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PRELIMINARY SURVEY ON INTESTINAL HELMINTHS IN ASAHAN AREA OF NORTH SUMATRA, INDONESIA

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Abstract: Surveys on human intestinal helminths were conducted in Asahan Regency of North Sumatra, Indonesia, during a period between May 1979 and July 1980. The overall positive rates of eggs in fecal specimens were 77.5 per cent for *Ascaris lumbricoides*, 88.2 per cent for *Trichuris trichiura* and 60.5 per cent for hookworms. *Necator americanus* and *Ancylostoma duodenale* were detected in 75.7 and 29.1 per cent of 247 hookworm positive fecal specimens, respectively. Positive rates of eggs were correlated negatively in *A. lumbricoides* and positively in hookworms with ratios of rice field area to the gross area in each village. Candidates breeding pigs harbored higher rates of hookworm eggs than those without pig, while the former harbored lower rates of eggs of *A. lumbricoides* and *T. trichiura* than the latter. Positive rate of *A. lumbricoides* eggs was lower in rice farmers than that in fishermen while that of hookworm eggs was higher in the former than that in the latter. Contribution of sources of drinking water to positive rates of the three predominant helminth eggs was not clear.

INTRODUCTION

Asahan Regency is a typical rural area of North Sumatra Province, Indonesia, facing Malacca Strait. As social status of this area is changing with the construction of an aluminum smelter plant, unfavorable impacts on health have been warned. Thus, it was requested to obtain basic data on enteric diseases among inhabitants in Asahan area.

In recent years, some intestinal parasites were detected in northern Sumatra including a village of Asahan area (Cross *et al.*, 1976; Stafford and Joesoef, 1976). Some trials on treatments of *Ascaris* and hookworm infections in this area were also reported (Kosin, 1975). However, prevalences of helminths in area around the smelter plant are not yet elucidated.

Surveys on intestinal helminths were conducted at six villages selected as social and geographical representatives of the rural area in Asahan Regency surrounding the smelter site. Names of villages and periods of the surveys are as follows; Sei Buahkeras (surveys were conducted between May–July 1979), Limau Sundai (August–December 1979), Perupuk (February–March 1980), Guntung (March–May

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1980), Medang (April–June 1980) and Tanjung Muda (July 1980).

This paper describes our findings on risk factors, especially concerning to area of rice field, breeding pigs and kinds of water source, in relation to human intestinal helminths in this area, which might be basic informations to control helminthiases in North Sumatra.

Materials and Methods

Study area: The area is located at 3°20′ latitude north and 99°40′ longitude east in a low flat land facing Malacca Strait (Figure 1). This area is included in a climate area of tropical rain forest which has no distinct dry and rainy seasons. Annual rainfall is 1,400–1,800 mm. Estimated populations of Sei Buahkeras, Medang, Limau Sundai, Tanjung Muda, Perupuk and Guntung are about 2,000, 2,900, 3,000, 1,350, 6,000 and 2,500 respectively. Christians are about 95, 70 and 28 per cent of inhabitants in Limau Sundai, Sei Buahkeras and Tanjung Muda respectively. The others are Moslems. Inhabitants are mainly engaged in rice farming, while majority in Perupuk and parts in Sei Buahkeras and Medang are engaged in fishery. There are three kinds of drinking water sources, deep and semi-deep wells, shallow wells and rivers. A deep well with about 250 m in depth is seen in Guntung. Five semi-deep wells with 70–90 m in depth are seen in Sei Buahkeras, Medang and Perupuk. Inhabitants having semi-deep wells sometimes utilize shallow wells or rivers when water spouting from the semi-deep wells is not enough. Toilets recom-

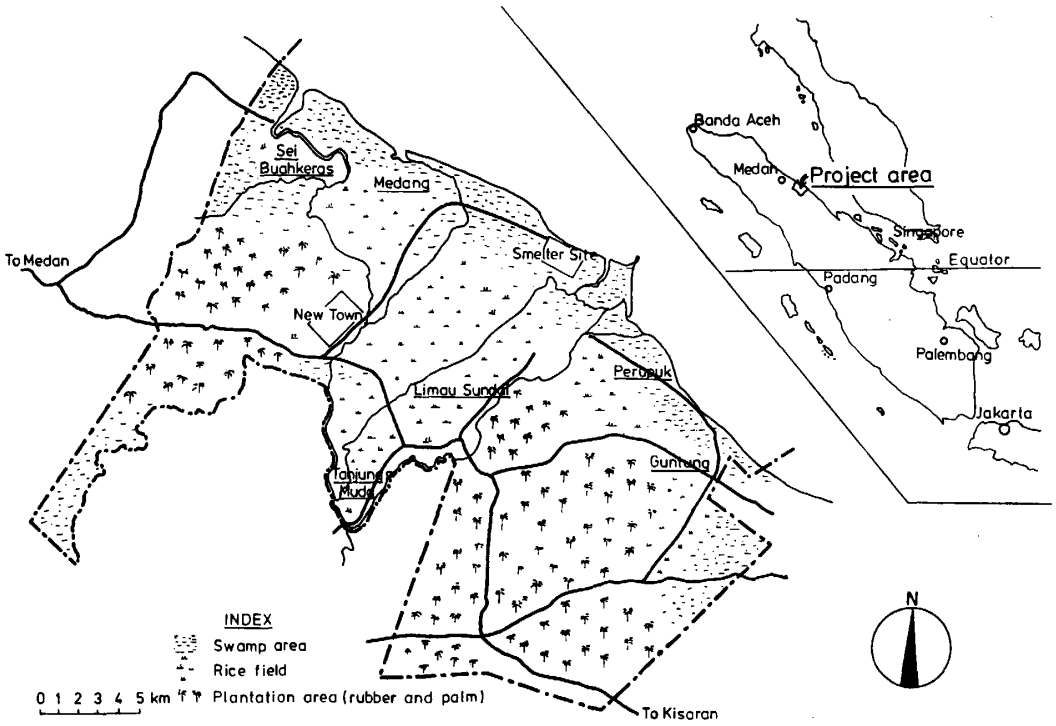

Figure 1 Location map of the pilot area.

mended by Government (swan neck type) are seen at a few houses in each village. Most inhabitants utilize no toilet but excrete in bushes or rivers around premises. They breed many chickens and few ducks, goats, buffaloes or cattle. Some inhabitants breed pigs, who wander around premises, eat and disseminate human feces, excrete freely and contact with children in the daytime. They are kept in cages in the nighttime.

Methods of examination: Fecal specimens were collected from candidates in the six villages, carried to Regional Health Laboratory in Medan and examined by Kato's thick smear technic (Komiya *et al.*, 1960) and flotation on saturated saline (Hunter *et al.*, 1967). Species of hookworm larvae were identified using test tube cultivation method (Sasa, 1969) for a part of hookworm egg-positive specimens. Informations on social factors for each individuals were obtained by hearing from them. Results were shown in age groups of every two years old for children, while those obtained from age groups of 15 years old or more were shown in two groups, 15–30 and 31 years old or more because of no reliable informations on exact age of adults and of insufficient number of specimens. Contributions of the epidemiological factors to positive rates of each helminth were evaluated using χ^2-test.

Results

Fecal specimens collected from 1,784 candidates in the six villages was examined and eggs of helminths were detected as listed in Table 1. The overall positive rates of eggs were 77.5 per cent for *Ascaris lumbricoides*, 88.2 per cent for *Trichuris trichiura* and 60.5 per cent for hookworms. Specimens collected from 1,741 candidates (97.6 per cent of total candidates) harbored at least one kind of helminth egg. Twenty eight in 43 helminth-free candidates were two years old or below. The overall coverage of candidates was 10.1 per cent of total populations in the six villages.

Table 1 Eggs of intestinal helminths detected in six villages

Village	Number of candidates	Helminth				
		Ascaris lumbricoides	*Trichuris trichiura*	Hookworms	*Hymenolepis nana*	*Enterobius vermicularis*
Sei Buahkeras	235	181 (77.0)*	200 (85.1)	102 (43.4)	0	1
Medang	301	231 (76.7)	278 (92.4)	235 (78.1)	1	0
Limau Sundai	512	383 (74.8)	447 (87.3)	406 (79.3)	1	0
Tanjung Muda	166	105 (63.3)	136 (81.9)	133 (80.1)	0	0
Perupuk	296	249 (84.1)	260 (87.8)	117 (39.5)	0	0
Guntung	274	233 (85.0)	253 (92.3)	87 (31.8)	2	1
Total	1,784	1,382 (77.5)	1,574 (88.2)	1,080 (60.5)	4	2

* Number in parentheses is a positive rate in per cent.

Table 2 shows results of cultures of some of hookworm positive fecal specimens, in which dominant hookworm species was *Necator americanus*, while *Ancylostoma duodenale* was less prevalent in this area.

Table 2 Results of culture against the feces of hookworm-ova positive

Village	*Ancylostoma duodenale*	*Necator americanus*	*Strongyloides stercolaris*	Total
Medang	35*	96	4	135 (126 cases)
Limau Sundai	14	22	0	36 (34 cases)
Tanjung Muda	16	50	2	68 (61 cases)
Perupuk	1	4	0	5 (5 cases)
Guntung	6	15	0	21 (21 cases)
Total	72	187	6	265 (247 cases)

* Number of positive specimens.

Figure 2 shows overall positive rates of three predominant helminth eggs in different age groups in the six villages. Most candidates in the age groups of three years old or more harbored eggs of *A. lumbricoides* and *T. trichiura*, though positive rates of hookworm eggs were delayed to reach the highest level in the age group of 11–12 years old. Positive rates of *A. lumbricoides* eggs fell in the age groups of 15–30 years or more. Positive rate of hookworm eggs fell in the age group of 15–30 years old and increased again in the age group of 31 years old or more.

Positive rates of the three predominant helminth eggs related with ratios of rice field area to the gross area in each village are shown in Figures 3, 4 and 5. Positive rates of *A. lumbricoides* eggs were negatively correlated with the ratios (r=−0.836,

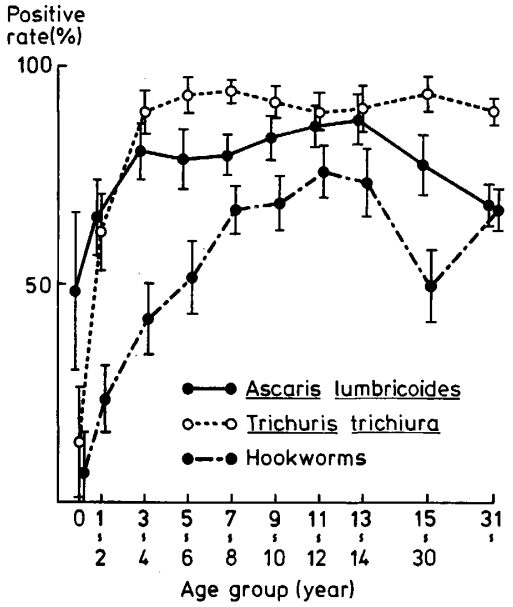

Figure 2 Positive rates of three predominant helminth eggs in the six villages. Vertical bars indicate 95 per cent reliable range of rates.

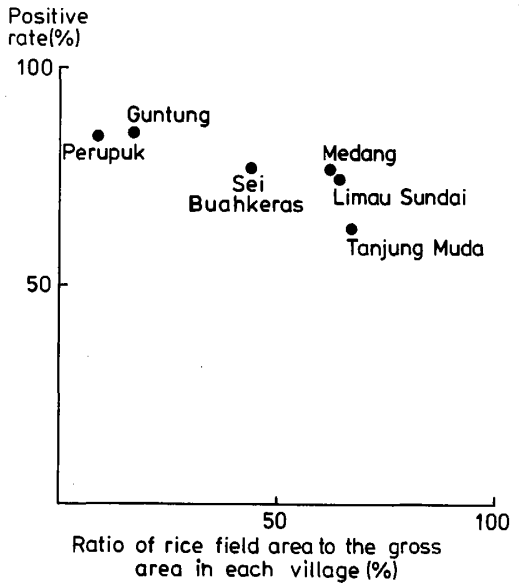

Figure 3 Correlation between positive rate of *Ascaris lumbricoides* eggs and ratio of rice field area to the gross area in each village.
r=−0.836, t=5.048.

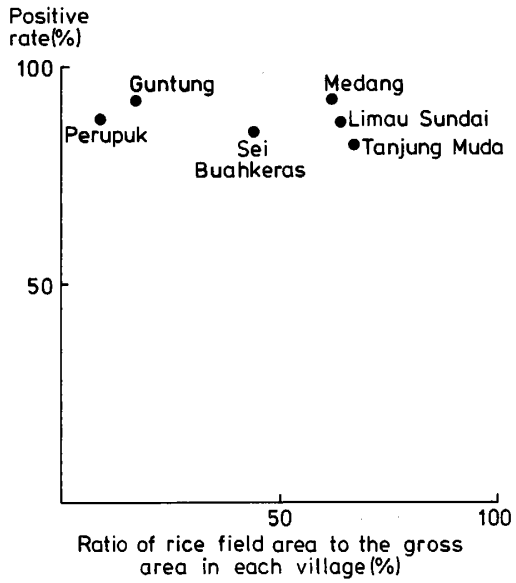

Figure 4 Correlation between positive rate of *Trichuris trichiura* eggs and ratio of rice field area to the gross area in each village.
r=−0.356, t=2.967.

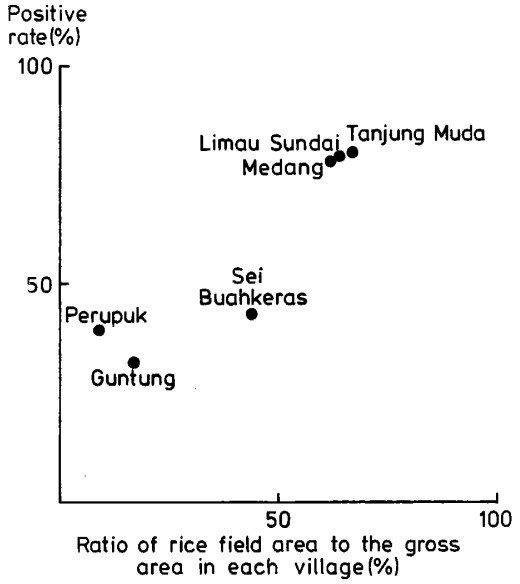

Figure 5 Correlation between positive rate of hookworm eggs and ratio of rice field area to the gross area in each village.
r=0.925, t=5.361.

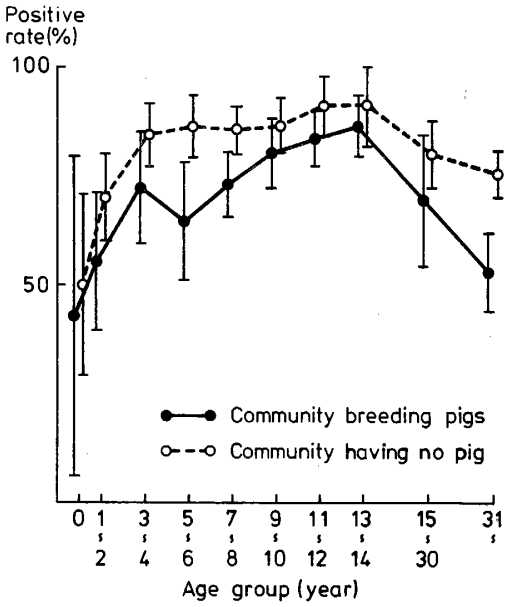

Figure 6 Positive rates of *Ascaris lumbricoides* eggs in communities breeding pigs and that having no pig. Vertical bars indicate 95 per cent reliable range of rates.

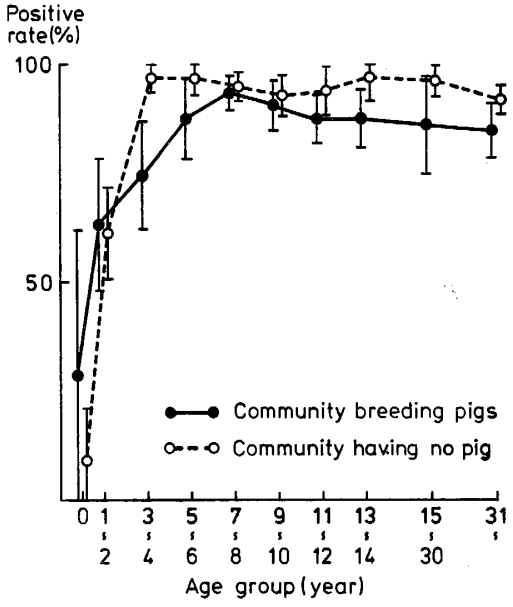

Figure 7 Positive rates of *Trichuris trichiura* eggs in communities breeding pigs and that having no pig. Vertical bars indicate 95 per cent reliable range of rates.

t=5.048) (Figure 3). Positive rates of *T. trichiura* eggs were high and not so much varied among villages with different rice field areas (r=−0.356, t=2.967) (Figure 4). Positive rates of hookworm eggs were positively correlated with the ratios (r=0.925, t=5.361) (Figure 5).

Positive rates of *A. lumbricoides* and *T. trichiura* eggs were not different significantly among candidates breeding pigs and those having no pig in most age groups (Figures 6 and 7), while those of hookworm eggs were higher significantly in those breeding pigs than in those having no pig in age groups of 5–6 years old or more ($P<0.05$) with the exception of an age group of 13–14 years old (Figure 8).

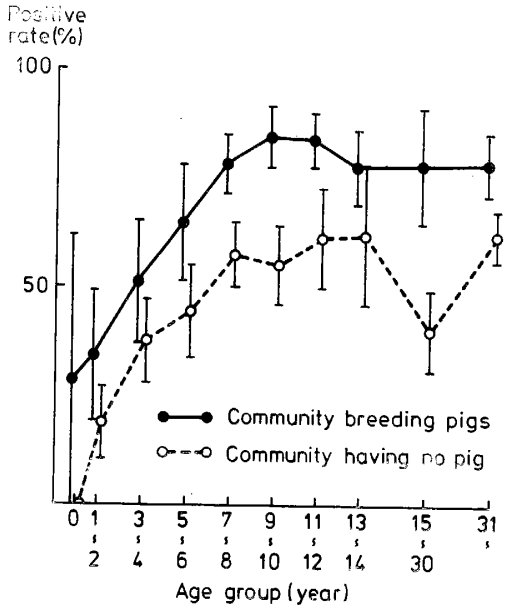

Figure 8 Positive rates of hookworm eggs in communities breeding pigs and that having no pig. Vertical bars indicate 95 per cent reliable range of rates.

Positive rates of the three predominant helminth eggs related with rice field, pig and drinking water source are summarized in Table 3. The rates of contributions of rice field and pig to the positive rate of *A. lumbricoides* eggs were similar level (χ^2= 19.62 and 17.03, respectively). Positive rate of *T. trichiura* eggs was lower in those breeding pigs than those without pig ($P<0.01$). However, contribution of pig to the positive rates of *A. lumbricoides* and *T. trichiura* eggs was not clear in most age groups as seen in Figures 6 and 7. Positive rates of *A. lumbricoides* and *T. trichiura* eggs in those utilizing deep and semi-deep wells (83.5 and 94.4%, respectively) was significantly higher than that in those utilizing shallow wells (76.5 and 86.6%, respectively) ($P<0.05$ and $P<0.001$, respectively), while that of hookworm eggs in those utilizing deep and semi-deep wells (36.5%) was significantly less than that in those utilizing shallow wells (65.2%) ($P<0.001$). However, the differences in the positive rates between those with different water sources were not significant when backgrounds in rice field and pig were matched (data not shown). The positive rates of the three

Table 3 Positive rates of three predominant helminth eggs related with three epidemiological factors

Community	*Ascaris lumbricoides*	*Trichuris trichiura*	Hookworms
Rice field			
Large	719/979 (73.4)*	861/979 (87.9)	774/979 (79.1)
Small	663/805 (82.4)	713/805 (88.6)	306/805 (38.0)
Breeding pigs			
Yes	563/774 (72.7)	665/774 (85.9)	579/774 (74.8)
No	819/1,010 (81.1)	909/1,010 (90.0)	501/1,010 (49.6)
Source of drinking water			
Deep and semi-deep wells	238/285 (83.5)	269/285 (94.4)	104/285 (36.5)
Shallow well	1,080/1,412 (76.5)	1,223/1,412 (86.6)	921/1,412 (65.2)
River	64/87 (73.6)	82/87 (94.3)	55/87 (63.2)

* Number positive/number examined (positive rate in per cent).

Table 4 Contributions of rice field and pig to positive rates of hookworm eggs

Area of rice field	Breeding pigs		Total
	Yes	No	
Large	491/615 (79.8)*	283/364 (77.7)	774/979 (79.1)
Small	88/159 (55.3)	218/646 (33.7)	306/805 (38.0)
Total	579/774 (74.8)	501/1,010 (49.6)	1,080/1,784 (60.5)

* Number positive/number examined (positive rate in per cent).

helminth eggs in those utilizing river waters were not different significantly with the other two groups.

Contributions of rice field and pig to positive rates of hookworm eggs were analyzed in details (Table 4). The rates were significantly higher in area with large rice fields than in area with small rice fields regardless of breeding pigs ($P<0.001$). In area with large rice fields, the rates were not different significantly between those breeding pigs (79.8%) and those having no pig (77.7%). However, in area with small rice fields, the rate was significantly higher in those breeding pigs (55.3%) than in those having no pig (33.7%) ($P<0.001$).

Positive rates of three predominant helminth eggs related with occupations of candidates are shown in Table 5. The positive rates were compared among those having no pig because no candidate other than rice farmers bred pigs. Positive rate of *A. lumbricoides* eggs was higher significantly in fishermen (92.6%) than those in rice farmers (80.8%) ($P<0.05$) and in marchants (63.6%) ($P<0.01$). Positive rates of *T. trichiura* eggs were not different significantly among those in rice farmers, fishermen and marchants. Positive rate of hookworm eggs was higher in rice farmers (52.6%) than that in fishermen (23.5%) ($P<0.001$) while that in marchants was not different

Table 5 Contribution of occupations to positive rates of helminth eggs*

Occupation**	*Ascaris lumbricoides*		*Trichuris trichiura*		Hookworms	
Rice farmer	727/900	(80.8)***	814/900	(90.4)	473/900	(52.6)
Fisherman	75/81	(92.6)	71/81	(87.7)	19/81	(23.5)
Marchant	14/22	(63.6)	19/22	(86.4)	9/22	(40.9)
Teacher	3/7		5/7		0/7	
Total	819/1,010	(81.1)	909/1,010	(90.0)	501/1,010	(49.6)

* Positive rates were compared among those having no pig because no candidate other than rice farmers bred pig.

** The results include those of all family members.

*** Number positve/number examined (positive rate in per cent).

significantly from those in rice farmers and fishermen. Specimens taken from teachers were not enough to analyze.

Positive rates of the three predominant helminth eggs were not different significantly among males and females in most age groups.

Discussion

Surveys on human intestinal helminths were conducted in rural areas of North Sumatra. At least one kind of helminth was detected in fecal specimens from 97.6 per cent of candidates, in which *A. lumbricoides*, *T. trichiura* and hookworms were predominant.

Prevalence of hookworm eggs was positively correlated with ratios of rice field area to the gross area in each village, which seems to be reasonable concerning the mode of transmission of predominant hookworm larvae being *Necator americanus*. The augmentation of the positive rates of hookworm eggs by breeding pigs was apparent in areas with small rice fields but not in areas with large rice fields, which suggests that factors related with rice farming in large rice fields, but not in small rice fields, might overcome the contribution of factors related with breeding pigs.

The role of pigs to helminth infections were suggested by Jones (1976) as that hookworm eggs might dug up with buried human feces and destroyed in pig intestine in Papua New Guinea, based on the fact that eggs of cat hookworm, *Ancylostoma tubaeforme*, but not eggs of *Ascaris suum*, were largely destroyed in pig intestine. In North Sumatra, candidates breeding pigs harbored higher rates of hookworm eggs than those without pig, while the former harbored lower rates of eggs of *A. lumbricoides* and *T. trichiura* than the latter. If pigs eat these worm eggs in North Sumatra, inhabitants breeding pigs should harbor lower rates of hookworm eggs and should not harbor lower rates of *A. lumbricoides* and *T. trichiura* eggs, which is inconsistent with our observations. Thus, further investigations would be necessary to elucidate sources and routes of these worm infections in this area.

Cross *et al.* (1976) reported intestinal parasites for those living in North Sumatra, in which positive rates of hookworms were 82 and 77 per cent in two villages where

ratios of rice field area to the gross area were more than 60 per cent and inhabitants bred pigs, 44 per cent in a village where the ratio was less than 30 per cent and inhabitants bred pigs, and 10 per cent in a village where inhabitants bred no pig and they had no rice field because this area was a small town along the coast. Their observations seem to be consistent with our observations because social and geographical conditions of their areas were similar to those of our pilot areas.

Contributions of rice field areas to positive rates of *A. lumbricoides* and *T. trichiura* eggs seem to be less than that of hookworms. High positive rates of *A. lumbricoides* and *T. trichiura* eggs regardless of rice field area are consistent with observations of Cross *et al.* (1976) and Stafford and Joesoef (1976) in Sumatra Island. Oral entrance nature of infection of these worm eggs and high positive rates might overcome the contribution of rice field area.

Differences in positive rates of the three predominant helminth eggs among those utilizing different water sources were also observed. However, the contribution of water sources seems to be less than those of the other two factors, rice field and pig, because most of the rates were not different significantly among those having the same epidemiological backgrounds other than different water sources.

Positive rates of the three predominant helminth eggs related with occupations of candidates (Table 5) seem to be consistent with our considerations on the contributions of rice field and pig and also with results of Cross *et al.* (1976). Positive rate of hookworm eggs was higher in rice farmers than in fishermen. Positive rate of *A. lumbricoides* eggs was lower in fishermen than in rice farmers. Positive rates of *T. trichiura* were not different significantly among those having three main occupations.

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The authors wish to express our gratitude to all staff members of parasitology and microbiology sections of Regional Health Laboratory in Medan for their technical cooperation and to all members who concern to the Asahan health project. We wish to express our gratitude to all Japanese experts for their kind cooperation.

