日本熱帯医学会雑誌

第22巻 第1号 平成6年3月

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FOUNDED 1959

日熱医会誌

日本熱帯医学会

Jpn. J. Trop. Med. Hyg., Vol. 22, No. 1, 1994, pp. 1-4

STUDIES ON CHILDHOOD DIARRHOEAL DISEASE IN GHANA

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Received October 18 1993/Accepted January 5 1994

Abstract: Studies on diarrhoeal disease were conducted in Ghana to understand the present status and problems of the disease in developing countries. The World Health Organization has been promoting the Programme for Control of Diarrhoeal Diseases in developing countries, however, the results in the studies showed that the treatment of diarrhoea cases with oral rehydration salts may not be well operated because of insufficient education or training to the public. Poverty was also preventing sick children from receiving adequate treatment at health facilities. Mortality due to the disease was closely correlated with the complications such as malnutrition and measles. A survey on enteric pathogen in childhood gastroenteritis demonstrated that singnificantly more rotaviruses were detected in diarrhoea cases than in those without diarrhoea. Enterotoxigenic and enteropathogenic *Escherichia Coli, Shigella, Salmonella* and *Campylobacter* were identified as the bacterial enteropathogens, however, statistical difference was not found in detection of any bacterial pathogen between children with and without diarrhoea. The results suggested that intensification of primary health care activities to spread appropriate oral rehydration therapy is important for the successful control of the disease.

INTRODUCTION

Diarrhoeal disease is very common illness in African children. The annual incidence in children under five years old was reported to be 4.5 episodes per child per year at rural communities in Ghana (2). Mortality due to the diseases is also still high in Ghana (7). The World Health Organization has intensified the efforts at controlling diarrhoeal diseases since 1980 through the Programme for Control of Diarrhoeal Diseases (CDD). This programme emphasizes the treatment of diarrhoea cases with oral rehydration salts (ORS). ORS has contributed much to reduction in mortality due to diarrhoea, however, the next step is required to achieve better conditions of diarrhoea-associated mortality, morbidity and malnutrition among infants and young children in developing countries.

To identify the problems in management of diarrhoeal disease, we conducted the following studies in Ghana; (I) survey on the knowledge on treatment of diarrhoeal disease in rural cummunities with different economical conditions, (II) epidemiological investigation on diarrhoeal disease in a rural community, (III) study on microbial aetiology of childhood diarrhoea in a children's hospital.

SUBJECTS AND METHODS

Study I. Survey on the knowledge on treatment of diarrhoeal disease

Ninety-three mothers in two rural communities in southern Ghana were investigated for the knowledge on health care such as oral rehydration therapy (ORT) and immunization with a standardized questionnaire by community health nurses in July, 1990. One community, Gomoa Mprumen (GM), is a farm village with high agricultural production by an agricultural project of the foreign organization, while another in the same farm area, Gomoa Onyadze/Otsew Jukwa (GO), is a typical Ghanaian village without affluent farm produce. The results were compared between the two village by x^2 tabulations at $p<0.05$.

Study II. Epidemiological investigation on diarrhoeal disease

Weekly disease surveillance had been conducted in

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a coastal village of southern Ghana, Gomoa Fetteh, during the period from Janaury to December of 1990. The deaths in children under five years of age were continuously recorded by a well-trained community health worker.

