physical examination was negative; no jaundice and anemia were found; the liver and spleen were not palpable. Routine laboratory test was normal except for the hematocrit (38.5%), the platelet count (59,000/mm^3), GPT (58 IU/*l*) and CRP (5+). On the fourth hospital day the platelet count reached to the lowest level of 41,000/mm^3 in the clinical course.

The morphological diagnosis of blood film was *P. ovale* (Po). The number of infected erythrocyte was 72 per 10,000. Sera were examined with the indirect fluorescent antibody test (IFAT). Antigens of Pf and Pv were used for the test. The antibody titer of Pf (1 : 1,024) was higher than that of Pv (1 : 64) at the beginning of the course but decreased faster than the Pv after chloroquine therapy. Ten months later, the titer of Pf became negative (<1 : 4) but the titer of Pv still remained positive (1:16).

This report indicates the importance of thrombocytopenia which may lead to DIC when the density of parasitemia is high, even though ovale malaria infection rarely becomes severe. The unusually high titer of Pf antibody is probably due to the previous Pf infections. Po infection might elicit the production of Pf antibody nonspecifically. In the diagnosis of species of malaria the morphology still remained with high priority at the present time, and the production of Po and *P. malariae* antigens remained to be clarified.

1 Third Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, Yahata-nishi-ku, Kitakyushu 807, Japan
2 Department of Parasitology and Tropical Public Health, School of Medicine, University of Occupational and Environmental Health, Japan
3 Department of Parasitology, Gunma University School of Medicine, Maebashi 371, Japan

Jpn. J. Trop. Med. Hyg., Vol. 20, No. 1, 1992, pp. 37-40

Research note

EFFECT OF CHLOROQUINE ON OXYGEN RADICAL PRODUCTION BY KUPFFER CELLS, BLOOD MONOCYTES AND PERITONEAL MACROPHAGES OF NORMAL GUINEA PIGS

R. N. PRASAD, K. J. VIRK, N. K. GANGULY
AND R. C. MAHAJAN

Received June 16 1991/Accepted November 20 1991

Abstract: The study was conducted to determine whether chloroquine has any effect on the oxygen radical production of Kupffer cells, blood monocytes and peritoneal macrophages. Exposure of these cells to chloroquine, using therapeutic concentrations, for 30 min at 37°C did not alter the viability of cells. The chemiluminescence (CL) response of all the cell populations was not effected after 10 min of exposure to drug. However, the CL response of all the three types of cells was significantly inhibited after 20 min exposure to chloroquine. The effect of chloroquine was reversible after washing out the drug from cuvates after 20 min of treatment. But when the cells were washed after 30 min it was only partially reversible. The effect of chloroquine on CL response of the cells have been discussed.

INTRODUCTION

Mononuclear phagocytic cells (MPC) play an important role in the host defense mechanism (Nelson, 1982). However, the over activation of these cells and the subsequent release of their secretory products can lead to various pathological consequences (Clark, 1987). In the recent years many workers have emphasized that elevated release of free oxygen radicals by immunologically activated MPC is responsible for tissue damage in a variety of disease (Bulkley, 1983; Allen and Egui, 1983). The reactive oxygen radicals have also been shown to cause malaria pathology by damaging vascular endothelium and erythocytes (Clark and Hunt, 1983; Weisiger, 1986). Chloroquine is the widely used antimalarial drug for the treatment of malaria. It has been shown that chloroquine inhibits the phagocytic capacity of monocytes in normal animals whereas in the infected animals it controls the hyperactivation of these cells (Prasad *et al.*, 1986). However, it's effect on the oxygen radical production capacity of phagocytic cells is not well understood. Since the oxygen radical production by leukocytes is dependent upon their phagocytic capacity (Allen *et al.*, 1972), it is very much

Department of Parasitology and Experimental Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh-160012 (U.T.) India
Present address to whom correspondence should be addressed: Dr. R.N. Prasad, Officer-in-charge, Malaria Research Centre, Khirni Bagh, Sadar Bazar, Shahjahanpur-242001 (U.P.) India

important to study the effect of chloroquine on the oxygen radical production capacity of these cells. The present study was, therefore, conducted to determine whether chloroquine has any effect on the oxygen radical production of Kupffer cells, blood monocytes and peritoneal macrophages.

Materials and Methods

Three to four weeks old normal healthy guinea pigs of eigher sex were obtained from Central Animal House of the Postgraduate Institute of Medical Education and Research, Chandigarh.

Kupffer cells and blood monocytes were separated by pronase digestion and Ficoll hypaque density gradient methods respectively as discribed elsewhere (Virk *et al.*, 1987). Peritoneal macrophages were obtained from peritoneal large without any prior activation with slight modifications as described in our previous publication (Virk *et al.*, 1987).

The concentration of chloroquine which is attainable during treatment (Prasad *et al.*, 1985) of malaria (230 $\mu g/ml$) was added to the cuvettes containing cells. The cells were exposed to chloroquine for 0, 10, 20 and 30 min before taking the chemiluminescence reading. In some of the experiments the drug was removed from cuvettes by washing the cells with minimum essential medium without phenol red.

The oxygen radical production by MPC was studied through chemiluminescence. Chemiluminescence (CL) was measured, after culturing the blood monocytes and peritoneal macrophages for 1 hr and Kupffer cells for 3 hrs, in LKB luminometer (Model, 1250). Latex particles were used for the stimulation of cells (Virk *et al.*, 1987).

Results

Exposure of cells to chloroquine, using therapeutic concentrations, for 30 min at 37°C did not alter the viability of cells. The CL response of Kupffer cells, blood monocytes and peritoneal macrophages recorded before and after chloroquine treatment is shown in Table 1. There was no significant effect on the CL response of all the cell populations after 10 min of exposure to drug. However, the CL response of all the three types of cells was significantly

Table 1 Effect of chloroquine on chemilumincscence response of Kupffer cells, blood monocytes and peritoneal macrophages

Time of chloroquine treatment in min	Peak CL response (mV)					
	Kupffer cells		Blood monocytes		Peritoneal macrophages	
	A	B	A	B	A	B
0	5.56±1.23	5.16±1.20	6.50±1.17	6.86±0.75	8.50±0.81	8.50±0.55
10	5.67±0.45	5.30±1.04	5.70±0.50	6.46±0.61	6.10±1.37	7.46±0.45
20	2.65±0.32*	4.20±0.30	3.30±0.80*	5.86±0.31	1.95±0.37*	6.90±0.26
30	0.66±0.11*	2.83±0.91*	1.34±0.81*	4.96±0.15	0.91±0.14*	5.56±0.66*

A = In the presence of chloroquine
B = In the absence of chloroquine, i.e. after washing
* = ($P < 0.01$)

inhibited after 20 min of exposure to chloroquine ($P<0.01$). The effect of chloroquine, was reversible, as no significant inhibition was noticed when the CL response was measured after washing out the drug from cuvettes after 20 min of treatment. When the cells were washed after 30 min of treatment, the inhibitory effect of drug on the CL response was only partially reversible.

Discussion

The data presented here shows that chloroquine inhibits the CL response of Kupffer cells, blood monocytes and peritoneal macrophages of normal guinea pigs. The depressed CL response of MPC may be due the prevention of recycling of surface receptors of macrophages by chloroquine (Brodsky, 1984). Toxic oxygen radical are important effector molecules in the microbicidal activity of MPC (Badwey and Karnovasky, 1980). *Plasmodium* species are sensitive and can be killed within erythrocytes and mononuclear cells by oxidant stress. But why the parasite is not eliminated at the time of peak oxygen metabolite production *in vivo* is still not well understood (Brinkmann *et al.*, 1984). It appears that parasite escapes from the toxic effect of oxygen radical produced by MPC (Allen and Egui, 1983). However, the enhanced release of these matabolites may cause extensive tissue damage in their immediate environment (Clark, 1987; Marx, 1987). Apart from this production of oxygen radicals by activated MPC at a higher quantus can effect the generating phagocytic cells also (Weisiger, 1986). Under these circumstance inhibition of oxygen radical production of MPC by chloroquine seems to be beneficial in protecting the host tissue from oxygen radical mediated damage. Antinflammatory and antiphagocytic activity of chloroquine, apart from its parasiticidal action, have already been shown to be helpful in the resolution of disease (Prasad *et al.*, 1986).

It is hypothesised that this inhibitory effect of the chloroquine might be protecting the host from the toxic effect of the oxygen radicals during malaria infection as enhanced release of these radicals is known to cause malarial pathology.

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PROCEEDINGS OF XXXIII ANNUAL MEETINGS OF JAPANESE SOCIETY OF TROPICAL MEDICINE

8-9 November 1991, Kyoto

President

Yukio Yoshida

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Prize winner's lecture

JSTM (Japanese Society of Tropical Medicine)
Young Investigator Award

IDENTIFICATION OF *LEISHMANIA* ISOLATES IN ECUADOR

TATSUYUKI MIMORI
Department of Parasitic Diseases, Kumamoto University Medical School

New World leishmaniases are widely distributed in Ecuador, where they present a considerable health hazard. Since the first human leishmaniasis was described in this country in 1920, many additional cases of the disease have been reported. Until now, the identification and taxonomy of these Ecuadorian parasites have been based mainly on their clinical manifestations in humans, epidemiological features, and differing growth patterns in the hamster and *in vitro*. However, it is difficult to identify species of parasites on those criteria. Recently, we compared 40 *Leishmania* isolates from Ecuador with well characterized WHO reference strains using isoenzyme electropholesis (zymodeme analysis), reactivities of monoclonal antibodies with parasite species-specific antigens (serodeme analysis) and restriction-endonuclease fragment patterens of kinetoplast DNA (schizodeme analysis). All of the human strains were isolated from the cutaneous lesion of patients. Eighteen isolates of humans from the Pacific coast were identified as *Leishmania panamensis*. *L. major*-like strains were isolated from 3 humans in Paute, Andean highland and 1 human in Quinide, Pacific coast. Nine isolates from Paute were identified as *L. mexicana*. In the Amazon, *L. braziliensis* isolate was found from 2 humans. With regard to isolates of reservoir host and vector, single isolates from *Sciurus vulgaris, Potos flavus* and *Tamandua tetradactyla* in Pacific coast were identified as *L. amazonensis,* while ones from *Choloepus hoffmani didactylus* and *Sciurus granatensis* in Naranjal were thought as new species. *L. mexicana* isolate was found from dog, and sand fly, *Lutzomyia ayacuchensis,* in Paute, Andean highland.

Special lecture

THERAPY OF AIDS: RECENT PROGRESS AND THE PERSPECTIVE

HIROAKI MITSUYA
Clinical Onchology Program, National Cancer Institute,
Maryland, USA

Since the discovery of human immunodeficiency virus (HIV) as a pathogenic retrovirus linked to acquired immunodeficiency syndrome (AIDS), a number of potentially useful stragies for the antiviral therapy of HIV infection have emerged (Mitsuya and Border, 1986, 1987; Mitsuya *et al.*, 1990; Yarchoan *et al.*, 1989). One such approach is the use of the broad family of 2', 3'-dideoxynucleosides to which the first prescription drug 3'-azido-2', 3'-dideoxythymidine (AZT or zido vudine) belongs (Fig. 1). AZT has been shown to reduce the replication of HIV-1 *in vivo* and to confer significant clinical benefits in patients in both early and advanced stages of infection. Other members of the family, 2', 3'-dideoxycytidine (ddC) (Mitsuya and Broder, 1986) and 2', 3'-dideoxyinosine (ddI or didanosine) (Mitsuya and Broder, 1986; Yarchoan *et al.*, 1989), and 2', 3'-didehydro-2', 3'-dideoxythymidine (d4T), have also been shown to be active against HIV-1 in short-term clinical trials.

In the past years, however, several new challenging issues have also emerged. They include (1) long-term drug-related toxicities (Mitsuya *et al.*, 1990; Yarchoan *et al.*, 1989), (2) partial restoration of immunological dysfunctions, (3) development of various cancers, in particular, as improved therapies result in prolonged survival (Pluda *et al.*, 1990), (4) emergence of drug-resistant HIV variants (Larder *et al.*, 1989), and (5) lack of methodologies to quantitate and monitor the effect of antiviral therapy (Murakami *et al.*, 1991). Much more effort is urgently required in these areas.

The armamentarium of antiretroviral agents is definitely and rapidly growing. We have learned that substitution of an atom at a certain position in the base or ribose moiety can drastically alter the antiviral activities or other properties of a given nucleosides. For example, 6-halogen-substitution in dideoxypurine nucleosides can increase the lipophilicity (Murakami *et al.*, 1991). A number of antiretroviral nucleosides without the dideoxyribose moiety such as analogues of 1-{(2-hydroxyethoxy) methyl}-6-(phenylthio) thymine

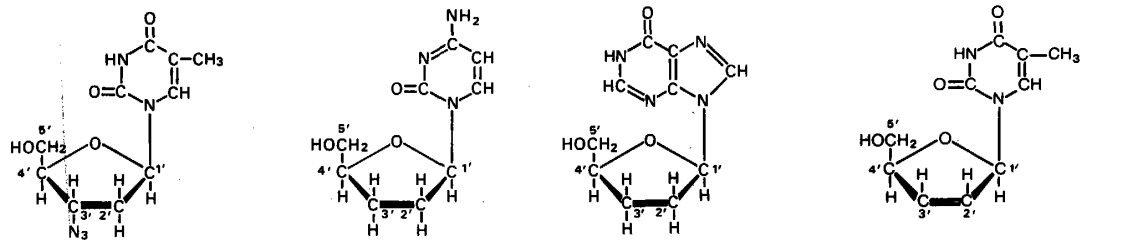

Figure 1 Structures of 2', 3'-dideoxynucleoside analogues now use in humans.

(HEPT) have also been developed as potential antiviral drugs.

HIV possesses at least nine known genes and is the most complex retrovirus studied thus far. However, the very comlexity of this virus could contribute to its defeat. Indeed, many steps in the replicative cycle of HIV have been considered as potencial targets for novel antiretroviral therapy. The use of viral protease inhibitors is an especially interesting strategy (Fig. 2) (Erickson *et al.*, 1990; Roberts *et al.*, 1990). Several non-nucleoside reverse transcriptase inhibitors such as TIBO compounds are now in preclinical or clinical development as well. A number of natural products have also been considered as potential drugs active against HIV.

For the immediate future, continued progress in structure-activity relationships and clinical effectiveness is likely with dideoxynucleoside analogues, and in particular with refinements in AZT, ddT, or related drugs now in use. However, in the future it seems certain

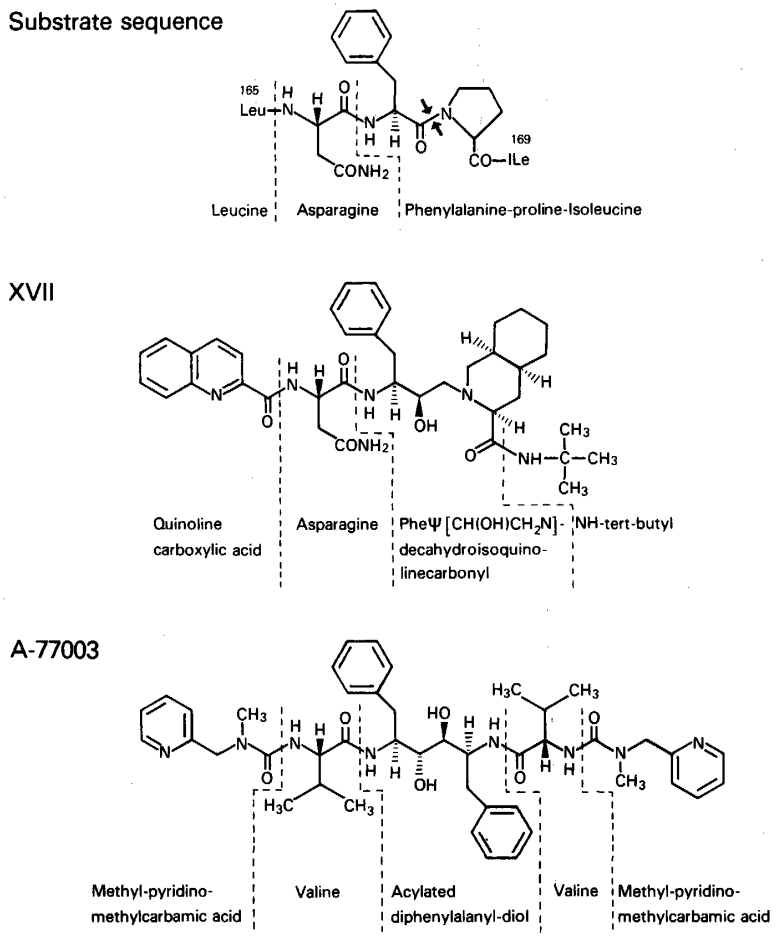

Figure 2 Structures of protease inhibitors XVII (Roberts *et al.*, 1990) and A-77003 (Erickson *et al.*, 1990). XVII, a transition state mimetic protease inhibitor, contains the transition state moiety PheΨ [CH(OH)CH_2N] Pro in place of the Phe^{167}-Pro^{168} scissile bond. A-77003, a symmetric inhibitor, has a two-fold symmetric (C_2) structure. The sequence of physiologic substrate for HIV protease is shown at the top.

that a number of non-nucleoside analogues affecting multiple steps in viral replication will be available, and combination therapy employing such agents with nucleoside analogues or other future drugs will exert major effects against the morbidity and mortality caused by HIV.

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Symposium
Human Ecological and Anthropological Approaches to Research on Tropical Medicine

1 INTRODUCTORY REMARKS

YOSHIKI AOKI[1] AND ISAO TADA[2]
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[1] and Department of Parasitology, Faculty of Medicine, Kyushu University[2]

Tropical diseases have treathen some billion of people in the tropics. Due to the recent expansion of global market, drastic increase of overseas tourism and the marked increase in the request of overseas technical collaboration, tropical diseases became a serious concern in Japan as well. However, the control programs for any tropical diseases in developing countries are encountering with big difficulties. The major factors will be economic recession and deficiencies in infrastructures and health staff. Further, drug resistance is rising up year by year in some pathogens and likewise insecticide resistance in vector insects. Even effective vaccines are not sufficiently implemented in tropical countries. Therefore, human ecological approach to research on tropical medicine is requested to be done to facilitate control campaingns against various tropical infectious and parasitic infections.

In many tropical countries, where traditional society has been built, people have developed their own characteristic concepts on endemic diseases. This tendency is especially marked in the areas in which illiteracy exists and witch doctors play importand role in social life. In such countries, modern medicine is hardly accepted and thus any modern treatment are frequently refused. An example is seen in Peru where epidemic cholera produced more than 70 thousand infected and inhabitants beleived Evil eyes as the cause of epidemic (Newsweek, May 6, 1991). In Nigeria, a symptom of schistosomiasis which cause hematuria and urogenital disturbances has been considered as the ritual symbol for adulthood in boy. Appearance of hematuria is beleived essential condition for marriage in this community (Akogun, 1991). Another unexpected example is the refusal for the construction of modern toilet by a Kenyan tribe people who were refractory to pile up feces in the bottom, because this action has been considered to symbolize incest taboo (Hamamoto, personal communication). The same observer reported in another community of Kenya the refusal of drinking iron water supplied by modernized tap-system despite the prohibition of natural spirit of water.

Recently the importance of KAP (knowledge, attitude, practice) studies of community people are stressed in the investigations and health activities in tropical regions. Human ecological analysis of disease transmission in the target community would introduce unique strategy in the control campaign. So far high priority was given to the development of advanced biological technologies. However, what we have done are environmental contamination, resistance against insecticide/drug in vectors/pathogens, less compliance of people to health campaign etc. In the malaria control campaign, the insecticide spraying strategy seems no more effective. At the present, WHO recommends to use pyrethroid-impregnated

bednets in the endemic areas. In the Central and South America, the strategy for Chagas' disease should be switched from traditional insecticide spraying to the construction of compact housing accomodations. Thus human ecological/anthropological approaches would analyze and modify human factors relating to disease transmission with less expenditure and resources in health sectors.

On this context, the present symposium was designed and realized by inviting 5 experts in human ecology, anthropology and tropical medicine. The chairs would like to thank to Dr. Y. Yoshida, the President of the Japanese Society of Tropical Medicine for the support.

2 THE UNDERSTANDING OF MAN-ENVIRONMENT SYSTEM IN MEDICINE

TAI-ICHIRO TAKEMOTO
Department of Public Health, Nagasaki University School of Medicine

Considering the tropical medicine is a study in tropical areas where various ethnics live in, the understanding of human-environment system seems to be essential. Moreover the idea of primary health care based on comprehensive health care consisted from the health promotion to social rehabilitation in a community has been promoting the understanding the interaction between man with socioeconomic and cultural attributes, and environment involving socioeconomic and cultural factors. The most classic idea on man and environment system in medical science is found in epidemiology in which the existance of particular pathogen (behaviour) is presumed as the cause of a disease. Human ecology or ecological anthropology tries to evaluate the man-environment system in a community for their survival through physical and cultural adaptive processes. Cultural or social anthropology in medical or health science has stressed the cultural aspects of human behaviour relating to health and illness. Tropical medicine which has been dealing with so called tropical diseases should paid more attention to man-environment relationship involving cultural and/or behavioral characteristics for the improvement of health condition in tropical areas.

3 ILLNESS AND HEALING: ANTHROPOLOGICAL APPROACH

EMIKO NAMIHIRA
Department of Design, Kyushu Institute of Design, Japan

In traditional societies disease is usually given cultural 'meanings' by the peoples. 'Illness' is used to express such a 'meaningful' disease in cultural anthropology.