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インドネシア共和国北スマトラ州アサハン地域における腸管寄生蠕虫調査

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インドネシア共和国北スマトラ州アサハン県の6カ村で，1979年5月より1980年7月まで，糞便検査による住民の腸管寄生蠕虫調査を行った。検査にはセロファン厚層塗抹法，飽和食塩水浮游法及び試験管内濾紙培養法を用いた。被検者1,784名のうち虫卵陽性率は，回虫卵77.5%，鞭虫卵88.2%，鉤虫卵60.5%で，他に矮小条虫卵と蟯虫卵がおのおの4名と2名より検出された。回虫卵の陽性率は〔水田面積/総面積〕比（水田占有率）の高い村が低く，鉤虫卵の陽性率は水田占有率の高い村が高い値を示した。また豚を飼育する地域では，豚を飼育しない地域に比較して回虫卵の陽性率が有意に低く，鉤虫卵の陽性率が有意に高かった。水田占有率の高い村では，豚の飼育の有無により鉤虫卵陽性率に著しい差は見られなかったが，水田占有率の低い村では，住民の鉤虫卵陽性率は豚を飼育する住民の方が，豚を飼育しない住民に比較して有意に高かった。回虫卵，鞭虫卵，鉤虫卵の陽性率は深井戸，浅井戸及び川の水を飲用に供している住民の間で，有意差が見られなかった。

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William Panjaitan（北スマトラ州衛生局）
Sumitro　（保健省リージョナルオフィス）

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尾辻　義人，原田　隆二，堂園　貞巳，
橋本　修治　（鹿児島大・医・第二内科）
徳永　正義，佐藤　栄一
（鹿児島大・医・第二病理）
上山　達典　（上山内科）

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田中　　寛　（東大・医科研・寄生虫）
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17 4例のマラリア患者に対するST合剤の臨床効果
山口　恵三，賀来　満夫，松瀬真寿美，
草野　展周，重野　芳輝，斉藤　　厚，
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（北里大・医・寄生虫）

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高岡　宏行　（大分医大・医動物）
多田　　功　（熊本大・医・寄生虫病）
鈴木　　博
（長崎大・熱帯医研・ウイルス）
野田　伸一　（鹿児島大・医・医動物）

Luis. Yarzábal, María. G. Basñáez
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Eugene O. Ogunba　（イバダン大・医）

25 タイ国チャンタブリ県における蚊の発生源調査
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（JICA, タイ国公衆衛生省医科学局; アース製薬）
Mongkol Chenchittikul,
Boonluan Phanthumachinda
（タイ国公衆衛生省医科学局・衛生昆虫部）
長谷川　恩　（JICA, タイ国医科学局）

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奥脇　義行　（女子栄養大・微生物）
杉山　雅俊　（順天堂大・医・衛生）
池田　照明，及川陽三郎
（金沢医大・医動物）
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中野　宏秋，細溝　浩二，市来　重光，
橋本　　智，阿部　久夫，浅野　信夫
（大阪空港検疫所）
小野　忠相，中林　敏夫
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奥脇　義行　（女子栄養大・微生物）
杉山　雅俊　（順天堂大・医・衛生）
池田　照明，及川陽三郎
（金沢医大・医動物）
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藤田紘一郎，月舘　説子，黒川　憲次
（長崎大・医・医動物）
杉山　雅俊　（順天堂大・医・衛生）
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村田　良介，松橋　　直，近藤　　了，
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渋谷　敏朗，亀井喜世子
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山本　典巳，木村　富紀，大山　昭夫
(関西医大・微生物)
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44 *Trypanosoma cruzi* と Sprague-Dawley ラット腹腔マクロファージとの相互作用の超微形態学的研究
三浦左千夫，竹内　勤，浅見　敬三
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45 マウス腹腔マクロファージ培養におけるデングウイルスの増殖に及ぼす回虫抗原の影響
Agus S. Wiharta, 堀田　博
(神戸大・医・微生物)
堀田　進
(神戸大・医・国際交流センター)
松村　武男　(神戸大・医・医動物)
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46 実験的トキソプラズマ感染マウスの胸腺萎縮について
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Norman Chen, Walter Stahl,
Brian Grimwood
(New York State Department of Health)

47 トキソプラズマ抗原接種によるマウス・バベシア感染に対する感染死防御効果とその機構について
小川　了，桜井　治久，齋藤　篤志，
鈴木　直義　(帯広大・獣医・生理)

48 トキソプラズマ性リンパ節炎の血清学的診断について
小林　昭夫，渡辺　直熙，鈴木　康弘，
牧岡　朝夫，片倉　賢，平井　徳幸，
浜田　篤郎　(慈恵医大・寄生虫)

49 酵素抗体法によるトキソプラズマ特異 IgM 抗体検出に関する研究
中島ひとみ，土橋　賢治，鈴木　寛，
松本　慶蔵　(長崎大・熱帯医研・内科)

50 IgM 酵素抗体法 (ELISA) によるトキソプラズマ (Tp) 急性感染妊婦の検索

土橋　賢治，鈴木　　寛，中島ひとみ，
松本　慶蔵　　(長崎大・熱帯医研・内科)

51 トキソプラズマ抗体保有状況と感染経路に関する1疫学調査

小西　英二，高橋　純子，松村　武男
(神戸大・医・医動物)

一 般 講 演

1 ホルマリン固定赤血球のアルボウイルス HA および HI 試験への応用

A. Sompop, N. Somboon, I. Wallapa, P. Krawan, C. Kanai
（タイ国公衆衛生省医科学局・ウイルス研）
B. Damrong （プラポクラオ病院）
伊藤 宝務 （武田薬品・中研）

アルボウイルス感染症は東南アジア地域において重要な疾患の1つにあげられている。本ウイルスに対する血清学的診断ならびに疫学的調査には Clarke and Casals (1958) が標準化した赤血球凝集阻止 (HI) 試験が広く使用されてきた。HI 試験は操作が比較的簡便で正確，感度も高く，しかも短時間内に判定できるなどの利点を供えている。しかし，従来の HI 試験ではガチョウまたは初生ビナの新鮮な生赤血球を使用するので，試験の都度採血，洗浄して反応用赤血球を調整する必要があった。また，アルボウイルスの赤血球凝集 (HA) 反応の特徴として，希釈液の pH に依存する傾向が著明で，予備試験による条件の設定が要求された。そこで，アルボウイルスの HI 試験をさらに簡便化，省力化する目的で，試験に繁用されているガチョウ赤血球をホルマリンで固定して，長期保存可能に安定化することを試みた。ホルマリン固定ガチョウ赤血球 (FGRBC) の JE, Dengue 1, 2, 3 および 4 型ならびに Chikungunya HA 抗原に対する感受性は新鮮ガチョウ赤血球のものとほぼ同等であった。上記 HA 抗原を使用して，ヒトおよびブタ血清中の HI 抗体価を測定したところ，これも FGRBC と新鮮ガチョウ赤血球とで，ほぼ同等の抗体検出感度を示した。

以上の結果から，FGRBC が新鮮ガチョウ赤血球に代ってアルボウイルス HI 試験に使用できる可能性を認めた。今後，FGRBC は東南アジア地域におけるアルボウイルス疫学調査，血清診断ならびにワクチン接種後の効果判定などに役立つものと考えられる。

2 タイ国チャンタブリ県における日本脳炎ウイルスの疫学的調査–飼育豚血清中の JE-HI 抗体の検出

伊藤 宝務 （武田薬品・中研）
宮崎 武夫，長谷川 恩 （チャンタブリ計画本部）
N. Somboon, K. Watana, A. Sompop （タイ国公衆衛生省・ウイルス研）
B. Damrong （プラポクラオ病院）
奥野 良信，深井孝之助 （阪大・微研）

日本脳炎の流行が近年益々重要な問題になりつつあるタイ国において，JEV の感染環の中での豚の役割についての情報を得ることを目的として，東タイ，チャンタブリ県において1982年7月から1983年6月にわたり，毎月一定期日に30頭の屠場豚から採血し，その血清中の HI 抗体を測定，HI 抗体陽性率を指標として豚におけるウイルス伝播の分析を試みた。

HI 抗体陽性率は現地における雨期（5月中旬から9月まで）前の数カ月は30%あるいはそれ以下に低下したが，その他の月ではほぼ100%で，ベクターであるコガタアカイエカ数の消長に密接に関連した季節性を示す。雨期前の陽性率の低下は蚊の多発時期（＝雨期）を経過しない豚個体の増加の反映であり，低い陽性率からほぼ100%の陽性率への転化は雨期の始まりと共に急速に起こる。

陽性率の消長には，通年ゆるやかな変動を示す市街地近郊型と，雨期（ベクターの消長）に伴って著しい変動を示す農業地区型との2つの型があるようで，一般的にはこの2つの型の複合として観察される。

以上から，タイ国においても豚の日本脳炎ウイルス感染は，ベクターの生能と結びついた周期性（季節性）をもつことが示唆され，日本脳炎のコントロールに役立つ基本的情報の一つを明らかに

することが出来た。

3 1977-1982年のタイ国におけるポリオの血清疫学調査

山田　光昭，加藤伸之輔，渡理　英二，
鈴木　　博，草間みちる，山地　幸雄
（日本医大・微生物）
三木　　康　（山梨県衛研）
Jiraporn Supawadee,
Somboon Suprasert　（チェンマイ大）

我々は，1977年-1982年に，タイ国チェンマイ，およびバンコックを中心とする地域において採取した1,318検体のヒト血清試料について，ポリオウイルス抗体価の測定を，ウイルス中和テストにより行った。

中和テストは，微量中和反応術式により行った。100 $TCID_{50}$ Mahorey strain (Type 1), MEF 1 strain (Type 2), Sankett strain (Type 3) を希釈した血清に加え 37°C 3時間，炭酸ガス培養器内におき，さらに4°C 1夜後，サル腎またはHeLa細胞に接種し細胞変性効果を観察して判定し抗体価≥4を中和抗体陽性とした。

抗体陽性率，平均抗体価は，χ^2 test および t-test により解析を加え，以下の結果を得た。

1) 母児免疫の失われる時期の1歳児では抗体価保有率は，0～40%に低下するが，6歳以上では，70～90%以上の抗体保有者がおのおのの集団においてみられた。

2) 各タイプの抗体保有率を9歳以下，10歳以上に分けて検討したところ，それぞれのタイプの抗体保有状況は地域により異なるが，山間部では Type 1, Type 2, チェンマイ周辺では Type 1, Type 3, バンコック周辺では Type 1, Type 3 の抗体保有率がやや低いと示唆された。

3) 抗体保有率が50%以下であったのは，山間部の一部の集団の9歳以下において，Type 1 および Type 2 でみられた。

4) ワクチン接種歴の明らかなものでは，居住地域による抗体保有率の差を認めなかった。

5) ワクチン接種による抗体価の上昇は，Type 2, Type 3 では Type 1 に比べ顕著であった。

4 タイにおけるデング出血熱患者血清の免疫酵素測定法

分藤　桂子，五十嵐　章
（長崎大・熱帯医研・ウイルス）

東南アジアにおいては，毎年多数のデング出血熱 (DHF) 患者が発生し時にはショック症状を経て死に致ることから，小児のウイルス感染症として最も重要視されている。最近新しい血清反応として免疫酵素測定法 (ELISA) がウイルス血清診断に導入されているが，今回，この方法を DHF の患者血清に応用した。

DHF 患者血清は，1982年五十嵐らによりチェンマイ地区において採集された血清，1978年福永らによりチャンタブリ地区で採集された血清を用いた。対照としてチェンマイ地区の脳炎患者，日本の日本脳炎 (JE) 患者，およびチェンマイと北海道の正常人血清を用いた。

ELISA はデングウイルス1型 Hawai 株，2型 New Guinea B 株，3型 H-87 株，4型 H-241 株を C 6/36 細胞に感染させた培養液からポリエチレングリコールと超遠心により濃縮したもの，及び阪大微研観音寺研製 JE ホルマリン不活化精製濃縮ワクチン原液を抗原とした微量間接法を使用した。HI は Clarke-Casals の微量変法を用いた。

DHF と診断された患者血清165について HI 抗体価の対数 (X) と IgG-ELISA 抗体価の対数 (Y) は相関係数0.82～0.88で相関した。DHF 患者血清66対のうちデング及び JE のどれか1つの抗原で HI が4倍以上の上昇を示したものは47検体あり，そのうち34検体は IgG-ELISA でも4倍以上の上昇を示し残りの13検体もすべて IgG-ELISA 抗体価16,000以上を示した。一方 IgG-ELISA では39検体が4倍以上の上昇を示し，このうち5検体は HI で陰性または判定不能であった。脳炎及び DHF 患者血清の IgM-ELISA を D1 と JE 抗原で測定したところ，IgM-ELISA 抗体価は初感染時で高く同種の抗原により強く反応した。これらの結果から ELISA は DHF の血清診断，特に JE との鑑別に使用できると思われる。

5 マラウイ共和国における供血者血液のHBs 抗原-抗体に関する調査

高岡千代子，菊池　典子
（元青年海外協力隊員）
鹿住　祐子　（上尾中央総合病院）
赤尾　信吉　（防衛医大・寄生虫）

マラウイ共和国の国立病院 Queen Elizabeth Hospital と Kamuzu Central Hospital の検査室における輸血の検査の状況をまとめ，特に供血者の HBs 抗原-抗体を調査した。

供血者の条件は年齢18歳以上，血色素量 12 g/dl 以上。原則として患者の家族・親戚・友人から1回 500 ml の血液が採血される。

HBs 抗原陽性は供血者433名中23名 (5.3%)，HBs 抗体陽性は供血者297名中73名 (24.6%)であった。このことにより健康なマラウイ人20人に1人が HBs 抗原をもち，4人に1人が HBs 抗原の感染をうけたことになる。

現在マラウイ共和国において HBs 抗原-抗体検出の検査は行われておらず，現在もこのように HBs 抗原陽性の血液が患者に輸血されていることになる。

血液型に関しては，Queen Elizabeth Hospital において約1万人を対象とした調査では，O型53%，A型24%，B型20%，AB 型3%で，Kamuzu Central Hospital における692名の血液型の結果の，O型54%，A型23%，B型19%，AB 型4%とほぼ一致する。

マラウイ共和国だけでなく発展途上国において，患者はもちろんのこと医療従事者，長期滞在者にも HBs 抗原感染の危険性は高く，大きな問題となってくるのではないかと思われる。

6 発展途上国における肝炎罹患の危険性について

海老沢　功　（東邦大・医・公衆衛生）
大谷　杉士，大利　昌久，表　光代，
佐藤　幸子
（日本青年海外協力隊診療室）
小原　博　（金沢医大・医動物）

海外に長期間滞在する日本人の代表として日本青年海外協力隊 (JOCV) の肝炎罹患状況を調べて次の結論を得た。

1) 1978-1982年の5年間の平均罹患率は，年間3.25%で男女の罹患率に差はなかった。

2) 隊員の2年間の滞在中における肝炎罹患率は東南アジア（フィリピン，ネパール，バングラデシュ），地中海沿岸諸国（シリア，チュニジア，モロッコ），アフリカ（ガーナ，ザンビア）等で多かった。全隊員の5%以上が肝炎にかかった国は28カ国中11カ国にのぼる。

3) 現地到着後6～9カ月と13～15カ月に患者集積の傾向があるが，全期間にわたり発生している。

4) HA が主であるが HNANB もこれに次いで多い。HB の血清反応は派遣前後で陽転したものと陰転した者がありその差は1.1%と少なかった。

5) JOCV は同年代のビルマ人より30倍以上の肝炎罹患率を示す。

7 コレラ菌型別用ファージの近縁性検討とケニア国分離エルトールコレラ菌の型別

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1981年カルカッタの National Institute of Cholera and Enteric Diseases で分与を受けた Mukerjee のクラシックコレラ菌型別用ファージ4種 (I-IV)とエルトールコレラ菌型別用ファージ5種 (1-5) について，各群の増殖用菌株 (154と757) から各ファージへの抵抗性変異株を分離，それらのファージ感受性を精査した。またエルトール用ファージではケニア国分離株の型別を試みた。

抵抗性変異株の分離は軟寒天重層法で行い，分離困難な場合は親株を変異誘起剤 N-メチル-N′-ニトロ-N-ニトロソグアニジンで処理した。クラシック系の場合 H218 も用いた。

757/1の9株はすべて5にも抵抗性，2には感受性低下，10株の 757/2 は1と5への抵抗性と2への感受性低下，757/3 の9株は4への感受性低下，10株の 757/4 は1, 2, 5への抵抗性と3への抵抗性ま

たは感受性低下，757/5の9株は1への抵抗性と2への感受性低下を示し，1と5は近縁とみられた。

154からは154/I 8株と154/II 4株のみが分離でき，前者のうち，4株はII, III, IVにも抵抗性，残る4株はIのみに抵抗性であった。154/IIはIII, IVへも抵抗性であった，H218の場合分離できた8株と10株のH218/IではII, III, IVに対して前者は感受性，後者は抵抗性，10株ずつのH218/II, /III, /IVは全株が全ファージ抵抗性を示した。この結果I-IV特にII-IVは極めて近縁と考える。

性状試験を行った757由来の47株とH218株由来の38株では，前群の一部でVP反応，ポリミキシンB感受性が，後群の一部で血清型，VP反応，ポリミキシンB感受性が親株と異なっていた。

ケニア国分離エルトールコレラ菌434株の型別では，2回目の成績と1回目のそれが一致したもの67.7%，3回目の成績が前2回のいずれかと一致したもの27.0%，残る23株も4回目を実施した後の一致した2成績でと，全株型別可能であった。その結果4型44.0%，3型27.2%，1型11.3%，5型11.1%，6型4株，2型1株となり，残る23株は型別表の型に一致しなかった。この成績から，再現性は実用に耐えるものであり，ファージ型は疫学的に利用できる標識であろうとみられた。

8 インドネシアにおける土壌中嫌気性菌の研究

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藤田紘一郎，月舘 説子，黒川 憲次
（長崎大・医・医動物）
杉山 雅俊 （順天堂大・医・衛生）
朝倉 健夫 （日本熱帯医学協会）

インドネシアにおいては，依然として，新生児破傷風患者は5～6万人はいるものと推定されているし，また，小児における破傷風罹患率は3%以上で，その致命率は45%にものぼるであろうという報告が得られている。

このような観点から，我々は，インドネシア各地で土壌を採取し，その土壌中より嫌気性菌の中でも特にヒトとの疾病に因果関係をもつ *Clostridium* 属に注目し，検出を試みている。

第1回目の報告を，第23回の本学会において行ったが，その成績は必ずしも満足すべきものではなかった。そこで，引き続き，1982年6月から7月にかけての調査時に，再びインドネシア各地より合計21検体の土壌を採取し，帰国後，分離培養し，さらに菌種の鑑別を行った。

その結果，合計20菌種というように，前回の検討に比較して，多岐にわたり菌種の検出がなされた。その中で，最も多く検出されたのは *Clostridium perfringens* で14検体から分離された。ついで多かったのは，それぞれ8検体から分離された *Clostridium cadaveris* と *Clostridium aurantibutyricum* であった。一方，最も注目される *Clostridium tetani* は4検体から分離された。

今後，より多くの土壌を集めて検討を加えることにより，インドネシアにおける *Clostridium* 属の分布図を作製できればと考える。

9 ケニア共和国、クワレ地区におけるビルハルツ住血吸虫症の流行像について

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我々は現在進行中の伝染病研究対策プロジェクトにおいて，ビルハルツ住血吸虫症の流行像を解析しその対策に資するために，住民の寄生虫学的検査，伝搬貝の調査，住民の水との接触状況の調査等を，パイロット地区を設けて長期に行っている。今回はその一部の成績について報告する。

1982年5-6月の住民検診では，得られた851の検体中581に虫卵を認め，男女それぞれの陽性率は62.4%と69.4%で，平均排泄虫卵数は時間あたりそれぞれ52.4，47.4個であった。性差はこれらを年齢別にみる時に明らかで，陽性率，排泄虫卵数共に男性では15-19歳を過ぎて急激に低下するのに対し，女性では徐々にしか下がらない傾向が

認められた。

1982年6-9月に行われた住民と水との接触調査では1人あたりの水との接触時間は男性で16.9分，女性では21.4分であった。これを男女それぞれ年齢別にみると異なる年齢分布を示し，男性では5-19歳での接触時間が長いのに対し，女性では0-4歳と30-39歳のふたつの年齢層にピークがみられた。

以上の結果から，ビルハルツ住血吸虫流行地の年齢別陽性率，排泄虫卵数の解釈にあたり水との接触状況の把握が不可欠であることが示唆される。本報告にみる男女差は水との接触の違いから説明できるかもしれない。また，女性の20-39歳の年齢層で水との接触時間が年齢と共に増加してゆくにもかかわらず，陽性率，虫卵排泄数が低下してゆくのは，ヒトにおけるビルハルツ住血吸虫の再感染に対する抵抗性を示唆する現象かもしれない。今後さらにくわしく解析するために，これら調査の継続とセルカリオメトリーなどが必要と考えている。

10 精製虫卵抗原を用いた日本住血吸虫症のELISA

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今井　淳一　（宮崎医大・寄生虫）

住血吸虫症の免疫診断法としてELISAが有望視されている。虫卵可溶性抗原（SEA）は感度の点ですぐれているが，肝蛭症，旋毛虫症，肺吸虫症などと交差反応性がある。この交差反応性のない抗原がより精度の高いELISAの確立に必要と考えられる。今回は日本住血吸虫虫卵抗原よりDE52陰イオン交換クロマト及びSephacryl S200のゲル濾過により得られた精製抗原J-1（分子量26万，pI 4.9）をELISA用の抗原として用いた。ELISA用平底マイクロタイタープレート（Dynatech A129M）にSEA（2.5 μg/m*l*）及びJ-1（1 μg/m*l*）をコートした。ラベル抗体はペルオキシダーゼ標識抗ヒトIgGを用いた。基質はo-phenylene diamineを用い，H_2SO_4で反応を停止後OD492 nmを測定した。

結果：ゲル内沈降反応においてJ-1は感染マウス血清との間に明確な1本の沈降バンドを形成した。またFCAを用いてウサギに免疫して得た抗J-1血清はCOP陽性であった。J-1はCOP反応に関与する主要抗原の1つであると考えられる。J-1を抗原としたELISAでは，Sj患者についてはSEAを抗原とした時とほぼ同じ感度が得られた。Sm, Sr患者ではJ-1抗原の使用により交差反応性の減少が見られたが，完全に除去することはできなかった。一方SEAを抗原とした時，肺吸虫及び肝蛭症に見られた交差反応は，J-1を使用することにより完全に除かれたが，旋毛虫症では逆に交差反応性が増加した。以上の結果よりJ-1は他の吸虫症との交差反応を除くことができる点ですぐれた抗原であるが，線虫症との交差反応性の点はさらに検討の必要があると考えられる。

11 ELISA法によるマラリア抗体の検出

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田辺　清勝　（東大・医科研・内科）

今回我々は邦人の輸入マラリア症例の血清を入手し，IgGおよびIgM抗体の検出を試みた。症例は東アフリカに1カ月間滞在し帰国後悪感・発熱をもって発病し（潜伏期間37～67日）発病7日目に熱帯熱マラリアと診断された。患者は帰国後1回だけ抗マラリア剤（内容不明）を服用したのみである。診断当日よりクロロキン塩基総量1,500 mgによる治療を型のごとく行い，3日目には末梢血中のマラリア原虫は消失し，体温も平熱となった。

この患者の治療開始前の血清を用いてELISA法でIgG, IgM抗体の測定を行った。標識酵素はアルカリフォスファターゼ，また酵素基質としてリン酸パラニトロフェニル（pNPP）を用いた。

結果：IgG ELISAのODは0.082で2例の正常対照（0.044および0.060）と有意の差がなかった。IgG ELISAのODは0.503で正常対照（0.058および0.089）に比し充分高値を示した。比較のためにタイで採取した熱帯熱マラリア患者2例の血清と熱帯熱マラリアの実験感染を数回くりかえしたヨザル血清につき同様のテストを行ったとこ

ろ，これら3検体においては IgG 抗体価が有意に高く，IgM 抗体はヨザルのみに検出された。また本患者血清につき螢光抗体法により抗マラリア抗体を調べたところ，IgG および IgM の両テストで血清希釈256倍まで螢光を認めた。

以上，マラリア初感染の本患者では本法で IgG 抗体は検出されず IgM 抗体のみ高値を示した。慢性のマラリア感染と考えられるヒト血清では，これと逆に IgG 抗体のみ高値を示し，IgM 抗体は検出されなかった。実験感染をくりかえしているヨザルの血清では IgG, IgM 抗体が同時に検出された。本患者の螢光抗体法によるテストでは IgG, IgM 両者の抗体価が高く，ELISA 法と異なる結果を得たが，その原因については今後の検討が必要である。

12 マラリア原虫の検出法に関する検討

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マラリア原虫の形態学的な検出法に血液塗抹標本検査法（薄層および厚層）があるが，マラリア原虫を検出する機会の少ない国内の検査技術者にとっては通常行われている一般的なその検出法では心許なく，原虫を誰もが容易に検出できる方法が改良あるいは開発されれば，増加傾向にある輸入マラリアに対しもっと速やかな診断や対処が可能となる。

①原虫数を濃縮できる。②原虫の形態を変形させない。③原虫を顕微鏡下に確実に見い出すことができる。以上の3点を満たす方法としてマウスマラリア *Plasmodium berghei* を材料に用いてアクリジンオレンジ染色を利用した原虫の検出法の検討を行った。

PBS で希釈した感染血液を CM-Cellulose column（溶出液は10% calf serum 加 PBS）を通して白血球成分を分離し，Tris-buffered ammonium chloride で溶血してその沈渣の AO 染色を行った。

その結果，CM-Cellulose column ならびに Cellulose powder を加圧して充填した Cellulose column も同様に白血球成分を完全に分離し得た。赤血球成分は CM-Cellulose の場合はほぼ100%を，Cellulose の場合は90%を回収でき，column を通したことによる赤血球成分の損失は僅少であった。同時に赤血球感染率の変化もみられなかった。また薄層塗抹標本作製の要領で溶血後の沈渣をスライドグラスに塗抹した場合，厚層塗抹標本と同程度の原虫の濃縮率を得た。原虫は元の形態を維持したまま黄緑色（DNA）と赤色（RNA）とに明瞭に核酸染色され，しかも暗視野中に原虫のみが螢光を発するので400倍の低倍率でも容易に原虫を見い出すことができた。

以上のように AO 染色による原虫の検出法は先に述べた3つの条件を満たしており，ヒトマラリア検査への応用も可能であると考える。ただし熱帯地方の流行地フィールドでは設備上本法を利用できないし，また原虫種の鑑別もむずかしいなどの難点がある。

13 東アジア地域におけるマラリア媒介者の生物学的ならびに疫学的研究

神田　錬蔵
（聖マリアンナ医大・病害動物）

マラリア駆除の達成が，今までのところ系統発生にはじまる生物学的性状の解明に不充分なところが多いために，他の要因とも併せて困難に直面している現状をふまえ，媒介者の生物学的基礎研究の取り残されてきた数々の解明に努力してきた。今回は東アジア地域分布のマラリア媒介者について調査し得られた情報を報告する。*hyrcanus* 群シナハマダラカはオオツルハマダラカと共に北東アジア地域での媒介者であり，現在，中国，ベトナムに至る地域で三日熱媒介の猛威をふるっているが，両者との交配実験で交雑 F_1 のX染色体が対合するかどうかの相違でわけられる。日本では対合しない動物吸血性のものが多く分布し，三日熱マラリア原虫に感染しないという実験結果と合わせて，置換えによる駆除への方向の導入の可能性が考えられる。*leucosphyrus* 群は熱帯熱マラリアの強力な媒介者であるが，台湾系と東南アジア8つの諸系との調査で遺伝的分化の程度の差こそあれ同一種 *balabacensis* に含まれることがわ

かった。*minimus* はタイ国で広大な地域にわたる媒介者で，カンチャナブリ省サトウキビ栽培流行地での雨期終りから乾期の始まる3カ月間の調査によると，採集蚊8,257頭中74%がハマダラカでうち60%が本種で他の16種はごくわずかであった。蚊の自然感染率は5/646，1/156と2つの部落でみられた。経産率は3部落で0.539，0.462，0.707であった。時間による吸血頻度は開けた野外が最高で植物密生地，屋外ベランダが次ぎ，屋内は最も少ない。吸血活動は動物吸血性で明方盛んなものが多く，夜半型，両者混合の3型がみられた。*maculatus* は形態上3型に分けられるものの間に8酵素系で11遺伝子座に関し2相違を見，遺伝分化がみられたが熱帯熱感染症には変化なく高率であった。*maculatus*, *minimus* の殺虫剤抵抗性で進展はないようであった。