Study III. Study on microbial aetiology of childhood diarrhoea

To identify causative agents of acute gastroenteritis in children under five years of age, 225 children with diarrhoea and 64 without diarrhoea were examined for enteric pathogens in their diarrhoea feces. Subjects were recruited at the outpatient department of Princess Marie Louise Children's Hospital in Accra, the capital city of Ghana, from September to November of 1992. Acute diarrhoea is defined as the conditions with episodes of three or more, loose or watery stool in preceding 24 hours and with duration less than 14 days. Specimens collected from the children were examined for bacteriology, virology and parasitology. *Salmonella spp, Shigella spp,* enteropathogenic and enterotoxigenic *Escherichia coli* (EPEC and ETEC), *Campylobacter spp, Yersinia enterocolitica* and *Aeromonas* were cultured by the method recommended by the WHO (10). All bacterial isolates were specified according to their biochemical and serological reactions with commercially available kits. Ratavirus was detected by electron microscopy (3) and enzyme linked immunoassay using commercially available kits (1, 4). *Giardia lamblia, Entamoeba histolytica, Trichuris, Hookworm* and *Ascaris* which can be causes of parasitic diarrhoea (12) were examined by direct smear method and formal-ether concentration method (8). Detection rates of the pathogens in the two groups were campared by x^2 tabulations.

RESULTS

Study I. Survey on the knowledge on treatment of diarrhoeal disease

89 (41/46) % of mothers in GO and 81 (38/47) % in GM were aware of the treatment for diarrhoea cases with ORS as well as immunization and family planning (Table 1). More than 80 % of the mothers in the two villages similarly understood ORS as a treatment for diarrhoea although more mothers in GO realized the benefit of immunization than these in GM ($p<0.03$).

89 % (42/47) of mothers in GM took their children to the health facilities near the village when they had diarrhoea, whereas only 35 (16/46) % in GO had their

Table 1 Mother's knowledge on treatment of diarrhoeal disease

Community	Knowledge on health care (%)			Mothers who take their children with diarrhoea to health facilities (%)
	ORS	Family planning	Immunization	
Gomoa Onyadze /Otsew Jukwa	41/46 (89.1)	44/46 (95.7)	46/46 (100.0) *	16/48 (34.8) +
Gomoa Mprumen	38/47 (80.9)	46/47 (97.9)	37/47 (78.7) *	42/47 (89.4) +

ORS; oral rehydration salts
*; $p<0.03$, +; $p<0.001$

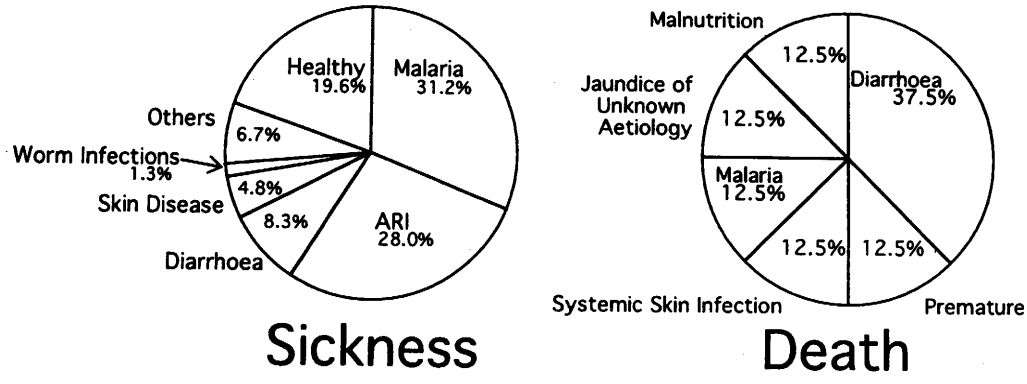

Figure 1 Major causes of ill health and death in children under five years of age in Gomoa Fetteh in 1990

Table 2 Characteristics of the cases who died of diarrhoeal disease

Case	Age at death (month)	Birth weight (kg)	Weight at death (kg)	Complications	No. of siblings alive/dead
1	16	3.0	4.9* (at 13th month)	malnutrition	1/0
2	14	3.2	8.4 (at 12th month)	none	1/0
3	3	1.7	3.4*	low birth weight malnutrition, twin	4/1
4	7	2.4	3.3*	low birth weight malnutrition, twin	6/1
5	11	2.9	7.7	measles	2/2

*; under −2 standard deviation from the average weight at each age in Ghanaian children

children with diarrhoea treated at the clinics/hospitals ($p<0.001$). In most of the cases treated with ORS at home, the duration of ORT or the volume of solution administered was not enough for the effective treatment in the both villages.