The reasons why diseases are so meaningful in such societies are as follows:

(1) diseases of the members give serious influences to the working and marriage systems since such societies are small scaled;

(2) members of such societies are related not only in kinship networks but also have close and frequent contacts each other.

Then, they are depressed when other members fall in serious illness;

(3) disease is thought to be one of misfortune which could come to everybody at any time, and it is understood in the whole misfortune context, not in biomedical science.

Causes of illness are thought to be witchcraft, sorcery, revenge of the dead spirits, punishment of gods, and so on. Such belief might be thought to prove they are not 'scientific' or 'rational'. But we have to understand their belief shows their cosmology in which human body, its activities, ecological situations and human relationship are concerned each other. That is, they think illness is a representation of disorder in the relationship.

Most important matter when we work with them in order to expell a specific disease, e.g. malaria, is to understand the disease with their whole life. Therefore, the strategy must be planned in the context of their life, i.e., family life, productive activity, community system, belief, social prestige and so on.

To be concrete the following are important to practice a plan for stamping out a certain epidemics:

(1) to search and make clear the whole feature of the diseases which afflict the people;

(2) to make clear the priority or raking of the misfortune and the diseases the people are most concerned about;

(3) to make the plan adjusting the people's traditional ways of life, and not to changing them too rapidly.

4 ETHNOGEOGRAPHICAL ANALYSIS OF MALIGNANT TUMORS IN WESTERN KENYA, EAST AFRICA

KAN TORIYAMA
Department of Pathology, Institute of Tropical Medicine,
Nagasaki University

It is well known that the genetic factors and environmental factors such as climatic conditions and life styles, living conditions and etc. play important roles in the causation of various diseases and modify their manifestations, especially in the developing countries. However, medical researches alone cannot analyse these factors nor understand the relationships between the diseases and these factors without the knowledges of geography, social and cultural anthropology and human ecology.

We are carrying on the ethnogeopathological study on malignant tumors in western Kenya, east Africa, since 1979. This area experiences a wide variation of climatic conditions such as the dry desert and tropical savannah in the most northern and southern parts, the cool tropical highlands along the great Rift Valley and the moist tropical savannah around Lake Victoria. Also various ethnic groups who are descendants from different origins inhabit in this area. Certain malignant tumors in this area show considerable variation in incidence over small distances; a high incidence of penile cancer is seen among the Luo and Turkana who have no customs of circumsion. Esophageal cancer is highly spread among the Kikuyu and Luo who are mainly peasants in the agricultural area. The Luo, an inhabitant of the moist tropical savannah shows the highest incidence of Kaposi's sarcoma and very few cases

of Kaposi's sarcoma are found among the inhabitants of the dry desert area. Burkitt's lymphoma shows similar distribution to Kaposi's sarcoma. Also several other tumors, such as, fibrosarcoma and retinoblastoma show specific ethnogeographical distributions. These findings suggest that some etiological factors, such as, genetic factors, natural environments, human behaviours and etc. might have some relations with causation of these tumors. This is the reason why the cooperative study among medical sciences, geography, social and cultural anthropology, human ecology and others must be a clue to cope with the various kinds of diseases, especially in the developing countries.

5 ADAPTIVE MECHANISMS OF HUMAN POPULATIONS IN REGIONAL ECOSYSTEMS OF PAPUA NEW GUINEA

RYUTARO OHTSUKA
Department of Human Ecology, Faculty of Medicine,
University of the Tokyo

Malaria has been the most important determinant of population change and distribution in Papua New Guinea. The long-term survival mechanisms of the Gidra-speaking Papuans, who are fewer than 2,000 and inhabit inland, riverine, and coastal villages (only one coastal village was recently moved from the riverine environment), were revealed by our repeated human ecology surveys. Their intergenerational population reproduction rate in the period without manifest modernization influence, or before approximately 1950, was estimated at only 1.05, using their genealogical records; this rate corresponds to 0.2% population increase rate per year. Largely because of highly malaria endemicity in the riverine villages, the increase rate based only on mothers of these villages was below zero.

For 183 blood samples collected in 1989 from four environmentally different villages, serum antibodies against *Plasmodium falciparum* and *P. vivax* were measured with fluorescent antibody test. The results demonstrate that the level of titers is correlated with the village environment. The highest was in the coastal village, followed by the riverine villages (two in number) and then the inland villages; the median was 1:256, 1:64 and 1:16, and 1:4 for females, and 1:64, 1:16 (both villages), and 1:4 for males.

The reconstruction of the Gidra Village locations in the past revealed that, in the initial stage (presumably, until one or two centuries ago), they were concentrated in the inland, and then the gradual population increase pushed some of them to the riverine and then coastal area. It is noted that the coastal and riverine villagers have been able to modernize their lifestyle since these villages are close to the town of Daru, the center of modernizing influence on the Gidra, despite the fact that the people dislike such mosquito-rich environment. These facts suggest that the Gidra people's entrance to the malarial holo-endemic zone was caused by their long-term population expansion, which is, in general, considered as an indicator of adapteness, and by their convenience of access to the town. Thus, any medical interventions should be planned, taking into account their survival history on the one hand and their preference about village locality, in a wide sense, on the other hand.

6 MEDICAL PLURALISM IN NORTHWEST AMAZON

HIDEO TAKEI
Oyasato Research Institute, Tenri University

Medical pluralism is a phenomenon which is found everywhere in the world. However, this condition is relatively new one among the ethnic minorities in the third world, who have kept on their traditional medicine. In the course of introducing modern scientific medicine to those people it has been understood that native cultural concepts of diseases and socioculturally shaped behavior of the people have made it difficult for them to accept the health system based on medical science in the way the health professionals expected. Hence, in a sense, has been formed Medical Anthropology.

In Japan, Medical Anthropology is still in formative stage, and the information exchange between health professionals who have worked in the third world countries and cultural anthropologists hardly has taken place until now, and the former has experienced many difficulties which may have been relieved by anthropologists' intervention.

In this paper I present my observation of a case of primary health care project which was going on during 1980s among native communities in Colombian Vaupés, northwest Amazon. The field research was carried out from December, 1986 to January, 1990, with several intervals (Research Grant from The Toyota Foundation, 1985 and 1988).

The cosmological concepts about disease causation among the native people in the upper Río Tiquié region proved not to be an absolute obstacle to the project. The concepts formed a rigid barrier for them not to identify the tuberculosis bacilli under microscope as the causative agent of tuberculosis, because they must have been invisible from their cosmological point of view. However, in case of constructing water supply system which utilizes collected rain water, they could overcome the difference between cosmological meanings attributed to rain water, dangerous, and river water, relatively safe.

Most problematic part of the project, I think, was the educational program, both in capacitation of native health personnel and in health education for whole community members. In both cases the teaching staff, who belong to politicoeconomically dominant "whites", always treated the natives in a paternalistic way, and the materials used in seminars hardly included native ones, even when treating nutritional values of foods. Thus the education always carried the same message: "White" things are all valuable and native ones not. Natives were clearly aware of it, feeling powerlessness of their own.

The presence of politicoeconomically powerful outsiders can always produce similar reactions. If we respect the people's taking the initiative and want to help them do on their own, we should be so careful about the way of our presence as not to impair their self-esteem.

General presentation

1 MEDICAL SURVEYS IN THE KINGDOM OF TONGA

HISAE TAKAHASHI[1], YOSHIYUKI OKUWAKI[1], MIYUKI ADACHI[2],
NOBUKO MURAYAMA[2], TAEKO OHUCHI[3], TILITILI PULOKA[4] AND SIAOSI AHO[5]
Department of Microbiology[1] and Department of Human and Food Ecology[2],
Kagawa Nutrition College, Kanagawa Prefectural Junior College of
Nutrition[3], Ministry of Health[4] and Viola Hospital[5],
the Kingdom of Tonga

It is reported that we serveyed inhabitant medical examination of adult Tongans in urban (Kolofoou) and rural (Uiha) areas.

A positive sugar reaction in urinalysis was detected in only 6 out of 250 Tongans, which found in only urban area. The number of a positive protein reaction in urinalysis was 17 persons (3 males and 14 females) out of 250 Tongans.

Hematocrit test in blood examination showed the range of 37-56% in males and the range of 35-50% in females.

As to fourteen items of blood chemistry, the significance of the difference between values for Kolofoou and Uiha was assessed on the basis of Student's t-test. In males, there was a great difference in γ-GTP, ChE, TG, LDH, BUN, and UA between Kolofoou inhabitant and Uiha ones. In females, there was also a great difference in γ-GTP, TG and LDH between Kolofoou inhabitant and Uiha ones.

Comparison of the medical examination of Tongan adults in urban and rural area, abnormal findings in urine sugar reaction were only found in urban inhabitants, and TG value in urban inhabitant tended to be higher than that in rural ones.

2 SEROEPIDEMIOLOGICAL STUDY OF HEPATITIS B VIRUSES INFECTION IN THE KINGDOM OF TONGA

HISAE TAKAHASHI[1], YOSHIYUKI OKUWAKI[1], MIYUKI ADACHI[2],
NOBUKO MURAYAMA[2], TAEKO OHUCHI[3], TILITILI PULOKA[4] AND SIAOSI AHO[5]
Department of Microbiology[1] and Department of Human and Food Ecology[2],
Kagawa Nutrition College, Kanagawa Prefectural Junior College of
Nutrition[3], Ministry of Health[4] and Viola Hospital[5],
the Kingdom of Tonga

We studied for serological evidence of current or past infection with hepatitis B virus, and relationship between liver function (GOT, GPT) and hepatitis B virus markers.

As to the results of HBV examinations, 48 out of 249 subjects (19.3%) were found to be positive for HBsAg, and 11 out of 39 in them had HBeAg, 25 subjects had Anti-HBe antibody, and remaining 3 subjects did not have both.

One-hundred and ninety-four out of 249 subjects (77.9%) were found to be positive for Anti-HBs antibody. Five out of 7 subjects that HBsAg and Anti-HBs antibody negative respectively were tested for Anti-HBc antibody. Three out of them were found to be positive, and remaining 2 did not detected. Overall, 97.5% of these subjects showed the evidence of hapatitis B virus infection.

As to the results of GOT and GPT, GOT value over 42 IU/*l* shown 2.4% of subjects. GPT value over 37 IU/*l* was showed 16.8% of subjects. Only 1 out of 249 subjects was maked a diagnosis of liver injury.

These results indicate that hepatitis B infection in Kingdom of Tonga is healthy carrier.

3 VIROLOGICAL STUDIES ON POLIOMYELITIS IN KARACHI, PAKISTAN

SHIN ISOMURA[1], DURE-SAMIN AKRAM[2] AND MUBINA AGBOATWALLA[2]
Aichi Prefectural Institute of Public Health[1] and
Civil Hospital, Karachi[2]

Enterovirus isolations from fecal samples were carried out on 58 poliomyelitis patients in their acute stages of the illness at Civil Hospital Karachi during a period of November 1989-October 1990. Feces of age matched 203 diarrheal children and 64 healthy children living in the same area were studied as control. Clinically, ages of the patients were primarily around one year old and onset of the illness was distributed all the year around, mostly September 1990. Main clinical manifestation was acute flassid paralysis of their extremities.

Enteroviruses were isolated from 86.2% of the polio cases, from 49.3% of diarrheal children and from 42.2% of healthy children, respectively. More than half of the viruses isolated from the polio patients were polio virus, and all these polio viruses belonged to wild type. Isolates from diarrheal and healthy children were consisted of various types of enteroviruses, including many strain of CoxA, CoxB, ECHO, Entero 71 viruses and several strain of polio virus of wild type. Vaccine type polio viruses were isolated from only a few healthy and diarrheal children.

Virological examinations and serological tests including polio-specific IgM antibody disclosed that dominant causative agent of the epidemic was wild type Polio type 1.

4 CURRENT SITUATION OF JAPANESE ENCEPHALITIS AND PRODUCTION OF PREVENTIVE VACCINE IN VIETNAM

AKIRA IGARASHI[1] AND MITSUO TAKAGI[2]
Department of Virology, Institute of Tropical Medicine,
Nagasaki University[1] and Kanonji Institute, Research Foundation of
Microbial Diseases Osaka University[2]

Japanese encephalitis (JE) has been prevalent in several developing countries in Asia. In

Vietnam, the first outbreak of acute encephalitis syndrome (AES), clinically compatible with JE, was reported in 1964 in Bac Thai Province in the North, and JE virus was isolated from patient's blood. Serodiagnosis by the hemagglutination-inhibition test showed positive reaction with JE antigen for 1/3-2/3 of AES patient's sera, while JE virus was isolated from wild birds, swine and vector mosquitoes.

For the control of JE, Vietnamese government has requested WHO to supply JE vaccine, and 148,000 doses of the vaccine were donated through international organizations. A part of these vaccines was given to high risk age groups in a selected study area, Dong Anh District in Hanoi, in pre-epidemic season in 1986. The number of AES cases in this pilot area was greatly reduced in the following years.

In response to the recommendation of the first author as a WHO consultant, the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi started to produce JE vaccine of international standard by technical transfer from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University. All the pilot products in NIHE were proved to be satisfactory by the current Japanese Standard for Biological products.

Although current production facilities at NIHE are quite insufficient, the Ministry of Health as well as NIHE regarded production of JE vaccine as a high priority target.

The production method of JE vaccine at NIHE was basically satisfactory, and should be strengthened by supplying essential equipments and consukable items. Due to limited amount of mouse supply, current production capacity was estimated as maximum 40 liters/year, which is approximately 1/12 of the amount required to immunize 80% of target population (600,000 children under 3 years old) in JE-endemic areas in Vietnam.

For the time being, quality control on the final product, particularly biological tests, requires monitoring by appropriate Japanese Institution until the test at NIHE will provide reproducible results. Financial support is required to construct an integrated vaccine production facility, which can produce not only JE but also other vaccines such as polio and hepatitis B which are currently produced at NIHE.

5 HISTOPATHOLOGY OF LIVER IN DENGUE INFECTIONS: A FACET OF THE PATHOGENESIS OF DENGUE HEMORRHAGIC FEVER/SHOCK SYNDROME (DHF/DSS)

SUSUMU HOTTA
Department of Tropical Medicine, Medical Research Institute,
Kanazawa Medical University

Monkeys infected intracutan, or subcutan, with DV show pyrexia and viremia which however are variable and which therefore cannot necessarily be used as markers of infection. Histopathological alterations of the liver of infected monkeys are inevitable and constant; changes of varying degrees such as fatty degeneration and cellular infiltration appear. It seems that the cellular damages are parallel with virulence of infecting virus; when an "attenuated" strain of DV, e.g. Mochizuki strain, is inoculated, the pathological pictures manifested are mild, whereas the changes shown in monkeys inoculated with a newly isolated

(human-virulent) virus are much severer. The liver damages determined by a "scoring" method may be a "marker of virulence" of DV.

Tissues from fatal patients exhibit changes of varying sorts and degrees. Particularly, changes of liver are marked. "Midzonal necrosis" appear. Kupffer cells contain eosinophilic granular structures which cannot be distinguished from Councilman bodies seen in the liver of yellow fever patients. DV antigen can be detected by immunoenzyme antibody stain method. By use of anti-DV type-specific monoclonal antibodies, types of infecting viruses can be determined. Pictures of "macrophage activation" are revealed in various tissues.

Mice succumb to encephalitis after being ic-inoculated with DV. However, liver damages of ic-infected mice are not marked. An extraneural (e.g. ip) inoculation of DV can hardly infect ordinary mice. Contrarily "nude mice" can be infected with DV through ip route. The active virus is detected in various tissues including liver. The liver cells reveal degeneration and Kupffer cells contain eosinophilic granular structures which resemble the Councilman bodies found in the liver of humans. Specific DEN antigen is detected in those cells by applying immunofluorescent or immunoenzyme stain techniques. Similar changes are seen in liver of nude mice inoculated ip with YF virus 17D strain.

In summary, the liver damage is one of the characteristic signs of DV infection commonly in monkeys, humans and nude mice. Hence DV may be called a "hepatotropic" agent and the damage of liver is perhaps a key phenomenon underlying the pathogenesis of dengue. The hepatotropism is also shared by YF virus. It has already been known that both viruses are very near in antigenic properties as well as in molecular-biological characteristics such as nucleotides and amino acids sequences of genomic RNA (The human specimens were taken from the collection of USA-AFIP, Washington, D.C. during the speaker's stay there. He is very grateful to Dr. Wear, Mr. Duckett and Mr. Bratthauer for their kindness in providing the materials. His deep thanks are also due to Dr. Russell and the scientific staff of WRAIR for their helpful suggestions and favorable arrangements).

6 TRANSOVARIAL TRANSMISSION OF ARBOVIRUS IN *AEDES ALBOPICTUS* MOSQUITOES CONCURRENTLY INGESTING MICROFILARIAE OF *DIROFILARIA* SPP.

EIMAN M. ZYTOON[1], HUSSEIN I. EL-BELBASEI[2] AND TAKEO MATSUMURA[1]
Department of Medical Zoology, Kobe University School of Medicine[1]
and Kobe Women's University[2]

Vertical transmission, direct transfer of virus from parent to progeny can occur in arthropods by a variety of mechanism including transovarial transmission (TOT) or transovum transfer. In 1987 the possibility of TOT of chikungunya virus (CHIK) in *Aedes albopictus* mosquitoes was studied by Dr. Mourya and his results indicated absence of TOT of CHIK in *A. albopictus* mosquitoes after oral and intrathoracic infection.

From our previous studies, evidence has been accumulated to show the dissemination of CHIK in mosquitoe's legs and salivary glands in *A. albopictus* mosquitoes that concurrently ingesting microfilaria (Mf) of *Dirofilaria* spp. From that purpose there has been considerably

recent interest in the possible way of TOT of CHIK in *A. albopictus* (Miki strain) concurently ingesting Mf.

In order to evaluate the importance of this mechanism for arbovirus transmission and maintenance, experiments were carried out by allowing these mosquitoes for ingesting CHIK and Mf to investigate the potential effect of a variety of the mechanical factors on TOT.

Mosquitoes were kept in an insectary at approximately 28°C with a photoperiod of 16 hrs of light and 8 hrs of darkness. Experiments were carried out for two groups of parent mosquitoes by allowing them to become fully engorged with the sheep difibrinated blood mixed with 3×10^3 PFU per m*l* and 40-50 Mf per mosquito and compared the recovery of CHIK from eggs, larvae, pupae, F_1 and F_2 in these mosquitoes with those ingesting CHIK alone as control. Fourty per cent of eggs from first ovarian cycle after embryogenesis was complete, were emmerged in water to induce hatching while the remaining eggs were dried and stored at 28°C until virus assay. Each pool of eggs, larvae pupae, F_1 and F_2 was titrated by plaque assay technique. The above experiment shows that CHIK was detected from pools of eggs, pupae and F_1 of first ovarian cycle but not detected from any pools of larvae of first ovarian cycle and F_2 of second ovarian cycle during two repeated experiments. None of parents, eggs, larvae, pupae, F_1 and F_2 of control mosquitoes that ingested CHIK alone showed the presence of CHIK. Biological TOT in arboviruses in its arthropod vector at an adequate rate appears to be an extremely efficient mechanism to insure a survival way of the agent during adverse environmental condition.

7 PREVALENCE OF LYME *BORRELIA* IN TICK FAUNA OF HOKURIKU DISTRICT WHERE THE DISTRIBUTIONS OF NORTHERN AND SOUTHERN SPECIES OVERLAP EACH OTHER

NOBUHIRO TAKADA[1], FUBITO ISHIGURO[2] AND YASUHIRO YANO[1]
Department of Parasitology, Fukui Medical School[1] and
Fukui Prefectural Institute of Health[2]

Recently Lyme borreliosis has been known to occure arround the world, and some tick species as definite or possible vector are nominated in USA, Europe and Northern Asia. Since 1987, this disease have been reported also in Japan.

So, to clarify the epidemiological situation of this disease in Hokuriku District, we surveyed tick fauna and the prevalence of the pathogen *Borrelia burgdorferi* in 14 mountaineous points of Fukui Pref. and 6 similar points of Gifu Pref. during April to July in 1991. In this time, 4 genera and 13 species belonging Family Ixodidae were found. Of these, most of common species including *Ixodes ovatus* were collected throughout the hilly or lower mountaineous areas, and also the southern species, *Ammblyoma testudinarium* and *Dermacentor taiwanensis* were first recorded in Fukui Pref. The famous northern species, *I. persulcatus* and *I. angustus* were collected only at high mountains over 800 m. Three-hundred-thirty-seven adults consisting of 4 genera and 7 species were individually examined to isolate *Borrelia*. As results, 34 and 5 strains were isolated from *I. ovatus* and *I. persulcatus*, respectively, such as the former was positive in 20.8% from Fukui Pref. and 12.5% from Gifu

Pref., and the latter positive in 11.1% from Fukui Pref. and 15.4% from Gifu Pref. It should be discussed which species of ticks is the most potential vector of this disease in southwestern Japan, as *I. persulcatus* is a rare one in human habitat south of Hokuriku District.

8 INVESTIGATION ON THE PREVALENCE OF LEPTOSPIROSIS AMONG CATS IN OKINAWA, JAPAN

YOSHIKATU YONAHARA[1], KATSUMASA TOKUMURA[1], EIZO KINJO[2], YOSHIO SHINGAKI[2], MASAJI NAKAMURA[3], MASASHI TOGUCHI[3] AND YOSHIO CHIBA[4]

Okinawa Prefectural Institute of Public Health[1], Okinawa Prefectural Animal Management Center[2], Okinawa Prefectural Meat Hygiene Inspection Center[3] and Okinawa Prefectural Livestock Experiment Station[4]

Leptospirosis is a typical zoonosis and one of the important diseases in public health. Rodents are important animal reservoirs for its transmission, and human infection is caused by direct contact with water and soil contaminated with the rodent's urine, but recent studies have suggested that human leptospirosis may occur by contacting with an infected pet itself.