14 インドネシア共和国北スマトラ州の1海岸村落における *Anopheles sundaicus* 幼虫の生態学的研究

今井長兵衛　（大阪市環科研）
高木　正洋　（三重大・医・医動物）
William Panjaitan
（北スマトラ州衛生局）
Sumitro
（保健省リージョナルオフィス）

インドネシアと日本の技術協力の一環として，北スマトラ州アサハン県の1海岸村落でマラリア媒介蚊 *Anopheles sundaicus* 幼虫の生態を1982年3月から1年間観察した。ヒトおとりによる成虫調査定点から半径 600 m 以内の大部分の池41カ所を対象に月2回，すくいとりによる幼虫密度，水位，面積，透視度，塩分濃度，長繊維状浮遊藻類群落，魚類の生息状況などを調べた。

発生源の面積は5-6月と10-12月にピークをもつ2峰型の消長を示し，乾季の末の3月には23カ所の池が，小乾季の8月には9カ所の池が干上った。藻類群落は主として低塩分濃度の池において季節の推移とともに0～51%の池で観察された。魚類は3-4月には半数未満の池に，5月以降は大半の池に出現した。

幼虫は高塩分濃度の池の多い Sub-area で多く，他の2つの Sub-area では少なかったが，水位が急増した3-4月に密度が高く，以後は低密度で推移するという傾向がいずれの Sub-area においても認められた。

全データを各要因についてクラス分けし，各クラスに属するデータ数を分母にして，幼虫が採集された場合の比率（出現頻度）と密度の平均値を求めたところ，塩分濃度が高くて陽当たりの良い池で水位が急増した場合に幼虫が多く，その逆の条件や水が濁って悪臭を放つ池には少ないことが判明した。魚類の生息する池の密度は低かったが，出現頻度には差が認められなかった。これらの結果から，幼虫の生息に好適な要因の組合せと不適な要因の組合せをつくって出現率と密度を求めると，予測通りの結果が得られた。

成虫調査定点から 150 m 以内の Sub-area について池の面積と密度の積として求めた幼虫発生指数の消長は Man Hour Density のそれとほぼ対応し，4月と9-10月にピークをもった。第1のピークは高密度により，第2のピークは広い発生源がその原因である。

15 急激な経過をたどった熱帯熱マラリアの1例

尾辻　義人，原田　隆二，堂園　貞巳，
橋本　修治
（鹿児島大・医・第二内科）
徳永　正義，佐藤　栄一
（鹿児島大・医・第二病理）
上山　達典　（上山内科）

私達は最近ナイジェリア・エヌグ地区で医療活動に従事し，熱帯熱マラリアに感染，死亡した症例を剖検する機会を得たのでその概要を報告する。

患者は36歳の男性で，職業は医師。

主訴は発熱で，家族歴ならびに既往歴に特記すべきことなし。

現病歴：昭和58年6月20日より6月28日までナイジェリア・エヌグ地区で医療活動に従事。58年6月29日 帰国，7月10日 悪感戦慄を伴って 発熱 (体温 39°C)，7月11，12，13日も同様，午前中は

下熱，夕刻発熱を繰返した。軽度の頭痛，筋肉痛，軟便，下痢便あり。7月14日黄疸，肝障害を認めたのでマラリアまたはA型肝炎を疑って入院をすすめた。7月14日15時，歩行入院。

入院時所見：体温 37°C，熱感，頭重感，全身倦怠感を訴える。皮膚黄染を認めるも意識は正常。19時突然全身痙攣頻発，意識混濁，39°C 発熱，呼吸不全，乏尿となったので，挿管して呼吸管理を行うとともに強心処理を行った。熱帯熱マラリアの脳症を疑い末梢血を検索，熱帯熱マラリア原虫を証明した (parasite density 351,900/mm³)。直ちにキニマックス 4 ml (塩酸キニーネ 400 mg 含有) を開始したが (点滴終了直後の parasite density 28,880/mm³)，7月15日2時40分熱帯熱マラリアによる多臓器不全 (脳，心，肝，腎，脾など) で死亡した。

剖検所見および病理診断：病理組織学的には全身の小血管及び毛細血管の著明な拡張を来たし，赤血球内のマラリア原虫や遊離したマラリア色素が充満していた。病理診断としては熱帯熱マラリアで，①クッパー細胞の反応を伴う肝腫大，②組織球反応及び赤脾髄の拡張を伴う脾腫，③著明な血管拡張を伴う脳浮腫，④肺及び腎のうっ血水腫，⑤出血傾向などが目立った所見であった。

16 三日熱マラリアの再発した2症例

田辺 清勝 (東大・医科研・感染症)
田中 寛 (東大・医科研・寄生虫)
海老沢 功 (東邦大・医・公衆衛生)

過去3年間に当研究所病院に入院して治療したマラリア患者数は29名であり，三日熱マラリアは19名であった。その中の2名が帰国後治療して再発した。2例とも感染地はタイ-カンボジアの国境周辺であった。プリマキン低感受性三日熱マラリアの疑いがあり報告した。

症例1：37歳の男性。予防薬にキニーネを服用しながら福祉活動に従事していたが，帰国3カ月前 (カンボジアに移動して3週目) にマラリアに罹患し，2度現地の病院で入院加療を受けた。その後は帰国前まで定期検査でマラリア原虫を指摘されていないが帰国後まもなく発熱し，当院に1回目の入院となった。三日熱マラリアの診断でクロロキン3日間総量 1.5 g 塩基とプリマキン1回 15 mg，14日間の型通りの治療をしたが2カ月後に再発した。2回目の入院時治療は先ずクロロキン 1.5 g 塩基を投与してその1週間後に再度クロロキン 300 mg 塩基を2日間投与し，その翌日からプリマキン 45 mg 塩基を週1回，8週投与したが投与後9カ月目にまた再発した。3回目の入院時にはプリマキン8週間投与と共にクロロキン 300 mg 塩基を毎週服用させた。

症例2：40歳の男性。予防薬にファンシダールを服用してタイ農村部にて調査活動を行った。帰国後2週間目に発熱し，手持ちのファンシダールを服用してまもなく入院してきた。クロロキン総量 1.5 g 塩基とプリマキン 15 mg 塩基，14日間投与を受けたが，2カ月後に再発した。2回目の入院前にも手持ちのファンシダールを服用して入院しており，入院後クロロキンは初回と同量，プリマキンは1回 30 mg で7日間に変更して投与した。1年を経た現在でも再発はみていない。2症例ともプリマキン投与後の血中濃度は測定していない。

17 4例のマラリア患者に対する ST 合剤の臨床効果

山口 恵三，賀来 満夫，松瀬真寿美，
草野 展周，重野 芳輝，斉藤 厚，
原 耕平 (長崎大・医・第二内科)

目的：マラリアの流行地では依然として，その発生率は高く，これらの国を旅行し，帰国後国内において発病する患者が近年増加の傾向にある。

本疾患の治療剤としては，通常はクロロキン製剤が用いられる場合が多いが，わが国においては本剤が限られた施設にしか用意されておらず，入手迄に時間を要する場合が少なくない。そこで，私達は日常の診療の場で入手が極めて容易で，且つすでに抗マラリア薬としての評価を受けている ST 合剤による治療を4名の患者を対象に試み，良好な成績が得られたので報告する。

成績：現在迄に私達が経験したマラリア患者は7例で，その内訳は熱帯熱マラリア4例，三日熱

マラリア1例，熱帯熱マラリアと三日熱マラリア，四日熱マラリアと卵型マラリアの混合例がそれぞれ1例ずつであった。全例国外で罹患したと考えられ，7例中6例はアフリカで感染したものと思われた。

治療剤としてはクロロキン，プリマキンが3例に対して投与され，残りの4例を対象にST合剤が投与され，そのうち1例のみはクロロキンが追加された。STが投与された4例は熱帯熱マラリアが3例，三日熱マラリアが1例で，末血中の原虫数は 1.7×10^4～$6.7\times10^5/\mu l$ であった。STの総投与量は12～36錠で投与日数は2～6日間であった。4例中3例はSTの単独投与であったが，全例とも速やかな末梢血中原虫の消失と症状の軽快がみられた。残りの1例ではST投与後直ちに解熱傾向がみられたが，症状の軽快が少し遅れたため，熱帯熱マラリアであることを考慮して，2日目にはクロロキンに変更したが，その翌日には原虫は消失していたことから，本症例においても有効であったものと考えられた。

副作用としては1例に軽度の胃腸障害を認めただけであった。

18 名古屋市における広東住血線虫の分布状況 -市内のネズミ集団における感染率

真喜屋 清　（名大・医・医動物）
鬼武 一夫　（名大・医短・生物）

名古屋市内のドブネズミ（以下ドブ）から昨年広東住血線虫（以下広住虫）が見つかったが，この場所は港湾部から約14 kmも離れた内陸部であった。そこで，本虫の定着状況を知るために，その後港湾地区も含めてその分布を調べた。

その結果，港湾地区の港区船見町で捕獲された8匹および港湾部から数kmはなれた南区松城町で捕獲された4匹のドブから，それぞれ163匹および104匹の広住虫成虫（合計雄142，雌125）が採集され，最初の発見場所名東区猪子石でこれまでに得られた120匹（雄62，雌54，不明4）を加えると，380匹以上の同成虫が採集されたことになる。

広住虫が寄生していたのはドブだけであり，クマネズミ（以下クマ）には認められなかった。ドブ集団の感染率は港区・南区・名東区でそれぞれ25.8%（8/31），25.0%（4/16），16.7%（6/36）と港湾部に近いほど高く，同線虫の移入ルート・定着状況を考察する上で興味深い。また，市内10数地域で行った住家性ネズミの蠕虫調査の結果から見ると，市の東よりに分布する傾向が認められた。

広住虫以外の蠕虫類の寄生もクマよりドブ集団で著しく高く，それぞれ93%（91/98）および27%（23/84）であり，また2種以上の混合寄生率もクマよりドブで著しく高く，ドブでは蠕虫陽性個体の89%に2種以上の蠕虫寄生を認めたが，クマでは22%と低率であった。

広住虫のネズミ1匹当たりの寄生頻度は，他の蠕虫と同様負の二項分布に適合する集中型の傾向を示した（$k=0.0456$, $0.30<P<0.50$）。しかし，分布の集中度を示す k の値が他の5種の蠕虫（0.2457～0.0702）よりさらに小さいことから，本虫のネズミへの感染がより集中的に起こっているものと推定された。

19 近畿地方におけるブラックバス（オオクチバス）の寄生蠕虫類の調査

正垣 幸男　（京大・医・病理）

ブラックバスは大正11年（1925）に北米オレゴン州より芦の湖に移殖され，ブルーギルは昭和35年（1960）に北米・シカゴより伊豆半島に移入されて以来，一時は絶滅に瀕したが，後によく繁殖力を恢復して生態的地位を獲得した。現在では本州，九州，四国全域の湖，沼，池，淵などに繁殖を来たし，その水域に生息していた在来の淡水魚の大部分が食害されて，養魚家に大きな被害を与えている。今回はブラックバスについて報告したい。ブラックバスはルアー，餌釣り共に可能な極めて魅力のあるゲーム・フィッシュであり，また，その肉は白味で，淡白であり，さしみ，塩焼き，バター焼き，フライなどの何れにしても美味である。従って，本種がヒトに感染する寄生虫を持たず，また魚毒のないことが判明すれば，将来必ず食料魚として重要視されるであろう。

昨秋（1982）11月より本調査に着手し，本魚の

胸部（幅 3 cm），腹部（幅 3 cm），尾部（幅 3 cm）における鱗（各部300枚）及び筋肉を剖検して，寄生蠕虫幼虫の有無を検査した。更に内部諸器官（鰓，腹腔，消化管，腸間膜，肝，膵など）を剖検して，寄生蠕虫幼虫及び成虫の有無を検査した。滋賀県・琵琶湖産のブラックバス11匹（大きさ16.5×5.5～28.5×8.5 cm），宇治市・喜撰山ダム産の5匹（19.0×6.8～28.0×7.5 cm），兵庫県・円山川産の5匹（15.2×3.7～29.0×8.5 cm）を剖検した。調査したブラックバス20匹（琵琶湖産11匹，喜撰山ダム産5匹，円山川産4匹）には何らの寄生蠕虫幼虫成虫を認めず，円山川産の1匹（雌，体長29.0 cm, 体高 8.5 cm）の腹腔小腸上部外壁に鉤頭虫 Acanthocephala の被嚢幼虫2個体を検出した。本幼虫（雌）はバーリーゼ氏液封入，カバーグラスによる加圧標本にて吻の大きさ 0.43×0.33 mm, 虫体の大きさ 2.80×0.84 mm, 鉤縦列10個であり，虫体前部の体壁に小棘の縦列が見られた。

本鉤頭虫幼虫を犬，ラット，ミニ豚或は鶏などに感染実験を実施して得た成虫について精査した上で種が決定されるが，虫体前部の体壁に棘を持つ特徴により Polymorphidae 科に属するものと思われる。本幼虫が人体内で成虫になるか否かは不明である。本種及びブルーギルの剖検，円山川畔のブラックバスより採集した鉤頭虫の発育史を検討中である。

20 マウス内被嚢旋毛虫幼虫 (USA *strain*) に対するフルベンダゾールとメベンダゾールの効果

牧　　純，中島美佐保，柳沢十四男
（北里大・医・寄生虫）

ベンズイミダゾール系の薬物の旋毛虫に対する治療効果が近年一層注目されている。今回演者らはフルベンダゾール (FB) とメベンダゾール (MB) のマウス内被嚢旋毛虫幼虫に対する治療効果を比較検討したので発表する。感染マウスの筋肉をペプシン消化して得た幼虫を♀ ICR (6週齢) のマウスに体重 10 g 当たり 50匹経口投与した。感染後35日, 35–37日または70–72日に, 1% Tween 80に懸濁させた FB または MB の原末を経口投与した（10, 50, 100 mg/kg/day×3 days または 300 mg/kg/day×1 day)。対照群には 1 % Tween 80 のみを与えた。投薬終了後 3 週間または 6 週間を経て横隔膜の圧平標本を調べた。横隔膜に於ける幼虫数の減少から他の筋肉に於けるそれが推定できることが予備的検討で確認されているので今回は横隔膜のみについて正常な幼虫の残存数を算定した。横隔膜の生鮮重量 100 mg 当たりの正常幼虫数を投薬群と対照群の間で較べて減少率(%)を求めこれを更にプロビット変換して ED_{50} の大小を推定した。感染 5 週後の投薬ではいずれの投薬群に於ても極めて高い虫体減少率（82～100%）が認められた（$P<0.01$)。300 mg/kg を感染35日後に投与した場合は MB が FB よりも有意に効果的であったが（$P<0.1$) 他の投与では FB と MB の間に差がみられなかった（$0.1<P$)。FB, MB ともに分服（100 mg/kg/day×3 days）が頓服（300 mg/kg/day×1 day）よりも高い効果をもたらした（$P=0.05$)。感染後10週間を経て投薬した実験結果は以下の通りであった。いずれの薬物も 50 mg/kg/day×3 days または 100 mg/kg/day×3 days の投与で高い虫体減少率（79～100%）をもたらした（$P<0.01$)。FB の 10 mg/kg/day×3 days では殆ど効果がみられなかったが（$0.5<P$), MB のそれでは中程度（60%）の効果が認められた（$0.01<P<0.05$)。FB と MB の総投与量の ED_{50} はそれぞれ115及び 21 mg/kg で MB が FB より効果的であった（$P<0.05$)。

21 Bithionol による *Giardia lamblia* の呼吸阻害と致死活性について

小林　正規，竹内　　勤，河村　康司，
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我々は先に肺吸虫症の治療薬であるビチオノールが赤痢アメーバ栄養型の内因性呼吸を阻害し，また致死活性もあることを報告した。

今回は赤痢アメーバと同じ嫌気性原虫であるランブル鞭毛虫についてビチオノール及び近縁化合物の効果を検討したので報告する。

ランブル鞭毛虫は米国 NIH 由来の無菌培養株

Portland strain で，これを TYI-S-33 改良培地にて培養した。呼吸活性の測定には Clark 型の酸素電極を用い，intact な虫体について基質を加えることなしに呼吸を測定し薬剤の阻害効果を検討した。薬剤によるランブル鞭毛虫の増殖阻止活性については各種濃度の薬剤を添加した培地内での増殖曲線を対照と比較することで検討した。また薬剤により作用を受けたランブル鞭毛虫の形態的変化を電子顕微鏡により観察した。その結果ビチオノールのランブル鞭毛虫の内因性呼吸50%阻害濃度は 0.50 mM であり，近縁化合物であるジクロロフェン及びヘキサクロロフェンではそれぞれ 0.75 mM 及び 0.50 mM であった。致死活性については，ビチオノール 100 μg/ml の濃度では72時間以内にランブル鞭毛虫は全て死滅した。ジクロロフェン，及びヘキサクロロフェンについてもほぼ同様な結果を得た。ビチオノールを作用させたランブル鞭毛虫の形態学的変化としては通常の細胞死の一連の変化と同じ様であり特異的な変化は見い出せなかった。今回 *in vitro* の結果より得たランブル鞭毛虫に対するビチオノールの有効濃度は赤痢アメーバに対するものとほぼ同程度であり，また *in vitro* における肺吸虫に対する有効濃度より低いことから実際のランブル鞭毛虫症にも応用が可能なものと考えられた。よってさらに *in vitro* の実験系の確立を検討中である。

22 グアテマラ・ブユ撲滅対策地区住民のオンコセルカ-ミクロフィラリア陽性率の4年間における変動

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多田　　功　（熊本大・医・寄生虫病）
林　　滋生　（予研・寄生虫）

1976年よりグアテマラにおいて実施されてきたオンコセルカ症撲滅対策事業において，その効果を調べるために対策の実施された地区住民を対象として毎年1回，定期的にオンコセルカ症の疫学調査が行われてきた。そこで，資料の整っている1978年-1982年の4年間におけるミクロフィラリア (mf) 検出状況につき検討した。

方法: San Vicente Pacaya のオンコセルカ流行地の内6地区 (Santa Cruz, los Ríos, Patrocinio, Berlin, Guachipilin 及び Hamburgo) の住民を検査対象とした。調査は毎年1回7-8月に実施した。Holth 型パンチを用いて2カ所より皮膚片を採取し，生食水に浸し，遊出する mf を検鏡した。

結果: (1) mf 陽性率の年次変動は，各地区とも漸減の傾向が認められ，特に Berlin, los Ríos で減少の傾向が著しかった。しかし，ブユ撲滅対策の施行されていない Hamburgo でも減少傾向の認められたことから，この減少が撲滅対策の影響と考えることが出来なかった。(2)各調査時に腫瘤保有者の腫瘤摘出術を施行してきた。そこで前年度腫瘤摘出術を受けた者の mf 陰転率を調べたが，わずか5%にすぎなかった。このことは腫瘤摘出術施行後1年間は皮膚内の mf が陰転することがなく，また，減少傾向が腫瘤摘出の結果でないことを示している。(3) 4年間で4回ないし5回の検査を受けた総数700名の住民の内，いずれの検査でも陽性であった例が115名，全て陰性であった例が393名，合計508名 (72.5%) が常に同一の検査結果を示した。このことは mf 検査法の安定性を示している。(4)1980年を境に前2年が mf 陰性，後2年が陽性の例を新感染例，前2年が陽性，後2年が陰性の例を自然治癒例とみなして，その率を求めた。全体としてみると，6地区の対象者52名の内16名(2.9%)の自然治癒例と13名(2.4%)の新感染例が認められた。この両者の価の差を求めることによってその地区の感染状況を推察し得るものと考えられた。

23 ベネズエラ国アマゾナス州におけるオンコセルカ症伝搬に関する研究

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多田　　功　（熊本大・医・寄生虫病）
鈴木　　博
（長崎大・熱帯医研・ウイルス）
野田　伸一　（鹿児島大・医・医動物）
Luis Yarzábal, María G. Basáñez
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文部省科研海外学術調査「南米型および中米型

オンコセルカ症とその伝搬機構の比較研究」の一環として1982年10-11月，南米ベネズエラ国アマゾナス州のオリノコ河上流域に広がるオンコセルカ症流行地数地区において本症の総合調査を行った。本大会では，本症の伝搬に関して行った昆虫学的調査の結果を報告した。人吸血ブユは *Simulium pintoi*, *S. pseudoantillarum*, *S. yarzabali*, *S. exiguum* および *S. amazonicum* グループの4種1種グループであった。これまで自然感染の結果から媒介種として報告されている *S. pintoi* と *S. amazonicum* グループはおのおの標高250-1,000 mおよび標高約200 mにおいて最優先種であった。人体吸血部位は前者ブユ種が下半身，また後者ブユ種が上半身と異なっていた。*S. pintoi* を *O. volvulus* ミクロフィラリア保有者から吸血させた感染実験により次の結果を得た。本種のmfとりこみ数は人体部位で異なり脚で0-77（平均9），背中で58-495（平均245）であった。24時間後の死亡率は脚を吸血したブユ群2%であったが，背中から吸血した群では47%と高かった。飼育温度16-24°Cの条件下で幼虫発育は正常に，しかも同調して進行した。第Ⅲ期幼虫は吸血後8日以降死亡したブユから見出された。8日以上生存したブユのうち第Ⅲ期幼虫保有率は脚および背中を吸血した群でおのおの55.3%と62.5%と高かった。今回の生態調査によりアマゾナス州の主要伝搬ブユ種は地域により異なることが示唆された。また，感染実験により *S. pintoi* はmf多数とりこみによる死亡がみられるものの *O. volvulus* 幼虫を効率よく伝搬しうることが明らかになった。

24 ナイジェリアにおけるロア糸状症の疫学的研究 2. Epeにおける microfilaria 調査と媒介アブの調査

堀 栄太郎 （埼玉医大・寄生虫）
Eugene O. Ogunba（イバダン大・医）

1982年10月，11月の2カ月間，ナイジェリア西南部のロア糸状虫症流行地において，住民のmicrofilaria保有状況の調査と媒介アブ類の調査を行った。

住民のMf調査：調査を行った地区はラゴス州の1都市，Epeの周辺で，被験者は3つの中学・高校の学童（11歳-23歳）と給水施設住民（20歳-70歳）であった。Mfの検査は午前11時から午後2時までの間に行い，母指頭採血，血液厚層塗抹標本を作り，ギムザ染色して検出した。また，陽性者の問診による症状も調査した。Mfの保有状況は3つの学校で学童440名，陽性率平均6.6%（5.8～10.4%），職員（20歳-48歳）107名，陽性率平均7.5%（4.5%～11.4%）であった。給水施設住民は38名で陽性率28.9%の高率であった。問診による症状調査ではMf陽性者（46名）は非陽性者（39名）に比較し，Calabar swelings (63%), pricky sensation (71.7%) が高率であった。

媒介アブ種の調査：西南部熱帯雨林域のLagos, Ogun, Oyo, Ondo, Bendelの5州で調査し，成虫，幼虫と蛹，卵塊を採集した。アブ類成虫は計179個体採集，うち，*Chrysops* 属は47.8%で *C. silacea* 77.6%, *C. dimidiata* 18.9%, *C. longicornis*, *C. distinctipennis* であった。幼虫と蛹は計474個体採集し，うち，*Chrysops* 属は61.8%で *C. silacea* 8.6%, *C. centurionis* 13.3%, 同定保留9.4%, *C. longicornis* 57.6%, *C. distinctipennis* 10.9% であった。卵塊は計224個体採集，*Chrysops* 属が大部分で *C. cilacea* group (*C. silacea*, *C. dimidiata*, *C. centurionis* を含む) 20.7%, *C. longicornis* 72.5%, *C. distinctipennis* 6.8% であった。また採集した成虫27個体（*C. silacea* 25個体，*C. dimidiata* 2個体）を解剖し，*C. silacea* と *C. dimidiata* のそれぞれ1個体からロア糸状虫第2期幼虫をいずれも腹部から検出した。

25 タイ国チャンタブリ県における蚊の発生源調査

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Mongkol Chenchittikul,
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長谷川 恩 （JICA，タイ国医科学局）

1981-83年，チャンタブリ県において蚊の生態学的研究を行った。本報では，当地方の蚊相と種族維持の立場からみた発生水域の重要性を明らかにする目的で，主として人為的に開けた環境の水域を対象として行った蚊の発生源調査の結果を報告する。

調査した水域は，人工容器，地表の水溜り，湿原，水田，井戸，ピット，ロックプール，竹株，ゴム林の受皿，葉腋など145カ所で，計9属67種が採集された。

Aedes 属は8種類を得たが，人家周辺の人工容器にはネッタイシマカ，ヒトスジシマカが普遍的に発生し，特に水がめ，放置されたゴムタイヤなどはデング出血熱の予防対策上重要な発生源とみられた。ネッタイシマカの発生場所は人家に近接したところに限られたが，ヒトスジシマカとの混棲場所が約30%もみられた。ゴム林ではヒトスジシマカの高密度の発生がみられた。また，海水を含むロックプールでは多数のトウゴウヤブカの発生が認められた。

Culex 属は29種が得られた。ネッタイイエカとコガタアカイエカが優占種で，後者は地表の水溜り，湿原，水田，浅井戸など広範囲にわたり，しばしばシロハシイエカと混棲していた。*C. sitiens* は海岸ぞいの塩分を含む水溜り，ロックプールに多数棲息していた。

Anopheles 属は14種を得たが，*peditaeniatus*, *sinensis*, *barbirostris*, *philippinensis* が dominant な種類であった。*A. balabacensis* は清冽な水溜り，井戸，渓流のロックプールなどから採集されたが，個体数は少なかった。しかし，この地方に多い果樹園の敷地内には本種の好発生環境が散見されたことは，マラリア対策上考慮されるべきである。

竹株からは3種の *Tripteroides* 属が得られ，*aranoides* が優占種であった。*Uranotaenia* 属は8種を得たが，*edwardsi* 以外は未同定である。

26 上海市内におけるアカイエカ産卵活動の1982年の季節的推移

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刘　維徳，繆　建吾，徐　薇
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アカイエカは上海においては下水溝や汚れた池，あるいは，各種の人工的容器から多発している。我々は1981年8月から1982年12月まで，上海市内の1公園で，この蚊の産卵活動の季節的推移について調べたので，今回は1982年の調査結果について報告する。

調査を行った上海市は北緯32度付近にあり，鹿児島市とほぼ同じ緯度に位置している。この都市は中央部の市区と周辺の県部にわかれているが，今回の調査は市区の南寄りにある復興公園内で行われた。この公園の一角で，人家に隣接した木蔭に，容量約10 *l* の水がめを置き，水をはり，適宜少量の麩を投入して水を汚染させ，オビトラップとした。この水がめを毎朝点検し，水表面に産み落されているすべての蚊卵塊を採集して実験室へ持ち帰り，卵塊別に汲み置きの水を入れた容量90 ml のプラスチック容器に移して 27°C の恒温室内で2～3日間放置し，その孵化状況を調べた。