Study II. Epidemiological investigation on diarrhoeal disease

Diarrhoeal disease was approximately 8 % of all sickness in children under five years of age and caused 37.5 % of all deaths (Figure 1). Five children died of the disease during the survey period (Table 2). The fatal cases were complicated by low birth weight (40 %, 2/5), malnutrition (60 %, 3/5), twin (40 %, 2/5) or measles (20 %, 1/5). Three of 5 fatal cases had the dead siblings.

Study III. Study on microbial aetiology of childhood diarrhoea

Rotavirus was found significantly more in diarrhoea patients than in controls ($p<0.05$) (Table 3). More ETEC, *Shigella, Salmonella* and *Campylobacter* were detected in children with diarrhoea than those without diarrhoea, but statistical difference was not observed. EPEC and ETEC were isolated at similarly high incidence in the both groups. *Yersinia enterocolitica* and *Aeromonas* were not observed in any group. Detection of parasites was relatively lower as compared to other kinds of pathogen in the both groups. More *Giardia Lamblia* and *Entamoeba histolytica* were likely to be found in diarrhoea patients more than controls.

Table 3 Enteropathogens detected in children with and without diarrhoea

	Children with diarrhoea (%)	Controls (%)
Bacteria		
EPEC	40/225 (17.8)	12/64 (18.8)
ETEC	27/225 (12.0)	6/64 (9.4)
Shigella	13/225 (5.8)	0/64 (0.0)
Salmonella	2/225 (0.9)	0/64 (0.0)
Campylobacter	11/225 (4.9)	1/64 (1.6)
Yersinia	0/225 (0.0)	0/64 (0.0)
Aeromonas	0/225 (0.0)	0/64 (0.0)
Virus		
Rotavirus	34/225 (15.1)	3/64 (4.7)
	*	
Parasite		
Giardia Lamblia	8/225 (3.6)	1/64 (1.6)
Entamoeba Histolytica	4/225 (1.8)	0/64 (0.0)
Trichuris	1/225 (0.4)	0/64 (0.0)
Ascaris	12/225 (5.3)	5/64 (7.8)
Hookworm	0/225 (0.0)	0/64 (0.0)

*; $p<0.05$
EPEC; enteropathogenic *Escherichia coli*
ETEC; enterotoxigenic *Escherichia coli*

DISCUSSION

The results demonstrated that ORS may not be well operated at family level because of the insufficient knowledge on the treatment. The education is an important factor in the treatment for diarrhoea cases. The development of functional system accompanied by social mobilization is essential to promote the programme for CDD successfully. PHC activities may be one of the effective measures to educate the public.

Financial condition seemed to make some gurdians hesitate to take their sick children to health facilities. Mothers in the two villages with different financial conditions showed the different attitudes in treatment for their sick children at health facilities. Poverty is an important factor preventing the adequate treatment for diarrhoeal disease. Construction of infra-structure in each village will improve the villagers' health conditions through the hygienic, dietic and economical improve-

ments.

The investigation on the fatal cases showed that some complications had the influence on the severity of the disease. Measles and malnutrition are known to be closely correlated with mortality of the disease. Persistent diarrhoea and malnutrition form the visious circle of ill health in infants and young children in developing countries. Comprehensive approach including nutritional and hygienic education is necessary for the control of the disease complex.

As reported in developing countries (5, 9, 11, 12), *Shigella, Salmonella, Campylobacter,* ETEC, EPEC, rotavirus, *Giardia Lamblia* and *Entamoeaba histolytica* were detected as enteric pathogens responsible for childhood diarrhoea in this study. Especially, the result proved that rotavirus is an important pathogen of the disease. At present, rehydration therapy is only an effective treatment for viral gastroenteritis. ORT must be properly applied to the children with dehydration due to viral gastroenteritis as a primary treatment at home. EPEC and ETEC were most commonly detected in children without diarrhoea as well as diarrhoea cases. Immunological or nutritional status in the children may be related to the pathogenesis and severity of the disease caused by the bacteria. The study also demonstrated that approximately 40 % of children with diarrhoea had enteropathogenic bacteria. To treat these children with adequate antibiotics, the simple and economical methodology for detection and identification of the bacterial pathogens should be developed and supplied to the developing countries.