Eight-hundred-twenty-two stray cats from 6 health centers and Kadena Air Base were examined for leptospirosis. Eight-hundred-three kidneys and 48 blood samples were cultivated in order to isolate the *Leptospira*. Eight-hundred-sixteen serum samples from stray cats were tested for antibody against *Leptospira* by microscopic agglutination test (MAT). The titers of *Leptospira* antibody, 1:80 or greater, were considered positive. A portion of kidney that remained after the isolation of *Leptospira* was examined by pathologically.

No positive blood cultures were obtained. *Leptospira* were isolated from 12 samples out of 803 kidney cultures (1.5%); 7 strains isolated were serovar *canicola* and 5 serovar *javanica*. Sixty-nine out of 816 serum samples (8.5%) showed positive reaction by MAT. The most prevalent serovar was *javanica*, and followed by serovar *canicola* and *pyrogenes*. But there were no positive antibody against serovar *bataviae* and *grippotyphosa*. Cats in the central and northern areas of Okinawa showed a higher positive rate of *Leptospira* antibody in comparison with other areas. There was no statistical difference between both sexes in the positive rates, although 9.4% of male and 7.4% of female showed a positive reaction. The presence of *Leptospira* were comfirmed inside the uriniferous tubules of cat's kidney.

These results suggest that cats can be one of the important reservoir of *Leptospira*. Further studies are required from the point of preventive measures, because cats can be a source of human leptospirosis.

9 ANALYSIS OF DRINKING WATER IN AIRPORT, AIRPLANE AND PUBLIC WATER FACILITY

ETSUSHI OKUMURA[1], KEISUKE HASHIMOTO[2], NARUHIKO HASHIMOTO[2],
MITSUTAKA HATA[3] AND YASUSHI OKURURA[4]
Department of Health Science, Kochi Gakuen Junior College[1],
Association of Tropical Medicine, Osaka Medical College[2],
Department of Clinical Technology, Minami Osaka General Hospital[3]
and Okumura Medical Clinic[4]

At this time, 10 years of 1981 to 1991, we opperated the examination of drinking water which the tourist can easily get from the airport, airplane and public water facilities in foreign countries. Through the result of bacteriological examination, we mainly studied to prevent the infection from the drinking water during the traveling.

The result were; 4 out of 9 airports, the ratio of 44.4%, and 2 out of 9 airplanes, the ratio of 22.2% showed the positive result on ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen. The consumption of the potassium permanganate which showed more than 20 mg/*l* were, 8 out of 14 airports, the ratio of 57.1%, 8 out of 12 airplanes, the ratio of 66.7% and 10 out of 16 public institutions, the ratio of 62.5%.

The hardness which showed more than 200 mg/*l* of hard water were, 5 out of 16 airports, the ratio of 31.3%, 3 out of 16 airplanes, the ratio of 18.8% and 3 out of 20 public institutions, the ratio of 15.0%.

The total bacteria which showed more than 1.0×10^2/m*l* were, 6 out of 9 airports, the ratio of 66.7%, 7 out of 11 airplanes, the ratio of 63.6% and 11 out of 18 public institutions, the ratio of 61.1%. The water which showed positive results of coli-form group were, 5 out of 8 airports, the ratio of 62.5%, 6 out of 10 airplanes, the ratio of 60.0% and 4 out of 11 public institutions, the ratio of 36.4%.

The drinking water which did not prove the existence of remained the residual chlorine were, 9 out of 16 airports, the ratio of 56.3%, 2 out of 5 airplanes, the ratio of 40.0% and 4 out of 7 public institutions, the ratio of 57.1%.

We studied also the bacteriological examination on 29 object of the total bacteria and the coli-form group. *Escherichia coli* were the predominantly detected bacteria, and after that *Acinetobacter calcoaceticus, Aeromonas hydrophilia, Pseudomonas* sp., *Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae* and *Citrobacter freundii* was followed.

We made the comparison of following water, the drinking water which showed the positive result of total bacteria and coli-form group, the water which proved positive in ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen, the drinking water which showed the high value of the consumption of potassium permanganate and the water which showed the negative result of residual chlorine. Their correlation was high.

10 BACTERIOLOGICAL SURVEYS OF DRINKING WATER IN INDIA

HISAE TAKAHASHI[1], YOSHIYUKI OKUWAKI[1], KOICHIRO FIJITA[2],
SETSUKO TSUKIDATE[2], YASUKO OGASAWARA[3] AND TAKEO ASAKURA[3]
Department of Microbiology, Kagawa Nutrition College[1],
Department of Medical Zoology, Faculty of Medicine,
Tokyo Medical and Dental University[2]
and Japan Association of Tropical Medicine[3]

In order to know the influence of the degree of the pollution of the drinking water, we carried out surveys in way of bacteriological examination of the drinking water in various regions of India. The investigation was done in November of 1990.

Tap water, well water, boiled water, filtrated water, commercial mineral water and green tea were collected and examined.

We carried out a quantitative test of total colonies and coli-form group counts, using URICULT set that houses CLED and MacConkey media.

Upon returning to Japan, we ran a culture, using agars of SS, MacConkey, modified Drigalski, DHL for the purpose of isolating enteric bacteria.

Total colonies were detected in 32 out of 41 samples. Coli-form groups were detected in 17 out of 41 samples.

Enterobacter sp., *Enterobacter cloacae* and *Klebsiella pneumoniae* were found in all samples of the green teas, one of the boiled water and one of the tap water.

11 PREVALENCE AND DRUG RESISTANCE OF ENTEROPATHOGENIC BACTERIA IN CHILDFOOD IN RURAL GHANA

TAKASHI NAKANO[1], MINORU SAKURAI[1], HITOSHI KAMIYA[2],
DALETH AGBODAZE[3] AND EDWIN AFARI[3]
Department of Pediatrics, Mie University School of Medicine[1],
Mie National Hospital[2] and Noguchi Memorial Institute for
Medical Research, Ghana[3]

A survey was carried out over 1 year in a rural area of Ghana on the isolation of enteropathogenic bacteria from children under 5 years of age. From June 1987 to May 1988, 465 faecal specimens were examined; 196 from the diarrhoeal and 269 from the non-diarrhoeal children. The survey was conducted in the village which was about 70 km west of Accra. *Shigella* species were the most frequently isolated bacteria, followed by *Campylobacter,* EPEC and ETEC. Isolation of *Salmonella* species and *Aeromonas hydrophilia* was rather infrequent. *Vibrio cholerae, Vibrio parahaemolyticus* and *Yersinia enterocolitica* were not isolated during the survey period. The isolation rate of *Shigella flexneri* was significantly higher in the diarrhoeal children.

We tested our isolates against antimicrobial agents for susceptibility using the agar

diffusion method with sensitivity disc. Kanamycin (KM), sulfamethoxazole-trimethoprim (ST), nalidixic acid (NA), and minocycline (MINO) were the effective drugs against the strains of *Shigella* isolated from our survey. *Campylobacter jejuni* strains were susceptible to erythromycin (EM), fosfomycin (FOM), MINO, and other kinds of antibiotics. The susceptibility of EPEC and ETEC to orally medicative antibiotics was not good enough, but KM was acceptable among them (65% strains susceptible).

The efficacy of antibiotics for shigellosis is established, but controversial for other bacteria. It may be useful for severe or persistent cases caused by them. You cannot always find laboratories where they can isolate and identify bacteria in developing countries. When you encounter children of bacterial enteric infection who need antibiotic therapy, KM can be the first choice drug.

12 SERUM ANTIBODY TITERS OF AMAZON INDIANS TO THREE MAJOR PATHOGENIC ORGANISMS OF RESPIRATORY INFECTION (*H. INFLUENZAE, B. CATARRHALIS* AND *S. PNEUMONIAE*)

YOSHIAKI UTSUNOMIYA[1], SHIGEYUKI KANO[2], TAKESHI ALFRED HONDA[3], MILTON MASATO HIDA[4], ATSUSHI TAKAHASHI[1] AND KEIZO MATSUMOTO[2]
Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University[1], Department of Parasitology, Gunma University School of Medicine[2], Hospital Amazonia[3] and Departamento de Oftalmologia, Universidade Estadual Paulista[4], Brazil

Haemophilus influenzae, Streptococcus pneumoniae and *Branhamella catarrhalis* are now important as major causative organisms of respiratory infection in Japan. We have reported that these organisms are also common in Thailand by our collaboration studies with Chiang Mai University. Although there are few reports about respiratory causative organisms in tropical area. To examine IgG antibodies in sera to *H. influenzae, S. pneumoniae* and *B. catarrhalis* we collected blood samples from twenty Kaiapos' Indians aged from 8 to 74, where they live in a small village located about 800 km to the south of Belem in Brazil and compared with those of healthy Japanese, frequent *H. influenzae* infection group, frequent *S. pneumoniae* infection group and frequent *B. catarrhalis* infection group (healthy Japanese: n=10, frequent *H. influenzae* group: n=9, frequent *S. pneumoniae* infection group: n=8, frequent *B. catarrhalis* infection group: n=8). Two strains of *H. influenzae* and one strain of *B. catarrhalis* were isolated from the patients with chronic bronchitis in our department. Outer membrane protein (OMP) and lipopolysaccharide (LPS) were obtained from each strain and prepared as antigen for measurement of IgG antibodies in sera to *H. influenzae* and *B. catarrhalis*. Also 23-valent pneumococcal polysaccharide (pneumovax™: Banyu corporation) were used as antigen for measurement of IgG antibodies in sera to *S. pneumoniae*. Serum antibodies were measured by ELISA. The average serum IgG antibody titers (fold) of healthy Japanese, each frequent infection group and Kaiapos' Indians were respectively (1) to OMP of *H. influenzae*: 4,222, 4,703, 3,805 (2) to LPS of *H. influenzae*: 220, 732, 362 (3) to OMP of *B. catarrhalis*: 857, 2,167, 968 (4) to LPS of *B. catarrhalis*: 71, 283, 198 (5)

to Pneumovax™: 919, 1,744, 791. There were no significant differences between healthy Japanese and Kaiapos' Indians. Also antibodies in sera of Kaiapos' Indians were not correlative with age. These results demonstrated that Kaiapos' Indians in Amazon are exposed by *H. influenzae, B. catarrhalis* and *S. pneumoniae* as well as healthy Japanese.

13 A SURVEY OF MEDICAL PROBLEMS AMONG REFUGEES IN IRAN

KIYOKATSU TANABE
Department of Medical Zoology, Faculty of Medicine,
Kagoshima University

The allied forces ceased offensive operations against Iraq on February 28 of this year however, in late March hundreds of thousands of Kurdish fled to the mountains of neighboring Iran. The Iran officials prepared temporary refugee centers, and the Japanese government sent the rotating (1st to 5th) medical teams of the JDR (Japanese Disaster Relief) to assist a field clinic in Oshnaviyeh Town from April until June. I participated in the 4th team and performed tent clinic for 14 days. In this period 2,851 patients visited the clinic, on an average of 210 patients per day. More than a half of them were middle or old ages.

Concerning the profile of their illnesses, infectious diseases occupied 38% of the total. The infectious diseases are classified as bronchopneumonia (46%), entero-colitis (10%), systemic dermatitis (12%), otitis media (8%), urinary tract infection (6%) and conjunctivitis (5%). Parasite infections were detected among school children. Pinworm infection was popular but ascariasis was less frequent. Giardiasis was suspected as a cause of diarrhea. As an endemic disease simple goiter was frequently seen among women. Urolithiasis was also usual illness by drinking Ca-rich water.

Each facility provided by each nation seemed to be of great help to the Iraqi refugees however, more developed relief systems may be possible to operate the refugee field clinic. In the future cooperation among each clinic will be successful by providing professional medical informations and equipments which would otherwise not been available.

14 *CONUS*-STINGS IN OKINAWA

YASUTETSU ARAKI[1], YASUHIRO TOMIHARA[1], KEISUKE FUKUMURA[1]
AND SHIGEO YOSHIBA[2]
Department of Habu, Okinawa Prefectural Institute of
Public Health[1] and Department of Hygiene,
Jikei University School of Medicine[2]

The conches of Conidae are classified into about 500 species, and are globally distributed in the warm-current sea areas, of which 120 species live in the sea around Japan (Habe and Kosuge, 1967). They are all carnivorous and guard themselves with a poisonous organ.

Moreover, they have a special food-catching apparatus well developed to meet their own way of eating.

So far, 18 cases of *Conus*-stings have occurred in Okinawa including 4 death cases (22.2%). They are divided into 13 cases (72.2%) by *Gast ridium* (*Conus*) *geographus,* and 1 case each (5.6%) by *Strioconus striatus, Darioconus textile* and *Pionoconus magus.* The remaining 2 cases (11.1%) are caused by unknown species. All of the death cases are due to *Gastridium geographus.*

The injured parts are mostly the finger (11 cases, 61.1%), followed by the palm (3 cases, 16.7%) and the chest (2 cases, 11.1%). Also, there has been reported 1 case each (5.6%) on the forearm and the sole.

Many species of the Conidae shells have beautiful appearance, and most cases of *Conus*-stings occur when people are led to touch them without knowing their poisonousness. As for other stinging cases, the process of *Conus*-stings remain unknown. It is therefore necessary to campaign for hygienic education to warn the public against the dangerous nature of the shells.

15 A QUESTIONNAIRE SURVEY ABOUT LIFE-STYLE RELATED TO PARASITIC INFECTIONS IN HUEY KEAW VILLAGE, NORTHERN THAILAND

MIKI MORI[1], MUTSUMASA YOH[1], MASANORI NAKAGAWA[1], TAKAO KAWAI[1],
KAORI KOGA[2], SHIRO KASUYA[2], RASSAMME KEAWVICHIT[3]
AND CHIRASAK KHAMBOONRUANG[3]
Medical Students[1], Department of Parasitology[2], Gifu University
School of Medicine and Research Institute for Health Sciences,
Chiang Mai University, Thailand[3]

We have studied about primary schoolchildren for their intestinal parasitic infections since 1986 in Chiang Mai Province, nothern Thailand. From the difference of infection rates among the various areas, we supposed that the parasitic infections were closely related with the life-style, the climate and/or the culture of the area.

In this study, we have researched about life-style related to parasitic infections by questionnaire to the villagers of Huey Keaw Village, northern Thailand who had already undergone stool examinations by Koga *et al.* (see next presentation). One hundred and forty-three villagers ranging from 5 to 86 years answered a questionnaire. The questions and answers were mentioned below.

(Questionnaire about your health)

1. What is your occupation?
   a. farmer 29% b. labour 6% c. student 62% d. officer 1%
2. Do you farm? a. every day 20% b. sometimes 65% c. no 15%
3. Do you walk outdoors barefoot?
   a. always 8% b. sometimes 41% c. rarely 10% d. never 41%
4. Do you use human feces as manure?

a. always 1% b. sometimes 2% c. no 97%

5. What kind of toilet do you use?
   a. type 1: 85% b. type 2: 2% c. type 3: 4% d. no 8%
6. How do you get your drinking water?
   a. rain 6% b. well 83% c. deep well 8% d. pond 3%
7. Do you bathe in the river? a. yes 66% b. no 34%
8. Do you eat raw vegetables?
   a. every day 21% b. sometimes 66% c. rarely 10% d. never 3%
9. Do you eat raw fish?
   a. every day 1% b. sometimes 18% c. rarely 24% d. never 57%
10. Do you eat raw crab?
    a. every day 0% b. sometimes 9% c. rarely 13% d. never 78%
11. Do you eat raw snail?
    a. every day 0% b. sometimes 3% c. rarely 8% d. never 89%
12. Do you eat these dishes?
    Nahm a. yes 65% b. no 35% Larb a. yes 62% b. no 38%
    Larp-pla a. yes 33% b. no 67% Koi-pla a. yes 0% b. no 100%
    Pla-som a. yes 45% b. no 55%
13. Have you ever eat Maklua? a. yes 13% b. no 87%

And we compared the answers from parasite-positive villagers with the ones from negative villagers. *Strongyloides*-positive people answered to farm and walk barefoot more than negative people. Hookworm-positive people answered to farm more, but to walk barefoot less than negative people. *Opisthorchis*-positive people answered to farm and eat raw fish more than negative people.

Most of the villagers were farmers. Some of them still walk barefoot, bathe in the river and eat raw animals. However, most of the household already have toilets and wells as fundamental conditions for sanitary life.

16 INTESTINAL PARASITIC INFECTIONS AMONG VILLAGERS IN HUEY KEAW VILLAGE, NORTHERN THAILAND; STOOL EXAMINATION INCLUDING AGAR PLATE METHOD

KAORI KOGA[1], SATOE TOMITA[2], SHIRO KASUYA[1], RASSAMEE KEAWVICHIT[3], CHIRASAK KHAMBOONRUANG[3] AND HIROSHI OHTOMO[1]
Department of Parasitology[1] and Medical Student[2], Gifu University School of Medicine and Research Institute for Health Sciences, Chiang Mai University, Thailand[3]

We have studied about primary schoolchildren for their intestinal parasitic infections since 1986 in Chiang Mai Province, nothern Thailand. Generally, the present state of parasitic infections in this area is; *Ascaris lumbricoides* infection has drastically decreased, under the Intestinal Helminthiasis Control and Eradication Project of Thailand, however, *Opisthorchis viverrini* infection, probably one of the causes of liver cancer, is now increasing.

Moreover, we have been reporting that the agar plate method is much more reliable to detect *Strongyloides* than other traditional stool examination methods, also in this area.

In this study, stool examination from not only children but also adult villagers in Huey Keaw Village, Chiang Mai Province, northern Thailand, was performed to determine the present state of parasitic infections in the village.

The positive rate of intestinal parasites seen among 254 villagers was 61.4%. The most common parasite was *Strongyloides* (33.9%) followed by hookworm (24.4%), *Opisthorchis viverrini* (16.1%), *Giardia lamblia* (6.3%), *Entamoeba coli* (5.9%), *Taenia* spp. (3.9%). Ascariasis was not found. By using the agar plate method, it was shown that *Strongyloides* was highly and most prevalent parasite in this area. *O. viverrini* infection rate was also much higher than the rate in our preliminary studies, mainly because of difference of the ages of examined persons.

Prevalence rate of *Strongyloides* according to age groups increases steadily, showing over 50% in 40-59 years groups. Rate of both hookworm and *Opisthorchis* were different between under and over 20 years old groups. Adults have these worms more than children.

Enterobius vermicularis infection rate was also examined by using the cellophan tape method among 49, 1-5 year children. The positive rate was 34.7%.

The agar plate method could detect 59.7% hookworm-positive cases. And 10 cases was detected only by this method. From these results, it was supposed that this method could be used also for hookworm.

17 LONG-TERM FECAL EXAMINATION AFTER ADMINISTRATION OF ANTHELMINTICS FOR STRONGYLOIDIASIS

RYUJI ASATO[1], TAMIO NAKASONE[1], CHOKEI YOSHIDA[1], TAMIKI ARAKAKI[2], ATSUSHI SAITO[3], KOSHIN SHIKIYA[3], SHOKO SHIROMA[4], SEIKO SHIROMA[5] AND SHOSEI ISHIHARA[6]

Okinawa Prefectural Institute of Public Health[1], Department of Bacteriology[2] and 1st Department of Internal Medicine[3], School of Medicine, University of the Ryukyus, Izumisaki Hospital[4], Nanbu Hospital of Okinawa Prefecture[5] and Nakagami Hospital[6]

The therapeutic efficacy of several anthelmintics for strongyloidiasis was evaluated by using the agar-plate culture of feces up to 2 years after administration. The anthelmintics used and dosage were as follows: Group 1. pyrvinium pamoate 5 mg/kg/day×5 days×3 courses (57 cases); Group 2. thiabendazole 1.5 g/day×3 days×(1-4) courses (79 cases); Group 3. thiabendazole 1.5 g/day×5 days×(1-4) courses (131 cases); Group 4. thiabendazole 1.0 g/day×3 days×3 courses (77 cases); Group 5. mebendazole 200 mg/day×(15-28) days (116 cases); Group 6. mebendazole 200 mg/day×18 days+thiabendazole 1.5 g/day×10 days (24 cases); Group 7. ivermectin 6 mg/day×2 (49 cases).

By the post-treatment fecal examinations, reappearance of *Strongyloides* larvae was observed in 11, 4, 1 and 2 cases after 6 months, 1, 1.5 and 2 years, respectively, in Group 1, in 3 cases after 6 months in Group 2, in 4 cases after 6 months in Group 3, in each 4 cases after

6 months and 1 year in Group 4, in 16, 7 and 1 cases after 6 months, 1 and 2 years, respectively, in Group 5, and in 4 and 1 cases after 2 and 6 months in Group 7. The final cure rate was 68.4% in Group 1, 96.2% in Group 2, 97.0% in Group 3, 89.6% in Group 4, 79.3% in Group 5, 100% in Group 6 and 89.8% in Group 7.

Although thiabendazole and mebendazole showed high cure rate, they often accompanied with severe side effects. Thus, these anthelmintics are considered to be unsuitable for mass treatment of apparently healthy *Strongyloides* carriers. On the other hand, ivermectin was highly efficacious and had little side effects, and may be the most suitable anthelmintic for mass treatment of *Strongyloides* infection. Because the recurrence of infection was observed at 2nd year after the treatment in some cases, the complete cure should be judged after careful long-term fecal examinations.