アカイエカの産卵活動は1982年には最高及び最低平均気温がそれぞれ 24°C および 15°C を示した5月上旬に開始された。その後次第に活発となり，7月上旬の梅雨期前に最も多くの卵塊が採集された。梅雨期の後半から盛夏にかけてその活動はやや低下するが，8月下旬には再び活発となり，9月上旬に第2の山を示した。9月中旬に入り，最低平均気温が 20°C 以下になると産卵活動は急に衰えるが，11月中旬まで産卵がみられた。しかし，11月下旬になり，最高平均気温が 20°C 以下になってからは全く産卵がみられなかった。この消長の傾向は8月下旬から9月上旬にかけての山が前年よりかなり低かった点を除いて，概ね前年の調査結果と一致していた。

27 世界各地の発展途上国に在留する邦人の腸管寄生虫感染率と飲料水汚染についての経年的観察

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上田　正勝，森　　章夫，小田　　力
（長崎大・医・医動物）
奥脇　義行　（女子栄養大・微生物）
杉山　雅俊　（順天堂大・医・衛生）
池田　照明，及川陽三郎
（金沢医大・医動物）
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第23回熱帯医学会総会において，我々は世界各地に在留する邦人の腸管寄生虫感染率について報告し，それが，邦人の在留地域に供給されている飲料水から大腸菌群が検出される度合と有意な（$P<1.5\%$）正の相関（$r=0.957$）を示すことを証明した。今回は世界各地に在留する邦人の寄生虫感染率と飲料水汚染度について経年的変化を調べ，さらに，最近のデータにより再度両者の相関を求めたので報告する。

インドネシアにおいては，邦人の寄生虫感染率は1978年から1980年にかけて，8.0%から1.8%にまで低下したが，それ以降は増加に転じ，1983年には再び8.0%にまで達した。また，彼らが使用している飲料水からは70%から50%の高い割合で大腸菌群が検出されていた。フィリピン，タイも同じような傾向で，邦人が感染している寄生虫の種類では，回虫，鞭虫，ランブル鞭毛虫が多く，次いで異形吸虫，テニア属条虫，鉤虫などであった。一方，マレーシア，シンガポール，ホンコンなどに在留する邦人の寄生虫感染率は低く，飲料水もほとんど汚染を受けていなかった。パキスタンをはじめとする南西アジア，中近東，東アフリカおよび中南米の在留邦人の寄生虫感染も比較的低く，経年的変化もあまり認められなかった。しかし，飲料水の汚染度は，調査する年によって多少異なる結果が得られた。

次いで，1983年度における各地の在留邦人の寄生虫感染率と飲料水の汚染度との相関を求めた。在留邦人の寄生虫感染率と飲料水の大腸菌群検出の割合が，今回も有意な（$P<2.5\%$）正の相関を示した（$r=0.925$）。しかし，寄生虫感染率と飲料水の過マンガン酸カリウム消費量陽性率の間には全く相関が見出せなかった。

28 海外旅行者下痢症からの腸管原虫の検査成績について

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中野　宏秋，細溝　浩二，市来　重光，
橋本　　智，阿部　久夫，浅野　信夫
（大阪空港検疫所）
小野　忠相，中林　敏夫
（阪大・微研・原虫）

海外旅行者下痢症の実態把握を目的に，これまで，主として細菌学的検査を行い，海外旅行者下痢患者の約半数は細菌性下痢症であることを明らかにしてきた。残る半数は下痢原因を知るため，下痢の原因となる腸管原虫の検索を始めたので，今年1月から6月までの成績について報告する。

大阪空港から入国した海外旅行者下痢患者のうち，旅行期間が5日以上で，自然排便を採取できた318名を検査対象として，直接塗抹法，培養法（田辺・千葉培地），MGL 法の3者を併用して腸管原虫の検索を行った。

その結果，ランブル鞭毛虫を2月，3月，4月，6月の各月にそれぞれ1名，合計4名（1.3%）から検出した。ランブル鞭毛虫陽性者は，いずれも5週間以上にわたる長期間旅行者で，うち3名はインド・ネパールでの感染であった。2名は赤痢菌との混合感染であったため隔離病院に収容されたが，他の2名は軽度の下痢を3～7日間隔でくり返し，うち1名は帰国後1カ月以上続き，アテブリンの投与によって駆虫できた。6月にインド・ネパールを7週間にわたって旅行した者1名から大腸アメーバを検出した。

以上，今回の6カ月間の成績では，ランブル鞭毛虫と大腸アメーバの2種類，合計5名（1.6%）しか検出できなかった。しかしランブル鞭毛虫陽性者は，下痢症状を一定間隔でくり返し，長期間

にわたることから，検出率が低率であっても，今後十分な注意が必要である下痢症の1つと考える。

29 世界各地の発展途上国の飲料水についての化学的および細菌学的検査成績

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（長崎大・医・医動物）
奥脇　義行　（女子栄養大・微生物）
杉山　雅俊　（順天堂大・医・衛生）
池田　照明，及川陽三郎
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世界各地の，いわゆる発展途上国に在留する邦人の数は著しく増加している。それと同時に，各地でA型肝炎，腸チフスやアメーバ赤痢など重篤な経口感染症に罹患する邦人の数も増加している。我々は，その主な原因が飲料水の汚染にあると考え，世界各地の発展途上国の飲料水について，特に糞便系汚染との関連について調査した。

我々は，1982年より1983年にわたり，東南アジア，南西アジア，中近東，アフリカおよび中南米の国々を訪問し，主として在留邦人が利用している飲料水を採取し，細菌学的および化学的水質検査を行った。なお，一部の調査は1977年より1980年にかけて行われた。あらかじめガス滅菌してある検水用器を，検水で何度も洗い，飲料水を採取し，これを検体とした。ただちに，この検水をマッコンキー培地およびCLED培地が塗布されているウリカルトセット(第一化学)に十分浸し，それを37°C，24時間保存し，翌日発生した集落の数を算定し，1 m*l* 中の生菌数とした。化学的検査としては，過マンガン酸カリウム消費量，アンモニア性窒素，亜硝酸性窒素，硝酸性窒素，塩素イオン濃度，総硬度，pH，残留塩素および鉄，銅，鉛を水質基準に関する省令で定められている方法によって，それぞれ測定した。その他，色度，濁度，臭い，味，沈殿の有無についても調べた。

その結果，世界の発展途上国に供給されている飲料水の水質は極めて不良であり，一部の地域を除き，ほとんどの飲料水が大腸菌により汚染されていることがわかった。特に，東南アジアでは，インドネシア，タイ，フィリピン，南西アジアではパキスタン，中近東ではイラク，アフリカではケニア，モンザビーク，ナイジェリア，中米ではメキシコ，南米ではエクアドル，ペルーの飲料水が糞便系汚染にさらされていた。殺菌剤としての残留塩素が末端から見出された地域としては，シンガポールとマレーシアで，その他，パナマとエジプトの一部の地域にみられ，他の国では全く検出することはできなかった。

30 インドネシア各地の飲料水の細菌学的研究

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藤田紘一郎，月舘　説子，黒川　憲次
（長崎大・医・医動物）
杉山　雅俊　（順天堂大・医・衛生）
朝倉　健夫　（日本熱帯医学協会）

今回は，1982年の6月から7月にかけて，インドネシア各地で採取した飲料水75検体について行った一般細菌数と大腸菌群数の算定，腸内細菌およびその類似菌の検出，およびその検出菌の化学療法剤に対する感受性試験の結果についてを報告する。

現地で，ウリカルトセットを用いて行った一般細菌および大腸菌群数の結果では，一般細菌数は43検体（59%）で陽性（10^3/m*l* 以上）を示し，このうち，カリマンタン，スラウェジ，スマトラでの18検体は全てで陽性であり，最も検体の多かったジャワ島での57検体については水道水で28検体中11検体（39%），井戸水で29検体中14検体（48%）で陽性を示した。一方，大腸菌群数の成績では，37検体（49%）で陽性（10^3/m*l* 以上）がみられ，そのうちカリマンタン，スラウェジ，スマトラでの18検体は全て陽性であり，またジャワ島での57検体は，水道水28検体中6検体（21%），井戸水29検体中13検体（45%）で陽性を示した。

検水より分離された腸内細菌およびその類似菌は，*Enterobacter cloacae* と *Citrobacter freundii* が

最も多く，それぞれ16検体から分離された。次いで多かった菌種は，6検体より分離された *Klebsiella pneumoniae* と *Pseudomonas aeruginosa* であった。し尿汚染との関連からその指標として最も重要視される *Escherichia coli* は5検体から分離された。この中には，ジャカルタ市内の水道水の2検体から分離されたものが含まれている。また，今回の調査で特に注目されるのは，*Salmonella* C_1 群がジャカルタ市内の1件の井戸水より，さらに *Salmonella* E_1 群がジャカルタ市内，カリマンタン，スラウェシ，スマトラのそれぞれ1件ずつの井戸水からというように全島から分離されたことである。

分離した菌株の57菌株について，Kanamicin, Gentamicin, Tetracyclin, Chloramphenicol, Carbenicillin, Cephaloridine, Cephalothin, Colistin および Nalidixic acid に対する感受性試験をディスク法で行った結果，特に顕著な傾向や耐性菌の出現はみられなかったが，*Salmonella* sp. についてのみ，Cephaloridine と Cephalothin でジャカルタ市内で検出されたものは感受性陰性，一方他の3島でのものは感受性陽性というように差がみられたことは興味深い。

31 1982年における奄美大島のハブ咬症の現況について

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（日本蛇族学術研）

我々は1957年以来，奄美大島のハブ咬症患者の疫学及び治療，予防，予後に関する調査を行って来たが，今回は前年に引続いて1982年の調査について報告する。患者数161名（死亡1）で前年より8名増加した。その内，徳之島が105名（8増）で全咬症の65%を占め，奄美本島が56名で35%であった。

月別発生数では，6月が34で最も多く，5月が22，8月が20，4月が17，9月が16，3月が14，7月が12であった。即ち3月から10月までの8カ月間に146（91%）が受傷していた。年齢別，性別受傷数では最も多いのは50代の45（28%）で，40代の31（19.3%）60代の23（14.3%）がこれについだ。また，男性の受傷は120（74.5%）で女子の41（25.5%）に比べると2倍以上にのぼっている。

次に受傷場所では，田畑で農作業中に受傷したものが92（57.1%）で最も多く，屋敷内が29（18.0%）でこれに次いでいたが，両者が全咬症数の75%を占めていた。これを地区別にみると，田畑の受傷率は徳之島が本島より明らかに高い傾向を示している。受傷部位では上肢が86（52.8%），下肢72（44.2%）で受傷の大部分を占めていた。最も多いのは手指の56（34.4%）で，手の21（12.9%）を加えると50%に近く，下肢では下腿が36（22%）で最も多い。

受傷時刻では，午前6時-午後6時までの明るい時刻に121（75.6%），午後6時-午前6時までの暗い時刻に39（24.4%）が受傷しているが，田畑では93.5%が昼間に受傷しているのが特徴的である。これに反して，屋敷内及び道路では夜間の受傷が目立っている。

ハブ咬症の予後は受傷数161（死亡1）の内，軽症が134（83.2%），重症27（16.8%）でその内高度の腫脹，嘔吐，嘔気，血圧低下，下痢などの全身症状を呈した9例，受傷局所の壊死のみを起こした15例と全身症状を合併した3例計18例（11.2%）中，後遺症は4例（2.5%）にみられた。1名は死亡した。致命率は0.6%であった。壊死を伴った18例中9例は手指，4例は下腿等で後遺症の4例は手指運動障害であった。

32 沖縄県における1982年のハブ咬症

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近年，沖縄県においてはハブによる咬症が減少し始めているが，今回はサキシマハブ咬症とともに1982年の調査について報告する。

ハブによる咬症者は，戦後初めて200名以下を記録し，179名，1,000人当たりの受傷率は0.17となった。最近10年間（1972-81年）の平均受傷者は270.1名，受傷率は0.28であり咬症者が急減している。サキシマハブの咬症者は80名（八重山78名，沖縄島の2名を含む），八重山の受傷率は

1.86であった。最近10年間の平均は51.1名，受傷率は1.09である。なお，ハブ・サキシマハブともに死亡者はいなかった。

月別発生数では，ハブで10月が33名（18.8%）で最も多く，3月26名，11月21名，6月19名であった。サキシマハブでは，3月11名（13.8%），4月10名，6・11月9名であった。

ハブの受傷場所で最も多かったのは，田畑68名（38.6%）で，次いで不明36名，屋敷内21名，道路18名，山野14名，屋内13名となっており，サキシマハブでは田畑38名（47.5%），屋敷内17名，道路9名，屋内・山野・水辺各5名であった。

受傷部位は，ハブで上肢95名（53.4%），下肢76名（42.7%）となり，受傷の大部分を占めていた。最も多いのは手指58名（32.6%）で，次いで手の21名を加えると半分近くを占めている。下肢では足が32名で最も多い。サキシマハブの場合は上肢65名（81.3%），下肢13名（16.3%）で，ハブに比べ上肢の受傷の比率が高かった。

ハブ咬症の大幅な減少は，多くの市町村においてハブ対策の事業が始まっていることや住居環境の整備，特に木造家屋が鉄筋コンクリートの家屋，古い石垣がブロック塀になっていること，それに農地改良事業の進展，各種団体に対する衛生教育の普及などが，発生の減少につながっていると考えられる。

33 ハブトキソイドの野外接種（第10報）

福島　英雄，水上　惟文，鳥入　佳輝，
古賀　繁喜，東　勝観，川畑　英機，
山下　正策，香月　恭史，坂本　宗春
（鹿児島大・医・熱疫研）
村田　良介，松橋　直，近藤　了，
貞弘　省二　（予研・細菌第二部）
大井　清，近藤　久
（千葉県血清研）

昭和45年から奄美において，ハブトキソイドの接種によるハブ咬症の重症化予防に関する研究を行い，その成績は，第16回総会以来逐次発表してきているが，今回は，総括して述べる。

昭和45年から57年3月までに，成人9,618名に，ハブトキソイド（沈降ハブトキソイド Lot 12, 13, 14, 15, 18, 20, 36, 37, C-1, C-2, アルコール沈殿ハブトキソイド Lot 1）を，基礎免疫としては，トキソイド 0.5 m*l*（～0.1 m*l*）あて4週間隔で2～3回接種し，その後追加免疫としては，数カ月～1年～2年数カ月の間隔で，6回接種した。

その結果，有効と考えられる血中抗毒素価1単位1 m*l* 以上の者が，抗出血1価においては，基礎免疫終了4週後61.8%，追加免疫4週後88.9%に認められた。このうち 18 ImU/m*l* より高力価のトキソイド（Lot 12, 13, 15, 36）接種群においては，基礎免疫終了4週後76.2%，追加免疫4週後96.0%に認められた。抗出血2価は，総てのトキソイドにおいて，基礎免疫終了4週後，追加免疫4週後も共に，殆ど全例において1単位/m*l* 以上を示した。

ハブトキソイド接種時の副反応としては，接種部位の疼痛，腫脹などが主であるが，その他，硬結，掻痒，発赤などを訴える者もある。稀には蕁麻疹を訴える者（0.043%）もあったが，重篤なアレルギー反応，発熱などを呈する者は認められなかった。

昭和56年7月までのトキソイド接種後のハブ咬症者176例（181回）中，咬傷時，全身症状3例（1.7%），壊死20例（11.0%），後遺症11例（6.1%），死亡者1例（0.55%）である。ただし，この死亡例は不完全接種者であった。

これらの成績から，ハブトキソイド接種は有効な血中抗毒素を産生させ，かつ，副反応も軽微で，また，ハブ咬症の重症化を減少させ，臨床的にも有効と考えられる。

34 スリランカ，アヌラダプラ地区における毒蛇咬症の死因について

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（ペラデニヤ大・医）

スリランカでは毒蛇咬症による死亡は年間800

名前後が記録されているが，特にアヌラダプラ地区では人口10万に対して18人という死亡数が記録された。今回は文部省科学研究費海外学術調査の補助をうけて行われたアヌラダプラ地区における毒蛇咬症の死因に関する調査について報告した。

調査方法は同地区の政府統計局から1980年及び81年の毒蛇咬症による死亡患者のリストを入手し，それによって直接各部落を訪問し，患者の家族等に会い，患者の受傷から死亡に至るまでの経過をカードに記録した。また一方では患者が入院したとみられる病院を訪問して調査を行った。

調査された死亡例110の中で，毒蛇が鑑別された99例のうち44例はインドアマガサヘビ，28例はラッセルクサリヘビ，26例はインドコブラによるもので，他にヒプナレマムシによるものが1例であった。

死亡は年間を通してみられたが季節により発生のかたよりがみられた。年齢別にみると，最高は10代の28例（25.5%）で71例（85%）が0-29歳に集まっていた。また男子は女子の2倍以上多く死亡した。受傷時間ではアマガサヘビでは夜間，クサリヘビは昼間，コブラでは昼夜を問わず咬症が発生していた。受傷部位でもヘビによって特徴がみられた。

臨床的には79%の患者は受傷後，部落内の民間療法師の手で治療をうけ，血清注射なしで死亡していた。残りの21%は病院にたどりつき，中には血清の注射をうけた者もいたが，手おくれのため死亡した。血清はインドから輸入された4種混合の多価血清が用いられているが，その普及度は低い。

35 沖縄ハブ毒中のプロティナーゼ：精製と酵素的性質

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沖縄ハブ毒から出血因子を分離する過程で一種のプロティナーゼを分離・精製し，その酵素的性質を明らかにした。

2つの出血因子（HR1 と HR2）を相互分離するために，粗ハブ毒を Sephadex G-100 でゲル濾過して得た粗 HR2 画分を出発材料とした。これを Bio Rex-70 でクロマトグラフし，HR2a と 2b に先行して溶出される主要なプロティナーゼ画分を集め Sephadex G-75 でゲル濾過した後，Bio Rex-70 で再クロマトグラフした。精製標品は SDS-ポリアクリルアミドゲル電気泳動で均一で，比活性は粗 HR2 画分から10倍上昇していた.

本酵素は分子量約25,000の単一ポリペプチドで，至適 pH を9付近に持ち，EDTA やシスティンで阻害されたが NEM や DFP では阻害されなかった。また Zn^{2+} と Mn^{2+} で若干失活したが，Ca^{2+}，Co^{2+}，Mg^{2+} は阻害も活性化も起こさなかった。

数種のタンパク質基質に対する作用を奄美ハブ毒の主要なプロティナーゼの結果と比較し，Hide powder azure と Azoalbumin を特に好んで分解する事を明らかにした。

インシュリンA鎖やB鎖，各種の合成ペプチドを加水分解した時に生ずる新しいN末端をダンシル法で同定し，分離したペプチドのアミノ酸分析の結果とあわせ，基質特異性を調べた。その結果，インシュリンA鎖では -Ser_{12}-Leu_{13}-，B鎖では -Ala_{14}-Leu_{15}- が切断され，Substance P と Physalaemin では -Gly-Leu- が切断される事が分った。一方，レニンに対する合成基質中の -Leu-Leu- は切断されなかった。dipeptide や tripeptide は切断されなかった。

36 熱帯地在留邦人の健康管理

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海外在留邦人を対象として巡回健康診断を行う場合，限定された期間内における問診，診療，臨床検査，生活指導などを効率よく行うためには，予め仕事量を考慮しつつ手順，検査項目の選定などを行うことが必要である。そこで今回は臨床検査面について，特に熱帯地の現状から見て重要と考えられる2・3の項目を取り上げて検討した。

1. 血液検査：熱帯地において重要な急性ないし慢性感染性のスクリーニングに役立つ検査の中で最も基本的なものとして白血球数，白血球百分

率を取り上げ同時に赤血球数，ヘモグロビン，ヘマトクリットにつき検討を加えた。上記5項目のうち，赤血球，Hb, Ht は簡易検査キットとして発売されているが，白血球数算定用キットは発売されていないので希釈法を考案した。原理は通常のメランジュールを用いた方法と同様であるがEDTA 加凝固阻止血液をマイクロピペッターで0.02 m*l* 採取し，これをあらかじめ96穴のマイクロプレートに用意した9倍量のチュルク氏液と混和して血算盤を用いて算定した。この方法による結果はメランジュール法およびコールターカウンターによる測定値とほとんど同様の値を示した。マイクロプレート法ではピペッターに採取する血液量が極微量なのでチップの外側に付着した血液のふきとりが不充分である場合大きな誤差がでる。マイクロタイタープレートによる希釈法では百分率算定の薄層標本を同時に作成しながら35検体処理するのに1時間15分を要した。

2. 糞便検査：虫卵検査は直接塗抹法，飽和食塩水浮遊法を行い，潜血反応は便潜血スライドを用いて行った。当然のことながら浮遊法は直接塗抹法より発見率が高く，また浮遊法では35名分の検体処理に3時間，潜血反応は同数の検査に2時間を要した。

37 アフリカ在留邦人の健康・衛生に関するアンケート調査

笠置　綱清　（鳥取大・医・小児科）
原　　宏　（鳥取大・医・第二外科）
大儀　敏雄　（日本国際医療団）

昭和57年度アフリカ在留邦人の巡回健康相談を機会に，健康と現地での衛生状態についてのアンケート調査を行い，次のような結果を得た。赴任前の健康診断の受診率は82%で，必ずしも高いとはいえず，赴任後も定期的に受診していない人も多かった。巡回医師団による健康相談は高く評価されていたが，風土病的疾患はもっと現地の医療機関を利用すべきであった。特に，マラリアは恐るべき風土病であるが，副作用を恐れて予防薬の定期的内服をしていない人が多く，さらに啓蒙が必要であった。

現地の衛生状態に対しては生水の飲用，野菜など食物の生食はほとんどなく，充分警戒心があった。また，現地到着後に発病した下痢，不明熱，マラリアなど風土病的疾患は到着後6カ月以内に発症するものが過半数を占めており，現地到着後6カ月は現地の生活に馴化する準備期間といえる。

38 中近東・北アフリカ滞在日本人の健康調査

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中近東・北アフリカ諸国に滞在する日本人の健康状態，滞在中に罹患した疾患等につき調査を行い検討した。調査の対象となった者は，総数254名で，平均滞在日数は248日である。

滞在中に罹患した主要疾患としては，下痢性疾患が93例で最も多く，胃炎，胃・十二指腸潰瘍36例，腰痛症18例が上位を占め，以下急性肝炎7例，交通事故6例，高血圧6例という順位であった。高血圧，糖尿病，胃・十二指腸潰瘍，心疾患等については，出国前よりすでに異常所見またはその疾患を示唆する症状を呈したことがある例が多く，既往歴または検査の異常が有る者と無い者との間には，発症率に関して有意差が認められた。

帰国時に実施した諸検査の結果では，異常所見として，好酸球増多症5.3%，HBs 抗原陽性3.1%，寄生虫陽性3.8%，血沈亢進3.1%などが多く認められた。調査対象者中52名については，現地滞在中あるいは帰国時における抗A型肝炎ウイルス抗体を測定し，9名が陽性であるという成績が得られた（17.3%）。12名については出国時と比較して抗A型肝炎ウイルス抗体を検索することができ，2名は現地滞在中に陰性から陽性へ転化したものであることが判明した。

リビアでは，日本人102名につき糞便検査を実施したが，鞭虫2例，回虫1例が認められたのみであった。同時に現地人126名について実施した糞便検査では，大腸アメーバ20名，鞭虫12名，回虫12名，ランブル鞭毛虫4名が陽性であるという成績が得られた（感染率35.7%）。

本調査を実施した地域は，比較的衛生状態が良い地域であり，特に問題となるような風土病も存

在しない地域であったため，感染症の比率は低かったが，既往の疾患が悪化もしくは再発する例，身体的および精神的疲労を訴える者の多いのが目立った。また，A型肝炎ウイルスおよび各種寄生虫に感染する機会は決して少なくなく，注意を怠ることは極めて危険であると考えられた。

39 巡回医療からみた東南アジア・インド亜大陸における在外邦人の疾病

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我々は，外務省から派遣されて東南アジア・インド亜大陸を巡回し，在外邦人の健康相談を行ったので，その結果を報告する。

1. 巡回地・相談数：期間は58年1月25日より2月21日迄28日間，1カ所3～4日滞在した。相談数はハノイ17，ビエンチャン17，ラングーン89，ダッカ133，カルカッタ44，マドラス38，コロンボ98，計436，他に予防注射51，総計487（男243，女193）であった。年齢的には30歳台の家族同伴が最も多く，40歳台の男は単身赴任が多い。これは子供の教育事情を反映しているものと考えられる。

2. 現有疾患：1）日本にも存在する一般疾患と，2）熱帯特有の感染症に分けられる。

1）に関しては心疾患，肝障害，糖尿病，消化器潰瘍等日本から持ち越した慢性成人病と感冒等の急性疾患がある。小児では扁桃腺疾患と皮膚疾患が多かった。後者は現地の高温多湿の気候が影響しているように思われる。尚受診者の50%が何らかの疾患を有していたが，これは訴えのあるものが，特に集合してきたためではないかと考える。2）に関しては，A型肝炎，細菌性赤痢，アメーバ赤痢，マラリア，フィラリアが数例認められた。

3. 既往症としての熱帯感染症：熱帯感染症は急性一過性であるため，検診時数例しか遭遇しなかったが既往症を尋ねるとかなりの人が罹患していた。1）蚊の媒介する感染症としては，マラリアは大都市には少ないが，田園に接する小都市のビエンチャンで受診者の18%が，またデング熱はビエンチャンで受診者の12%が，コロンボで23%が罹患しており，2回罹患した者も6人いた。地理と気候が関係あるように思われる。特に成人女子に多いのは服装，生活と関連するものと考えられる。2）消化器感染症としては，A型肝炎，チフス，パラチフス，細菌性赤痢，アメーバ赤痢，ランブリア症の既往者が受診者の6%に，激症または長期下痢の経験者は14%であった。他に過去に狂犬に咬まれた者が2名があった。

4. 結論：在外邦人の疾患としては日本人でもみられる疾患と，熱帯性感染症の2つが存在する。そして後者の場合，蚊の媒介する感染症と消化器感染症が多く，予防が重要であると考える。

40 タンザニア国 Moshi 市 Kilimanjaro Christian Medical Centre における医療状況

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Kilimanjaro Christian Medical Centre (KCMC) では通常の診療の他，rural 診療所医師の卒後研修，医療技術者養成，特定地域の栄養改善，防疫活動等を行っている。規模は420床，常勤医師11名，看護師45名，他の医療従事者4名で，医療活動範囲は 120 km^2，医療対象人口は8万人前後，診療科目は内科，外科整形外科，小児科，産科，眼科，放射線科，歯科で，1日の外来診療件数は400名前後，年間入院総数は12,512名，うち退院者総数 11,735名，死亡者は755名で死亡率は6.03%である（1981年度の統計）。

伝染性疾患の罹患状況はマラリアが第1位を占め，次いで下痢性疾患，麻疹，回虫症，肺結核，アメーバ赤痢，腸チフス，鉤虫症，ジストマ，ポリオ，癩と続く。

予防接種は BCG, DPT, Polio, 麻疹ワクチンなど施行されているが対象人員の0.2%前後にすぎない。

入院患者の上位10疾患は循環器疾患が第1位で，

糖尿病，高血圧，マラリア，肺結核，アメーバ赤痢，消化性潰瘍，ジストマ，気管支喘息，貧血と続き，寄生虫疾患及び結核が上位を占めるのが特徴的である。小児科領域で特徴的なことは麻疹の罹患率が最も高く，死亡率も第1位で，クワシオコール，腸管寄生虫，マラリアと続く。産科では流産が多い。

これらの事実に基づき，KCMC では疾病予防の為の地域医療活動を行っており，動物性蛋白生産産業育成による栄養改善，母子保健サービス，医療教育等が sketch-outline とされ，それぞれの project staff により少額予算ながら活動が続けられている。

41 過去5年間におけるベトナム難民の罹病状況

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我々は来日するベトナム難民の健康状態に関して大きな関心を寄せ，これまでもその検診結果を当学会に発表して来た。1982年2月に，長崎県大村市に大村難民一時レセプションセンターが開設され，今まで各施設毎に放任されていた難民に対する入国時の健康診断が確実に実施されるようになった。したがって，今後は，各施設における難民の罹病状況にも変化をもたらすことが期待される。そこで今回，それ以前のベトナム難民の疾病を総括する目的で受診状況を調査してみた。対象は1977年5月から1982年4月までの5年間に滋賀県日野町の施設に入所したベトナム難民322名（大人192，小児130；男210，女112）である。平均年齢18.0歳（男18.9歳，女16.5歳），平均施設内生活者数68.13名であった。外来受診延総数1,465件で，入院患者25件，21名であった。またこの間に9名の出生があった。科別では，内科，小児科の受診者が多く42.7%を占め，加えて，歯科（26.7%）および泌尿器科（11.0%）受診者もかなりの数にのぼる。呼吸器系，消化器系の疾患が圧倒的に多く，循環器系疾患，貧血は見られなかった。梅毒が11例認められるが淋疾は皆無であった。また尿路結石症の多いことが1つの特徴であった。入院患者では，マラリア4例（全て三日熱マラリア），アメーバ赤痢および肝膿瘍2例，結核症3例，尿路結石症4例などであった。結核症のうち1例は大量喀血により死亡していた。医療費として年平均3,558,357円が出費されていた。受療率が人口10万対1,105で，1980年日本人15-19歳のそれ（2,740）の0.40倍であり，一人当たり医療費は50.8千円で，1980年日本人15-44歳のそれ（55.8千円）と同程度であるので，疾患当たりの医療費がむしろ高値であると言えよう。

42 温度順化の形成過程における局所脳血流の変化

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暑熱・寒冷順化ウサギにみられる脳血流の変化から温度適応や体温調節機序を検索している。(1)温度 25°C，湿度60%の環境条件で飼育したウサギ（非順化群）の視束前野（PO/AH），中脳網様体（RF），脊髄の各局所血流量は 36～37 ml/100 g•min と有意差は無いが，LPS 発熱物質投与による高体温時の PO/AH，RF の各血流は共に20%の有意な増加を示したが，この有意変化は暑熱（30°C，60%）・寒冷（10°C，60%）条件にて1カ月間飼育した温度順化ウサギの LPS-発熱期には殆ど消失した。(2)暑熱順化群の PO/AH，RF の血流量は 25°C•60% 条件ではそれぞれ 38.8，39.9 ml/100 g•min で非順化群（既述）の値との間に有意差は無かった。寒冷順化群では 31.7，28.9 ml/100 g•min で暑熱順化群との間に有意差があった。(3)以上3群のウサギに皮膚加温（気温 40°C）・冷却（15°C）を行って PO/AH の血流量を測定すると非順化群は加温で18.8%，冷却で−8.0%；暑熱順化群では加温で1.6%，冷却で−5.4%；寒冷順化群ではそれぞれ5.5%，−2.8%の血流変化を認め，これらの変化は非順化群の加温時の18.8%（$P<0.005$）を除いてすべて有意の変化ではなかった。(4)この事は LSP 発熱や皮膚加温による高体

温時には非順化ウサギの PO/AH, RF の局所血流量は共に20%の増加を認めるが暑熱や寒冷に順化したウサギでは，この量的変化が消失または減少する事を示し，所謂，Habituation の現象を示唆している。以上の結果の生理学的解釈や血流量変化の機序については温度順化形成過程における経時的観察，特に各種体温調節反応指標や脳内組織の器質的変化などの同時記録・照合が必要と考えられる。

43 蚊培養細胞 (*Toxorhynchites amboinensis* 171株) における Dengue virus の増殖並びに形態学的な観察

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非吸血性の *Toxorhynchites* 属蚊を用いた Arbovirus の分離，同定法は，従来の方法に比較し，分離率の優れている事が報告されている。我々も蚊培養株化細胞を用いて，JEV および Dengue virus の増殖に関する研究を行い，報告してきた。今回 *Toxorhynchites amboinensis* の株化細胞 (TRA 171 細胞) を用い，Dengue virus type 2 (D-2) の増殖の様相を検討した。virus 株として，教室保存マウス脳継代馴化株 (Tr 1751株)，および，ビルマの DMR にて DHF 患者 (BR 006株)，並びに DSS 患者 (BR 116株) より分離，D-2 と同定された継代歴の極めて少ない virus 株を用いて比較，検討した。

TRA 171細胞における virus の増殖は，何れの virus 株とも感染48時間後より細胞凝集を伴う CPE を認め，螢光抗体法による観察でも核膜周辺部，および細胞質内に特異螢光陽性部位を認めた。Vero 細胞を用いた plaque 法による定量は，感染5日後に BR 006株では $10^{6\sim7}$ PFU/m*l*, BR 116株では $10^{5\sim6}$ PFU/m*l* に達し，10日目までこの値を維持した。一方，Tr 1751株では6日後に 10^{8} PFU/m*l* に達した。plaque 形成は1.2% methyl-cellulose を含む培養液で7日間，37°C で培養した場合，Tr 1751株では直径 1.2～1.5 mm とほぼ均一な plaque を形成したが，BR 006株では直径 1.4～1.8 mm; 19%, 0.8～1.2 mm; 50%, 0.4～0.6 mm; 31% の割合，BR 116株では直径 1.4～1.8 mm; 10%, 0.8～1.2 mm; 29%, 0.4～0.6 mm; 61%の割合で形成され，大小不均一であった。また，各 virus 株の温度感受性を検討するため温度 (39～35°C) 変化による plaque 形成の差を検討した。Tr 1751株ではほとんど影響は認められなかったが，BR 006株，BR 116株では低温で plaque 数は増加し，35°C の場合 37°C に比較し，10～100倍高く認めた。D-2 感染 TRA 171細胞の電顕観察では他の蚊培養細胞と同様な所見を観察した。しかし，非感染細胞の細胞質に 10 nm，および 15～20 nm の粒子の配列した構造，核内には 100 nm の電子密度の高い粒子も観察した。

以上の如く TRA 171細胞は Arbovirus の分離，同定には優れた培養細胞と考えられるが，非感染細胞の電顕観察で virus 様粒子を認めた事は今後，virus 増殖の宿主として疑問を投ずるものと考える。

44 *Trypanosoma cruzi* と Sprague-Dawley ラット腹腔マクロファージとの相互作用の超微形態学的研究

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Trypanosoma cruzi (Tulahuen strain) (以下 *T. cruzi*) は Sprague-Dawley (以下 SD) 系ラットに感染しないが，同系のヌードラットは強い感受性を示す。従って SD 系正常ラットとヌードラットを対比して検討する事により正常ラットの示す *T. cruzi* に対する強い抵抗性のメカニズムの解明が可能になるものと思われた。従来の検討においては SD 正常ラットでは *T. cruzi* 腹腔内接種に対して液性抗体の出現，白血球遊走阻止等が観察された。そこで免疫担当細胞等のヌードラットへの移入実験も行ったがいずれも *T. cruzi* 感染防御は発現しなかった。今回は *T. cruzi* 感染当初，生

体防御反応の上で重要な役割を演じうる腹腔マクロファージに注目し，*T. cruzi* とラット腹腔マクロファージとの相互作用について超微形態学的に検討した。その結果 SD 正常ラットとヌードラットの腹腔マクロファージの間では Parasitophorous vacuole 内の虫体処理能力に差がある事が示唆された。また正常ラットマクロファージの虫体処理能力は carrageenan 投与により抑制され，ヌードラットマクロファージ内に観察される虫体増殖像と同様の所見が得られた。

45 マウス腹腔マクロファージ培養におけるデングウイルスの増殖に及ぼす回虫抗原の影響

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我々はマウス腹腔マクロファージ (Mø) とデングウイルス (DV) との関連を追求し，Mø の培養を細菌菌体成分 (LPS, 細胞壁成分等) で処理すると，培養細胞からの DV 産生が増大することを知った (Microbiol. Immunol., 26, 665, 1982; ICMR Annals 2, 65, 1982; Infect. Immun., 41, 462, 1983)。それに引き続いて寄生虫 (*Ascaris suum* および *Parascaris equorum*) 抗原の効果を検討した。

Mø 培養術式は上記論文に既報の通りで，抗原は *A. suum* では虫体体腔液，*P. equorum* では細切虫体の 0.1% NaCl 液抽出物を粗抗原とし，Sephadex G-100 濾過により 4 種の分画 (I, II, III, IV) を得た。抗原の蛋白，糖の定量，limulus amebocyte lysate test などは定法の通り行い，また Mø の latex あるいはブドウ球菌食作用を既報の通り実施した。

得られた成績の要点は次の通りである。

(1) Mø 培養に DV (2 型，Trinidad 株) はほとんど増殖しないが，これを上記回虫抗原で処理すると DV 産生が増大した。

(2) この効果は抗原の添加量と添加時間に応じて増大し，carrageenan の添加により抑制された。しかしマウス脾細胞との co-culture により特に増強されなかった。Limulus test の成績により，細菌 LPS の関与の可能性は除外された。

(3) 抗原処理により DV 感染 Mø の数が増加したが，一方，Mø 自身の latex 粒子またはブドウ球菌食作用の増強も認められた。

(4) Sephadex 濾過による分画も同様の効果を示したが，見掛け上 I, III の効果が II, IV にくらべて強い傾向が示された。

46 実験的トキソプラズマ感染マウスの胸腺萎縮について

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慢性 *Toxoplasma gondii* (Tp) 感染マウスの胸腺萎縮について報告する。実験には Nya: NYLAR マウスを使用し，Tp は強毒 (RH) および弱毒 (CS) 株を用いた。弱毒 CS 株を感染させたマウスの胸腺の変化を経日的に観察した。胸腺湿重量は感染 1 週から 5 週にかけて減少し，5 週から 7 週にかけて一時的な回復がみられ，その後再び減少した。この変化にほぼ一致して胸腺の蛋白および DNA 含量の低下が認められた。病理組織学的には，皮質小リンパ球の消失および皮質・髄質の境界不鮮明化が観察された。胸腺の *in vivo* DNA 合成能は感染の 1 週から 4 週において有意に低下していた。また感染の 7 日から14日におけるマウスの胸腺細胞は *in vitro* DNA および蛋白合成能の有意な低下を示した。また，感染マウスの胸腺内チミジンキナーゼ活性の顕著な低下も観察された。一方，Tp 感染マウスの血漿中コルチコステロン含量は対照に比較して低下する傾向を示した。そこで，次に非感染系モデルを用いて，この Tp 症における胸腺萎縮の病原機構の解析を企だてた。Tp が感染しているマウスの血清・

腹水，あるいは培養 BHK 細胞に Tp を感染させて得たメディウム上清を濾過滅菌し，正常マウスに投与した。この結果，投与後5日から7日目には胸腺湿重量の低下，蛋白・DNA 含量の低下，チミジンキナーゼの活性減少，胸腺細胞の *in vitro* DNA および蛋白合成能の低下等が観察された。しかしながら，腹水・血清あるいはメディウム上清いずれにおいても，正常胸腺細胞への直接的な影響は見出せなかった。以上の成績は，Tp 感染マウスの胸腺萎縮が Tp 由来の何らかの物質に起因していることを推測させる。この点について今後さらに検討を加える予定である。

47 トキソプラズマ抗原接種によるマウス・バベシア感染に対する感染死防御効果とその機構について

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鈴木　直義　（帯広大・獣医・生理）

バベシア感染に対する宿主の発症軽減，あるいは予防効果賦与物質の検索を目的として以下の実験を行った。

まず，トキソプラズマ可溶性抗原（TLA）と共に，マウス・バベシア・リンホカイン(B-LKs)および，マウス・トキソプラズマ・リンホカイン(Tp-LKs)を用いて，マウス・バベシア原虫（*Babesia rodhaini*）感染に対する，免疫アジュバント効果の有無について基礎的検討を行った。

TLA と FIA の乳剤（TLA 乳剤）を投与する群，TLA 乳剤と B-LKs あるいは Tp-LKs を併用投与する群とし，14日おきに2回腹腔内投与した。初回処理から28日目に，*B. rodhaini* 感染赤血球 10^2 個を腹腔内接種した。

無処置群では，感染後12日目までに全例死亡した。TLA 乳剤投与群では14例中5例，B-LKs 併用群で15例中8例，Tp-LKs 併用群で15例中11例が20日目以降も生残し，これらの処置が，マウス・バベシア感染に対し宿主抵抗性を賦与することが示唆された。

以上のことから，我々は，このような感染死防御の機構について1，2の検討を行った。

TLA 乳剤によって感作されたマウスは，白血球の軽度な増加とマクロファージの O_2^- 産生の増加が認められたが，脾臓細胞培養上清と血清の MIF 活性は変化しなかった。Tp-LKs との併用投与によって感作されたマウスは，MIF 活性の上昇と，マクロファージ貪食能の亢進が認められた。

以上のことから，TLA-FIA 乳剤の投与，あるいは，リンホカインとの併用投与により，マウスはバベシア感染に対する感染死防御能が亢進し，その機構の一部に，マクロファージの活性化が関与するものと推察された。

48 トキソプラズマ性リンパ節炎の血清学的診断について

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後天性トキソプラズマ(Tp)症の代表的病型である Tp 性リンパ節炎に対する，血清反応による診断基準を求めることを目的とし，リンパ節の病理組織学的所見から本症と診断された10例，対照として悪性リンパ腫13例，上記以外の一般外来患者368例につき，各種血清反応を施行，結果を検討した。

色素試験 (DT): Tp 性リンパ節炎全例が抗体価1,024倍以上，80%が4,096倍以上を示したのに対し，悪性リンパ腫では全例が64倍以下，一般外来患者では殆どが256倍以下であり，最高1,024倍を示した者は僅かに1.9%に過ぎなかった。間接ラテックス凝集反応 (ILA): Tp 性リンパ節炎全例が512倍以上を示し，57%が4,096倍以上であったのに対し，悪性リンパ腫では全例256倍以下，一般外来患者では4,096倍以上はなく，512倍以上は12.8%に過ぎなかった。間接赤血球凝集反応 (IHA): Tp 性リンパ節炎全例が1,024倍以上を示し，89%が4,096倍以上であったのに対し，一般外来患者においても1,024倍以上28%，4,096倍以上13%と高い抗体価を示す例がみられた。間接螢光抗体法 (IFA)-IgM: Tp 性リンパ節炎全例が16倍以上を示し，63%が32倍以上であったのに対し，悪性リンパ腫は全例8倍以下，一般外来患者は16倍以下20.8%，32倍以上4.3%に過ぎなかった。

以上から次の結論が得られた。リンパ節腫張患

者で DT, IHA 1,024倍以上, ILA 512倍以上, IFA-IgM 16倍以上の場合, Tp 性リンパ節炎の可能性がある。さらに DT, ILA 4,096倍以上, IFA-IgM 32倍以上の場合, 本症の可能性は極めて高い。

49 酵素抗体法によるトキソプラズマ特異 IgM 抗体検出に関する研究

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妊婦が妊娠中にトキソプラズマ (Tp) 初感染をうけると, 流・死産, 奇形児出産の可能性があると言われ, 妊婦の Tp 感染症診断においては, 感染時期を知る事が重要である。現今, 急性感染か否かを判定する際には, Tp 特異 IgM 抗体価の測定が不可欠である。そこで我々は, Tp 特異 IgM 抗体検出法としての, 間接赤血球凝集反応 (IHA 法)・ラテックス凝集反応 (LA 法)・酵素抗体法 (ELISA) を比較検討した。

検査対象は, 長崎大学熱研内科の Tp 陽性患者血清195検体と長崎市医師会より検査依頼をうけた妊婦血清3,176検体, 合計3,371検体である。まず, 全検体を IHA 法にて測定し, 主に高い抗体価を示した 189検体に対して プロテインA処理を行い以下の実験に用いた。

成績としては, IHA 法では12検体, LA 法では16検体で IgM 抗体の存在が疑われたが, さらにショ糖密度勾配遠心法による確認試験を行った結果, IHA 法では全く検出できず, LA 法では 2 検体で検出した。ここで, 感度・特異性が高いと言われる ELISA を試みた。本法には, IgG 抗体価測定用キットを IgM 抗体価測定用に転用する方法を用いた。高い ELISA 値を示した検体についてショ糖密度勾配遠心法を行った結果, IgM 分画にのみ抗体が存在する事が証明された。さらに, 特異性確認のために Tp 虫体による吸収試験も実施したが, 明らかに Tp 由来の IgM 抗体である事が示された。

以上より, ELISA は, Tp 特異 IgM 抗体検体法として IHA 法や LA 法に比しはるかに感度・特異性が秀れている事が明確にされた。現在我々は, IgM-ELISA を用い, Tp 抗体陽性妊婦やその新生児の臨床経過を追っている。

50 IgM 酵素抗体法 (ELISA) によるトキソプラズマ (Tp) 急性感染妊婦の検索

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妊婦が妊娠初期に Tp 症に感染した場合, 先天性 Tp 障害児出産の可能性が指摘されている。現在我々は Tp-急性感染の血清診断に, ELISA 法による Tp-IgM 抗体の検体を行っているが, 今回 Tp-IgM 抗体保有妊婦における妊娠経過及び, 新生児への本症の影響を調べた。また本 IgM ELISA 法によって検出された Tp-IgM 抗体の意義に関しても詳細に検討を行った。

昭和51年12月より昭和58年 9 月までに検査した血清3,728検体の中で, 急性 Tp 感染症が疑われた 283検体 をプロテインA処理 した後, Tp-IgM ELISA 法にて Tp-IgM 抗体価を測定した。10倍以上の高値を示したものは35症例・66検体であった。妊婦は31症例であり, この内経過を知り得た23症例では, 人工中絶, 自然流産が各 1 症例ずつ見られたが, 残りの21症例では母児共に異常は見られなかった。高い Tp-IgM 抗体価を示した 8 症例で, さらに精査を行った。その結果, 新生児には先天性の Tp 感染は 1 例も発見されなかった。その他, この検査の中で母親の血清中に, 感染より数年を経た今日においても Tp-IgM 抗体が存在していることが判明した。詳細に検討した結果, 急性感染によって上昇した Tp-IgM 抗体は, その後次第に減少するが完全には消失せず, ほぼプラトーの状態にて患者血中に長期間検出されることが推測された。また慢性 Tp 症の急性増悪においては, IHA 及び Tp-IgG ELISA 値は著明に上昇するが, Tp-IgM ELISA 値は上昇せず, 従って, Tp-IgM 抗体の上昇の有無によって初感染か, 慢性 Tp 症の急性増悪かの鑑別が可能であると考えられた。今後の課題として, 本 IgM ELISA 法を用いる場合, 急性感染と見なしうる, また先天

性 Tp 障害児出産の可能性を示す，Tp-IgM ELISA 値を明確にする必要がある。

51 トキソプラズマ抗体保有状況と感染経路に関する1疫学調査

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トキソプラズマ（Tp）は先天性奇形や免疫力低下時の日和見感染を引き起こす原虫であり，ヒトへの主要な感染源はネコ糞便中に排出される卵嚢子や食肉中の嚢子と考えられている。今回，兵庫県三木市住民を対象とした抗体保有調査を実施しTp の感染経路について考察した。

調査に供した血清は1982年7月から9月にかけて健康診断で集められた総数891検体で，Tp 抗体を酵素抗体法（ELISA）により定量した。

陽性率は年齢とともに上昇し，50歳代以上の男女には有意差が認められた（男：42.5%，女：22.8%，$P<0.001$）。抗体量の頻度分布は典型的な2峰性を呈し，陽性率の違いにかかわらず陽性群の抗体量は ELISA 値の 0.5–0.7 をピークとし男女間・年齢間で有意差は認められなかった。また市街部と農村部とでは抗体保有率，保有量ともに有意差はなかった。アンケート調査の結果，ネコ飼育経験の有無は陽性率と有意に関係しなかったが，一方，50歳代の女性と60歳以上の男女において生肉摂食経験者の陽性率は非経験者に比べて有意に高かった。

一度感染を受けると宿主は Tp を容易に排除できないために年齢とともに陽性率は上昇するが，抗体保有量に差のないことから宿主の免疫において一種の平衡状態が存在するものと思われる。陽性率の男女差については生肉摂食経験率（男，41.6%，女，17.2%）の差が一因であろう。一般にネコから排出される卵嚢子は感染力が強いが，現在の三木市の環境下ではヒトの感染機会はネコの飼育とは関係しないことが明らかにされた。

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General presentation

1 APPLICATION OF FORMALINIZED GOOSE RED BLOOD CELLS TO ARBOVIRUS HEMAGGLUTINATION (HA) AND HEMAGGLUTINATION-INHIBITION (HI) TESTS

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Arthropod born (arbo) virus infection is a serious problem in Southeast Asian countries. In serodiagnosis and seroepidemiological studies on arbovirus infection, the HI-test, developed by Clarke and Casals (1958), has been widely used as a standard test. Erythrocytes collected from chicks or geese have been routinely used. A major difficulty associated with freshly prepared erythrocytes, however, is the instability of the red blood cells, that is, the use of fresh erythrocytes involves frequent bleeding of the fowl, subsequent treatment of erythrocytes, and standardization of HA against assay (HI-test).

In order to simplify the arbovirus HI-test which is widely used in Southeast Asian countries, an attempt was carried out to stabilize erythrocytes of goose by formalinization, so that they can be stored for a long time and this trial was successful. Comparison of fresh and formalinized goose erythrocytes (FGRBC) in HI assays for JE, Dengne type 1, 2, 3 and 4 and Chikungnya virus antigens showed that antibody titers of human and pig sera were the same, or within one dilution of each other. Namely, FGRBC was found to be useful for the routine serodiagnosis or epidemiological HI-test to arbovirus infection.

2 SERO-EPIDEMIOLOGICAL INVESTIGATION ON JAPANESE ENCEPHALITIS VIRUS TRANSMISSION AMONG PIGS IN EASTERN THAILAND (CHANTHABURI PROVINCE)

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Sera obtained from monthly bleedings of pigs in Chanthaburi municipal slaughter

house were determined for HI titer against JE Nakayama antigen during the period from July 1982 through to June 1983.

Seasonal periodicity in the fluctuation of HI antibody positive rate was observed. The positive rate was nearly 100% in rainy season (June to September, 1982) and in the following several months (October to December, 1982). HI negative rate gradually increased from January 1983 and became highest in May 1983 (67%). This high negative rate abruptly dropped to 0% (=100% positive) in June 1983. The marked sero-conversion seems to be closely related to the starting of rainy season which resulted increased vector population.

The results of observations suggest that there is a seasonal peak in transmission of JE virus in pigs in Chanthaburi similar to the observations in temperate zones such as Japan.

3 SEROEPIDEMIOLOGICAL SURVEY OF POLIO IN THAILAND, 1977–1982

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Sera from 1,318 persons aged 0–72 years old were collected at Chiang Mai Province and Bangkok in Thailand by the Society for Medical Research in Southeast Asia, Nippon Medical School in 1977–1982. These sera were examined by the micro cell culture method for determination of the Polio neutralizing (NT) antibody titer using Vero and HeLa cells.

Polio antibodies were found in 0–40 per cent of children at the age of one year when they had lost maternal antibody but 70–100 per cent of the antibody positive rate was observed in each age group over six years.

Distribution of the antibody by age were somewhat different in different geometrical areas. Children younger than nine years of the Karen tribe and of employees of the Raming Tea plantation living in high land showed lower antibody positive rates against type 1 and type 2 than the adult of the each group. Antibody positive rate of children in Chiang Mai against type 1 and type 3 were lower than those of adults in the same area. In Bangkok a low antibody positive rate against type 1 and type 3 were observed in children younger than nine years.

Difference of the antibody positive rates was not observed between group of children living in rural areas and those in urban areas, if they had been immunized with the oral polio vaccin (OPV).

After immunization of the trivalent OPV, increase of the NT antibody against the type 2 and type 3 were more different than the type 1.

4 ENZYME-LINKED IMMUNOSORBENT ASSAY ON SERA FROM DENGUE HEMORRHAGIC FEVER PATIENTS IN THAILAND

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Dengue hemorrhagic fever (DHF) is a viral infection with greatest public health concern in Southeast Asian countries attacking many children with severe manifestations of shock syndrome. Recent advance in the diagnostic virology has introduced enzyme-linked immunosorbent assay (ELISA) to the serodiagnosis and seroepidemiological study on various viral diseases. We attempted to apply the ELISA to sera from DHF patients in Thailand.

Sera were obtained from Chiang Mai and Chanthaburi in Thailand. As controls, encephalitis sera from Chiang Mai and those from Japanese encephalitis (JE) patients in Japan, as well as from healthy adults in Chiang Mai and Hokkaido were examined. ELISA antigens of dengue viruses were concentrated from culture fluids of *Aedes albopictus*, clone C6/36, cells infected with one of the four types of dengue viruses; type 1, Hawaiian, type 2, New Guinea B, type 3, H–87, and type 4, H–241 strains, by using polyethylene glycol precipitation and ultracentrifugation. Antigen of JE virus was formalin-inactivated purified JE vaccine concentrate (Research Foundation for Microbial Diaseses of Osaka University), and indirect micromethod was used in the ELISA. Hemagglutination-inhibition (HI) titers of sera were assayed by Clarke and Casals' method with modification to microtiter system.

Correlations were obtained between the logarithm of the HI (X) and that of the IgG-ELISA (Y) for sera from DHF patients, with correlation coeficients between 0.82 to 0.88 according to the assay antigens. Forty seven out of 66 pairs of DHF patients' sera showed 4-fold or more titer rise by any of the four types of dengue or JE antigens, and 34 of the latter also showed 4-fold or more titer rise by the IgG-ELISA, and the remaining 13 specimens possessed the IgG-ELISA titer over 16,000. On the other hand, IgG-ELISA could detect 4-fold or more titer rise in 39 pairs of the specimens, five of which did not show positive results by the HI test. IgM-ELISA was performed on sera from DHF, as well as from encephalitis patients, and the results showed that the patients with primary type of HI antibody response possessed higher IgM-ELISA titers than those with secondary responses, and also that the higher IgM-ELISA titers were obtained by the homologous than the heterologous assay antigens.

These results and the frequency distribution of ELISA titers indicated that the ELISA can be applied to the serodiagnosis or seroepidemiological survey on DHF and also to the differential diagnosis on JE from dengue infections.

5 STUDIES ON THE POSITIVE RATE OF HBs-ANTIGEN OF DONORS IN THE REPUBLIC OF MALAWI

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In blood transfusion, it is easy to infect in case of the donor having HBs-antigen. However, there is no laboratory system to check the HBs-antigen of donors in the Republic of Malawi.