18 NIGERIAN ONCHOCERCIASIS: EPIDEMIOLOGICAL PERSPECTIVE

KUNINORI SHIWAKU[1], BETRAM E.B. NWOKE[2] AND HIROSHI TAKAHASHI[3]
Department of Environmental Medicine, Shimane Medical University[1],
School of Biological Science, Imo State University, Nigeria[2]
and Nerima-ku, Tokyo[3]

Onchocerciasis is a widespread filarial disease in Nigeria that produces grave socio-economic effects. The great majority of the communities are mesoendemic for onchocerciasis while only few are hyperendemic especially in the savanna zone. Sex-related infection depends on the degree of endemicity while age-infection increases gradually with advancing age. Visible and palpable nodules which are more abundant around the pelvic region are countered more abundant around the pelvic region are encountered more in the rain forest zone even when microfilarial density (MFD) is moderate while it is less numerous in the savanna form with high MFD. The microfilariae in concert with host's immune response precipitate various skin lesions. The resulting pruritus, scratching and itching among other lesions are generalized in the rain forest and localized in the savanna. In the eye, various ocular lesions are associated with the death of microfilariae. However, there is high incidence of eye lesion in the savanna zone than in the forest zone, especially the anterior lesions. The epidemiological picture presented by onchocerciasis in Nigeria is the summation of a complex array of contributing factors, both intrinsic to the microfilariae and resulting from the host-immune response, bioclimatic factors and vector species complex.

19 SECULAR TRENDS IN PREVALENCE OF ONCHOCERCIASIS IN SAN VICENTE PACAYA, GUATEMALA

TAKESUMI YOSHIMURA[1], MASATO IKEDA[1], YOSHIHISA HASHIGUCHI[2], YOUICHI ITO[3], ISAO TADA[4] AND G. ZEA FLORES[5]
Department of Clinical Epidemiology, University of Occupational and Environmental Health[1], Department of Parasitology, Kochi Medical School[2], Department of Parasitology, Kitasato University School of Medicine[3], Department of Parasitic Diseases, Kumamoto University School of Medicine[4] and Ministry of Health, Guatemala[5]

Present paper aims to evaluate the effect of vector control program on prevalence rate on Onchocerciasis in San Vicente Pacaya, Guatemala.

The vector (simulium) control by larvicide has been operated since 1979 in San Vicente Pacaya (pilot area), Guatemala. Almost all of the residents in the pilot area, have been examined almost annually by two skin snip biopsies since 1977.

Total examinees between 1977 and 1988 in the area were 4,003 in male and 4,581 in female. Prevalence rates of microfilaria positives for the persons aged 0 through 59 and for those aged 0 through 9 by sex in each year examined were calculated for valid comparison among years examined. Direct standardization method was adopted for the former prevalence rate.

The prevalence rates of microfilaria positives tended to be decreased from 40% to 20% after 1984, for male aged 0-59 years old. For female aged 0-59 years old, rates also tended to be decreased. If the vector control is successful, the effect on prevalence rate of onchocerciasis should be seen in early age group. Therefore, prevalence rates were calculated for those aged 0 through 9. For male decreased trend of prevalence was observed after 1982 and for female the same tendency was seen after 1984.

However, it should be considered that if some of the residents diagnosed as microfilaria positives selectively refused to be examined in the following year, decreased trend observed would be expected, even if no effect of vector control on the prevalence exist. It would be also possible that the prevalence rates in the control area would be decreased naturally due to social development not due to vector control.

In conclusion, even present data showed that the prevalence rates of microfilaria positives tended to be decreased after the vector control operations, conclusive statement for the effect of vector control program on the prevalence of onchocerciasis in the pilot area should be reserved. Further data analysis should be required for final conclusion.

20 *MILLARDIA MELTADA*, A NEW HOST FOR *ACANTHOCHEILONEMA VIETAE* AND THE SEPARATION OF MICROFILARILARIAE FROM THEIR PERIPHERAL BLOOD

HIROKO SUGAYA[1], KENTARO YOSHIMURA[1] AND KIMIYUKI TSUCHIYA[2]
Department of Parasitology, Akita University School of Medicine[1]
and Experimental Animal Center, Miyazaki Medical College[2]

Acanthocheilonema vietae has been used as a model parasite for the immunological study of human filariasis. Recently, we have reported that *Apodemus sylvaticus* serves as a suitable host for *A. vietae* (Int. J. Parasitol., 21, 105-107, 1991). The current study demonstrated that *A. viteae* could develop to adult worms in *Millardia meltada,* showing microfilaremia with an extremely high microfilarial (mf) density, and also that a large number of microfilariae could be separated from the peripheral blood of infected *M. meltada.*

Eight *M. meltada* of each sex, weighing 70 g (♀)-91 g (♂), were infected by subcutaneous injection of 50 third-stage larvae recovered from infected *Ornithodorus tartakovskyi* ticks, and monitored for microfilaremia from day 47 post-infection (p.i.) onward. Five male jirds, weighing 60 g, served as controls. On day 47 p.i., 15 out of 16 *M. meltada* developed microfilaremia, indicating successful maturation of *A. viteae* in this animal species. Mean mf density at this date was 40/30 μl blood in male *M. meltada* and this value was higher than that (1/30 μl) of male jirds. Male *M. meltada* then showed gradually increasing microfilaremia during the course of the study with a peak level of 7,000/30 μl blood at week 20 p.i., which was higher than that (3,000) of male jirds. No appreciable clinical signs were noted in infected *M. meltada,* and their mortality rate was low as compared with that of jirds. In contrast, mf density of female *M. meltada* was low, yielding a peak value of 200/30 μl at weeks 10-12 p.i. Since infected male *M. meltada* provoked markedly high microfilaremia, we attempted to separate microfilariae from their peripheral blood. A male *M. meltada* infected with 100 infective larvae, showing an extraordinarily high mf density $\{(5\text{-}7)\times10^4/30\ \mu l\}$ at weeks 25-35 p.i., was used as a donor for microfilariae. One ml of blood taken by ophthalmic venous puncture was diluted with 9 ml of saline solution containing 0.4% citric acid. Five milliliter of the diluted blood was layered over the same volume of Lympholyte-M (Cedarlane Lab.) and centrifuged at $500\times g$ for 10 min at room temperature. After centrifugation, microfilariae were harvested from a Lympholyte-M layer formed between a leukocyte layer and a pellet (red blood cells), and thoroughly washed with a large volume of RPMI 1640 medium. Contaminated erythrocytes, if any, were lysed by hypotonic treatment with 0.2% NaCl for 30-40 sec. After further washing twice, approximately $(10\text{-}30)\times10^5$ viable microfilariae could be harvested. In conclusion, *M. meltada* is a useful animal model for filariasis, especially for microfilarial study, since it is possible to repeatedly collect a large number of microfilariae from the peripheral blood of the same individuals.

21 CLINICAL MALARIA IN JAPAN; A STUDY OF JAPANESE LITERATURES FOR PAST 25 YEARS

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

We have some problems with imported clinical malaria in Japan including difficulty in obtaining anti-malarial drugs and death cases due to delayed treatment. In this report we intended to summarize these problems of malarial cases reported in Japanese literatures for the past 25 years. The total number of Japanese literatures on malaria from August, 1965 to July, 1990 was 1,282, from which we collected 245 cases (male 212, female 25, unknown 8) for analysis. Cases of age under 14 and over 61 year-old were 9 and 8, respectively. Identified malaria species were *Plasmodium falciparum* (Pf, 108 cases), *P. vivax* (Pv, 101 cases), Pf+Pv (13 cases), *P. ovale* (Po, 10 cases), and *P. malariae* (Pm, 8 cases). The cases included 27 of foreigner, 6 transfusion malaria, 1 cogenital malaria and 2 of not imported cases. Severe cases were 50 (46 Pf, 3 Pv and 1 Pf+Pv), and death cases was 19 (all Pf) including 15 autopsy. Double infections with other endemic diseases were seen in 11 cases. As severe complication, 20 cases of renal failure, 23 of cerebral malaria, 16 of DIC, 3 of black water fever and 16 of liver function disorder and/or jaundice were experienced, and liver biopsy in 7 cases was done. Some interesting hematological complications such as hemoglobinuria, reticulosis like reaction, leukemoid reaction, bone marrow hypoplasia, idiopathic thrombocytopenic purpura and acute progranulocytic leukemia were a matter of discussion. Problems of morphology (2) and importance of IFA (4) were pointed out with relation to diagnosis. Self-treatment and prophylactics, difficulty of obtaining anti-malarial drugs (4), no response and/or drug-resistance (13) and new drugs treatment; Fansimef (3 cases), qinghaosu (2 cases), sulfamethoxazole/trimethoprim (3 cases), sulfa-drug alone (1 case), quinidine (1 case), FOM (1 case) were subjects of malarial therapy. Fever, splenomegary, anemia and thrombocytopenia were seen in 98.3%, 52.9%, 39.0% and 73.5% of available cases, respectively.

22 EXPERIENCES IN THE TREATMENT OF 10 FALCIPARUM MALARIA PATIENTS WITH FANSIMEF

ISAO EBISAWA[1], MIKIO KIMURA[2] AND KAORU SHIMADA[2]
Keihin Railway Clinic[1] and Department of Infectious Diseases,
The Institute of Medical Science, The University of Tokyo[2]

In view of the increasing number of chloroquine- and other drugs-resistant falciparum malaria patients in recent years, particularly in the African continent, the role of mefloquine in the treatment of this disease is increasing. We report here our experiences in the treatment

of 10 falciparum malaria patients with Fansimef®, a combination of sulfadoxine-pyrimethamine plus 250 mg of mefloquine, as this combination was the only drug available in place of tablets of mefloquine.

All 10 patients were males, 9 were Japanese and 1 was a Ruwandan, their age ranged from 26 to 60 years, with a mean of 36, 8 of them were infected in Africa (4 in Nigeria, 1 each in Ruwanda, Malawi, Zambia and Tanzania) and 1 each in Pakistan and Indonesia. A patient from Ruwanda was complicated by AIDS. The parasite density before treatment ranged from 100 to 277,500 per μl of the blood. Four were given after chloroquine R1(2), R2(1) and R3(1) resistance were confirmed. Six patients were given Fansimef from the beginning of treatment. Two tablets of Fansimef were given on Day 1 and 1 tablet on Day 2. The total dose of mefloquine was 750 mg. Three patients vomitted and the treatment was repeated later.

The asexual parasite clearance time ranged from 1 to 4 days, and the fever clearance time was 2 to 7 days in 9 and 13 days in 1 patient who had hyperglycemia.

23 FOUR CASES OF MALARIA SUCCESSFULLY TREATED WITH MEFLOQUINE

TSUYOSHI YAMAGUCHI[1], ATSUSHI AJISAWA[1], MASAYOSHI NEGISHI[1], GOHTA MASUDA[1], SHIGEYUKI KANO[2] AND MAMORU SUZUKI[2]
Department of Infectious Diseases, Tokyo Metropolitan Komagome Hospital[1] and Departemnt of Parasitology, Gunma University School of Medicine[2]

Mefloquine (Roche), a recently developed antimalarial agent which is not commercially available in Japan at present, was used in the treatment of four cases of malaria (three cases due to *Plasmodium falciparum* and one case due to *Plasmodium vivax*) which contracted malaria in Thailand and returned to Japan in 1991. The administration of mefloquine was performed with the informed consent of each patient. All the four cases were males, three Japanese and one Australian, aged 20-29 years. Two had prophylactic administration with chloroquine or sulfadoxine-pyrimethamine (Fansidar) irregularly and the rest had no prophylactic regimen. All the four cases had no past history of malaria. Mefloquine, 500 mg orally in one dose for two days, was started singly or in combination with other antimalarial agents, chloroquine, Quinimax, Fansidar or qinghaosu. In three patients with *P. falciparum* the therapy was started from days 9, 10 and 12 after the onset of fever and in the patient with *P. vivax,* mefloquine was started from day 5 of fever. Two patients underwent hemodialysis due to acute renal failure. After the start of mefloquine, the fever subsided within a couple of days, and *Plasmodium* parasites in the peripheral blood disappeared within 3 to 6 days of treatment. All patients had a favorable course and no relapse was seen.

Of the four patients, two complained of upper abdominal discomfort, abdominal pain or vomiting soon after taking oral mefloquine and the other two had no side effects. Vertigo, dizziness or any kind of psychotic manifestations were not recorded.

24 TWO CASES OF VIVAX MALARIA TREATED WITH HALOFANTRINE

SHIGEYUKI KANO[1], SATOSHI YODONAWA[2] AND MAMORU SUZUKI[1]
Department of Parasitology, Gunma University School of Medicine[1]
and Department of Internal Medicine, Horie Hospital[2]

Halfan, SK & F Ltd., is a new drug for the treatment of malaria and reported to be effective against all parasite species, and in cases of drug resistance, simple dosage regimen and very well tolerated. We report two imported cases of vivax malaria which were successfully treated with Halfan.

[Case 1] The patient was a Pakistani male, 26 years of age, who came to Japan in 1989. The last malaria episode was in Pakistan about 5 years prior to this date. At the beginning of April 1991, he began to manifest fever. On admission to Horie Hospital on April 15, he had a body temperature of 40.4°C, chills, nausea and diarrhea. Anemia, jaundice and hepatosplenomegaly were not observed. Spike fevers which were resistant to antibacterial medicine were recognized on the 17th, 19th, 21st, 23rd and 25th. Vivax malaria parasites were first detected from thin blood smears taken on the 24th of April at the density of 0.05% of RBCs. Antibody titer against *Plasmodium vivax* (Pv) and *P. falciparum* (Pf) antigen were shown at 1:1,024 and 1:256 respectively by the indirect fluorescent antibody test. Two tablets each of Halfan (halofantrine hydrochloride 233 mg base/tablet) were administered orally at 18:00 on 26th, 0:00 and 6:00 on 27th, followed by primaquine at 15 mg per day for 14 days. Parasites were cleared by May 1 and body temperature fell to normal on April 28 (36.7°C). No adverse reactions were recognized except slight nausea.

[Case 2] This patient was a 44 year old Japanese male who had previous vivax malaria history in July 1988. He visited Indonesia between March 26-April 3 and April 13-25 in 1991. Although he manifested fever at 39°C on May 5, his general condition was good. But his fever and chills on May 9 reminded him of malaria then. On May 10, parasitemia at 0.03% of RBCs was observed and his Pv and Pf antibody titers were 1:4,096 and 1:256 respectively. Halfan was given at 10:30, 16:45 and 22:35 at the same dosage as mentioned previously. Just after the first two tablets of Halfan the patient recognized slight hematuria, felt malaise and later sleepy. This was followed by primaquine treatment, and no relapse has been shown since then.

Halfan has been used for the treatment of human malaria since 1984 but in Japan these reports are thought of as the first cases treated with Halfan. As has been reported, clinical side effects or symptoms after the drug use on these two patients seem to have been very mild. Parasite clearance was rapid and fever dropped dramatically. Imported drug-resistant malaria has been showing up in Japan and the general use of Halfan is expected.

25 ULTRASONOGRAPHIC DETECTION OF SPLENOMEGALY IN A MALARIOUS AREA IN SOLOMON ISLAND

HIROSHI OHMAE[1], FUMIHIKO KAWAMOTO[2], AKIRA ISHII[3], MANABU SASA[4], J. RAEFASIA[5] AND N. KERE[6]
Nagoya Kyoritu Hospital[1], Department of Medical Zoology, Nagoya University School of Medicine[2], Department of Parasitology, National Institute of Health, Tokyo[3], Toyama International University[4], Ministry of Health, Medical Services[5] and Medical Training and Research Institute[6], Honiara, Solomon Island

The spleen rate is a useful index of malaria endemicity. Palpation has long been used to measure spleen size and the proportion of individuals, especially children, with palpable splenomegaly is often estimated before control measures are introduced. However, palpitation is subjective; it needs considerable experience and skill, and children are often frightened, showing some resistance in the form of abdominal muscle stiffness. Ultrasonography (US) has been used to evaluate the spleen size in the childhood, and we have some indexes to detect slight splenomegaly by US. Our study was in the Solomon Islands, where malaria is highly endemic. The aims were to try out a simple portable US machine and to compare parasite rates with measured spleen size.

We examined 84 clinic patients with symptoms or signs of malaria such as fever and chills. Their ages ranged 1 to 50 years and 34 were less than 15 years old. The US equipment weighed only 6 kg (Yokogawa type KLT-50). Subcostal, intracostal and sagittal scans of the spleen were done with the patient lying on his and her left side. After adjustment to give optically the largest section area in the longitudinal and transverse planes, spleen length, depth, and/or breadth were measured, and volume was calculated by Dittrich's formula. Thick and thin blood films were examined immediately by a fluorescent dye staining combined with acridine-orange and interference filters.

Among patients less than 15 years old, only 3 were detected by palpitation to have splenomegaly. Two were positive for malaria parasites. Among the remaining 31 patients, 12 were positive for malaria parasites.

According to Dittrich, calculated spleen volume by US increases in relation to height during childhood. In 16 of the 34 children, spleen volume exceeded the upper limit of normal range for height. Twelve of these children were positive for malaria parasites, the other 4 having a history of recent malaria infection. In adults, spleen volume showed no correlation with malaria parasites in the bloodstream.

In this study, most patients under 15 years old with malaria were detected by US examination. Since US is simple, quick and non-invasive, and since it can measure even a low to mild degree of splenomegaly, it should be useful in assessing spleen rate.

26 RAPID DIAGNOSIS OF MALARIA BY FLUORESCENCE MICROSCOPY USING AN INTERFERENCE FILTER AND A DAYLIGHT-ILLUMINATED MICROSCOPE

FUMIHIKO KAWAMOTO[1], SAHOKO MIZUNO[1], HIROSHI OHMAE[2], AKIRA ISHII[3] AND MANABU SASA[4]
Department of Medical Zoology, Nagoya University School of Medicine[1], Department of Parasitology, Tsukuba University School of Medicine[2], Department of Parasitology, National Institute of Health, Tokyo[3] and Toyama International University[4]

Fluorochrome stainings is much easier for inexperienced workers and less time-consuming for detecting malaria parasites in the dark field than conventional stains. In the acridine orange (AO) staining method, differential colouration of the nuclear DNA and the cytoplasmic RNA permits reliable and easy identification of the protozoan parasites using dark field illumination. The major drawback of fluorochrome staining methods is their reliance upon an expensive fluorescence microscope. The application of interference filter to standard light microscope is an approach for making a cheaper system: 'transmission' fluorescence microscopy. This idea has been developed successfully using a conventional light microscope and a new interference filter designed specially for AO. Stained parasites were observed with their nuclei fluorescing yellowish green and cytoplasm fluorescing red.

In our evaluation of the AO filter system using 100 thin smears obtained from undiagnosed outpatients in Tanzania, we compared the time required for the AO and Giemsa stainings to detect the first parasite at various parasitaemia. Detection of the parasites using the AO filter system was much faster than the Giemsa staining at lower parasitaemia. However, it was impossible to distinguish large rings of *P. falciparum* from rings of *P. malariae* since RBCs infected with these parasites were of similar size, and the associated pigments were not seen. In some cases a few typical stages such as band forms, schizonts and gametocytes were found using the AO system, but their presence in six cases of parasitaemia, $\geqq 0.003\%$ and all cases (7) of parasitaemia $< 0.003\%$ could not be confirmed in Giemsa stained smears after more than 30 min observation.

In another evaluation at the Solomon Islands, rapid diagnosis was also possible using daylight-illuminated microscopes. In particular, urgent diagnosis of malaria were required for several outpatients who had a fever, and this was achieved within a few minutes, then anti-malarial drugs prescribed immediately. In addition, a new 'thick smear method' (direct haemolysis and simulataneous staining with AO) which was made by mixing 5 μl of patient's blood with 10 μl of AO was applied to some patients, and we could also detect the malaria parasites within a couple of minutes. These results strongly suggest that the AO filter system combined with simple microscopes may be used as a rapid diagnostic technique in tropical countries endemic for malaria. Rapidity in the AO system may also indicate the ease of confident detection of the parasites, which may facilitate the diagnosis of malaria by inexperienced microscopists, such as community health workers in the primary health care systems.

27 DNA DIAGNOSIS OF FALCIPURUM MALARIA USING DOUBLE PCR

MEIJI ARAI[1], CHIYOKO MIZUKOSHI[2], TOSHIBUMI KAKUTANI[2],
SATOSHI NAKAGAMI[3], TATSURO KAWASOE[3], AKIO YAMANE[3], NOBUO OHTA[1],
AKIRA ISHII[4] AND YUSUKE WATAYA[2]
Department of Parasitology, Okayama University School of Medicine[1],
Faculty of Pharmaceutical Sciences, Okayama University[2],
Central Research Laboratory, Wakunaga Pharmaceutical Co. Ltd.[3]
and Departemnt of Parasitology, National Institute of Health, Tokyo[4]

We have developed DNA diagnosis system using double polymerase chain reaction (double PCR) for rapid detection of *Plasmodium falciparum* parasites in human blood. We chose the junction part of DHFR-TS (dihydrofolate reductase-thymidylate synthase) gene as a target for detection of malaria parasites, because the junction gene is specific for *P. falciparum* and that gene is not existent in human. In the parasite, there is only one copy of the target sequence, therefore, the target sequences were amplified by double PCR to increase the sensitivity. In first PCR, the 410 bp of the junction part was amplified, and in second PCR, the 226 bp of the inside sequence of the 410 bp was reamplified. With agarose gel electrophoresis of the PCR products, as little as 10^3 parasites per m*l* in human blood gave a visible band.

We have tried the DNA diagnosis sytem in the epidemic area of Solomon Islands, and the system proved to be useful for a judgement of the effects of antimalarial drugs.