This report describes the positive rate of HBs-antigen and antibody of the donors in Kamuzu Central Hospital. In case of taking a blood transfusion, HBs-antigen becomes an issue. It is important for the patients, Malawian medical staff and other people. According to the JOCV, about 100 JOCV members stay in the Republic of Malawi up to date.

The positive rate of HBs-antigen is 5.3 per cent, and the positive rate of antibody is 24.6 per cent on the donors. This report suggests that there is a great possibility of HBV spreading.

6 THE RISK OF HEPATITIS INFECTION IN DEVELOPING COUNTRIES –FOREIGN SOJOURNERS *VERSUS* LOCAL RESIDENTS–

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The yearly incidence of hepatitis among the Japan Overseas Cooperation Volunteers (JOCV) was 3.25 (range 2.0 to 4.9) per 100 persons per year during 1978–1982. The incidence was high in Southeast Asian (Philippines, Nepal and Bangladesh), peri-Mediterranean (Syria, Tunisia and Morocco) and African (Ghana and Zambia) countries. The incidence of hepatitis in two years of assignment reached five to 32 per cent of volunteers during 1978–1980 in those countries. They contracted hepatitis throughout two years of sojourn, although a small accumulation of the patients was seen between seven and nine months after arrival in the assigned country. Hepatitis A (HA) predominated, but hepatitis non-A non-B (HNANB) and hepatitis B (HB) were also diagnosed.

The yearly incidence of hepatitis in the JOCV was compared with that of the

Burmese people of the same age group. It was found that the JOCV ran more than 30 times higher risk of hepatitis infection than the Burmese youths.

7 SPECIFICITY OF THE TYPING PHAGES FOR *VIBRIO CHOLERAE* BASED ON THE RECEPTORS OF PROPAGATING STRAINS AND APPLICATION OF PHAGE TYPING TO *V. CHOLERAE* BIOVAR *ELTOR* ISOLATED IN KENYA

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Using the bacteriophages for typing *V. cholerae* and their propagating strains, Phages 1–5 and Strain 757 for *eltor* and Phages I–IV and Strains 154 or H–218 for classical, the sensitivities to the phages of phage-resistant mutants isolated from propagating strains were tested to clarify specificities of the phages. From Strain 757, several strains of phage-resistant mutants for each phage were isolated by direct method or from N-methyl-N′-nitro-N-nitrosoguanidine treated parent strain. Summarizing the sensitivities of mutants to each phage, it can be said that Phages 1 and 5 are identical and Phages 2, 3 and 4 are independent each other. From Strain 154 treated by NTG, only 154/I and 154/II were isolated. The half of 154/I were only resistant to Phages I, and the others were resistant to all phages. All of 154/II showed sensitivity to Phage I. In the case of Strain H–218, resistant mutants to phages were also isolated by NTG treatment. Nearly half of H–218/I remained sensitivities to Phages II–IV, and the remainings and all other mutants were resistant to Phages I–IV. These results indicate that there is a close relation among Phages II–IV.

A total of 434 strains of *V. cholerae* biovar *eltor* isolated in Kenya were typed by Phages 1–5. Phage types of 294 strains (67.7%) were decided by the same results after the second typing and 83.6 per cent of the remainings were classified after an additional test. After the forth typing, the remaining 23 strains were classified by two of the same patterns in four results. From these results, it can be said that the reproducibility of the phage typing for *eltor* was good enough to apply. The resulted phage types were as follows; Type 4: 191 (44.0%), Type 3: 118 (27.2%), Type 1: 49 (11.3%), Type 5: 48 (11.1%), untypable: 23 (5.3%), Type 6: 4 (0.9%), Type 2: 1 (0.2%). Untypable strains showed following patterns; −+−+: 2, −−+−+: 1, −−+−+: 10, +++−−: 2, ++−−−: 3, −+−+−: 1, +−−−−−: 1, −−−−−: 3. From the geographical distribution of phage types for tested strains, it can be said that the phage typing will give more information than serotype or prophage type for epidemiological observation of cholera.

8 STUDY ON ANAEROBIC BACTERIA IN THE SOILS IN INDONESIA

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Tetanus is expected to affect 50,000–60,000 newborns in Indonesia. Current morbidity of tetanus in children is greater than three per cent and the mortality of those affected reaches 45 per cent.

We sampled soils in various places of Indonesia in an attempt to isolate anaerobes. Special emphasis was given to genus *Clostridium*.

Our first report was presented before the 23rd meeting. The results of our report were unsatisfactory. Therefore, we obtained a total of 21 samples of soil from various places of Indonesia. The time interval of this survey included June to July, 1982. On returning to Japan, the differential culture for type species were made from the 21 soil samples.

As a result, 20 type species were detected, which predominates the previous detection as to variety of type species. The most frequently detected was *Clostridium perfringens*, isolated from 14 samples. This was followed by *Clostridium cadaveris* and *Clostridium aurantibutyricum* which were isolated from eight samples. *Clostridium tetani*, which has attracted the greatest attention, was isolated from four samples.

We plan to make a distribution map of the genus *Clostridium* in Indonesia by collecting and studying more Indonesian soil.

9 SCHISTOSOMIASIS *HAEMATOBIUM* IN KWALE DISTRICT, KENYA

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Since 1981, we have been studying several aspects of the infection of *S. haematobium* in Kwale district, Kenya, under the Communicable Diseases Research and Control Project, supported by KEMRI (Kenya Medical Research Institute) and JICA (Japan International Cooperation Agency). This first report on schistosomiasis

describes the egg output pattern and the water contact pattern of inhabitants in the study area.

Of the 1,338 people registered by census, 851 suitable urine specimen were collected successfully to be examined for the ova of *S. haematobium*. 581 or 68 per cent were determined to be positive in the study carried out in May and June, 1982. The overall prevalence was higher in females than in males. However, there was no statistically significant difference between them. The age-prevalence distribution of males showed a typical pattern of *S. haematobium* infection usually reported in other studies. The infection rate increased with age in the first decade of life, and had a clear peak of 100 per cent at 10–15 years of age. After the peak, the prevalence dropped down rapidly and remained at about 60 per cent consistently in the older groups. However, this usual pattern of age-prevalence was not observed in females. Although the prevalence also increased rapidly, and had a peak of 98 per cent at the same age group, it declined gradually in contrast to that of males.

The overall geometric mean egg count of total population was 50.2 eggs per hour, that of males was 52.4 eggs per hour and that of females was 47.4 eggs per hour. No statistical difference was observed. The age-intensity distributions showed almost the same patterns as that of age-prevalence distributions in both males and females.

The water contact study was carried out by the direct observation method. A total of 323 people were observed at the observation sites in the first four months from June to September, 1982. Of these, 164 were males and 159 were females. On the geometric mean of duration of contact according to age and sex, there was a remarkable difference between males and females. Among males, the children of age 5–19 years showed a high level of water contact with a peak of 20.74 min at 10–14 years of age. The geometric mean of contact decreased gradually with age after the peak to the lowest level of 4.73 min at age 40–49, then increased again. On the other hand, the geometric mean for females showed two peaks; at 0–4 years of age, and at 30–39 years of age.

An unusual pattern of age-prevalence distribution of *S. haematobium* infection was observed in females in contrast to males in this study, which might be explained by the difference of the water contact pattern between males and females. This suggests the importance of study on the water contact pattern in the epidemiological study of schistosomiasis. Furthermore, in spite of the increase of the degree of water contact in female at age 30–39, the prevalence and intensity of the same age-group still continued to decrease. This fact may suggest the existence of resistance to reinfection of *S. haematobium*.

10 EFFICACY OF A PURIFIED EGG ANTIGEN FOR ELISA IN THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

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ELISA is a useful technique for immunodiagnosis of schistosomiasis. Though soluble egg antigen is an excellent antigen for its sensitivity, it has some degree of cross-reactivity with facioliasis, trichinosis or paragonimiasis. Purified antigen is needed to get further specificity in ELISA. In this study, we obtained a purified antigen (J–1, MW 260,000, pI4.9) from *S. japonicum* SEA by DE52 anion-exchange chromatography and gel filtration on Sephacryl S200, and examined its efficacy for ELISA in schistosomiasis japonica. J–1 showed a single clear precipitin band against *S. japonicum*-infected mouse serum in Ouchterlony immunodiffusion. In addition to this, anti-J-1 serum caused significant COP reaction. From these result, J–1 is likely to be one of the main antigens which cause the COP reaction. J–1 (1 μg/m*l*) showed similar sensitivity with crude SEA (2.5 μg/m*l*) in ELISA. Decrease of cross-reactivity with *S. mansoni* or *S. haematobium* was observed by the use of J–1. Cross-reactivity with *P. westermani* or *F. hepatica* was completely abolished. On the contrary, cross-reactivity with *T. spiralis* was augmented by the use of J–1. These results suggested that J–1 is a promising antigen for serodiagnostic purpose.

11 MALARIAL ANTIBODY DETECTION BY ENZYME LINKED IMMUNOSORBENT ASSAY

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Serum was obtained from a imported case of falciparum malaria and was examined for serum IgG and IgM level using enzyme linked immunosorbent assay.

A patient presented himself to our clinic with a complaint of fever with chill which had continued to occur every evening for seven days. He returned home from East Africa after sojourn of one month. Blood examination revealed *Plasmodium falciparum* and venous blood was taken for further serological study. The incubation period was estimated to be between 37 and 67 days. This was thought to be primary infection of malaria considering his past history. Soon after blood taking, chloroquine treatment was started and after three days no plasmodium was found in the blood and fever subsided also.

Optical density of this serum read at IgG ELISA was 0.082 and showed no difference from those of two normal controls (0.044 and 0.060). IgM ELISA with

the patient's serum revealed very high optical density of 0.503 as compared with those of normal controls (0.058 and 0.089).

For comparison, IgG and IgM ELISA was performed similarly on two falciparum malarial cases in an endemic area in Thailand, and also on the serum from an owl monkey which had received several experimental infections of *Plasmodium falciparum*. IgG antibody titers were higher in these three cases, but high IgM titer was observed only in the sample from owl monkey.

It is interesting that immunofluorescence technique revealed high titer of both IgG and IgM in this patient. The difference between the results obtained by ELISA and by IF needs further analysis.

12 A METHOD FOR DETECTING MALARIA PASASITES

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In standard medical practice, a method routinely employed to detect malaria parasites is the microscopic examination of thick and thin blood films. However, in a non-malarious country such as Japan, even such a routine procedure proves difficult for the unexperienced technician. It is, therefore, necessary to devise a method which is both reliable and simple, allowing even an unexperienced technician to reach a definite diagnosis and thus, permitting the immediate administration of a suitable treatment.

Such a new method must satisfy the following criteria; (1) the parasites must be concentrated; (2) the morphology of the parasite must not be altered in any way; and (3) the parasites must be demonstrated in a microscopic field.

We have examined a method for detecting malaria parasites using blood obtained from *Plasmodium berghei* – infected mice.

0.1 m*l* of infected blood was diluted with PBS containing 10 U/m*l* of heparin at a ratio of 1:5 and then passed through a CM-cellulose column (2.0×3.0 cm). PBS supplemented with 10 per cent calf serum was used as eluent. Flow was regulated at a constant rate of 6 m*l*/min. The leukocyte-free erythrocyte suspension eluted out was centrifuged and the pellet was then lysed with Tris-buffered ammonium chloride (Boyle, 1968). This solution was again centifuged to wash out the ammonium chloride. The pellet obtained from the second centrifugation was stained with Acridine Orange.

Microscopic examination of the solution eluted from the CM-cellulose column, as well as the one eluted from a tightly-packed cellulose column, revealed a complete absence of leukocytes. Approximately 100 per cent of the erythrocytes could be collected through the CM-cellulose column. On the other hand, only 90 per cent of the erythtrocytes could be collected through the cellulose column. Blood parasitaemia before and after passage through the column remained basically the same. Although a thin blood smear was made from the stained pellet, the parasites

seen were concentrated at a degree almost similar to that of a thick blood smear. No alteration in the morphology of the parasites was observed. The nucleic acids were stained very brightly with the DNA (nucleus) appearing yellowish and the RNA (cytoplasm) appearing reddish. In dark field only the parasites could be seen very well so that microscopic examination can be easily done at a magnification of 400×.

It is clear, therefore, that the method presented above meets all the criteria previously mentioned, and thus could be of practical value in detecting cases of human malaria. However, such a method cannot be used in the field studies of malarious areas because of lack of instruments. Moreover, this method does not allow for distinction among various species.

13 PHYLOGENY, POPULATION GENETICS AND BIONOMICS WHICH CONCERN TO EPIDEMIOLOGY OF VECTORS OF MALARIA AND THEIR RELATED ENTITIES OF ANOPHELES IN EAST ASIA

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Due to overlap in their morphological variation there exist many biological critical problems unresolved among the malaria vectors and their related species of East Asia for their future control. Among strains from geographically separate area within same species of malaria vectors and their related entities, it seems that there exist some grades of genetic divergence in their cytogenetic observations, hybridization tests and allozyme variations in their electrophoretic analysis. Therefore, the relationships between these genetic characters and ecological ones related to epidemiology have been revealed, then these informations will be reported herewith. The *Hyrcanus* group: Some vector entities of the group are distributed in northern East Asia. Hybridization and experimental and field transmission of vivax malaria indicated that apparently there does exist a close genetic relationship between Korean and Chinese populations of *sinensis* and the strains of *lesteri* from both Japan and China. When cross-mating was performed between the both species, their F_1 hybrids showed synaptic X-chromosome but asynaptic autozomes in their salivary gland polytene chromosomes. This type of *sinensis* is a vector of vivax malaria in these countries. Crosses between another type of *sinensis* and *lesteri* yielded asynaptic progeny for all their chromosomes, this type did not infect with *Plasmodium vivax* in our experiments, distributes in most part of Japan, and can be found in China, too. These facts suggest that replacemental possibility of these two types of *sinensis* can be tried for future control. The *Leucosphyrus* group: Cytogenetic observations, hybridization experiments and isozyme-system analysis by adopting electrophoretic method revealed that there exist the evolutionary facts of the process of divergence among geographically separated species. With a

direction of genetic affinities, TSG–KTD–IMR–CTB–KCH. These nine strains are included in a taxon *balabacensis*. The *Minimus* group: Ecological and bionomical investigations were performed in the Province Kanchanaburi, Thailand, from September 1982. Twenty five strains at the six localities were set. A total of 8,257 were collected, 74 per cent of them were *Anopheles* belonging to 17 species. The predominant species was *An. minimus* with 60 per cent of the total catch. *An. minimus* density was strongly decreasing with the oncoming lower temperature season. A decrease of 925 was recorded between September and November. Regarding to the natural infection, the rate was 5/646 at Tha-lamyai, 1/158 at Pong Chang, and their sporozoite rates were 4/646 and 1/158 respectively in these villages. Physiological age composition was not changed by the time during the night. The attractiveness of various habitates was investigated at Tha-lamyai (TLY) and Wat Seri Somchai (WSS) where are sugarcane areas *An. minimus* attacked most frequently in open field, followed by dense vegetation, verandah, high vegetation fringe and indoor. The attractiveness of out door stations did not change during the night. Biting cycle were variable. Evidence was found that this variability was due to two different types of biting cycles. In a first type peak biting activity occured in the early morning, in the second type around mid-night. Often a mixture of the two types was observed. The two biting cycle types were neither related to time, stations nor localities. The occurence was at random. It is suggested that the biting cycle types are due to two behavioural subpopulation of *minimus*. The biting cycles of highly anthropophilic *minimus* population is comparable to one with a mixture of anthropophilic and zoophilic individuals. An influence of spray of insecticides also considered that the mosquito bite mostly outdoor and even when they go indoor for bite, they go out, to rest outside. However, this species and *maculatus* were tested for their resistance against DDT, malasion, smithion and abate, etc. and the results showed little resistance against these insecticides. The *Maculatus* complex: Three morphological types of the mosquito vector *Anopheles maculatus* are known to occur sympathetically at Kanchanaburi, Thailand. Although we previously reported that these types were interfertile upon hybridization, analysis for 11 electrophoretic loci of F_1 raised from wild-caught females that one type, known as variety willmori, is fixed or nearly fixed for electromorph differences at two loci. The significance of this discovery is examined in the light of hybridization experiments and with respect to what is known about mosquito systematics and evolution. This report is summerized from our laboratory work and collaboration with SEAMEO-TROPMED and WHO collaborating centre in China and Vietnam.

14 AN ECOLOGICAL SURVEY ON LARVAE OF *ANOPHELES SUNDAICUS* IN A COASTAL VILLAGE OF NORTH SUMATRA, INDONESIA

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An ecological survey on the larvae of *Anopheles sundaicus*, the vector of coastal malaria, was carried out under the cooperation between Indonesia and Japan in a village of Asahan Regency from March 1982 to February 1983. The larval density, size of larval habitat, salinity, the presence of floating algae and fishes etc. were recorded biweekly on 41 ponds within 600 m from a fixed point for adult survey.

The size (m^2) of larval habitat fluctuated with two seasonal peaks in May–June and in October–December. Twenty three ponds and nine ponds dried up in March, the end of dry season and in August, the small dry season, respectively. In March–April, just after the depth of water increased rapidly, the seasonal peak of larval density was observed. In late May, when the increase of water depth was terminated, the density decreased, even though the water depth itself was still high. Afterwards the density remained at low level towards February.

The effect of each environmental factor on the larval density was examined, based on total 943 data. The density was high when the water depth in ponds increased rapidly, or in ponds with sunny surface or high salinity. On the other hand, low density was observed in ponds with shady surface, low salinity or door. The density was lower in fish-present ponds than fish-absent ones, but the frequency of larva-present cases was not affected by the presence of fishes. Based on the above results, combinations of these factors for the breeding of the larvae were worked out, and the density and frequency were calculated from data set under each condition. The results almost coincided with the estimation based on tendency on each single factor.

The index of larval abundance was estimated as $\sum d_i \cdot S_i$, where d_i was the density in pond i and S_i was the size (m_i) of pond i. The index in ponds near the fixed point for adult survey fluctuated with two seasonal peaks in April and in September–October, somewhat corresponding to the seasonal prevalence of man hour density of adults. The first peak of the index was due to the high density and the second peak was caused by large size of habitat.

15 A CASE OF RAPIDLY-DEVELOPED FALCIPARUM MALARIA

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We have recently had an opportunity of necropsing a male who was infected with *Plasmodium falciparum* parasites while he had been engaging in medical activities in Enugu district, Nigeria and died later. The result of the course and necropsy is reported briefly herein.

The patient is a 36-year-old male, physician by profession. His chief complaint was fever and there was nothing particular with his family and past history.

Present history: For a period of June 20–28, 1983 the patient had engaged in medical activities in Enugu district, Nigeria. He returned home on June 29. On July 10 fever of 39°C developed, accompanied with shaking chill which persisted through July 11, 12 and 13. The fever lowered to normal in the morning and then rose higher again toward the evening. Slight headache, myaglia and loose feces and/or diarrhea occurred as well.

On July 14 jaundice and liver dysfunction were recognized. As a result malaria or hepatitis A was suspected and he was advised to be hospitalized. The patient checked into a hospital at 3:00 p.m.

Findings on hospitalization: Body temperature was 37°C. He complained of feverish sensation, heavy-headedness and general malaise. The skin was yellowish and consciousness clear. A sudden spell of generalized convulsion attack occurred around 5:00 p.m. accompanied with turbid consciousness, high fever of 39°C, respiratory insufficiency and oliguria, which condition eventually required respiration management by means of endotracheal tube insertion. Cerebral malaria due to *Plasmodium falciparum* was suspected and the peripheral blood examination revealed *Plasmodium falciparum* parasites (parasite density 351,900/mm^3). Intravenous administration of 4 m*l* of quinimax (contains 400 mg of quinine) was instituted immediately The parasite density in the peripheral blood after first administration of the drug was found to be 28,880/mm^3. However, at 2:40 a.m., July 15, the patient died of multiple organ failure with the brain, heart, liver and kidneys involvement.

Necropsy findings and pathological diagnosis: Whole small blood vessels and capillaries were dilated remarkably and filled with malarial parasites in the red blood cells as well as with isolated malarial pigments. Pathologically the case was diagnosed as falciparum malaria.

Noticeable pathological findings were: 1) Hepatomegaly with Kupffer cells' reaction. 2) Splenomegaly with histocytic reaction and expansion of red splenic

medulla. 3) Cerebral edema with marked blood vessel dilatation. 4) Congestion and edema of the lung and kidneys. 5) Hemorrhagic tendency, etc. .

16 TWO CASES OF RECURRENT VIVAX MALARIA

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In these three years (Sep. 1980–Aug. 1983), 29 patients suffering from imported malaria have been treated in our hospital. Nineteen cases were diagnosed as vivax malaria by identifying the species. Two cases were recurrenced after the chemotherapy including primaquine.

Case 1: I. K., a 37-year-old male. Oral administration of quinine was used for malaria prophylaxis. Three weeks after arrival in Cambodia, he became febrile and was hospitalized in the region. After the therapy by antimalaria drugs, no parasites had been detected in blood smears until his departure from the endemic area. Soon after his arrival in Japan, he developed a fever of 40°C with rigors. As *Plasmodium vivax* ring forms were seen on blood slides, he was admitted to our hospital. A standard course of chemotherapy for vivax malaria was given; 600 mg chloroquine base orally on admission (day 1), followed by 300 mg six hours later and on day 2–3 he received 300 mg chloroquine base daily. Two months after the therapy, fever returned with rigors and malaria parasites appeared in his blood. During his second admission a modified protocol for the antimalaria was followed, that is, total amounts of 1.5 g chloroquine base was given on day 1–3, then on day 6–7 300 mg was administrated daily for two days and on the 8th day 45 mg primaquine base for one dose was prescribed. The same dose was repeatedly administrated for eight weeks, once a week. In spite of the preceeding therapy, next recurrence could not be avoided. Nine months after the second admission, parasites of vivax malaria reappeared.

Case 2: A. T., 40-year-old male. Fansidar (25 mg pyrimethamine plus 500 mg sulfadoxine) were used for prophylaxis of malaria. Two weeks after his return to home, he became febrile. Self-prescribed two tablets of Fansidar were ingested one day before his first admission to this hospital. A standard course of chemotherapy for vivax malaria was given. Two months after his discharge, he was hospitalized again because of the recurrence of malaria. The second protocol for the therapy was the same as the first one except the twice doses of primaquine for a half period. No recurrence has been observed since the second chemotherapy.

Both cases are imported malaria by which non-immune Japanese were infected near the boundary between Cambodia and Thailand. Decreased sensitivity to primaquine may be suggested, but the serum concentration of the drugs has not been measured yet.

17 CLINICAL EVALUATION OF SMX/TMP ON TREATMENT OF PATIENTS WITH MALARIA

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The case of malaria carried back from endemic areas is increasing in Japan with the increase of travelers abroad and the progress of transportations. On the other hand, it is rather difficult to obtain immediately the common anti-malarial drugs such as chloroquine or primaquine for the urgency since these drugs are kept at only limited institutes or hospitals in our country.

Therefore, we tried to administer the Smx/Tmp to the patients with malaria for the treatment instead of those antimalarial drugs and evaluated its clinical efficacy by reasons that this drug is so popular among the general hospitals and its effectiveness is already established in the endemic countries.

The Smx/Tmp were administered orally to four cases of malaria patients (three cases of falciparum and one case of vivax malaria) with the parasitemia of 1.7×10^4 to $6.7 \times 10^5/\mu l$ and total doses were 12 to 36 tablets for one to six days.

Disapperrances of parasite from the peripheral blood and symtoms were observed within four days after the start of administrations in three out of four cases. The drug was changed to chloroquine after one day administration in the remaining one case with most severe parasitemia due to *P. falciparum*, because the patient was not released from severe headache although fever and parasitemia were rapidly improved. But, it seemed that Smx/Tmp was probably effective in this case, because parasites disappeared from the blood on the next day after change of drug.

Remarkable adverse reations were not observed in the cases except one case with gastric disturbance such as anorexia and vomiting.

As the results, it was concluded that the Smx/Tmp was very useful for the treatment of malaria, particularly when chloroquine or primaquine was not available at the hospitals.

18 DISTRIBUTION OF *ANGIOSTRONGYLUS CANTONENSIS* IN NAGOYA CITY -INFECTION RATE OF HOUSE RAT POPULATIONS

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The rat lungworms, *Angiostrongylus cantonensis*, were detected last year from a house rat, *Rattus norvegicus*, in an inland area, Meitoh-ku (Area T) of Nagoya City, some 14 km apart from the seaport.

Subsequent surveys were made to know possible establishment of the rat lungworm populations around the seaport area. As a result, 163 and 104 worms were collected from eight and four house rats (*R. norvegicus*) in a port-side area, Minato-ku (Area P) and in a residential quarter, Minami-ku (Area M) several km north of the seaport, respectively. The number of the worms were more than 380 in total including 120 specimens so far collected in the inland residential area (Area T) where this worm was first detected.

All of these worms were collected not from *R. rattus* but from *R. norvegicus*. The infection rate of the worm in *R. norvegicus* populations was in decreasing order of 25.8 per cent in Area P, 25.0 per cent in Area M, 16.7 per cent in Area T along a direction from the seaport (Area P) towards the inland area (Area T). This result seems interesting from the viewpoint of immigration route and establishment of the worm populations in the areas. And it can be concluded from the infection rate in various areas that this worm is distributed in eastern side of the city.

The infection rate of other helminths was far higher (93%) in *R. norvegicus* than in *R. rattus* (27%), and the co-infection rate by more than two helminth species was also higher (89%) in the former rat species than in the latter (22%).

Frequency distribution of the rat lungworm per host showed a good fit to the negative binomial as well as five other helminth species. However, the parameter of overdispersion (k=0.0456) was smaller than the range for other helminths (k= 0.2457–0.0702), indicating that infection of this worm might occur more aggregatively than that of other helminth species.

19 A HELMINTHOLOGICAL SURVEY ON BLACK-BASS, *MICROPTERUS SALMOIDES* IN THE KINKI DISTRICT AREA

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The freshwater fishes, black-bass, *Micropterus salmoides* and blue-gill, *Leptomis*

macrochirus were imported to Japan from North America; the black-bass to Lake Ashino-ko, near Mt. Fuji in 1925, and the blue-gill to Lake Ippei-ko, on the Izu Penninsula. Both species have subsequently increased ponds, lakes and bays of streams, and are now found all round Honshu, Kyushu and Shikoku area, where they have eaten many of the indigenous species of Japanese freshwater fishes. Both species are very important as game fishes and very useful as cooking fishes.

I am currently engaged in a helminthological survey on black-bass, *Micropterus salmoides*. Black-bass collected since November 1982 were dissected and examined for the larvae and adults of helminthes in the scales, muscles, gills, abdominal cavities, digestive organs, mesenteries, liver and pancreas. Eleven fishes from Lake Biwa ($16.5 \times 5.5 \sim 28.5 \times 8.5$ cm), five fishes from Kisenyama Dam, Uji City ($19.0 \times 6.8 \sim 28.0 \times 7.5$ cm) and five fishes from Hyotan-ike Pond, bank of Maruyama River, Hyogo Prefecture ($15.2 \times 3.7 \sim 29.0 \times 8.5$ cm) were dissected. No parasitic helminthes were found in these black-bass. However, I found two encysted female larvae of Acanthocephala in the mesenteries of one black-bass (female, 29.0×8.5 cm) at Hyotan-ike Pond, bank of Maruyama River. One of these females was fixed by hot 70 per cent alcohol and mounted with Berlese's mounting media under a cover glass. This cylindrical female larva was 2.80×0.84 mm in body size and 0.43×0.33 mm in proboscis size. The proboscis bears 10 hooks in a longitudinal row. The anterior parts of the trunk bear small spines. I had previously been engaged in the survey of the life cycle of this larval Acanthocephala, and also studied Hoffman's literature (1967), "Parasites of North American freshwater fishes".