28 EFFECT OF INHIBITOR OF POLYAMINE SYNTHESIS ON MALARIA (*PLASMODIUM BERGHEI*) GROWTH

YUTAKA MIDORIKAWA[1], KATSUHIKO ANDO[2], YASUO CHINZEI[2] AND
HIROSHIGE HIBASAMI[3]
Department of Public Health[1] and Department of Medical Zoology[2],
Mie University School of Medicine
and College of Medical Sciences, Mie University[3]

The effect of 15-deoxyspergualin (DSG), an inhibitor for polyamine biosynthesis was tested on rodent malaria. *Plasmodium berghei* Anka strain, was injected in BALB/c mice and the parasitemia became 2 or 3%, then steriled DSG (dose 2.5 and 5.0 mg/kg) solution was injected i.p. into mice for six days. This drug was effective in limiting erythrocytic schizogony of *P. berghei* in mice. DSG markedly reduced parasitemia, prolonged survival time and cured the malaria in dose dependent-manner. When DSG administration was started at 2% parasitemia, all the infected mice were cured by the doses of both 2.5 and 5.0 mg/kg DSG, while in the case of administration starting at 5% parasitemia, all the infected mice were cured only by 5.0 mg/kg DSG. However, even in 2.5 mg/kg DSG, survival time of mice were prolonged. DSG, at the dose of 5 mg/kg, depleted putrescine, spermidine, and spermine in the erythrocytes of the *P. berghei*-infected mice to 50, 51 and 55% of the controls,

respectively, and cured the malaria by suppression of parasitemia in the erythrocytes. This first demonstration of the effectivity of DSG on plasmodial erythrocytic shizogony *in vivo* and suggests that interference with polyamine biosynthesis may, in fact, be a viable chemotherapeutic target in erythrocyte malaria.

29 THE EFFECTS OF *PLASMODIUM YOELII NIGERIENSIS* INFECTION ON ACTIVITIES OF INFECTED MOSQUITOES

TAKESHI KURIHARA AND TETSUSHI KIKUCHI
Department of Medical Zoology, Teikyo University School of Medicine

The effects of *Plasmodium* infection upon the life functions of mosquito vectors were studied. Mosquitoes examined were a laboratory colony of *Anopheles stephensi*. At day 5, these were exposed to a mouse parasitized with *P. yoelii nigeriensis*. Fully engorged mosquitoes were then divided into four groups of 100 females, and introduced into four wire cages of 20×20×20 cm. The mosquitoes in the first batch were examined daily and the mortality rate was recorded. The mosquitoes in the second batch served for the study of blood feeding activity. They were exposed to a mouse every second day and engorged females were then dissected. The other two batches were used for midgut examination. Two other cages of non-infected mosquitoes, 100 females each, served as controls for the mortality observation and blood feeding studies.

The cumulative daily survival rates of infected mosquitoes were compared with those of non-infected mosquitoes, and rates for the two populations were found similar in four paired replications. Although survival was unchanged, blood feeding activity of the infected population was always lower than uninfected females. In the first test, 239 females were exposed to mice and 35 were engorged. Of these, five had oocysts present and 30 did not. Of the total 239 females, it was estimated that oocysts were present in 83 and not in the other 156. Thus, blood feeding of infected females was observed in 6.0% out of the 83. In contrast, blood feeding of oocyst-absent females was found in 19.2% out of 156, and non-infected feeding controls were 19.4% out of 165 females. Through all the replications, the rate of feeding of oocyst-present mosquitoes was always lower than that of oocyst-absent and non-infected insects. It appears certain that the *P. yoelii nigeriensis* infection has a deleterious effect which diminishes the feeding activity of host mosquitoes. Oocyst development, however, did not influence mosquito mortality in this study.

30 INTRACELLULAR MIGRATION OF *PLASMODIUM GALLINACEUM* OOKINETES THROUGH *AEDES AEGIPTI* MIDGUT

MOTOMI TORII[1,2], MASAMICHI AIKAWA[2] AND LOIS H. MILLER[3]
Department of Parasitology, Ehime University School of Medicine[1],
Institute of Pathology, Case Western Reserve University, USA[2]
and Laboratory of Parasitic Diseases, NIAID, NIH, USA[3]

There are two theories regarding the route by which ookinetes pass through the mosquito midgut epithelium from the luminal side to the haemocoel side. *Plasmodium falciparum* was thought to pass between the epithelial cells without invading them. Ookinetes of the other *Plasmodium* spp. were seen both in the epithelial cells and between the epithelial cells. We studied the migration of *P. gallinaceum* ookinetes through the midgut epithelium in *Aedes aegypti* by transmission electron microscopy. *P. gallinaceum* ookinetes were observed both intracellular and intercellular position in the mosquito midgut epithelium. After epithelial cell invasion, ookinetes lacked a parasitophorous vacuolar membrane and were surrounded solely by its own pellicle. Thus, the ookinete in the midgut epithelium of the mosquito differs from erythrocytic and hepatic stages in that the parasite in the vertebrate host is surrounded by a vacuole. The midgut epithelial cytoplasm around the apical end of invading ookinetes was replaced by the fine granular material deprived of normal organelles. Membranous structure was observed within the fine granular area. Most ookinetes were seen intracellularly on the luminal side and intercellularly on the haemocoel side of the midgut epithelial cells, suggesting that epithelial invasion is followed by exit to the intercellular space.

31 DIAGNOSIS OF LEISHMANIASIS USING NON-ISOTOPE kDNA MINI-CIRCLE PROBES

TAKESHI AGATSUMA[1], MASATO FURUYA[2] AND YOSHIHISA HASHIGUCHI[1]
Department of Parasitology[1] and Institute for Laboratory Animals[2]
Kochi Medical School

kDNA hybridization techniques have been used successfully for the characterization of *Leishmania*. In this study, non-radioactive DNA labeling method (biotinylation) was used for identifying whole blotted DNA of *Leishmania* parasites. Parasites (10^9) harvested were treated with NET solution containing 1% Sarkosyl and 100 μg/m*l* of proteinase K for 1 hr at 56°C, extracted with a phenol: chloroform mixture (1:1) and precipitated with ethanol. The resultant whole DNA was blotted on Hybond N membranes, denatured and fixed by floating the filter on the 0.5 N NaOH/1.5 M NaCl solution. kDNA was obtained from the pellet after microcentrifugation for 1.5 hrs at 12,000 rpm, and digested with EcoRI. kDNA minicircle bands were purified using the gene clean solution, after agarose gel electrophoresis of the EcoRI digested-kDNA. The minicircles were ligated with a plasmid vector, pGEM. Six recombinant DNA probes were established, using six *Leishmania* species, *L. braziliensis*

complex (*L. braziliensis, L. panamensis* and *L. ecuatorensis*) and *L. mexicana* complex (*L. amazonensis, L. mexicana* and *L. pifanoi*). Those probes were labeled by nick translation with Bio-11dUTP. The Hybond N blotted *Leishmania* DNA was hybridized with the biotinylated minicircle DNA probes in hybridization solution (5×SSPE, 5×Denhardts, salmon sperm DNA) at 65°C overnight. The filters were washed extensively as follows; twice with 2×SSPE-0.5% SDS, twice with 1×SSPE-0.5% SDS and twice with 0.1×SSPE-0.5% SDS for 15 min each. In total, 38 *Leishmania* stocks were examined, including both international reference stocks and Ecuadorean isolates. Out of six probes, two probes (*L. ecuatorensis* and *L. pifanoi*) were found to show complex specificity of *L. braziliensis* and *L. mexicana*, respectively. Base sequence analysis on these two probes is in progress, using ABI 373A DNA sequencing system, which is a non-radioisotopic detection system.

32 DETECTION OF *LEISHMANIA* PARASITES BY DNA AMPLIFICATION USING POLYMERASE CHAIN REACTION

YUKI ESHITA[1], TOSHIHIDE FUKUMA[1], KOUSHI YAMAGUCHI[2], HIROSHI USHIJIMA[2], TOSHIRO SHIBUYA[3], MASATO FURUYA[4] AND YOSHIHISA HASHIGUCHI[5]
Department of Parasitology, Kurume University School of Medicine[1], AIDS Research Center, National Institute of Health, Tokyo[2], Department of Parasitology, Teikyo University School of Medicine[3], Institute for Laboratory Animals[4] and Department of Parasitology[5], Kochi Medical School

Epidemiological survey on new world leishmaniasis had been done in Ecuador, 1990. Polymerase Chain Reaction (PCR) techniques have been applied for detection of *Leishmania* DNA which had been isolated from human cutaneous lesions and wild animals.

Sensitivity and specificity of synthesized oligonucleotide primers which were derived from partial sequences of *Leishmania braziliensis* and *L. peruviana* have been tested by the PCR techniques using WHO reference strains, *L. braziliensis, L. panamensis, L. guyanensis, L. mexicana, L. amazonensis, L. pifanoi, L. garnhami* and *L. chagasi*. Titrated parasites (ranged from 10^4 to 2×10^0) were impregnated in filter papers and dried completely. And the DNA was extracted by using lysis buffer with proteinase K. For amplifications of the DNA, thirty cycles were repeated under the following conditions: denaturation at 94°C for 1 min, hybridization at 54°C for 2 min, 1 extension at 72°C for 3 min. Specificity of the amplified products was checked by Southern blot hybridization tests.

Results were summarized as follows: (1) The oligonucleotide primers led to amplify only *L. braziliensis* complex DNA of the WHO reference strains. (2) At least, two parasites were enough numbers to make amplification of the DNA. (3) Specificity of the primers has been tested by a double blind test using already identified Ecuadorian *Leishmania* parasites. DNA fragments of *L. braziliensis, L. panamensis* and *L. guyanensis* identified by isozyme pattern analyses were amplified specifically. But those of *L. mexicana* and *L. major*-like parasites were not amplified. (4) Fifty nine of unidentified Ecuadorian isolates were examined by the

PCR. DNA products of the fifty two isolates were found to be amplified. Out of unamplified isolates, two seemed to be morphologically *Leishmania* and five seemed to be *Trypanosoma* parasites.

These results indicate that the PCR techniques using the specific primers may differentiate *L. braziliensis* complex parasites from others including *L. mexicana* complex ones.

Collaborating researchers: Dr. Gomez, E.A. (Dept. Trop. Med., Catholic Univ., Ecuador), Dr. Coronel, V.V. (Dept. Parasitol., INHMT, Ecuador), Dr. Alexander, J.B. (Fundacion Centro Internacional de Entrenamiento e Investigaciones Medicas, Colombia), Dr. Lopez, M. and Dr. Arevalo, J. (Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Peru).

33 TRIALS OF TOPICAL TREATMENT FOR CUTANEOUS LEISHMANIASIS

SHIGEO NONAKA[1], YOSHIHISA HASHIGUCHI[2], KEN KATAKURA[3] AND EDUARDO A. GOMEZ L.[4]

Department of Dermatology, Nagasaki University School of Medicine[1],
Department of Parasitology, Kochi Medical School[2],
Department of Parasitology, Jikei University School of Medicine[3]
and Department of Tropical Medicine, Catholica University
School of Medicine, Ecuador[4]

There are many forms of treatment for cutaneous leishmaniasis, but these are either lacking in potency or produce various side effects. Therefore, an effective treatment for this condition remains to be found. Recently, topical chemotherapy for cutaneous leishmaniasis has been reported. In this study, we undertook an evaluation of topical treatment for cutaneous leishmaniasis.

A total of 132 cutaneous leishmaniasis patients living in the village of Ciento Tres, Manabi, Ecuador were recruited for this study. Paromomycin ointments were prepared in two concentrations, that is, 10.0% and 2.0%. Meglumine antimonate solution was prepared as follows; 250 m*l* of 30% meglumine antimonate, 750 m*l* of physiological saline and 1,000 m*l* of Mercurochrome. The ointment or solution was applied two or three times a day using a cotton applicator. The effect of topical application was judged primarily by the clinical features, that is, it was graded from (−) to (++).

Among 19 patients treated with 10.0% paromomycin ointment, two patients showed marked improvement, 10 a good reaction, four a slight reaction and four no reaction. On the other hand, the treatment with 2.0% paromomycin ointment produced a marked improvement in five patients, a good reaction in 11, slight reaction in 18 and no reaction in 10. Some patients with large ulcerative lesions complained of a burning sensation during application, while the patients with plaques or the nodulous type of lesion did not. Meglumine antimonate solution was given to 61 patients with cutaneous leishmaniasis. Among these 16 showed a definite improvement and 11 showed a slight improvement in their lesions. The solution was not effective in three patients. The dryness of lesions was more marked after the application

of meglumine antimonate solution than after the application of paromomycin ointment.

Almost complete healing of the lesions was obtained in several patients by topical treatment with paromomycin ointment alone. Paromomycin is usually used topically in high concentrations such as 12.5%. Furthermore, it is used as an ointment in combination with methylbenzethonium chloride. We compared the effect of a high (10.0%) and a low concentration (2.0%) of paromomycin. We selected a low concentration for large ulcerative lesions and a high concentration for dry, nodulous or plaque-type of lesions. We also tried the use of topical meglumine antimonate solution for the treatment of cutaneous leishmaniasis. This solution was combined with Mercurochrome solution to enhance the reduction of secondary infections in the lesions. The concentration of meglumine antimonate was 3.75%. The lesions tended to dry better by the application of meglumine antimonate solution than by that of paromomycin ointment.

34 CHARACTERIZATION OF ASYMPTOMATIC CHAGAS' DISEASE IN NORTHEAST BRAZIL

SACHIO MIURA[1], SEIKI TATENO[1,2], JOSE F. SILVA[2], JARBAS MALTA[3], ADRIANA M.S. TELES[2], MAURICEIA T. QUEIROS[2] AND MOZART M. MEDEIROS[2]
Department of Parasitology, Keio University School of Medicine[1]
and LIKA Universidade Federal de Pernambuco[2] and
Hospital das Clinicas da Universidade Federal de Pernambuco[3], Brazil

Between April to May in 1991, we conducted a serologic study (IFA and ELISA) of Chagas' disease on outpatients at the Hospital of Federal University of Pernambuco at Recife Pernambuco in Northeast Brazil.

ECG was performed with FUKUDA automatic ECG analyzer (CARDIOSUNY α 600 BX) by standard 12 leads and the data analyzed automatically. These asymptomatic patients were divided into 3 groups, 4 who had been already diagnosed as Chagas' disease by serologic test and xenodiagnosis, 8 positive for *T. cruzi* antibodies but asymptomatic and 2 negative for *T. cruzi* antibodies. For the IFA titer over 1/20 dilution and OD$\geqq$0.023$\pm$2 SD in ELISA were considered positive. Among the 8 serologically positive cases, there were two with inconsistent serologic results; namely one showed IFA(+), ELISA(−) and the other one IFA(−), ELISA(+). However, the former changed to negative by IFA after our absorption test using *Leishmania donovani* antigen. In addition, his ECG showed only CCW-rotation and right ventricular dilation with no symptoms and subsequent clinical diagnosis denied Chagas' disease. The other case, IFA(−), ELISA(+), showed a positive result by HA at the laboratory of this hospital and was also positive by IFA at HEMOPE. Subsequently our follow-up test with IFA using the fresh antigen showed the titer higher than $\times$160, and ECG showed a marked left-axis deviation. The other 2 cases with positive but low titer of IFA and ELISA, had no ECG abnormalities. The remaining 4 cases who had no treatment against Chagas' disease despite of positive IFA and ELISA showed complete right bundle branch block (CRBBB).

One of them also had abnormal Q, left anterior hemiblock (LABBB) and A-V block.

The other one who had abnormal Q showed relative low voltage, the 3rd one lateral infarction and A-V block, and the 4th only CRBB with IFA titer of ×20.

In contrast the 4 cases who had already been diagnosed as Chagas' disease and administered with Benznidazole, were still positive by serodiagnosis, and ECG showed CRBBB for three of them. Moreover, the other one showed left anterior hemiblock (LABBB) and the 3rd supraventricular premature beat. But 4th case showed CCW-rotation and relative low voltage but not CRBBB. The antibody titer of these cases tend to decrease to ×(80-320) when treated with Benzonidazol. The IFA titer of these patients whose ECG showed abnormalities ranged from 1/80 to 1/1,280.

The low antibody titer by IFA ×(20-40) of some of these cases might be due to insufficient safekeeping of antigen.

It should be necessary to do follow-up test including absorption test by *L. donovani* antigen, when serologic diagnosis of Chagas' disease is attempted.

35 CAUSATIVE ORGANISMS OF BACTERIAL RESPIRATORY INFECTIONS IN NORTHERN THAILAND

ATSUSHI TAKAHASHI[1], HIDEHIKO HIROSE[1], TSUYOSHI NAGATAKE[1],
YOSHIAKI UTSUNOMIYA[1], MISAO TAO[1], HIRONORI MASAKI[1],
NAOTO RIKITOMI[1], KEIZO MATSUMOTO[1], PARASIT THARAVICHITKUL[2],
THIRA SIRISANTANA[2], TAWORN KASOMSON[3] AND RONNATRAI RUENSVIRAYUDH[3]
Department of Internal Medicine, Institute of Tropical Medicine,
Nagasaki University[1], Chiang Mai University[2] and
Maesod General Hospital[3], Thailand

Respiratory infections, especially community acquired which caused by bacteria are one of the most important diseases in tropical area. But it is not known which pathogenic organisms are predominate in northern Thailand. This study was carried out to identify the causative organisms.

The aim of this study are, 1) To identify the causative organisms responsible for respiratory infections. 2) To identify suitable antibiotics available in Thailand, discussing with physicians of Mae Sod General Hospital. 3) To compare the results with those in Nagasaki.

Patients that were visited to Mae Sod General Hospital during November 1989-January 1990, July 1990-August 1990, and November 1990-December 1990. Gram-staining and acid fast staining and culture of expectrated sputum were done to determine the causative organisms. Isolated strains were stocked at the Department of Bacteriology of Central Laboratory, Chiang Mai University.

H. influenzae, S. pneumoniae and *B. catarrhalis* were three major pathogen on community acquired respiratory infections including acute bronchitis, acute pneumonia. Minimum Inhibitoratory Concentration (MIC) was measured. The susceptablities to various antimicrobial agents were almost same compared with the strains isolated in Nagasaki. In Mae Sod General Hospital, the physicians frequently used tetracycline antibiotic for community

acquired respiratory infections. According to our MIC test, there are many resistant strains of *H. influenzae, S. pneumoniae* and *B. catarrhalis* against tetracylcine antibiotic. Our results show that tetracycline is not so useful antibiotic for treatment on respiratory infections in Mae Sod area. In this hospital, penicilline is selected as first choice antibiotic on community acquired respiratory infections.

36 THE PROJECT OF THE TREATMENT FOR OPPORTUNISTIC INFECTIONS IN PATIENTS WITH HIV INFECTION IN KAMPALA, UGANDA

KAZUNORI OISHI, SATOSHI MITARAI, TSUYOSHI NAGATAKE
AND KEIZO MATSUMOTO
Department of Internal Medicine, Institute of Tropical Medicine,
Nagasaki University

The prevalence of HIV infection in Uganda, East Africa is among the highest in the world. We are now developing the project of the treatment for pulmonary tuberculosis, cryptococcal meningitis, oropharyngeal candidiasis in patients with HIV infection in Kampala, Uganda. The aim of this project is to control these opportunisic infections in patients with HIV infection, and to elongate their precious asymptomatic period.

1) The treatment protocol for *M. tuberculosis* infection in HIV-seropositive patients consists of two regimens; Regimen 1 (3 REH/6 RH) and Regimen 2 (3 REH/9 TH). The Uganda Tuberculosis Control Programme standard therapy (2 STH/10 TH) was excluded, because a high incidence of drug reactions in HIV-seropositive patients was noted. After the treatment, all the cured cases will be involved in the study of prophylaxis with isoniazide for 6 months.

2) The treatment protocol for *Cryptococcal* meningitis consists of 200-400 mg of fluconazole daily taken as a single oral dose for 2 months. After the treatment, all the cured patients will be involved the study of prophylaxis with fluconazole at a dose of 100 mg twice per week for 4 months.

3) The treatment for oropharyngeal candidiasis consists of 1,200 mg of amphotericin B syrup daily taken as oral three doses for two weeks.

These studies may provide new therapeutic strategies for these opportunistic infections in patients with HIV infection in Uganda.

37 COMPARISON OF MEASUREMENTS OF *ANGIOSTORONGYLUS* FOUND IN KITAKYUSHU CITY

KIYOSHI MAKIYA[1], KYOKO SAWABE[1] AND RYUSUKE MATSUOKA[2]
Department of Medical Zoology, University of Occupational and Environmental Health[1] and Osaka Airport Quarantine Station[2]

We reported at 60th Annual Meeting of Japanese Society of Parasitology (April, 1991) that *Angiostrongylus* collected from *Rattus norvegicus* in Kitakyushu City was identified as *A. malaysiensis*. This species was described by Bhaibulaya and Cross (1971) and has been found in Malaysia, Thailand and Indonesia.

Measurement was made on specimens from Kitakyushu and compared with *A. malaysiensis* specimens which were kindly sent by Dr. John H. Cross, one of the authors of the above-mentioned original description. The measurement data for Kitakyushu specimens are as follows (Mean±SD). Male: Body length (18.87±1.96 mm), body width (0.29±0.03 mm), esophagus length (0.34±0.07 mm), esophagus width (0.05±0.01 mm). Female: Body length (27.45±2.91 mm), body width (0.39±0.05 mm), esophagus length (0.32±0.05 mm), esophagus width (0.06±0.01 mm), tail tip-anus distance (0.07±0.01 mm), tail tip-vulva distance (0.22±0.03 mm).

As for the most important diagnostic characters, the results for Kitakyushu specimens were as follows: Spicule length (0.97±0.14 mm), separation of ventro-ventral (VVR) and latero-ventral rays (LVR) (48.9±11.6%, i.e. central part of whole length of ventral ray), inner minute projection of tail tip (5.52±1.51 μm long×9.41±2.19 μm wide).

In case of *Angiostrongylus* from Nagoya City (*A. cantonensis*), spicule (1.24±0.04 mm) was longer than Kitakyushu specimens, and junction of VVR and LVR (32.7±6.6%) was at 1/3 near the tip. The inner projection of tail was clearly seen in 98% of female worms from Kitakyushu, and the size was overlapped with that of *A. malaysiensis* (John Cross specimens) (6.07±1.79 μm long×9.41±2.20 μm wide). The projection was not observed in specimens of *A. cantonensis* (Nagoya specimens). Based on these results, Kitakyushu specimens were confirmed to be *A. malaysiensis*.

The infected wild rats were captured in two separate areas: 1) recently reclaimed seaport area, and 2) urbanized area which seems to be seaside in the past. Important differences were not found between the two groups of *Angiostrongylus* worms from these two separate areas.

Infection rate (8%) of *A. malaysiensis* (Kitakyushu origin) was lower than that (27%) of *A. cantonensis* (Nagoya origin) in exposure experiments of first-stage larvae (L_1) to *Biomphalaria glabrata*. Approximately 3,000 L_1 were exposed to one snail host in order to ensure infection density of 100 L_1/snail. After giving third-stage larvae to a experimental rat, L_1 were found in the feces of the rat on 37th day after infection.

38 EFFECTS OF FLUBENDAZOLE AT A LOW DOSE ON THE INFECTION OF ADULT *ANGIOSTRONGYLUS CANTONENSIS*

JUN MAKI
Department of Parasitology, Kitasato University School of Medicine

Effects of flubendazole on adult tissue-parasitic nematodes have interested the present authors. The drug at 10 mg/kg/day for 10 consecutive days was found to be highly effective in the elimination of adult *Angiostrongylus cantonensis,* one of the tissue-parasitic nematodes. Effects of flubendazole at a subcurative dose on *A. cantonensis* adults, however, still remain to be clarified. The present communication describes preliminary data in regard to this matter.

Nine or 11 weeks after inoculation with 50 third-stage larvae (L3) of *A. cantonensis,* the rats were each orally given flubendazole at 10 mg/kg for 3 consecutive days. The number of first-stage larvae (L1) in the faeces was counted one day before and till 13 days after the treatment, at which time the rats were autopsied for the recovery of adult worms. Until day 5 after the treatment no significant difference in the number of L1, which ranged $(2\times10^4)-(9\times10^4)$ per g of faeces, was seen between the medicated and unmedicated groups. On day 6, however, the number of L1 in medicated groups dropped drastically, and on day 7 and thereafter until the end of the experiment (day 13), L1 release was not seen in the treated rats. Autopsy at 13 days post-treatment revealed that 42-53% of the L3 inoculated developed to adult stage and all the worms were alive. Worms recovered from medicated rats had rather smaller dry weight (in mg/worm) than those from the untreated rats (in mg/worm), but the difference was mostly in-significant statistically.

This investigation, finantially supported with a grant of Ministry of Education, Science and Culture, Japan was carried out with coworkers, T. Yanagisawa, Emeritus Professor of Kitasato University and S. Kanda, Department of Pharmacy, School of Medicine, The University of Tokyo.

39 CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST EOSINOPHIL CHEMOTACTIC FACTORS DERIVED FROM THE YOUNG ADULT WORMS OF *ANGIOSTRONGYLUS CANTONENSIS*

KAZUTO ISHIDA AND KENTARO YOSHIMURA
Department of Parasitology, Akita University School of Medicine

Although *Angiostrongylus cantonensis* is well known as a causative agent of eosinophilic meningoencephalitis, the mechanisms of systemic and/or local eosinophilia still remain unclear. We have recently reported that there is a distinct difference in chemotaxis to eosinophil chemotactic factors derived from the young adult worms of *A. cantonensis* (ECF-YA) between rat-(permissive host) and guinea-pig-(nonpermissive host) eosinophils, and pointed out a possibility that there was a qualitative or quantitative difference in the

recognition mechanism (e.g. receptor) against ECF-YA between the two eosinophils (Ishida and Yoshimura, 1990). In an attempt to purify and isolate ECF-YA, therefore, we tried to produce monoclonal antibodies (mAbs) against ECF-YA.

Two kinds of mAbs were obtained by fusing spleen cells from mice immunized with ECF-YA, partially purified from whole worm extract (WWE) of YA, with P3-X63-Ag8-653 myeloma cells. An enzyme-linked immunosorbent assay (ELISA) indicated that both mAbs belonged to IgGl isotype. Western blot analyses demonstrated that one mAb recognized a 16.1 kD component of YA-WWE, and the other an 85 kD component. Both mAbs were reactive to ECF-YA, but did not show any cross reactions against other helminth antigens, as assessed by ELISA. The ECF activity of YA-WWE (500 μg/ml) was significantly inhibited by previous incubation of the extract with mAb (100 μg/ml) at 37°C for 20 min; the mAb recognizing 16.1 kD component inhibited 56-61% of the activity whilst the mAb recognizing 85 kD component inhibited 20-34% of the activity. A study on the dose-dependent (5-125 μg/ml) inhibitory effects of mAbs indicated that 25 or 125 μg/ml of either mAbs were significantly inhibitory against the ECF activity of YA-WWE (500 μg/ml), and also that their inhibitory effects reached a plateau level at the concentration of 25 μg/ml. The mixture of two mAbs (25 μg/ml each) exhibited a stronger (additive) inhibitory effect on both ECF-YA (% inhibition=85%) and YA-WWE (76%) than either mAb alone. These mAbs showed no inhibitory effect on the chemotactic activities of WWE of *A. cantonensis* first stage larvae, *Metastrongylus apri* adult worms, *Spirometra erinacei* plerocercoids and *Fasciola* sp. adult worms. These data suggest that both mAbs are useful for the isolation and purification of ECF-YA as well as for understanding the possible role of the ECF-YA *in vivo*.

40 BASIC STUDIES ON MONGOLIAN GERBILS AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (8) BASOPHILS

MASUMI SHIMIZU[1], KAZUHIRO SHICHINOHE[1], YOSHIHIKO NOROSE[2], TETSUO IEDOKORO[3], SETSUKO TSUKIDATE[2] AND KOICHIRO FUJITA[3]
Department of Laboratory Animal Science[1], Department of Microbiology and Immunology[2], Nippon Medical School and Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University[3]

The Mongolian gerbil (*Meriones unguiculatus*) has been used very frequently as a susceptible host to experimental filarial infection. Because of small numbers of reports about biological aspects of this animal, we have made basic studies on Mongolian gerbils. In those experiments, we confirmed that the Mongolian gerbil had some basophils in their peripheral blood in almost same propotion of those of human being. In this study, an increase of basophils in peripheral blood of the Mongolian gerbil could be induced by stimulations of a foreign protein.

Mature gerbils kept under conventional condition were used. They were injected 0.5 ml of horse serum per an animal per a day as a foreign protein intraperitoneally or subcutaneously at varying intervals and injection times. Blood samples were prepared from ether-

anesthetized animals from retro-orbital venous plexus. Total leukocyte counts and differential counts of leukocytes were performed. For the counting of basophils in the peripheral blood, the indirect method was used. The intial (normal) mean value for basophils was (0.1-0.4)%, $(21\pm2)/\mu l$. Basophil increase was not observed after a single intraperitoneal injection. The highest response in this experiment was obtained after daily subcutaneous injection for 8 days. In this injection schedule, total leukocyte counts began to increase immediately after the first injection. Basophil counts showed aslight increase from day 0 to day 8 and a much stronger increase to day 12. The peak of the mean basophil value was about $1{,}600/\mu l$, 8.7% on day 12. From this level, there was a sudden drop and it returned to the normal value on day 22. This experiment appeared that there were both basophils and mast cells in Mongolian gerbils and this animal showed a basophil response to repeated stimulations of a foreign protein. It was their own characteristics not observed in mice or rats. The mechanism of this induction of basophils was not fully understood, however, it suggested that the Mongolian gerbil had a considerable potential for an unique model of studying host-parasite relationship.

41 BASIC STUDIES ON MONGOLIAN GERBILS AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (9) SENSITIVITIES TO CHEMICAL MEDIATORS

KAZUHIRO SHICHINOHE[1], MASUMI SHIMIZU[1], YOSHIHIKO NOROSE[2], TETSUO IEDOKORO[3], SETSUKO TSUKIDATE[3] AND KOICHIRO FUJITA[3]
Department of Laboratory Animal Science[1], Department of Microbiology and Immunology[2], Nippon Medical School and Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University[3]

Monglian gerbil is widely used as a laboratory animal especially as a susceptible host of the experimental filariasis. However, it had not been clarified whether the gerbil had IgE or not. Recently, we investigated the existence of IgE like antibody in the gerbil using anti-rat IgE serum. Accerelations of capillary permeabilities in skins were observed after intracutaneous inoculation of anti-rat IgE sera to gerbils. It suggested that the existence of IgE like antibody in the gerbil.

In this report, we carried out skin tests in order to detect the chemical mediators which induce accerelations of capillary permeabilities in antigen-antibody reactions in gerbils. Acetylcholine, histamine, serotonin and leukotriene C_4 as chemical mediators and anti-rat IgE serum were used in this skin test. Sites on back skin were inoculated intracutaneously with 0.1 ml of these reagents in ten-fold serial dilutions. Immediately after intracutaneous inoculations, 0.5 ml of 0.25% Evans blue in saline were injected intravenously. The skin reactions were observed for 30 min after Evans blue injections and areas of blue spots were measured. For comparative study, skin sensitivities of SD rats were also examined by the same method. Differences between the dose of chemical mediators which induce the same size of blue spots in rats and gerbils were regarded as differences of sensitivities of capillary vessels to chemical mediators.

Only to histamine, skin sensitivity of gerbils were higher than those of rats. In reactions to the other chemical mediators, rats showed higher sensitivities than gerbils. The reactions to anti-rat IgE serum in gerbils were equal to those in rats. In the gerbil, the skin reaction to histamine was highest of all chemical mediators using this experiment.

In this study, it suggested that the chemical mediator inducing an accerelation of capillary permeability in an antigen-antibody reaction might be considered to be mainly histamine in the Mongolian gerbil.

42 COMPARATIVE STUDIES ON SENSITIVITIES AND RESPONSES OF PERIPHERAL EOSINOPHIL AND BASOPHIL TO *S. RATTI* INFECTION IN MONGORIAN GERBIL, SD RAT AND C57/BL-6 MOUSE

HIDEHARU SAEKI[1], NAOHIKO TAMURA[1], AKI OHTA[1], KYOKO SHIMOJIMA[1], YUKA NUMATA[1], TOSHIO ISHII[1], JINKICHI FUJITA[2], MASUMI SHIMIZU[3], KAZUHIRO SHICHINOHE[3] AND KOICHIRO FUJITA[4]

Nippon Veterinary and Animal Science University[1], Institute of Parasitology and Hygiene[2], Department of Laboratory Animal Science, Nippon Medical School[3] and Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University[4]

Strongyloides is one of parasitic nematodes that has recently been found very interesting for its association with adult T-cell leukemia in man or sudden death of calves and for its unique life cycle. Since *S. ratti* (SR) which infects *Ruttus* as a final host is easily reproducible using small laboratory animals, a large number of immunological and other studies have been undertaken. In this study, Mongolian gerbils (jirds), which have attracted much attention for their immunological characters because of unique response to infection with some parasites, were infected with SR to comparatively investigate the change in the numbers of eggs per gram (EPG), peripheral eosinophils and basophils in comparison with other rodents (SD rats and C57/BL-6 mice) likewise infected with SR. The following results were obtained.

1. The jirds, rats and mice were percutaneously infected with 10,000 larvae. Eggs were detected in all the animals. The EPG counts was highest in the jirds.

Of three color-mutant species of jirds, the black-color jirds showed the highest EPG count of 420,000 at 21 days after infection and maintained this high level until 150 days. In the rats, the EPG count continued to be increased from 8 to 16 days after infection, peaked 106,000 at 12 days and then almost disappeared at the 30th day. In the mice, the peak EPG count of 170,400 was demonstrated on the 8th day in males. However, the patent period was shorter compared to the other rodents. The periods in females and males were 3 and 10 days respectively.

2. In the jirds, the basophil count temporarily increased to 744 at 14 days after infection. The eosinophil count decreased at 14 days and then tended to gradually increase subsequently. The eosinophil count in the rats increased to the maximum of 1,330 with the increase in EPG at 7 days, and that in the mice to the maximum of 639 with the decrease in EPG. On the other hand, the basophil count showed no changes.

From these results it was concluded that the responses of the jirds to SR infection differed distinctly from those of the other rodents.

Histopathological and seroenzymological studies using jirds are now in progress.

43 ULTRASTRUCTURAL LOCALIZATION OF PHOSPHORYLCHOLIN ANTIGEN IN *TRICHINELLA SPIRALIS*

YUZO TAKAHASHI, NAOTO MIZUNO AND TSUNEJI ARAKI
Department of Parasitology, Nara Medical University

Phosphorylcholine (PC) can bind parasite constituents. Resulting PC-bearing molecules in parasites are strongly antigenic against the host. Wide-spread distribution of PC results in extensive cross-reactivity and hamperes the specificity of immunodiagnosis of the diseases. The aim of this study is to demonstrate the *in situ* localization of PC in *Trichinella spiralis*. Adult worms (5-6 day old) were collected from mouse intestines. Muscle larvae were collected by pepsin-HCl digestion. Those parasites were fixed in half-strength Karnovsky solution, dehydrated with alcohol, and embedded in LR White resin according to the established manner. Ultrathin sections were cut and used as a substrate for the subsequent postembedding-immunogold staining with two lines of monoclonal antibodies that share specificity for PC as determined in competition ELISA. Specific antigen-antibody reaction was visualised by two subsequent reactions; biotinated anti IgG antibody and avidine tagged colloidal gold. Major pool of PC-antigens in adult worms included the body wall (hypodermis, hypodermal glands, cord cytoplasm), hemolymph, intestinal gland cell granules, brush border of the midgut, reproductive systems (ovum, embryo sheath, intersperm space, sperms, exocrine granules). The presence of PC antigens in the alimentary tract and genital tract of adult worms suggests that excretory and secretory (ES) products of the adults are contaminated with PC. This may imply that adult ES products are devoid of specificity. Only small amounts of PC antigens were localized in the inner layers of adult cuticle. Adult stichocyte granules were devoid of PC antigens. Major pool of PC in muscle larvae icluded the body wall (inner layers of cuticle, hypodermis, cord granules), hemolymph, glycogen aggregates, intestinal gland cell granules, and discrete areas in the genital primordial cell, which are known to trigger antibody response in the early phase of the infection. PC antigens were absent from muscle larval stichocytes, cuticle surface, the esophagus occupying substance and the midgut occupying substance. This further supports our previous hypothesis that none of these structures contain non-specific antigens.

44 SERUM C-REACTIVE PROTEIN VALUE OF THE PATIENTS INFECTED WITH A *TAENIA SAGINATA*

KENJI OHNISHI AND MISAKO MURATA
Department of Infectious Diseases, Tokyo Metropolitan
Bokutoh General Hospital

Serum C-reactive protein (CRP) was measured in 5 asymptomatic Japanese male patients aged from 23 to 66 years old, infected with a single *Taenia saginata*. The CRP value of all patients was under 0.3 mg/d*l* and this value was in the normal range. We think that ordinary intestinal infection with a single *T. saginata* dose not bring about pathological elevation of serum CRP level, and if elevated serum CRP level is identified in an asymptomatic patient infected with a sigle *T. saginata* other abnormal accompanying conditions may be considered.

45 IMMUNODIAGNOSIS OF HUMAN CYSTICERCOSIS I. COMPARISON OF SCOLEX WITH CYST AND CYST FLUID ANTIGENS FOR THE DETECTION OF ANTIBODIES

KOICHI KOYAMA[1], YOICHI ITO[1], FUSAKO OSADA[2], DU JUN[2],
LIANG JIAN-AN[2] AND LI SHUHONG[2]
Department of Parasitology, Kitasato University School of Medicine[1]
and Department of Parasitology, Norman Bethune University of
Medical Sciences, the People's Republic of China[2]

Human cysticercosis is endemic in the northeast part of China, although its mechanism of infection and epidemiology have not been clear yet. To study the mechanism of infection, the immunodiagnosis, the ELISA system for human cysticercosis was developed.

In this study, two types of materials, scolex with cyst wall and cyst fluid derived from the pig cysticercus were used as the antigen scources. The cysticercus was cut into small pieces by scissors and centrifuged. Its supernatant was used as the cyst fluid antigen (CFA). The scolex with cyst wall antigen (SWA) was prepared from PBS-soluble proteins of the sediments. The reactivities of SWA and CFA to sera from human cerebral cysticercosis diagnosed with CT were analyzed by ELISA and Western blot analysis. When the concentration of 0.1 and 2.5 μg/m*l* CFA was adsorbed to the ELISA plate, the positive rates in the patient sera (28 cases) were 89.3% (0.1 μg/m*l*) and 100% (2.5 μg/m*l*) respectively. When the same concentration of SWA was adsorbed, their positive rate were 71.4% (0.1 μg/m*l*) and 75.0% (2.5 μg/m*l*). From these data, 2.5 μg/m*l* CFA was employed to prepare the ELISA plate. To assess the specificity of ELISA that 2.5 μg/m*l* CFA used as the antigen, more samples (79 patient sera) were examined and 83.5% of positive rate was obtained. Cross reactions were observed on the patient sera from sparganosis and diphyllobothriasis. To compare the antigenicity of SWA and CFA, their reactivities to the patient sera and the

sera from pig cysticercosis were analyzed by Western blot analysis. The antigens detected by the infected pig sera specifically were observed in SWA and CFA, whereas the specificity of the human sera was not clear because the same antigens were also detected by the normal human sera, although several bands were observed more intensively than normal human sera. Moreover, pig serum proteins in SWA and CFA were detected by anti-pig serum antibodies. The possibility was thought that the contamination of pig serum proteins in SWA and CFA inhibited the ELISA reaction and caused non-specific reactions.

From these results, the ELISA assay that CFA was used as the antigen is suitable for the diagnosis of human cysticercosis, although more serum samples must be examined by this system to improve its specificity and reactivity.

46 SEROLOGICAL ANALYSIS OF LIPID OF *ECHINOCOCCUS MULTILOCULARIS*

MAKOTO ITOH[1], SHIGEFUSA SATO[1] AND AKIRA ITO[2]
Department of Medical Zoology, Nagoya City Medical School[1] and
Department of Parasitology, Gifu University School of Medicine[2]

Lipids of *Echinococcus multilocularis* cyst obtained from liver of a echinococcosis multilocularis (EM) patient were studied on their reactivity with antibodies in sera of EM and other parasitoses patients.

Lipids were extracted from contents of the cyst with chloroform-methanol mixtures (1:2). Extracted lipids were applied to a thin layer chromatography plate and separated into eight fractions according to their mobility. Multi dot ELISA was used to detect antibodies to the fractions in patient sera. Each lipid fraction was spotted on a strip of casein coated tetrafluoroethylene membrane filter. The membrane was incubated in 100-fold diluted sera and then in 500-fold diluted peroxidase labeled anti-human IgG. Instead of sera, peroxidase labeled lectins were used to detect glycolipids in each fraction.

Fractions 2, 3, 4 and 5 were the major fractions reacted with antibodies in EM patient sera. Among the fractions, fraction 2 reacted with all the sera studied (22 serum samples), on the other hand, fraction 3 reacted with 11 sera and fractions 4 and 5 reacted with 18 serum samples.

Cross-reactivity of the lipid fractions with sera from other parasitoses patients was observed. The fractions which reacted with EM patients sera, fractions 2, 3, 4 and 5, cross-reacted with other patients sera. Especially, fraction 2 reacted with all the sera examined from the following patients: nine echinococcosis granulosa, four fascioliasis, five paragonimiasis, three taeniasis and five clonorchiasis patients. As there was no specific fractions which react only with EM patients sera, any fractions are not available for serodiagnosis of the disease.

Presence of glycolipids in those fractions was shown, since some lectins reacted with the fractions. RCA and LCA lectins reacted with fractions 2, 5 and 6 and WGA lectin reacted with all the fractions except fraction 7.

From patterns of the reaction with the fractions, it was suggested that EM patient sera

were divided into three groups: group 1 which reacted with fractions 1, 2, 3 and 4 (9 sera/22 sera examined); group 2 which reacted with fractions 2, 4 and 5 (9/22); group 3 which reacted with fraction 2 (4/22). Studies on relationships between the groups and symptoms of the patients are now going on.