20 EFFECT OF FLUBENDAZOLE AND MEBENDAZOLE ON ENCYSTED LARVAE OF *TRICHINELLA SPIRALIS* (USA STRAIN) IN MICE

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The aim of the present study was to evaluate effects of the two benzimidazole compounds, mebendazole and flubendazole (fluorine analogue of mebendazole) on encysted *Trichinella spiralis* larvae of early and late stages in mice.

After saline suspension containing 50 *T. spiralis* larvae of USA strain/0.1 m*l* was prepared, six-week-old females of ICR strain mice were each inoculated with 50 larvae/10 g of body weight via a stomach tube. The infected mice were randomly divided into experimental and control groups of 6–7 mice each. Pure powdered flubendazole and mebendazole (Janssen Pharmaceutica, Belgium) were suspended in one per cent (V/V) Tween 80 so as to give the following specified dosage in 0.1 m*l* of the suspension per 10 g body weight. These suspensions were orally administered to mice of the experimental groups at 10, 50 or 100 mg/kg/day for three days (35–37 days or 70–72 days post-infection) or a single dose of 300 mg/kg 35 days post-infection. The control mice were given one per cent Tween 80 alone.

Mice were autopsied 56–57 days or 111–112 days post-infection. Because worm reduction in the diaphragm was well correlated with that in the other muscles (Maki and Yanagisawa, 1983, Parasit., in press), only diaphragm was examined. The number of normal larvae/100 mg wet weight of the diaphragm was counted by the method of Maki and Yanagisawa (loc. cit.). Data were analyzed statistically using the Student's t-test. Differences between means were considered significant at P-values less than 0.1 unless otherwise noted.

Both the drugs given 35–37 days or 35 days post-infection significantly reduced 82–100 per cent of the larvae ($P<0.01$). No significant difference was observed between the number of larvae recognized from the mice treated with flubendazole and mebendazole at 10, 50 or 100 mg/kg/day for three days. At the single dose of 300 mg/kg administered 35 days post-infection, mebendazole was significantly more effective than flubendazole ($P<0.1$). Divided administration of the drugs (100 mg/kg/day for 3 days) was significantly more effective than the single administration (300 mg/kg) in both flubendazole and mebendazole at the P-value of 0.05. The two drugs given 70–72 days post-infection at 50 and 100 mg/kg/day eliminated 79–100 per cent of the larvae ($P<0.01$). Flubendazole at the dose of 10 mg/kg/day for three days was ineffective against the old encysted larvae while mebendazole at the same dosage regimen showed 60 per cent in worm reduction ($P<0.05$). From the comparison of the values of ED_{50} in the treatment of late-stage larvae, mebendazole ($ED_{50}=21$ mg/kg) was more effective than flubendazole ($ED_{50}=115$ mg/kg) ($P<0.05$). In conclusion, mebendazole is as effective as or more effective than flubendazole in the treatment of encysted *T. spiralis* larvae in mice. This is consistent with the data of Takada and Tatefuji (1982, Program and Summary of Sino-Japanese Seminar on Parasitic Zoonoses, 136–140), who reported that mebendazole was more effective than flubendazole from the viewpoint of degeneration, calcification and edematous changes of larvae in mice treated with the two drugs.

21 *GIARDIA LAMBLIA*: INHIBITION OF RESPIRATORY ACTIVITIES AND GROWTH *IN VITRO* BY BITHIONOL

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Endogenous respiration of intact trophozoites of *Giardia lamblia* (Portland strain) were inhibited by 2, 2′-thiobis (4, 6-dichlorophenol), i.e., bithionol, which is an effective chemotherapeutic agent for some trematode and cestode infections in man. Among other halogenated phenol derivatives examined, 2, 2′-methylenebis (4-chlorophenol), i.e., dichlorophene, and 2, 2′-methylenebis (3, 4, 6-trichlorophenol), i.e., hexachlorophene were also found to be highly inhibitory to the respiration of *G. lamblia*. Concentrations of bithionol, dichlorophene and hexachlorophene needed

for 50 per cent inhibition of this respiration were 0.50 mM, 0.75 mM and 0.50 mM respectively.

Bithionol, dichlorophene and hexachlorophene also inhibited *in vitro* growth of trophozoites (Portland strain) of *G. lamblia*. It took less than 72 hours to kill virtually all trophozoites in the cultures with 100 μg/m*l* of these compounds. These observations suggest that halogenated bisphenols such as bithionol inhibit some respiratory activities of *G. lamblia*, and that these compounds may be useful for the treatment of human giardiasis.

22 SOME RELATIVE CHANGES OF ONCHOCERCA MICROFILARIA POSITIVE RATES DURING FOUR YEARS SURVEY IN SIX ENDEMIC AREAS OF ONCHOCERCIASIS IN SVP, GUATEMALA

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An epidemiological survey of onchocerciasis was undertaken to study the effect of insecticidal treatment to the habitats of Simulium larvae in San Vicente Pacaya, Guatemala. The survey was conducted on the inhabitants of six endemic areas of onchocerciasis by parasitological and immunological examinations, once in a year from 1978 to 1982. Within these examinations, we discussed the results of microfilarial rate by skin biopsy in the representation. Results were summarized as follows;

1) Microfilarial positive rates decreased gradually with years between 1978 and 1982, although they were not statistically significant.

2) Only five (4.3%) of 117 inhabitants received onchocercal nodulectomy one year prior became microfilaria-negative by skin biopsy. This result has shown that the nodulectomy does not result in microfilarial negative at least within one year.

3) Out of 700 inhabitants who received four or five times of skin biopsy between 1978 and 1982, 115 showed positive and 393 showed negative at all times of examinations. These results suggested that skin biopsy was most reliable for the diagnosis of onchocerciasis.

4) New infection (2.4%) and cure (2.9%) rates were calculated from the proportion of people who converted from negative to positive and reversed from positive to negative between 1978 and 1982.

23 STUDIES ON THE TRANSMISSION OF ONCHOCERCIASIS IN THE TERRITORY OF AMAZONAS, VENEZUELA

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In relation to the transmission of onchocerciasis in the southern Venezuelan focus, blackflies in the Territory of Amazonas, Venezuela were investigated in October and November 1982. Adult females of four blackfly species and one species group (*Simulium pintoi*, *S. pseudoantillarum*, *S. yarzabali*, *S. exiguum* and *S. amazonicum* group) were captured using a human attractant at seven localities with different altitudes (180–1,000 m). Among these, *S. pintoi* was the predominant man-biting species in the region of the Parima mountain and its foothills (250–1,000 m in alt.), whereas this species was replaced with *S. amazonicum* group in the lowlands (ca 200 m in alt.) along the main channel of the Orinoco river. With regard to the biting preference, *S. pintoi* had a predilection for the lower parts of the human body, making a marked contrast to the biting preference of *S. amazonicum* group which exclusively bites the human body parts above the waist. On the other hand, it was clarified in our experimental infection study that under a temperature varying between 16°C and 24°C, microfilariae of *Onchocerca volvulus* developed to the infective larvae in *S. pintoi* on day nine after ingestion of microfilariae. Furthermore, *S. pintoi* has been proved to be highly susceptible to infection with *O. volvulus*, as larval development therein was synchronous, no abnormal and deformed larvae were observed, and nearly all the larvae recovered on day 10 or larter were in the third stage. It is thus suggested that this species is an efficient experimental vector of *O. volvulus*, despite high mortality following the excess intake of microfilariae.

24 EPIDEMIOLOGICAL STUDIES ON LOIASIS IN NIGERIA 2. SURVEYS OF MICROFILARIA AND SURVEYS OF VECTORS, *CHRYSOPS*

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From an epidemiological point of view, the surveys on loiasis and its vectors were carried out in the south-western region of Nigeria during the two months from

October to November 1982.

Prevalence of *Loa loa* was surveyed in Epe, Lagos State, by detecting the microfilaria in the thick films of the peripheral blood taken from the fingertip during the hours from 11 a.m., to 2 p.m. . In the three secondary and high schools in the town, a total of 440 students (aged 11 to 23) were examined and the positive rate for microfilaria was 6.6 per cent (5.8–10.4%). Among the stuffs (aged 20 to 48) of these schools, 107 were examined and 7.5 per cent (4.5–11.4%) of them were positive. In a Water Works of the town, 38 town inhabitants (aged 20 to 70) were examined and the positive rate, 28.9 per cent, were considerablly higher than those in the schools.

With regard to the clinical symptoms, a total of 85 persons (46 with microfilaria and 39 without it) were examined. In the group with microfilaria, carabar swelings (63%) and prickly sensation (71.7%) were more common than those in the group without microfilaria.

The surveys on the vector tabanids were carried out at many points within five states, Lagos, Ogun, Oyo, Ondo and Bendel, and the adults, larvae, pupae and egg-masses were collected. A total of 179 adults were obtained and 47.8 per cent of them were *Chrysops* species: Among the *Chrysops*, 77.6 per cent were *C. silacea*, 18.9 per cent were *C. dimidiata* and the rests were *C. longicornis* and *C. distinctipennis*. A total of 474 larvae (including some pupae) were obtained and 61.8 per cent of them were *Chrysops* species: Among the *Chrysops*, 8.6 per cent were *C. silacea*, 13.3 per cent were *C. centurionis*, 57.6 per cent were *C. longicornis* and 10.9 per cent were *C. distinctipennis*. With regard to the 9.4 per cent of the *Chrysops* larvae, the identification was reserved, though it was clear that they belong to the *silacea*-group. A total of 224 egg-masses were obtained and the most of them were those of *Chrysops*: Among the *Chrysops*, 72.5 per cent were *C. longicornis*, 6.8 per cent were *C. distinctipennis* and the rests (20.7%) were the *silacea*-group (*C. silacea*, *C. dimidiata* and *C. centurionis*).

During the survey, a total of 27 adult flies (25 of *C. silacea* and two of *C. dimidiata*) were dissected to examine for the larvae of *Loa loa*, and the second stage-larvae were found in the abdomen of two individuals (one of *C. silacea* and one of *C. dimidiata*).

25 SURVEY OF MOSQUITOES IN CHANTHABURI PROVINCE, THAILAND, WITH SPECIAL REFERENCE TO THE EPIDEMIOLOGY OF MOSQUITO-BORNE DISEASES

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During the period May 1981 to March 1983, a survey was made on mosquito breeding in Chanthaburi Province to determine the mosquito fauna in the area and the importance of each breeding habitats viewed as a place for species preservation.

A total of 67 species of mosquitoes were collected. *Aedes aegypti* and *Aedes*

albopictus, the principal vectors of dengue haemorrhagic fever, were dominant species breeding in the domestic containers in close associate to house of human beings in all localities in Chanthaburi. There are extensive rubber plantations, and *A. albopictus* larvae in large numbers were found in coconut shells which lie scattered there.

A principal Japanese encephalitis vector, *Culex tritaeniorhynchus*, were collected from a wide variety of ground water habitats including puddles, swamps, marshes, rice fields and shallow wells. It was suggested that rice field is the most important breeding sauce of *C. tritaeniorhynchus* in this survey.

A malaria vector, *Anopheles balabacencis*, was not common though its larvae were collected from puddles, wells and rock pools.

26 SEASONAL CHANGE IN THE OVIPOSITION ACTIVITY OF *CULEX PIPIENS PALLENS* IN SHANGHAI IN 1982

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The breeding places of *Culex pipiens pallens* in Shanghai are polluted water in ditches, ponds, and various kinds of containers etc. The seasonal change in the oviposition activity of this mosquito was observed at Fuxing Gongyuan (Renaissance Park), Shanghai from August 1981 through December 1982. An earthen jar of about 10 litters in size containing wheat bran solution was set up as an ovi-trap for house mosquitoes in a corner of the park. All egg rafts of mosquitoes, which were deposited on the water surface in the jar, were picked up every morning and examined for hatching in the laboratory.

The first egg rafts in 1982 were collected in the begining of May when the temperatures were 23.9°C (Mean Max.) and 15.7°C (Mean Min.). The oviposition activity of *Cx. p. pallens* in Shanghai seems to be rather low during the summer when the maximum temperatures are over 30°C. Two clear peaks of the activity were observed; higher peak was in early July and lower one was from late August through early September. The last egg rafts were collected in mid November. No egg rafts were found from late November through late December, when the maximum temperature was below 10°C. The pattern of seasonal change in the oviposition activity of *Cx. p. pallens* in Shanghai in 1982 is similar to the result in 1981 at the same place, except that the second peak in the fall of 1982 was rather lower than that of 1981.

27 TIME COURSE OBSERVATION OF THE PARASITOLOGICAL INFECTION OF JAPANESE INHABITANTS IN TROPICAL COUNTRIES AND THE POLLUTION OF THE DRINKING WATER

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As reported previously, the drinking water in tropical countries contained various kinds of microorganisms and was polluted by faeces and sewage. In order to know the relationship between the prevalence of the parasitological diseases and the degree of faecal pollution of the drinking water, we carried out the time course observation of the parasitological infection of Japanese inhabitants and the pollution of the drinking water.

First, we checked parasite eggs and protozoas in the stool samples of Japanese. Eggs of *Ascaris lumbricoides*, *Trichuris trichuira* and cysts of *Giardia lamblia* were found among Japanese. Infection rate of intestinal parasites was proved to be highest among Japanese in Indonesia, Thailand and the Philippines, and the rate was followed by those of people in Southwest Asia, in East Africa and in Central and South America. But the infection rate did not change so much in the past 10 years.

Drinking water samples from these countries were also tested with potassium permanganate consumed test and number of coliform bacilli. Relatively high proportion of samples was proved to be polluted by faecal matters.

The relationship between the infection rate of intestinal parasites and the degree of pollution of the drinking water was studied. Significant relationship was observed between the infection rate and the rate of the water samples containing coliform bacilli ($r=0.957$). However, no relationship between the infection rate of *Enterobius vermicularis* among the Japanese children in tropical countries and the degree of the pollution of drinking water was found.

28 DETECTION OF INTESTINAL PROTOZOA FROM OVERSEA TRAVELERS

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To investigate the actual circumstances of travelers' diarrhea, we had made bacteriological examinations before and demonstrated that half of the cases were caused by bacteria. However, since the nature and cause of the other half remained unknown, we began parasitological examination from January to June 1983. Free discharged stools of 318 travelers who stayed abroad for more than five days were examined using direct microscopic examination of stool smears, Tanabe-Chiba culturing technique and formalin-ether concentration technique. *Giardia lambria* was found in four patients (1.3%) and *Entamoeba coli* was found in one. The four patients who were infected with *G. lamblia* traveled abroad for more than five weeks. Three of them were infected in India and Nepal. Mixed infection of Shigella was ditected in two of the Giardia cases so that the patients were sent to hospitals. The other two patients suffered from slight diarrhea during their tour. In one of them, diarrhea continued for more than a month after he returned to Japan. The patient was treated and cured with Atebrin. *Entamoeba coli* was found in one tourist who visited India and Nepal for seven weeks.

Although the positive rate in the examination during this six months was low (1.6%), we intend to continue the detection to elucidate the nature of travelers' diarrhea caused by protozoa.

29 CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF THE DRINKING WATER OF THE TROPICAL COUNTRIES

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The number of Japanese stayed in tropical countries is increasing, as the Japanese technical cooperation with the countries is improved. The diseases from which Japanese suffers in these countries are infectious hepatitis, especially type A, amoebic as well as bacillary dysentery, typhoid fever, various kinds of intestinal helminthiasis and so on. These diseases are though to be orally infected, and among sources of infection, the drinking water is considered to be most important as the main route of infection.

From 1977, we started to examine the drinking water at various places in tropical countries. Results were shown that almost all water samples contained a large amount of coli form bacilli and were polluted by faeces and sewage. The degree of the pollution of water samples was proved to be highest in samples from Indonesia, Thailand and the Philippines of Southeast Asia, Pakistan of Southwest Asia, Iraq of Middle East, Kenya, Mozambique and Neigeria of Africa, Mexico of Central America, and Ecuador and Peru of South America. The residual chlorine as a bactericide was observed in the drinking water from Singapore and Malaysia, and some samples from Egypt and Panama, but, in the water samples from the other parts of tropical countries, no residual chlorine was found at all.

30 BACTERIOLOGICAL STUDY OF THE DRINKING WATER IN INDONESIA

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This time, we will present the results of a bacteriological study of 75 samples of drinking water collected in many regions of Indonesia for approximately one month (June to July 1982).

We carried out a quantitative test of total colony counts and coliform group counts, using URICULT set. The results of total colony counts were positive (more than 10^3/m*l*) in 43 samples. Eighteen samples out of these 43 samples which were positive were taken in Kalimantan, Sulawesi and Sumatra. Fifty-seven samples taken in Java, with Jakarta being the central location, showed the greatest number of positive samples; 11 out of 28 samples (39%) from tap water and 14 out of 29 samples (48%) from well water. On the other hand, the results of coliform group counts were positive (more than 10^3/m*l*) in 37 samples (49%). Eighteen out of 37 samples in Kalimantan, Sulawesi and Sumatra were positive. Fifty-seven samples in Java, with Jakarta being the central location showed positive results in six out of 28 samples (21%) taken from tap water and in 13 out of 29 samples (45%) taken from well water.

Enteric bacteria and related bacteria that are found in sample water were primarily *Enterobacter cloacae* and *Citrobacter freundii*. Both species were identified in 16 samples. The next most frequently identified bacteria were *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* found in six samples. *Escherichia coli*, which attracts the greatest attention in relation to fecal matter contamination, was found in five samples. This includes those discovered in two samples of tap water taken in Jakarta City. The most noticeable point in this investigation is that *Salmonella* C_1 group was detected from one well in Jakarta City; *Salmonella* E_1 group from another well in Jakarta City, and each type was found in wells in Kalimantan, Sulawesi and Sumatra showing the existence of the bacteria throughout the islands.

The drug sensitivity test for Kanamicin, Gentamicin, Tetracycline, Chloramphenicol, Carbenicillin, Cephaloridine, Cephalothin, Colistin and Nalidixic acid were performed by the sensitivity disk method, on 57 strains of the bacterial species identified with the following results: So far as *Salmonella* sp. only is concerned, *Salmonella* sp. detected in Jakarta City was resistant to Cephaloridine and Cephalothin in the sensitivity testing, whereas *Salmonella* sp. detected in the other three islands were susceptible.

31 SNAKEBITES IN THE AMAMI ISLANDS IN 1982

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In 1982, 161 bites were reported on the Amami Islands in which 105 (65%) were from the Tokunoshima and 56 (35%) were from the Amami Main Islands. During warmer months from March through October, 146 (91%) of the total bites were recorded in which the maximum number (34) of bites occurred in June. Although the age of bite is widely distributed from teen to seventies, 75 per cent of the bites occurred in ages from thirties to sixties in which the maximum number of bites occurred in fifties. Bites in male occurred two times as frequent as those in female. The most frequent bites (57.1%) occurred in agricultural field, whereas 18 per cent of the total bites occurred in residential area and 9.5 per cent of bites were on road. Those fact indicated that the human life and snakes are in close proximity.

Most bites occurred in extremities in which 56.1 per cent were in upper extremities and 42.6 per cent were in lower extremities. Most frequent bite occurred in finger (42.6%), whereas 22 per cent of bites was in lower leg. In daylight hours from 6 a.m. to 6 p.m., 102 or 75.5 per cent of the bites occurred mostly in agricultural field and 39 or 24.4 per cent of bites did at night time mainly in residences and on road.

The prognosis of habubites indicated that most patients recovered completely after the administration of antivenom except one death (0.6%) and four cases of motor disturbances (2.5%) of finger after the wound had healed.

32 SNAKEBITES IN OKINAWA PREFECTURE IN 1982

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In 1982, 179 bites of habu (*Trimeresurus flavoviridis*) and 80 bites of sakishima-habu (*T. elegans*) were reported in Okinawa Prefecture.

During 10 years, from 1972 to 1981, the average annual number of habubites was 270.1 and the average morbidity rate per 1,000 population was 0.28, with the both cases showing a continued decrease.

In the case of sakishima-habu they were 51.1 and 1.09 respectively.

Seasonal change of snakebites: Habubites occurred mainly during warmer months through March to November in which the highest number, 33 (18.8%) bites, was recorded in October. But in the case of sakishima-habu, considerable number of bite cases were reported during colder months, while the number decreased in July and August.

Locations where snakebites occurred: Sixty-eight (38.6%) of the habubites occurred in agricultural fields, 36 (20.5%) unknown sites, 21 (11.9%) in human residence, 18 (10.2%) on roads and 14 (8.0%) were in mountains. Thirty-eight (47.5%) of sakishima-habubites occurred in agricultural fields, 17 (21.3%) in human residence, 9 (11.3%) on roads, and each 5 (6.3%) in houses, mountains and at the waterside.

Body site of snakebites: Most of habubites occurred on the extremities; 95 (53.4%) and 76 (42.7%) were the number of bites on the upper and lower extremities, respectively. Sakishima-habubites occurred mostly on the upper extremities (65, 81.3%). Bites on the lower extremities were 13 (16.3%).

33 STUDIES ON PROPHYLAXIS AGAINST HABU SNAKE (*TRIMERESURUS FLAVOVIRIDIS*) BITE WITH HABU-VENOM TOXOIDS IN THE AMAMI ISLANDS, KAGOSHIMA PREFECTURE, JAPAN (10)

HIDEO FUKUSHIMA, KOREBUMI MINAKAMI, YOSHITERU TORIIRE, SHIGEKI KOGA, KATSUMI HIGASHI, HIDEKI KAWABATA, SHOSAKU YAMASHITA, YASUFUMI KATSUKI, MUNEHARU SAKAMOTO[1], RYOSUKE MURATA, CHOKU MATSUBASHI, TOORU KONDO, SEIJI SADAHIRO[2], KIYOSHI OH-I AND HISASHI KONDO[3]

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Between October 1970 and March 1982, 9,618 adults living in the Amami Islands in Kagoshima Prefecture were vaccinated with 0.5 m*l* each of Habu-venom toxoid (mixed toxoid lots 12, 13, 14, 15, 18, 20, 36, 37, C–1, C–2, APF toxoid lot 1).

Basic immunizations were given 2–3 times at four week intervals. Booster immunizations were given thereafter up to six times at intervals from several months to one and two years.

An effective anti-HR1 of higher than 1 u/m*l* in persons at the time of the basic immunization and four weeks after it was found to be 61.8 per cent, and at the time of the booster immunization and four weeks after it was found to be 88.9 per cent. Among these, when toxoids of 18 ImU/m*l* or higher were injected, the anti-HR1 of higher than 1 u/m*l* at the time of basic immunization was found to be 76.2 per cent, and at the time of the booster immunization was found to be 96.0 per cent. And at the time of both the basic and booster immunizations, the anti-HR2 in most cases was higher than 1 u/m*l*.

At the time of the Habu-venom toxoid vaccination, only mild side reactions were observed without any severe allergic reactions.

Among 176 (181 times) snake bite cases, there were three cases of generalized

symptoms (1.7%), 20 cases of necrosis (11.0%), 11 cases of sequelae (6.1%), and one death (0.55%) of a patient with an incomplete series of vaccinations.

From these results, it was found that the Habu-venom vaccinations produced effective serum antitoxins with only mild side reactions, and reduced the severity of the illness of snake bite victims, and was clinically effective.

34 THE CAUSE OF DEATHS DUE TO SNAKEBITE IN ANURADHAPURA DISTRICT OF SRI LANKA

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It is recorded that 800 deaths due to venomous snake occurred annualy in Sri Lanka in which the mortality per 100,000 population was 18 in Anuradhapura. This report concerns the study on cause of death due to snakebite in Anuradhapura.

According to the fatal statistics due to venomous snakes from the government agent of Anuradhapura, we have visited villages and hospitals where the fatal bites occurred during three years from 1981 to 1983. The snakes responsible for bites were identified in 99 cases out of the total of 110 cases in which 44 cases of bite were due to *Bungarus caeruleus*, 28 cases and 26 cases were *Vipera russelli* and *Naja naja*, respectively. One death due to *Hypnale hypnale* was also noted. Certain seasonal patterns in bites were found, although the bites occurred in each month of the year. The highest number of 28 cases or 25.5 per cent were recorded in teen agers and 71 cases or 85 per cent of bites were concentrated between the ages from 0 to 29 years. Bites of male were two times as frequent as those in females. The time of the day of bites showed a characteristic feature by the species of snake; most of the krait bites occurred at night while the victims were sleeping in their house, whereas Russell's viper bites occurred in daylight hours mainly in agricultural field and cobra bites occurred in both day and night time in residence or in field.

The body site of bites indicated characteristic choice of each species of snakes. Clinically, 79 per cent of the victims died at home after the treatment by traditional physician without antivenoms and 21 per cent of the victims visited hospital too late for medical treatment. The polyvalent antivenoms were imported from India, but the degree of their application is not yet satisfactory.

35 PROTEINASE IN THE VENOM OF OKINAWA HABU (*TRIMERESURUS FLAVOVIRIDIS*): PURIFICATION AND PROPERTIES

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A proteinase has been isolated from the venom of Okinawa Habu (*Trimeresurus flavoviridis*) and its enzymatic properties have been investigated.

Crude HR2 fraction obtained by gel filtration of crude venom of Okinawa Habu on Sephadex G-100 was subjected to column chromatography on Bio Rex-70, and the main proteinase fraction was purified further on Sephadex G-75 and Bio Rex-70.

The purified proteinase was homogeneous in SDS-polyacrylamide gel, and was shown to be a single polypeptide having molecular weight approx. 25,000. Its optimum pH was about 9. The enzyme hydrolysed Hide powder azure and azo-albumin more preferentially than H_2-proteinase, the main proteinase from Amami Habu.

Substrate specificity of the proteinase was investigated by the use of several piptide substrates such as oxidized insulin A and B-chains, substance P, physalaemin and synthetic peptides, and the proteinase was shown to be a typical endopeptidase having a relative narrow specificity.

36 HEALTH CONTROL STRATEGY FOR RESIDENTS IN THE TROPICS

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Visiting tropical countries for health control of residents necessitates medical personnels to elaborate selection of items for examination considering amount of works to be done within limited time. Some items of examinations are especially important in the tropics because of its special circumstances. Based on this fact, blood examinations, especially that of white blood cells, are picked up and simple technique for their counting was devised and evaluated.

The principle is based upon dilution method as in normally used counting technique. Peripheral blood is taken with EDTA as anticoagulant, 0.02 m*l* of blood is taken by micropipette and mixed with 0.18 m*l* of Türk's solution prepared in a well of microplate. After mixing thoroughly by micropipette, the mixture was put in the Neubauer's hemacytometer for counting the number of white blood cells. The results obtained by this method showed no difference from those obtained by traditional method and coulter counter.

In the field study, WBC counting by this technique was performed in parallel with preparation of blood films for differential counting of leucocytes. Time spent for all these procedures for 35 samples was 1 1/4 hour.