47 EFFECTS OF MEBENDAZOLE, ALBENDAZOL AND MILBEMYCINE OXIME ON SECONDARY HYDATID CYSTS MONGOLIAN GERBILS INFECTED WITH LARVAE OF *ECHINOCOCCUS MULTILOCULARIS*

TAMOTSU KANAZAWA[1], KEIJI MOCHIDA[2], KEIKO KATO[1] AND NOBORU KAGEI[1]
Department of Parasitology[1] and Department of Veterinary Medicine[2],
National Institute of Health, Tokyo

Echinococcosis multilocularis is one of serious parasitic diseases in the world. A surgical therapy is the best, but some cases are inoperable because of developed hydatid cysts. It has been reported that benzimidazole derivatives, mebendazole and albendazole, are effective against hydatid disease in human and experimental animals. These experiments presented here were conducted to clarify if the effects of the drugs are dependent upon the weight of hydatid cysts. Parasite material and animals: An isolate of *E. multilocularis* (kindly provided by Dr. M. Kumagai, Hokkaido Institute of Public Health) has maintained in jirds for several years in our laboratory. Male or female jirds aged over 8 weeks and weighing about 80 g were used. The jirds were infected by i.p. injection of about 100 protoscolex. Medication: Various concentrations of albendazole, mebendazole and milbemycine oxime were suspended in 0.5% methyl cellulose. Experimental design: Each group was treated with one of the test drugs. Control groups, infected and unmedicated, were given 0.5% metyl cellulose. The animals were administered p.o. each drug from three to 24 days, from 30 to 60, from 60 to 90, from 90 to 120 days after infection once a day. Necropsy was carried out within three days after the end of medication. All parasitic material was removed from the peritoneal cavity of each animal and weighed individually. Results and conclusion: The weights of the parasitic tissue recovered from the jirds treated from 30 to 60 days after infection were (2.8±1.9) g in the mebendazole-treated group (50 mg/kg) (reduction rate: 77%), (2.8±2.6) g in the albendazole-treated group (100 mg/kg) (reduction rate: 77%) and (12.2±3.6) g in the control group. On the other hand, those from the jirds administered from 90 to 120 days after infection were (31.7±2.8) g in the mebendazole-treated group (50 mg/kg) (reduction rate: 0%), (48±8.5) g in the albendazole-treated group (100 mg/kg) (reduction rate: −50%) and (32.0±6.9) g in the control group. These data suggest that albendazole and mebendazole exhibit no effect when they were given three months after infection.

48 SEROEPIDEMIOLOGICAL SURVEY FOR PARAGONIMIASIS AND GNATHOSTOMIASIS IN MIYAZAKI PREFECTURE

YUKIFUNI NAWA[1], JUN-ICHI IMAI[1], YOICHIRO HORII[1] AND WONG-YOUNG CHOI[2]
Department of Parasitology, Miyazaki Medical College[1] and
Department of Parasitology, Catholic University Medical College, Korea[2]

Miyazaki Prefecture had been known as the endemic area of paragonimiasis westermani and over 300 cases were discovered in 1955-1960. Although the prevalence of this disease in Miyazaki Pref. drastically decreased within next ten years, sporadic cases are increasing with time for recent 5 years mainly in the central to the southern part. Miyazaki Pref. is also known as the endemic areas of gnathostomiasis doloresi since the first confirmed human case of this disease in the world was recorded by Dr. Ogata, Dept. of Dermatol., Miyazaki Medical College, on 1988. Retrospective survey revealed that 1-2 cases were identified every year since 1985 and new cases are continuously occurring up to now in the central part of Miyazaki Pref. Paragonimiasis and gnathostomiasis are both food-born parasitic disease and their life cycle is closely related to wild boars. Since both diseases are endemic in the central part of Miyazaki Pref., we have carried out a preliminary study of sero-epidemiological survey for these diseases in the endemic area. The sera examined in this report were the stock collected from the habitants of Hae, Nishi-Mera Village, Koyu-County, Miyazaki Pref., on 1980 when general health survey was carried out by the Department of Public Health, Miyazaki Medical College. Sera obtained from healthy volunteers of the students of Miyazaki Medical College served as control. By IgG-ELISA against two parasite antigens, 8 (2.3%) out of 347 were positive for *P. westermani* and 27 (7.8%) were positive against *G. doloresi*. Two cases of *P. westermani*-positive males showed high ELISA value and were also positive by Ouchterlony's double diffusion test, indicating that they have active infection at that time. Agaisnt *G. doloresi*, one case (male) showed high ELISA value. These results indicate that more uncovered cases should exist in this area.

49 *ENTAMOEBA HISTOLYTICA* INFECTION IN INPATIENTS OF MENTAL HOSPITAL

AKIO KIMURA[1], YOSHIKAZU MINEKAWA[1] AND TOSHIO NAKABAYASHI[2]
Department of Virology, Osaka Prefectural Institute of Public Health[1] and Department of Parasitology, Fujita Health University, School of Medicine[2]

Since Janualy 1989, we have continually made the stool examination and serological tests (ELISA and IHA tests) of amoebiasis in patients admitted in a mental hospital in Osaka Prefecture. The examination was repeated 10 times in all the patients. To the patients

positive for stool and/or serological examinations, the examination was repeated 8 times after metronidazole treatment for 1 to 3 weeks. Eight (15.8%) of 51 examinees were positive in both the cyst detection and the serological test, while 7 (13.7%) were positive only in the serological test. Then 15 (29.4%) were totally counted as positive cases, among whom only 3 were symptomatic with diarrhoea. Twenty-two medical stuff were all negative. After treatment with metronidazole, 2 of 8 positive cases both for cyst and antibody were changed to negative for both and other 6 were changed to negative only for cyst, whereas only 1 of 7 seropositive cases was changed to negative. Ten of totally 15 positive cases were in the defect state of schzophrenic disorder and other 5 were mentally retardate in moderate or heavy state (IQ: 0-50). All the positive patients showed abnormal bihavior such as pica and handling stools. Such abnormal behaviors might be strolongly related to the high prevalence of amebic infection occurring among the inpatients of this mental hospital.

50 SEROLOGIC TYPING OF AMEBIASIS

EIICHI OKUZAWA, SEIKI TATENO AND TSUTOMU TAKEUCHI
Department of Parasitology, School of Medicine, Keio University

Clinical feature of amebiasis varies widely, from asymptomatic carrier, extra-intestinal amebiasis, acute imported case to chronic case. In GDP test, precipitation bands formed by different sera sometimes cross. This probably means patient sera contains multiple antibodies against different antigens. From these findings, we expect different serologic patterns distinct clinical feature. Creation of sensitive or selective serologic tests for acute amebiasis, chronic amebiasis, extraintestinal amebiasis, chronic colitis and subclinical cases are possible through differential titration of each antigen-antibody system.

The detail of modified GDP test were already discussed in the last year congress. P-type, defined by the presence of P band which surrounds the precipitated antigen fraction by salting out, was typically observed in liver involvement of chronic amebiasis. R-type, defined by the presence of R band which surrounds serum, was typically observed in chronic colitis cases. Q-type, defined by the absence of P and R bands, was typically observed in acute phase amebiasis.

We isolated the R band using semi-purified antigen by DEAE chromatography. This DEAE-fractionated antigen was served for modified ELISA. GDP positive subclinical cases responded well, while GDP negative cyst passing cases responded little. Chronic colitis cases responded well, while colitis cases within 90 days after onset responded less. Liver abscess cases with a past episode of infection or melena showed better response than recent imported cases. These data suggest this test might be specific for chronic colitis.

Early phase amebiasis (within 30 days after onset) responded little to the DEAE fractionated antigen. These cases responded better to a phenol extracted antigen. Moreover acute amebiasis (within 90 days after onset) also responded well to this phenol extracted antigen.

Presence of antibody against the phenol extracted antigen seems to be a index of acute phase of amebiasis. A portion of asymptomatic cases also responded to the phenol extracted

antigen. This antibody might appear before onset of appreciable clinical symptoms. This phenomenon would be investigated in more detail.

51 ANALYSIS OF PATHOGENICITY BY RESTRICTION ENDONUCLEASE DIGESTION OF AMPLIFIED GENOMIC DNA OF *ENTAMOEBA HISTOLYTICA* ISOLATED IN PERNAMBUCO, BRAZIL

HIROSHI TACHIBANA[1,2], SEIKI KOBAYASHI[1,3], KILMA C. PAZ[1], IVANIZE S. ACA[1], SEIKI TATENO[1,4], YOSHIMASA KANEDA[2], TSUTOMU TAKEUCHI[3] AND SEIJI IHARA[5]
Laboratório de Imunopatologia Prof. Keizo Asami, Universidade Federal de Pernambuco[1], Department of Parasitology[2] and Molecular Biology[5], Tokai University School of Medicine, Department of Parasitology, School of Medicine, Keio University[3] and National Medical Center Hospital[4]

The pathogenicity of 47 strains of *Entamoeba histolytica* isolated in Pernambuco, Brazil was examined by the polymerase chain reaction (PCR) followed with restriction endonuclease digestion. Electrophoretic patterns of PCR products digested with *Hin*fI revealed that all strains were nonpathogenic. The results were entirely in accord with phenotypic properties such as isoenzyme patterns and failure to bind a pathogenic isolate-specific monoclonal antibody. When the sensitivity of PCR was examined, amplified products could be detected from template DNA equivalent to 5 trophozoites. These observations indicate that PCR amplification of genomic DNA and subsequent digestion with restriction enzyme is a useful strategy for a sensitive and accurate diagnosis. The present study also demonstrates that nonpathogenic strains of *E. histolytica* predominate in northeastern Brazil.

52 BIOCHEMICAL AND MOLECULAR BIOLOGICAL CHARACTERIZATION OF *ENTAMOEBA HISTOLYTICA* ISOLATES (TOKAI STRAINS)

TOMOO TANAKA[1], MANAMI TANAKA[2] AND YOSHIMASA KANEDA[1]
Department of Parasitology, School of Medicine, Tokai University[1] and Institute of Basic Medical Sciences, University of Tsukuba[2]

Nine isolates of *Entamoeba histolytica* were recovered from 9 cyst passers, and cultivated axenically. Patients are all mentally retarded persons in a rehabilitation center near Tokai University. The biochemical characteristics, such as isoenzyme patterns, erythrophagocytic ability, and cytotoxicity to Chinese hamster ovary (CHO) cells were determined and compared with those of the highly pathogenic HM-1:IMSS and HK-9 strains. No differences were found between Tokai isolates and the known pathogenic strains. Isoenzyme patterns likewise did not reveal any differences when hexokinase, phosphoglucomutase, glucose phopshate

isomerase and malic enzyme were used as parameters. SDS-PAGE analysis of protein composition showed changes in band patterns in the high molecular weight region. The Tokai isolates showed one band at 129 kD, whereas 4 bands (108 to 121 kD) were detected in HM-1:IMSS and HK-9. DNAs and RNAs were extracted from the isolates and analyzed by electrophoresis and Southern and Northern blots. Distinct differences in rRNA band patterns were between the Tokai isolates and the virulent strains. All the Tokai isolates clearly showed 4 rRNA bands, molecular sizes of which were 1.7 kb, 1.1 kb, 0.8 kb, and 0.5 kb. As for HM-1 or HK-9 strain, there were 3 bands of rRNA (3.8 kb for 25S, 1.8 kb for 17S, and 1.4 kb for 16S rRNA). The basis for the differences in rRNA patterns and nucleotide sequences are under investigation.

53 CHARACTERIZATION OF THE ACTIN GENE OF *PNEUMOCYSTIS CARINII*

YASUSHI MIYAHIRA[1], YOSHIKI HIRAOKA[2], HIROSHI SAKURAI[3], YASUHIRO NOGI[3], TOSHIO FUKASAWA[2,3] AND TSUTOMU TAKEUCHI[1]
Department of Parasitology[1], Department of Microbiology[2] and Laboratory of Medical Molecular Genetics[3], Keio University School of Medicine

Several investigations have indicated that *Pneumocystis carinii* is a member of the Fungi using techniques to analyze rRNA gene sequences. But it seems necessary to investigate other genes to confirm this classification because molecular evolutionary classification still contains controversial aspects. We have chosen actin gene to achieve this purpose because this gene is ubiquitous in eukaryotes and profoundly analyzed in a variety of organisms.

After a partial purification of this organism from immunosuppressed Wistar male rats using Ficoll-Pague density gradient centrifugation, we extracted genomic DNA and performed Southern hybridization with *Saccharomyces cerevisiae* actin gene as a probe. Two bands considered specific for *P. carinii* were detected in *Eco*RI digested DNA lane compared to a healthy rat lung cell-derived genomic DNA as a control. One was a weakly reacted band of 6.6 kb length and the other a strongly reacted one of 5.3 kb. After *P. carinii* genomic DNA was fractionated on 0.7% agarose gel, we extracted part of the DNA from 4 to 10 kb and inserted them into an *Eco*RI arm in λ ZAPII, one of the available phage vectors, to construct a genomic DNA library. Two clones were screened out by plaque hybridization technique using *S. cerevisiae* actin gene as a probe. One clone gives a weak signal and the other a stronger signal. Length of the inserted fractions has been compatible with the specific bands in the Southern hybridization. We have named a longer insert pc1, and the other pc2. We have determined a partial sequence of pc2 gene primarily because of its stronger signal. A homology research has disclosed that pc2 gene is probably actin gene and this gene contains at least four introns in the length of 40-50 bp, relatively short ones which is consistent with the length of introns in thymidylate synthase gene of *P. carinii*.

We are planning to continue this work to get a complete sequence, make a molecular evolutionary tree using actin gene and determine whether *P. carinii* actin gene is similar to

those of Fungi.

54 CYTOCHEMICAL DEMONSTRATION OF GLUCOSE-6-PHOSPHATASE AND ACID PHOSPHATASE ACTIVITIES IN *GIARDIA LAMBLIA*

F. KNAIPPE AND YOSHIMASA KANEDA
Department of Parasitology, Tokai University School of Medicine

Ultrastructural localization of the enzymes glucose-6-phosphatase (G-6-Pase) and acid phosphatase (Ac Pase) was carried out in *Giardia lamblia,* Portland 1. G-6-Pase, one of the most important enzymes in intermediary metabolism, was assayed as outlined by Hulstaert and co-workers. In addition, sodium molybate (10 mM) quoted as a strong G-6-Pase inhibitor and non-specific substrates (2 mM fructose-6-phosphate and sodium b-glycerophosphate) instead of glucose-6-phosphate were used as negative controls. Tightly packed electron-dense reaction product was shown to be associated with some membranous organelles in the trophozoite dorsal surface. Frequently, these organelles displayed a Golgi-like shape, named a lengthy and flat single stack ending in rounded edges. Reaction product was also found in nuclear membranes and endoplasmic reticulum. Control samples showed do not have electron-dense granule deposits. Ac Pase activity was localized according to Robinson and Karnovsky methodology. Similarly with previous data, the reaction product was demonstrated in the peripheral vacuoles as well as in the endoplasmic reticulum and nuclear membranes. Encysted organisms, bearing the cyst wall, also showed to have electrondense granules in the vacuoles adjacent to the plasma membrane, besides the peritrophic space and cyst wall itself. Sodium fluoride (10 mM) drastically abolished the reaction product formation. Although some cytoplasmic compartments were shown to be common route for synthesis and/or transport of both enzymes, the final stages of protein sorting and storage seem to be directed to specific membrane-bounded organelles in the cell periphery. Furthermore, despite the current assumption that all enzymes of energy metabolism in *G. lamblia* are cytosolic, our morphological data evidenced that it might not be the case at least for G-6-Pase.

55 THE MOLECULAR BIOLOGICAL ANALYSIS OF THE GENE EXPRESSION AND ITS DEVELOPMENTAL REGULATION IN SCHISTOSOMES

MANAMI TANAKA[1], TORU SHIMIZU[1], HIDEO HAYASHI[1], HIROHISA HIRAI[2], TADASHI MATSU-URA[3] AND KAZUO SUGANE[3]
Institute of Basic Medical Science, University of Tsukuba[1], Department of Parasitological Diseases, Kumamoto University School of Medicine[2] and Department of Parasitology, Shinshu University School of Medicine[3]

We have studied the gene expression, especially of the oncoproteins, and its regulation in schistosomes. Schistosomes have a complex life cycle with a defined dimorphic lifestyle. The parasite are so far unique in biology in expressing oncogene products in their adult stage. In order to characterize the expression and developmental regulation, a lambda gt 11 cDNA library and lambda EMBL4 genomic DNA library of each growth stage of *Schistosoma mansoni* and *S. japonicum* was constructed, and was screened with various DNA or RNA probes, and monoclonal antibodies against oncogene products. The results obtained showed; 1. Drastic changes in rRNA gene structures between *S. japonicum* and *S. mansoni*. An entire ribosomal repeat approximately 8 to 12 Kbp in size from each species was isolated as an *Eco*RI fragment from a genomic library constructed in lambda EMBL4. Each part of the gene (large and small subunits, and internal transcribed spacer) were subcloned into plasmid vector and compared with their structures among species and developmental stages by Southern blot analysis. Drastic changes in the sizes of the restriction enzyme digested fragments were observed among species and stages. These changes were observed clearly in the part of internal transcribed spacer, especially between miracidia and adult stages of *S. mansoni*. 2. Changes in C-band formation, chromosome structures between *S. japonicum* and *S. mansoni*. There are also wide differences in chromosome structures between two species. *In situ* hybridization using rDNA probe from both species showed the genes are located on chromosome 3. 3. The existence of p53 gene in schistosome genomes. One positive plaque of lambda gt 11 phage, reacted to anti-p53 antibody (Ab-2, Oncogene Science, Inc.), was further analyzed. This fusion protein was about 120 kD in molecular weights, and expressed as 1.4 Kb RNA in the adult stage. P53 gene is well-known as the negative regulator of the cell cycle, and the mutations in the gene are turning out to be the most common genetic alterations in human cancers. The role and function of the gene is now under investigation.

56 A STUDY ON CROSS REACTION IN ELISA USING FRACTIONATED SEA: COMPARISON OF RESPONDING PROFILES AMONG FASCIOLIASIS, PARAGONIMIASIS AND SCHISTOSOMIASIS PATIENTS

W. MIN[1,2], TOSHIKI AJI[1] AND NOBUO OHTA[1]
Department of Parasitology, Okayama University School of Medicine[1]
and Department of Parasitology, Jiangxi Medical College, China[2]

To seek a simple and practical method that has high sensitivity to schistosomiasis but no or low reactivity to other trematode diseases, we performed ELISA using soluble egg antigen (SEA) frationated by high performance liquid chromatography (HPLC).

Eleven schistosomiasis sera from China, 8 schistosomiasis sera from Japan, 7 fascioliasis sera, 10 paragonimiasis sera and 26 healthy Japanese controls were tested in ELISA, and we observed the OD patterns of some diagnostic value. HPLC was carried out, and elution of every 0.25 min was consecutively and directly poured into 96 well plate from 2 to 22 min (0.25 m*l*/well) after passing through TSK G3000 SWXL gel column. Fractionated SEA was coated overnight. Dilutions of the first (patient serum) and the second (anti-human IgG-HRP) antibodies were 1:150 and 1:1,500 respectively. ABTS-H_2O_2 was used as substrate.

Results were summarized as follows: 1. OD patterns of schistosomiasis in ELISA were much different between Japanese and Chinese patients. Sera from Japanese patients reacted to 2,260-600 kD fractions. Sera from Chinese patients who had been treated more than 3 years before responded to 2,100-700 kD fractions, and both groups had tailing reaction to around 400 kD fractions. Sera from Chinese patients who were treated within these 3 years responded to 2,600-110 kD. 2. Five of 7 fascioliasis patients showed positive reaction to SEA, however, all the patients failed to respond to less than 750 kD fractions. 3. Three out of 10 paragonimiasis patients showed cross reaction to SEA. Comparing with the specific reaction of schistosomiasis, OD values of cross reaction were lower and only responded to high molecular weight fractions. 4. Among 26 healthy controls, 6 persons showed positive reaction to clude SEA in ELISA. All the false positive sera responded only to >2,000 kD fractions.

Our research confirmed that there was much cross reaction in trematodes and demonstrated that a crossreactive or false positive determinant(s) was expressed on high molecular fractions (>2,000 kD). Although it is still less discriminatory between old schistosomiasis and other trematode infection, reactivity to 400 kD fraction seems to be a specific target for schistosomiasis. It may be possible to overcome cross reaction in the diagnosis of schistosomiasis by using fractionated SEA.

57 *IN VITRO* GRANULOMA FORMATION WITH SPECIFIC T CELL LINES WHICH COULD RESPOND TO SOLUBLE EGG ANTIGENS OF *SCHISTOSOMA JAPONICUM*

TERUAKI AMANO
Department of Parasitology, Yokohama City University
School of Medicine

Schistosomiasis is caused by granuloma formation and subsequent fibrosis around the deposited eggs in the liver and intestine. It is also well known that cellular and/or humoral immune responses are correlated to regulate granuloma formation. In schistosomiasis mansoni, cellular immune mechanisms are more important than humoral immune system to modulate and regulate this disease. However, the functions of the cellular immune responses are not so clear in schistosomiasis japonica. As we can establish T cell clones to respond to soluble egg antigens of *S. japonicum* (SEA), the mechanisms of granuloma formation will be understood more well.

In this study, we tried to establish T cell lines or clones from C57BL/6 mice infected with *S. japonicum*. Spleens were aseptically removed from the 6 or 8 weeks infected C57BL/6 mice, which were infected with 20 cercariae of *S. japonicum*. Homogenized spleen cells (1×10^6 cell/m*l*) were cultured in tube with RPMI 1640, including 10% FCS, 3% penicillin-streptomycin and 20 μg/m*l* SEA. At 1 week later, only ten percent of the cells were recovered. The cell suspensions were diluted into wells of 96 well U-plate with SEA and irradiated spleen cells as feeder cells. As the cells were multiplying, the supernatant were removed and new complete medium, including 20% MLA-144 culture supernatant as IL-2, was added into each wells. As the cells were multiplying well, the cells were moved into big wells or bottle. On the way, the multiplying cells were checked to respond to SEA antigen which ^{3}H-TdR up take test. We could establish two T cell lines and 3 subcloning T cell lines with this method. All of these cell lines responded well to SEA and two of them responded also to IL-2. One of these T cell lines produced IL-2, as the culture supernatant was checked with CTLL cell lines. As these cells were cultured with SEA-coated polyacrylamide beads and irradiated spleen cells as antigen presenting cells, these T cells aggregated and multiplied around the beads. From this result, we may suspect that these T cell lines work as helper cells for granuloma formation around eggs. The surface marker of these T cells showed L3T4 (+) and Lyt2 (−). These helper T cells are considered to be a group of cells which consist in granuloma around the *S. japonicum* eggs.

58 PROLINE METABOLISM IN *SCHISTOSOMA MANSONI* EGG GRANULOMA

MASANOBU TANABE, NOBUAKI KANEKO AND TSUTOMU TAKEUCHI
Department of Parasitology, School of Medicine, Keio University

The fibrotic liver of *Schistosoma mansoni*-infected mice shows an extraordinary accumulation of collagen fiber which is primarily responsible for the mortality and morbidity in this endemic parasitic disease. The fibrotic liver also has much higher concentration of free proline than uninfected controls. Liver slice prepared from the infected mice can actively incorporate radiolabelled proline into protein-bound hydroxyproline. Although biochemical analysis indicates that pool size of free proline may be closely associated with a regulation of collagen biosynthesis, the mechanism involved in elevation of free proline level in the fibrotic liver remains unknown.

Our recent studies suggest that at least two different mechanisms seem to be responsible for elevation of free proline level in the liver of *S. mansoni*-infected mice. The first mechanism is an active production of free proline by *S. mansoni* egg granuloma. Freshly isolated egg granuloma actively utilized arginine, but not glutamic acid, and produced free proline as well as ornithine and pyroline 5-carboxylate. This finding was confirmed by tracer experiments using radiolabelled arginine or glutamic acid, and by biochemical determination of the enzymatic activities of this metabolic pathway. The second mechanism was demonstrated by the intraperitoneal implantation of intact egg granuloma or by intraperitoneal injection of the dialyzed $15,000 \times g$ 30 min supernatant fluid of their extracts into uninfected, normal mice. Hepatic concentration of free proline significantly increased in these animals during 3 to 5 days after administration. This finding suggests that the egg granuloma contains some soluble factor(s) which may be responsible for elevation of free proline in the fibrotic liver of murine schistosomiasis mansoni.

All of these findings suggest that the egg granuloma plays a central role in liver fibrogenesis during the course of *S. mansoni* infection in mice.

59 THE IMPORTANCE OF UNPARTICIPANTS IN THE SCREENING AND MASS TREATMENT FOR THE CONTROL OF SCHISTOSOMIASIS

MASAAKI SHIMADA[1], JUN-ICHI GYOTEN[2], SHIGEHISA HABE[3], EISAKU KIMURA[4], SHIN-ICHI NODA[5], KATSUYUKI SATO[6] AND YOSHIKI AOKI[7]
Department of Parasitology and Tropical Public Health, University of Occupational and Environmental Health[1], Department of Parasitology, Ehime University School of Medicine[2], Department of Parasitology, Fukuoka University School of Medicine[3], Department of Parasitology, Aichi Medical University[4], Department of Medical Zoology, Faculty of Medicine, Kagoshima University[5], Department of Pediatrics, Kumamoto University School of Medicine[6] and Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[7]

We introduced yearly mass chemotherapy follow the urine examination to a village of Kenya for the control of *Schistosoma haematobium* infection. The prevalence and the intensity of infection in the village decreased after the treatment, but one year later increased again. The rise of the prevalence indicates that the transmission of the disease was still active to have many new infections and reinfections. Unparticipants in the urine examination could play an important role in reinfection. Therefore, it is important to determine what kind of people are included in the unparticipated group for the urine examination.

Demographical and parasitological indices were compared between 805 participants and 153 unparticipants for the urine examination and treatment in 1989. The number of eggs per 10 m*l* of urine and the intensity of occult blood in the participants were determined by the urine examination. Positive occult blood patients were treated after the urine examination. The parasitological data of the unparticipated people were obtained by visiting them from house to house.

The results indicate that there is no significant difference in demographical and parasitological indices between the population who participated and those who did not in urine examination and mass treatment. If we could find other characteristics of the unparticipated group, the information would be useful for the further health education to encourage the people to participate in urine examination.

60 THE BIONOMICS OF *NEOTRICULA APERTA*, THE INTERMEDIATE HOST OF *SCHISTOSOMA MEKONGI*, DURING HIGH AND LOW WATER PERIODS

KAZUO YASURAOKA[1], HIDEKAZU HATA[2], KHAMLIENE PHOLSENA[3], BOUAPHANH SAYASENG[3] AND BOUASY HONGVANTHONG[3]
Institute of Basic Medical Sciences, University of Tsukuba[1],
Department of Parasitology, Chiba University School of Medicine[2]
and Institute of Malaria, Parasitology and Entomology,
Vientiane, Laos[3]

Schistosomiasis mekongi is endemic in the Khong District of Champassack Province, Laos. The intermediate host of *Schistosoma mekongi* is a tiny aquatic hydrobid snail, *Neotricula aperta*. Rainy and dry seasons are pronounced in the Mekong Basin. The dry season is from November to February essentially without rain. A marked decline of water levels is usual in late November and the lowest water is usually seen in April. Numerous small islands and rocks obstruct the river flow creating suitable aquatic habitats for *N. aperta*. Large numbers of the snails are observed on stone, rocks, twigs, or tin cans which rest on the mud substrate, usually under 10 to 60 cm of water, in the presence of a detectable current, slightly acid water and high temperatures.

The rainy season is from April to November, most rain falling in May to October. The river rises rapidly and becomes a raging torrent with extremely high turbidity. Information about the bionomics of *N. aperta* during the period of high water is very limited. The hypothesis was advanced by Upatham *et al.* (1980) that *N. aperta* populations dwindle down rapidly to zero as the water levels rise and that only the eggs are able to persist in a more or less unchanged state, protected as they are on the undersurface of stones, throughout the period of high water.

We made a search for *N. aperta* in late October 1990, at sites around Khong Island. The water level was still high and the river was a raging torrent. The water was heavily laden with silt. Surprisingly, however, *N. aperta* were found underneath the rocks at depths of 2 to 3 m in swift current having a surface water velocity of approximately 40 to 50 m/min, although the average velocity might be as low as 75% of that of the surface water. The snails could only be collected by diving for submerged rocks to which they adhere. The majority of the snails collected had 3.0 to 3.5 whorls and averaged 2.36 mm long and 1.73 mm wide. A few reached to 4 whorls and a length of 3.5 mm. More than half the snails showed marked apical erosion. It would seem that *N. aperta* might only have been staying and surviving on the bed of the Mekong River during high water and waiting for low water periods to reestablish their breeding colonies.

61 PRELIMINARY REPORT ON *PARAGONIMUS* AND PARAGONIMIASIS IN MANIPUR, INDIA

KENJIRO KAWASHIMA[1], TOSHIYUKI SHIBAHARA[2], HIROMU SUGIYAMA[3], T. SHANTIKUMAR SINGH[4], M. SAMARENDRA SINGH[5] AND Y. IBOTOMBA SINGH[4]

School of Health and Science, Kyushu University[1], Laboratory of Animal Research Center, Tottori University School of Medicine[2], Department of Parasitology, National Institute of Health, Tokyo[3], Department of Microbiology[4] and Department of Pathology[5], North Eastern Regional Medical College, India

A human case of pulmonary paragonimiasis in Manipur, India was first found in 1982 (Ibotomba *et al.*, 1982; Indian J. Chest Dis. Allied Sc., 24, 304-306). Thereafter, 39 cases of this disease were reported (Shantikumar *et al.*, 1986; Trans. Roy. Soc. Trop. Med. Hyg., 80, 967-971). Although paragonimiasis has become one of the most important public health problems in Manipur, very little attention has been paid to the causative agent. In 1990, Kawashima, Shibahara and Sugiyama visited Manipur, and studied on *Paragonimus* with Indian scientists.

Results obtained by Indian scientists were as follows: the intradermal test using VBS antigen of *P. westermani* in different localities showed a prevalence rate 6.7% (234/3,467). The numbers of clinical cases diagnosed in Microbiology Dept., RMC during 1982 to 1990 were 151 for skin test positive and 69 for sputum positive. Out of the 69 cases, 61 patients showed recurrent haemoptysis and pleural effusion, seven showed cutaneous symptoms and 1 cardiac involvement (expired). Mode of infection: eating of raw or inadequately cooked freshwater crabs infected with *Paragonimus* metacercariae.

The results of examination of freshwater crabs in 1990 were as follows: the crabs collected were identified as *Barytelphusa lugburis* and *Potamiscus manipurensis* by Dr. M. Takeda of National Science Museum, Tokyo. The crabs of the latter species were infected with 2 types of *Paragonimus* metacercariae. This is the first discovery of *Paragonimus* metacercariae in freshwater crabs in India. The first type seems to be *P. heterotremus* based on the metacercarial morphology and the second type seems to be *P. westermani* based on the metacercarial and adult morphology which was in good agreement with the description of *P. westermani* adults reported by Vevers in India (1923; J. Helminth., 1, 9-20). The results indicated that at least these 2 species might be related to, as the causative agents, paragonimiasis in Manipur, India. Further studies are in progress under the international joint research programme (These studies were supported by the Research Grant No. 02041066 from the Ministery of Education, Science and Culture, Japan and by the Government of Manipur, India).

62 EFFECT OF THERMAL ACCLIMATION ON BLOOD PRESSURE AND GROWTH IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

KATSUHIKO TSUCHIYA AND MITSUO KOSAKA
Department of Environmental Physiology, Institute of Tropical Medicine,
Nagasaki University

Male one-month old SHR and WKY were both divided into four groups. The rats were chronically exposed to 10°C and to 30°C. Systolic blood pressure was measured by tail cuff method. Total body surface area of the rat was calculated by a formula; $S=8.62\times BW^{0.67}$ (S: body surface area, cm^2, BW: body weight, g). Under anesthetic condition (sodium pentobarbital, 35 mg/kg, i.p.), the diameter of the tail was measured by slide callipers at intervals of 3 cm from the root of the tail. Tail surface area was calculated in terms of total amount of side surface areas of a row of several truncated cones, of which generating lines were 3 cm. $S=\sum_{i=1}^{n}(r_i+r_{i+1})\ \pi\cdot L$ (S: surface area of the tail, r: proximal radius of the truncated corne, L; the generating line of them, L=3 cm in this case, n=number of the truncated cornes) was applied. In the rats of 4 to 5 month old (N=6), systolic blood pressure (BP, M.±S.E.) was 182±6 mmHg in cold-acclimated SHR (SHR-C), 184±6 mmHg in heat-acclimated SHR (SHR-H), 143±2 mmHg in cold-acclimated WKY (WKY-C) and 114±6 mmHg in heat acclimated WKY (WKY-H). There was no significant difference between BP in SHR-C and SHR-H, while BP in WKY-C was higher ($p<0.01$) than that in WKY-H. Body weight was 340±10 g in SHR-C, 305±7 g in SHR-H, 349±6 g in WKY-C and 320±9 g in WKY-H. In SHR and WKY, body weight in cold acclimated group was great than that in heat acclimated one. The ratios of tail surface area to total body surface area were in the order of WKY-H > SHR-H > WKY-C > SHR-C. These results suggest that thermal acclimation has an effect on increases of body weight and tail surface area in SHR and WKY, but has only a weak effect on the development of hypertension in SHR.

63 PHYSIOLOGICAL CHARACTERISTICS OF PIKA (*OCHOTONA RUFESCENS RUFESCENS*) AS A WEAK HEAT TOLERANT ANIMAL

GUO-JIE YANG, MITSUO KOSAKA, TAKAAKI MATSUMOTO, MASAKI YAMAUCHI,
JIA-MING LEE AND KATSUHIKO TSUCHIYA
Department of Environmental Physiology, Institute of Tropical Medicine,
Nagasaki University

The pika, whistle rabbit, is an old-fashioned rabbit which had survived the Age of Ice. It is paleontologically primitive, and is considered to be "a living fossil". Since 1985, we have reared and bred Afghan pikas in the Animal Research Center for Infectious Tropical Diseases, Institute of Tropical Medicine, Nagasaki University. Adult pikas weigh 250 g, and exceptionally 300 g. They are covered by silky and short fawn fur. The very short neck supports a globulous head with black, prominent eyes and short, rounded ears. The tail is not

visible from the outside. In the present study, physiological and morphological characteristics of the pika as a weak heat-tolerant animal were investigated.

For an estimation of the life span, 101 pikas, 45 male and 56 female, which naturally died were used. Mean life span in our laboratory was 56.4±3.3 weeks, with a maximum of 144 weeks. There was no sex difference.

In rabbits, heat radiation from the large ear surface is a major heat loss mechanism, however, pikas have small rounded ears. The ratio of ear surface area to body surface area was 7.2±0.7% in pikas (n=15) which was significantly small compared to that of rabbits (17.0±0.5%, n=4).

Unanesthetized pikas (n=2) and rabbits (n=2) were lightly restricted in an environmental control room, and were heated by the stepwise elevation of ambient temperature from 28°C to 33°C, further to 37°C at constant relative humidity of 60%. Rectal and ear skin temperatures and respiratory rate were recorded. At 28°C of ambient temperature, respiratory rate in pikas was 140/min, and that in rabbits was 300/min. Marked increase in respiratory rate up to 500/min, thermal panting, was observed in rabbits during general heating at 37°C. In pikas, however, no change in respiratory rate was detected during 33°C heating, but a slight increase, less than 270/min, was noted during 37°C heating. Although rectal temperature in rabbits was regulated below 40°C, that in pikas rapidly elevated to above 42°C during general heating at 37°C, therefore heating was forced to stop. Thermal panting was not induced in pikas, though rectal temperature elevated above 42°C.

Because of its small size, high reproductive rate and relatively short life span, the pika is considered to be suitable for a laboratory animal. It was indicated that weak heat-tolerance in pikas was resulted from poor heat loss ability through a difficulty of thermal panting and less effectiveness of radiation from the ear pinnae.

64 WEAK HEAT TOLERANCE OF PIKA (*OCHOTONA RUFESCENS RUFESCENS*) —STUDY OF THERMAL SALIVATION—

TAKAAKI MATSUMOTO, GUT-JIE YANG, MITSUO KOSAKA, MASAKI YAMAUCHI, JIA-MING LEE AND KATSUHIKO TSUCHIYA
Department of Environmental Physiology, Institute of Tropical Medicine, Nagasaki University

The pika, whistle rabbit, is an old-fashioned rabbit which had survived the Age of Ice, and lives in cold zone and in high mountains. We have reared pikas since 1985 and previously reported their weak heat-tolerance due to weak heat loss responses such as thermal panting and radiation from the ear pinna previously.

It is well known that thermal salivation is one of the major heat loss responses in some species, therefore, in the present study, thermal salivation of the pika was examined to compare with that of rats, in which thermal salivation is one of the major heat loss responses.

In the environmental controlled room at 26°C and 60% of relative humidity, the animal was placed into the polyethylene cage of 5.6 *l* in volume, and heated by electric heater coil. The oxygen and nitrogen mixed gas (20% O_2 and 80% N_2) were flowed into the cage at

5 *l*/min of flow rate, and the relative humidity and temperature of the out-flow gas were measured, and perspiration rate was calculated.

Initial rectal temperature of pikas (n=9) was 39.58°C in average at 26°C of ambient temperature, and was significantly higher than that of rats (n=8). Mean perspiration rates of pikas and rats are almost same at 26°C. Mean duration of heating at 40°C was 12.22 min in pikas and 26.00 min in rats. Mean increase in rectal temperature after 40°C heat load, however, was about 2°C in both animal. It indicates pika's weak heat-tolerance. After 40°C heat load for each duration, 3 times and 6.5 times of increase in perspiration rate were observed in pikas and in rats, respectively. In rats, saliva spreading behavior was frequently observed during heating and the skin and the fur on the face, anterior chest, fore-paws and abdomen were wet after the heat load. In pikas, however, saliva spreading behavior seldom observed during heating and the skin and the fur were completely dry after heat load. The change in perspiration rate was related to the locomotive activity such like escape behavior, not to saliva spreading behavior in pikas.

It was clearly demonstrated that pikas do not dissipate heat effectively through thermal salivation and grooming, in addition to thermal panting and radiation from the ear pinna. Weak heat-tolerance in pikas might be attributed to the lack of effective heat loss mechanisms.

65 A REPORT ON THE MEDICAL EXAMINATION OF VIETNAMESE REFUGEE

TATSUYA KATSUMATA[1,2], ROKUSHI OKA[1,2], TSUNEO FUJIWARA[1,2],
SHIGERU KOHNO[2] AND KOHEI HARA[2]
Department of Internal Medicine, Omura Municipal Hospital[1]
and The 2nd Department of Internal Medicine,
Nagasaki University School of Medicine[2]

Medical examination was carried out with 932 Vietnamese refugees who were accomodated at the refugee center in Omura City, Nagasaki Prefecture, Japan in 1989-1991. Following examinations were carried out in Omura Municipal Hospital, 1) physical examination; 2) blood cell count; 3) biochemical examination (total protein, GOT, GPT, ALP); 4) hepatitis B surface antigen; 5) urine examination; 6) stool examination (occult blood, MGL method); 7) thick blood smear by Giemsa staining for diagnosis of malaria; 8) gel diffusion precipitin test for diagnosis of amoebiais; 9) microflocculation test and TPHA for diagnosis of syphilis; 10) chestradiograph. A variety of abnormalities were detected, including parasitic disease (78% in prevalence), anemia (6.6%), leucocytosis (12%), HBsAg positive (14%), liver dysfunction (9.8%), occult blood in stool (20%), hypertension (0.75%), pulmonary tuberculosis (3.4%) and syphilis (0.64%). Thus the high frequency of infectious diseases in Vietnamese refugees compared with Japanese community recommends medical examination and treatment for new-coming Vietnamese refugees.

66 A SURVEY OF INTESTINAL PARASITES OF THE FOREIGN LABORERS IN ISHIKAWA PREFECTURE

NOBUAKI AKAO[1], TAKAAKI OHYAMA[1], TSUTOMU OHKAWA[1], KAORU KONDO[1], SHIN-ICHI ITO[2] AND AKIO TAKEGUCHI[2]
Department of Parasitology, Kanazawa University School of Medicine[1] and Ishikawa Prefectural Health Service Association[2]

Recently, the increasing number of foreign laborers has become a major social problems in Japan. Although the immigration law has amended to regulate illegal workers, we are unconcerned with their health care whether they work legally or illegally. The aim of this study is to clarify the infectious status of intestinal parasites of foreign laborers in Japan.

An epidemiological survey of intestinal parasites was conducted on 198 foreign workers aged 19-27 years from Indonesia or Philippine. They work at a private company in Ishikawa Prefecture as technical training employee. On the basis of stool examination, 94 (71%) out of 133 Indonesians and 48 (74%) of 65 Filipinos had intestinal helminthiasis and/or protozoan infections. The prevalence of *Ascaris lumbricoides, Trichuris trichura* and hookworm was 4.5, 64.1 and 10.6%, respectively. And the positive rate of the cyst of *Entamoeba coli, Endolimax nana, Blastocystis hominis* and *Giardia lumblia* was 11.1, 5.6, 4.5 and 2.0%, respectively. No *E. histolytica* cyst was found; however, one out of 112 possessed the antibody against the antigen of HK-9 strain of *E. histolytica* with counter current immunoelectrophoresis. Sixty-three cases of trichuriasis were treated with mebendazole at a dose of 200 mg/day×3 consecutive days. Re-examination revealed that 53 (85.5%) of them were cured.

67 MALARIA INCIDENCE AND THE EFFECT OF MALARIA, CHEMOPROPHYLAXIS AMONG JAPANESE IN AFRICA

IKUKO SEKI[1], YONEATSU OSAKI[1], MASUMI MINOWA[1], HIROSHI NARUTO[2] AND MAMORU SUZUKI[3]
Department of Epidemiology, National Institute of Public Health[1], The Clinic of Japan Overseas Cooperation Volunteers, JICA[2] and Department of Parasitology, Gunma University School of Medicine[3]

A case-control study was carried out among 192 members of Japan Overseas Cooperation Volunteers who stayed in ten African countries—Ethiopia, Tanzania, Kenya, Rwanda, Malawi, Zambia, Niger, Ghana, Liberia and Senegal—between 1987 and 1989. Based on the medical examination and immunological tests, and a questionnaire distributed to the members, the malaria incidence was calculated and the efficacy of the malaria chemoprophylaxis used among them was evaluated: The diagnosis of malaria was considered verified either if a record of a bloodsmear examination that indicated the presence of *Plasmodium* parasites was available or if an indirect fluorescent antibody test showed positive; the questionnaire included personal data (age, sex and job), use of chemoprophylaxis and other preventive

behavior (i.e., use of mosquito nets, mosquito-coils and window screens).
Results are as follows:

1) Five types of malaria were found by serological examination: *Plasmodium falciparum,* 63 cases (54.8%); *P. vivax,* 20 (17.4%); *P. falciparum* and *P. vivax,* 5 (4.3%); *P. vivax* and *P. ovale,* 3 (2.6%); and *P. ovale,* 2 (1.7%).
2) The case group is constituted of 121 subjects and the control group of the remaining 71.
3) 98.3% of the case group and 100% of the control group used regular chemoprophylaxis. Chloroquine alone and the combination of chloroquine and proguanil, in this order, were most commonly used prophylactic regimens in both groups; no significant difference in the drug regimens and the compliancy was observed except that the use of Fansidar was more common in the control group.
4) The rate of side-effects accompanying the chemoprophylaxis measures, particularly of optical and dermal disorders, is higher in the case group.
5) No antimalarial preventive measures, other than chemoprophylaxis, and no personal data presented significant differences between both groups.
6) The number of those who complied fully with their regimens among the case group decreased as the duration of their stay became longer: to 93% in 6 month's time, 67.6% in 12 months, 67.3% in 18 months, and 26.4% in 24 months. This suggests that full compliance with chemoprophylaxis is likely to lesson the chance of contracting malaria.

ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

Vol. 20 No. 1 March 1992

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Jpn. J. Trop. Med. Hyg.

JAPANESE SOCIETY OF TROPICAL MEDICINE