At the same time, stool examinations were evaluated for their efficacy and time requirement. In examinations for parasite egg detection, positive rate was evidently higher in concentration method using saturated saline than direct smear. Time for processing 35 samples for concentration technique was three hours, and the test for detecting occult blood using commercially available kits of "Fecal Occult Blood Slide" required two hours for the same number of samples.

Other items of examination though to be important for the health control in the tropics were discussed.

37 ENQUETE STUDY FOR HEALTH OF THE JAPANESE, RESIDENT ABROAD IN AFRICA

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In 1982 we have conducted a traveling health consultation for Japanese residents in Africa. On that occasion, questionnairing on their health and sanitary conditions in the locales was held, and the following results were obtained.

The ratio of receiving health examination before coming to Africa was 82 per cent, not definitely so high. Many residents did not take periodic examinations even after coming there.

Health consultation by our team was highly evaluated, but concerning endemic diseases, medical institutions in the locales should have been utilized more. Especially malaria is a horrible endemic, but those who took no oral prophylactics periodically were many, suggesting the necessity of further enlightenment.

As to their precautions against sanitary conditions, they did not drink unboiled water or eat raw foods such as vegetables, keeping a full lookout.

Such endemic diseases as diarrhea, unknown fever and malaria taking place after arriving there were found to show the first onset within six months after arrival in the majority of cases involved. The duration of six months from arrival may be said to be a preparatory period to get accustomed to the life in the locales.

38 MEDICAL EXAMINATION OF THE JAPANESE STATIONED IN MIDDLE EAST AND NORTH AFRICAN COUNTRIES

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Major diseases and laboratory findings of Japanese staying in Middle East and North African countries were studied. The subject of this study consisted of 254 persons and average period of stay was 248 days.

Diarrheal diseases (93 cases), gastritis/peptic ulcer (36 cases) and lumbago (18 cases) ranked high among the diseases that occurred during their stay in those countries followed by acute hepatitis (7 cases), traffic accidents (6 cases), and hypertention (6 cases). Some diseases (hypertention, diabetes mellitus, peptic ulcer, heart diseases, etc.) seemed to have relation to the diseases contracted in the past or to be aggravation of chronic diseases.

Among abnormal laboratory findings eosinophilia (5.3%), HBs antigen (+) (3.1%) and intestinal parasites (3.8%) were detected frequently upon their return to Japan. Nine out of 52 persons were proved to have anti-hepatitis A virus antibody upon return to Japan and two of them were regarded to be converted cases from negative to positive during their stay.

Results of stool examinations performed in Libya showed that *Entamoeba coli* (15.9%), *Trichuris trichiura* (9.5%), *Ascaris lumbricoides* (8.7%) and *Giardia lambria* (3.2%) had high infection rate among local people, although only two cases of infection with *Trichuris trichiura* and one case of *Ascaris lumbricoides* were detected among 102 Japanese.

The sanitary conditions of the districts where the present investigation was carried out were, generally speaking, good and few serious endemic diseases were present. Although frequency of infectious diseases among Japanese were rather low in these countries as compared with other tropical or subtropical countries, cases of aggravation or recurrence of chronic diseases which were present before their oversea assignment were rather conspicuous.

39 DISEASES OF JAPANESE PEOPLE IN SOUTHEAST ASIA AND INDIA –REPORT OF THE TRAVELLING MEDICAL CONSULTATION SERVICE DISPATCHED BY THE MINISTRY OF FOREIGN AFFAIRS–

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We were dispatched from the Ministry of Foreign Affairs as travelling medical doctors and did health consultations to Japanese people living in Southeast Asia and India. The results are shown in this paper.

1. *Number of consultations and visiting places*

We have travelled for 28 days from Jan. 25 to Feb. 21, 1983 and stayed for 3–4 days in each place. The number of health consultations was 17 in Hanoi, 17 in Vientiane, 89 in Rangoon, 133 in Dacca, 44 in Calcutta, 38 in Madras and 98 in Colombo; the total was 436. In the meantime, vaccinations were done for 51 persons, and finally 487 persons, 243 males and 193 females, received the medical service. The largest population in ages was seen in their thirties, most of them were accompanied by their family, while most males in their forties was staying alone. These phenomena may reflect the educational problem for their children.

2. *Diseases seen in the health consultations*

Most of the diseases seen in the health consultations were the illnesses which also commonly existed in Japan: diabetes mellitus, cardiovascular diseases, chronic liver damage, peptic ulcer or cold etc. In children, hypertrophy of the tonsilles and skin diseases were frequently seen. The latter may be caused by the high temperature and humidity. Fifty per cent of the persons had more or less some disease and we suppose that those persons who had some physical complaint particularly came for the consultation. We observed several cases of tropical diseases: acute hepatitis, bacillary and amebic dysentery, malaria and filaria.

3. *Anamnesis of tropical infectious diseases*

Since tropical infections progress acutely and transitorily, we encountered only several cases in the examinations, but when we asked the anamnesis of the infections, we found that many people had suffered in the past. 1) Regarding mosquito-borne infectious diseases, past infections of malaria were rare in the big cities, but 18 per cent of the examined persons had the anamnesis of the infections in a small city, Vientiane. The anamnesis of dengue fever infection was observed in 12 per cent of the examined in Vientiane and 23 per cent in Colombo; six of them were infected twice. These infections may be related to the geographical feature and weather. In adult, the females were infected more than the males and it would be due to their dressing style and living condition. 2) About orally infectious diseases, six per cent of the examined had the anamnesis of the infections: acute hepatitis, typhoid and paratyphoid fever, bacillary and amebic dysentery and giardiasis, and 14 per cent of the examined had

severe or long-lasting diarrhea. 3) Two persons were bitten by a rabid dog in the past.

4. *Summary*

The diseases among Japanese people living in Southeast Asia and India can be divided into two types: one is the diseases commonly seen in Japan and the other is tropical infection. In the latter cases, mosquito-borne and orally infectious diseases are popular and the knowlege for their prevention is most important.

40 MEDICAL SITUATION OF KILIMANJARO CHRISTIAN MEDICAL CENTRE IN MOSHI, TANZANIA

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The Kilimanjaro Christian Medical Centre is doing ordinary medical therapy, medical training of doctor in rural clinic, education of paramedical staff, inprovement of nutrition in the undernutrition area and activation of communicable diseases control.

There are 420 beds and 11 doctors, 45 nurses and four paramedical staff are working in this hospital. The scope of therapeutic activity is approximately 120 km^2 and the subject population is about 80,000. They have eight departments i.e. internal medicine, surgery, orthopaedics, ophthalmology, gynecology, pediatrics, radiology and dentistry. The number of outpatients is about 400 per day. The total number of hospitalization is 12,512, and death toll was 755 persons in 1981, and the mortality rate was 6.03 per cent.

According to statistical analysis of communicable diseases in this medical centre, morbidity of malaria was significuantly higher. Then, enteritis and other diarrheal diseases, measles, ascaris, tuberculosis of respiratory system, amaebiasis, typhoid fever, anchylostomiasis, schistosomiasis, poliomyelitis and leprosy were reported.

Although they are doing vaccination (BCG, DPT, Polio, measles etc.), only 0.2 per cent of population are vaccinated in this area.

In the hospitalized patients, heart failure has highest morbidity, followed by diabetes mellitus, hypertension, malaria, pulmonary tuberculosis, amaebiasis, peptic ulcer, schistosomiasis, bronchial asthma, anemia. Parasitogenic disease and tuberculosis are outstanding.

In pediatric area, measles has highest morbidity, and kwashiorkor, intestinal helminthiasis, malaria are very common.

41 THE PROBLEMS ON THE HEALTH STATES OF VIETNAMESE REFUGEES DURING THE PAST FIVE YEARS

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The health care of Vietnamese refugees who lived in H. Camp, Shiga Prefecture from May 1977 to April 1982 were studied. The total 322 (adult 192, children under 14 year-old 130, male 210, female 112) of refugees were investigated, resulting as follows:

1) The total number of outpatients was 1,465 and inpatients 21. Moreover, the live births were nine in this period.

2) Forty-two point seven per cent of all outpatients have visited the departments of internal medicine and of pediatrics, and 26.7 per cent of dentistry, 11.0 per cent of urology.

3) Respiratory diseases and gastrointestinal diseases were most common diseases among the refugees, while cardiovascular diseases and anemia were not found. Although 11 cases of syphilis were registered, no case of gonorrhea was detected. It was the one of the most characteristic findings in the Vietnamese refugees' diseases that many cases of urolithiasis were found.

4) Among the inpatients, four malarial cases (all *P. vivax* cases), one amaebic dysentery, one amaebic liver abscess, three tuberculosis (one was died) and four urolithiasis were included.

5) The case rates (per 100,000 population) of outpatients was 1,105, which was lower than that of 15–19 year-old Japanese (2,740). But the cost of medical care (per one population) was 50.8 thousand yen, which was as much as that of 15–44 year-old Japanese (55.8 thousand yen).

42 LOCAL CEREBRAL BLOOD FLOW IN THE PROCESS OF THERMAL ACCLIMATION

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The role of hypothalamic and extrahypothalamic deep body thermosensitivity in central mechanisms of thermal acclimation was investigated in heat- and cold-acclimated rabbits. These animals were subjected to surgical procedures for measuring local metabolism, blood flow and temperature of the hypothalamus (PO/AH)

and mid-brain reticular formation. Blood flow was calculated from the initial slope of the hydrogen clearance curves. Local metabolism of the hypothalamus was detected by an electrical method. Blood flow of hypothalamus increased by 25 per cent during external heating as compared with cooling of control rabbits. After an intravenous injection of LPS-pyrogen, the local metabolism and blood flow of hypothalamus of normal (control) rabbits either increased or decreased, with similar biphasic shifts in hypothalamic temperature. However, the increased and decreased hypothalamic blood flow observed in thermally non-acclimated rabbits (control animals) vanished in heat- and cold-acclimated rabbits during both pyrogen-induced fever and external heating and cooling. This suggests that blood flow changes in central thermosensitive tissues may play an important role in both thermoregulatory mechanisms and in the process of thermal acclimation.

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43 THE STUDIES OF DENGUE VIRUS TYPE 2 IN *TOXORHYNCHITES* CELL LINE (TRA-171) REPLICATION AND MORPHOLOGICAL OBSERVATION

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Arbovirus isolation and identification by the inoculation into *Toxorhynchites*, a non-hematophagous mosquito, has been reported superior to the other methods such as suckling mouse brain or other hematophagous mosquitoes inoculation. Many mosquito cell lines have been established and used for the *in vitro* arbovirus researches. We have also reported some arbovirus replications in the cultured *Culex* mosquito cells.

In the present report we studied the replication of dengue virus type 2 (D-2) using a newly established *Toxorhynchites amboinensis* cell line TRA-171. Prototype strain Tr 1751 and wild type strains BR 006 and BR 116 were used. The former strain was highly passaged and adapted to suckling mouse brain. The latter two strains were isolated from DHF and DSS patients respectively, and identified as D-2 at the Department of Medical Research, Burma. These wild type strains were passaged in minimum and still unadapted and difficult to replicate in suckling mouse brain (ICR strain).

In the D-2 infected TRA-171 cells, cytopathic effects (CPE) acompanied with cell aggregation were observed at 48 hours after infection. These CPE were observed in each virus infected cells. Specific fluorescence was shown at the perinuclear

membrane at the same time. Thereafter these positive area had spread into cytoplasm.

Viral growth in TRA-171 cells was studied by plaque assay using Vero cells as the host cell. Viral titers were raised gradually from two days after infection and reached to 10^{6-7} PFU/m*l* for BR 006, 10^{5-6} PFU/m*l* for BR 116 at five days after infection. These titers were kept until 10 days after infection. The titer of Tr 1751 was reached to the plateau at six days after infection and the titer was 10^{8} PFU/m*l*.

White Tr 1751 showed uniform plaques of 1.2–1.5 mm in diameter, BR 006 and BR 116 showed heterogeneous plaques. The plaque diameter of BR 006 were 1.4–1.8 mm, 0.8–1.2 mm and 0.4–0.6 mm and their ratios were 19, 50 and 31 per cent respectively. Those of BR 116 were 10, 29 and 61 per cent respectively. Those plaques were formed at 37°C. Plaque numbers increased in BR 006 and BR 116 for 10–100 folds with the increased ratio of smaller plaques by changing the incubation temperature to 35°C. These phenomena were reversed at 39°C. The ratio of smaller plaque decreased causing the decrease of total plaque numbers. Plaque numbers and size of Tr 1751 were unchanged throughout the experiments.

Viral particles with 40–50 nm in diameter were found in the D-2 infected TRA-171 cells under the electron microscopy. They were distributed in the cytoplasmic vacuoles and in the tubular structures which were typically found in the arbovirus infected cells. These particles were found only in the D-2 infected cells. We also found some virus like particles unexpectedly both in the D-2 infected and not infected cells. Their sizes were about 10 nm, 15–20 nm and they were distributed in the cytoplasm and the cytoplasmic vacuoles. Larger size of particles of about 100 nm were found in the nucleus. Therefore, TRA-171 cell is useful for the isolation and identification but seems to be unfavorable for the morphological or biochemical studies of arbovirus replication.

The results were summerized as follows. These two wild type strains were heterogeneous in plaque size, were sensitive to temperature and grow in cultured cells but difficult in suckling mouse brain. The prototype strain was homogeneous in plaque size, was not affected by the temperature and grow in cultured cells and suckling mouse brain.

44 ULTRASTRACTURAL STUDIES ON THE INTERACTION OF PERITONEAL MACROPHAGES OF rnu/rnu AND rnu/+ OF SPRAGUE-DAWLEY RATS WITH *TRYPANOSOMA CRUZI*

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We have already found that Sprague-Dawley (SD) nude rats (rnu/rnu) are

highly susceptible to *Trypanosoma cruzi* (Tulahuen strain), although the littermates (rnu/+) had no sensitivity to this parasite. Accordingly, it has been expected that comparison of nude rats with the littermates may enable us to elucidate the mechanism of natural resistance of SD rats to *T. cruzi*. Previous investigations also demonstrated appearance of humoral antibodies, and inhibition of migration of leucocytes in the littermates. On the basis of these observations, immunological-competent cells were transferred from normal rats to nude rats. However, these cells did not elicit resistance to infection with *T. cruzi* in nude rats. Accordingly, in this study, ultrastractural aspects of the interaction of the parasite with macrophages of nude rats and the littermates were investigated, since macrophages appears to have a major role in the initial stage of host defense mechanisms against *T. cruzi* infection. Our studies suggested that there was a significant difference of the fate of the parasite in the parasitophorous vacuoles between nude rats and the littermates. The parasites in the vacuoles of the littermates were more readily killed than in those of nude rats. Moreover, addition of carrageenan diminished the rate of intracellular killing of the parasite inside the vacuoles of the littermates, which resembled *T. cruzi* inside the vacuoles of nude rats.

45 EFFECTS OF ASCARIS-PARASCARIS ANTIGENS UPON MULTIPLICATION OF DENGUE VIRUS IN MOUSE PERITONEAL MACROPHAGE CULTURES

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In our previous studies, we have reported that cultures of mouse peritoneal macrophages (Mø), when treated with certain substances of bacterial origin, such as LPS, peptidoglycans and other cell wall components, produced larger amounts of dengue virus (DV) compared with untreated control cultures of the same cells (H. Hotta, *et al.*, Microbiol. Immunol., 26, 665, 1982; Agus S. W., *et al.* ICMR Annals, 2, 65, 1982; H. Hotta, *et al.* Infect. Immun., 41, 462, 1983). In the present experiments, we examined similar effects, if any, of antigens from parasites (*Ascaris suum* and *Parascaris equorum*) upon the Mø cultures.

Mø's, collected from the peritoneal cavity of BALB/c mice injected intraperitoneally with methylcellulose, were cultivated *in vitro* and inoculated with DV.

Parasitic antigens were prepared as follows: (i) *A. suum*: The body fluid, harvested by puncturing the posterior end of each worm, was filtered through a filter paper and dialyzed against distilled water. (ii) *P. equorum*: The body of worm was cut into small pieces which were then subjected to freezing-and-thawing in 0.1 per

cent NaCl solution; the extract was centrifuged and the supernatant was dialyzed against distilled water. (iii) Those crude materials were passed through a Sephadex G-100 column and four fractions were separated.

The results obtained are summarized as follows:

(1) While the Mø cultures not treated with the parasitic antigens were poorly permissive to the DV infection, the same cultures treated with the antigens produced significantly large amounts of DV.

(2) In experiments in which infectious center assay and anti-DV immunofluorescent stain were performed, it was shown that number of DV-infected cells in the antigen-treated cultures were more than those in untreated control cultures.

(3) The virus production-enhancing effect depended upon the doses of antigens added and also upon the duration of treatment.

(4) The enhancement was suppressed by cotreatment with carrageenan, a specific Mø inhibitor; however, no additive effect was observed in cocultures of Mø and splenocytes from the same line of mice.

(5) The treated Mø's ingested larger number of latex particles and staphylococci as compared with untreated cells.

(6) The four Sephadex fractions showed similar effects as those by the crude extract; a tendency was noted that the fractions I and III were stronger in activity than were the fractions II and IV.

Virologic-immunological significance of the data are discussed.

46 THYMUS ATROPHY IN EXPERIMENTAL MURINE TOXOPLASMOSIS

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Thymi of Nya: NYLAR female adult mice infected with a non-lethal *Toxoplasma gondii* (Cornell strain) showed a marked involution throughout the course of observation period. In comparison with uninfected control, thymic DNA and protein contents reduced significantly in the infected animals. In *in vivo* thymidine incorporation into trichloroacetic acid (TCA)-soluble fraction of thymus, there was no appreciable difference between infected and uninfected animals, whereas thymidine uptake into DNA significantly reduced in the thymi of *Toxoplasma*-infected mice. Thymocytes from *Toxoplasma*-infected mice nine days after infection, incubated *in vitro*, also showed a lower incorporation of radioactive thymidine and leucine into TCA-insoluble fraction than did cells from control animals. In this experiment, there was no difference in cell viability and uptake of precursors into acid-soluble fraction between these two kinds of cells. Moreover, the activity of thymic thymidine kinase, a rate-limiting enzyme for DNA synthesis via salvage pathway, decreased significantly in *Toxoplasma*-

infected mice. However, plasma corticosterone level of infected animals showed no change or slightly lower value than that of controls.

Thymus involution was reproducible by intraperitoneal injection of parasite-free serum or peritoneal fluid prepared from infected mice with a lethal *Toxoplasma* (RH strain), but not with a non-lethal one (CS strain). The activity of thymidine kinase and protein content significantly reduced in the thymi of treated animals on the 7th day post-injection.

Intraperitoneal injection of the parasite-free culture medium of BHK (baby hamster kidney) cell infected with a lethal strain of *Toxoplasma* (RH) into the normal adult mice also resulted in a significant reduction of thymic weight, its DNA and protein contents, its thymidine kinase activity and *in vitro* DNA and protein synthetic activity of the thymocytes.

These observations probably suggest that the toxic substance(s), which may be derived from *Toxoplasma* trophozoite, play an important role on the pathogenic mechanism of thymic atrophy in the toxoplasmosis.

47 BODY DEFENCE MECHANISM IN MICE SENSITIZED WITH TOXOPLASMA LYSATE ANTIGEN AGAINST BABESIA INFECTION

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Toxoplasma lysate antigen (TLA), mouse Babesia lymphokines (B-LKs) and mouse Toxoplasma lymphokines (Tp-LKs), were used to examine the effect of immune adjuvant in reducing clinical symptoms of *Babesia rodhaini* infection in mice.

Normal mice were treated twice at an interval of two weeks an emulsion of TLA in Freund's incomplete adjuvants (TLA emulsion), or a combination of TLA emulsion and B-LKs or Tp-LKs. Two weeks after the second treatment, these mice were infected with *B. rodhaini* (10^2 parasitized erythrocytes/mouse, i.p.). 35.7 per cent (5/14, number of survivals/number examined) of mice treated with TLA emulsion, 53.3 per cent (8/15) of mice treated with a combination of TLA emulsion and B-LKs, and 73.3 per cent (11/15) of mice treated with a combination of TLA and Tp-LKs, survived for more than 20 days postinfection. None of the mice in the non-treated control group survived for more than 12 days postinfection.

The defence mechanisms possibly involved in the group treated with TLA emulsion and a combination of TLA emulsion and Tp-LKs, were examined with respect to macrophage activation. Mice treated with TLA emulsion showed a slight increase of leukocyte count, and O_2^- release of macrophages. The activity of macrophage migration inhibitory factor (MIF) of spleen cell culture supernatant and serum was not increased in this group. The mice treated with a combination of TLA and Tp-LKs showed increased MIF activity and greater macrophage phagocytosis.

These results suggest that treatment with TLA emulsion and a combination of TLA emulsion and B-LKs or Tp-LKs induces a degree of immunity in the host against babesia infection, and that macrophage activation is part of this defence mechanism.

48 SEROLOGIC DIAGNOSIS OF LYMPHADENOPATHIC TOXOPLASMOSIS

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Various serologic tests were performed on the patients and evaluated to determine the serologic criteria for the diagnosis of lymphadenopathic toxoplasmosis (lymph. toxo.). The serum specimens were those from 10 adult cases as lymph. toxo. in which lymphnode biopsies were performed and the histology was consistent with toxoplasmosis, 13 cases of malignant lymphoma and 368 general outpatients. In all but two patients of lymph. toxo., the biopsied tissue and serum specimens were obtained within three months from the onset.

The Sabin-Feldman's dye test (DT) titers of 1: 1,024 or higher were reached in all patients of lymph. toxo. and 80 per cent of them showed the titers of 1: 4,096 or higher. All patients of malignant lymphoma showed the titers less than 1: 64. In the great majority of general outpatients the titers were 1: 256 or lower, the highest titer of 1: 1,024 being shown in only 1.9 per cent.

In the indirect latexagglutination (ILA) test, all cases of lymph. toxo. had titers of 1: 512 or higher and 57 per cent of them had 1: 4,096 or higher. In both groups of malignant lymphoma and general outpatients, no one had a titer of 1: 4,096 or higher.

The immunofluorescent antibody test for IgM (IFA-IgM) titers were positive in all patients of lymph. toxo., and 1: 32 or higher in 63 per cent. The patients of malignant lymphoma were all negative. Although 20.8 per cent of the general outpatients showed the positive titers, those who had 1: 32 or higher were seen in only 4.3 per cent.

In the indirect hemagglutination (IHA) test, considerably large proportion of the general outpatients revealed high titers such as 1: 1,024 or higher.

From these results, serologic test titers for the diagnosis of possible lymphadenopathic toxoplasmosis would be DT$\geqq$1: 1,024, IHA$\geqq$1: 1,024, ILA$\geqq$1: 512, IFA-IgM$\geqq$1: 16, and the titers for the definite lymphadenopathic toxoplasmosis should be DT$\geqq$1: 4,096, ILA$\geqq$1: 4,096, IFA-IgM$\geqq$1: 32.

49 STUDY ON DETECTION OF TOXOPLASMA SPECIFIC IgM ANTIBODY BY ELISA

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The method of the IgM enzyme-linked immunosorbent assay (IgM-ELISA) for the detection of Toxoplasma specific IgM antibody was evaluated in comparison with that of the IgM indirect hemagglutinin test (IgM-IHA) and that of the IgM micro-latex agglutination test (IgM-LA). Toxo ELISA Test Kit (M. A. Bioproducts), which originary contains the alkaline phosphatase conjugated anti-human IgG, was modified, by utilizing the alkaline phosphatase conjugated anti-human IgM (Sigma).

One hundred and eighty-nine human sera, which had 178 IHA-positive sera, were preabsorbed with *Stapylococcus aureus* protein A. And then, they were measured using IgM-ELISA, IgM-IHA, and IgM-LA. The sucrose gradient centrifugation and absorption test with *Toxoplasma gondii* were performed to evaluate the specificity of the IgM antibodies detected. Although both IgM-IHA and IgM-LA revealed false-positive sera, the IgM-ELISA demonstrated no false-positive results.

It was concluded that the specificity and the sensitivity of the IgM-ELISA were higher than those of IgM-IHA and IgM-LA and that the IgM-ELISA method for the serodiagnosis of acute toxoplasmosis is very useful.

50 THE INVESTIGATION OF PREGNANT WOMEN INFECTED WITH ACUTE TOXOPLASMOSIS

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When pregnant women are infected with acute toxoplasmosis, it is said that abnormal delivery is seemed sometimes. But actual condition is not sure in our country still now. Therefore, we have measured toxoplasma IgM antibody (Tp-IgM) using the newly developted Enzyme Immunosorbent Assay (ELISA) for the purpose of serological diagnosis of acute toxoplasmosis, and investigated pregnant women and newly-born infants. 3,728 sera obtained in Nagasaki City from December 1976 to September 1983 were examined. 283 high titer sera were treated by Staphylococcus protein A, and then Tp-IgM was measured by IgM Toxo-ELISA system. Tp-IgM was detected from 66 specimens of 35 patients. 31 cases were pregnant women, and only 23 cases were confirmed their progress. One artifical and one spontaneous

abortion was found, but the rest of 21 pregnant women and children were all healthy. We could not find out the child infected with toxoplasmosis congenitaly. But the following were discovered newly.

(1) Tp-IgM, detected by Toxo ELISA system, was remained for a long term. It seems that, even if, Tp-IgM is detectcd by ELISA, it is not always acute toxoplasmosis.

(2) Elevation of Tp-IgM titer did not appeared in the exacerbation of chronic toxoplasmosis, so that we can judge which is toxoplasmosis or exacerbation of chronic toxoplasmosis by investigating the movement of Tp-IgM.

51 SOME EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMA INFECTIONS AMONG INHABITANTS IN JAPAN

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The total examined for antibodies to Toxoplasma was about 891 human serum samples collected at medical examinations in Miki City, Hyogo Prefecture, Japan, from July through September in 1982. The prevalence of antibodies determined by enzyme-linked immunosorbent assay (ELISA) method indicated its increase with age and its significant difference between males and females of age over 50 years (males, 42.5%; females, 22.8%; $P<0.001$). Irrespective of age and sex, however, mean antibody levels of positive cases were almost equal: the frequency distribution was bimodal and the peak of the positive group ranged from 0.5 to 0.7 in ELISA value for all age and sex groups. This suggests that an equilibrium relationship exists between host immunity and the parasite which is hardly eliminated from the host infected once. The sera collected in urban area had antibodies at a similar degree and frequency to those in rural area, indicating minor epidemiological importance of exposure to contamination from oocyst-infected soil.

To further clarify the factors relating to the acquisition of Toxoplasma infections, all the 891 people were requested to answer a series of questions involved in experiences in breeding cat(s) and in ingesting raw meat. While the prevalence of antibodies among the people who have bred cat(s) did not differ significantly from that of the inexperienced people in every age and sex group, consumption of raw meat significantly increased seropositivity in males more than 60 years old and females more than 50. Consequently, it is concluded that breeding cats itself, though infective oocysts in cat feces are still important, is not a major epidemiological factor under climatic, geographical and ecological circumstances in Miki City, but raw meat comsumption is closely related to the chance of acquiring the infection. Sex difference in antibody prevalence can be partly explained by a significant difference in the raw meat-ingested population between males (41.6%) and females (17.2%).

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