Finally, it is impressed through the studies that education to the public and proper treatment for individuals may be important for the successful control of the disease.

ACKNOWLEDGEMENT

This work was supported by a Grant for International Health Co-operation Research from the Ministry of Health and Welfare of Japan (Grant No. 5B-2).

We sincerly thank for the co-operation of Prof. F.K. Nkrumah, Dr. E.A. Afari, Dr. G.E. Armah, Dr. P. Akpedonu, Dr. M.E. Aryeetey and other staff members of Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana.

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Jpn. J. Trop. Med. Hyg., Vol. 22, No. 1, 1994, pp. 5-8

THE GELATIN PARTICLE INDIRECT AGGLUTINATION TEST, A MEANS OF SIMPLE AND SENSITIVE SERODIAGNOSIS OF CHAGAS' DISEASE

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Received November 1 1993/Accepted December 10 1993

Abstract: An indirect agglutination test using a *Trypanosoma cruzi* antigen-coated gelatin particles was employed to diagnose trypanosomiasis in Paraguay. Results with this test were quite comparable to those obtained with enzyme-linked immunosorbent assay (ELISA). Furthermore, nonspecific reaction to the gelatin particles alone was not found in either acute or chronic infection. This method is more convenient than the ELISA, since the antigen-conjugated particles is stable for at least 1 year at 4°C and since the test itself is short and simple to perform and does not require specialized equipment.

INTRODUCTION

Chagas' disease is a chronic parasitosis caused by *T. cruzi*. It is a serious health problem in Latin America where estimated 16-18 million people are infected and 100 million are at a risk of infection (WHO report, 1991). Acute Chagas' disease is generally characterized by fever, chagoma, blood parasitemia, lymphadenopathy and a low level of antibodies in serum. A few patients die during the acute phase. Some resolve to the chronic phase in which fatal cardiomyopathy, megacolon, megaesophagus and neuropathies may occur despite the relative absence of routinely demonstrable parasites in their blood or tissues (Garry and David, 1992), although high levels of antibodies have been detected (Krettli, and Brener, 1976).

T. cruzi infection is usually diagnosed by serological methods, except in the acute stage, where parasite isolation is feasible. The polymerase chain reaction (PCR) has been applied to the diagnosis of Chagas' disease (Russomando *et al.*, 1992) as have serological tests such as complement fixation (File and Kent, 1960), immunofluorescence (Camargo, 1966), hemagglutination (Camargo, 1973) and ELISA (File and Kent, 1960). ELISA and PCR have come into more common use because of their greater sensitivity. However, for ELISA and PCR, specialized materials and reagents such as enzyme-coupled antibodies, micro -ELISA reader, DNA synthesizer, analyzer and primer are required. Recentry, however, an indirect agglutination method using a gelatin particles was used in a screening survey of strongyloidiasis by Sato *et al.*, (1990). This test is sensitive, technically simply and can be performed rapidly without specialized equipment or facilities. This makes it useful for screening and serological diagnosis in endemic areas, especially those in which the electric power supply is unstable. In light of this, we attempted to evaluate whether this method could be used instead of ELISA for the serodiagnosis of Chagas' disease in endemic areas.

MATERIALS AND METHODS

Subjects

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Serum samples were obtained from 40 Paraguayan subjects. Informed consent was obtained from all patients before their involvement in this study. Of these 40 subjects, 10 had *T. cruzi* in their blood, 10 exhibited cardiomyopathy or megacolon but no parasites were detected in their blood, 10 showed only positive antibody response against *T. cruzi* antigen by ELISA and 10 were healthy subjects as controls.

T. cruzi antigen

T. cruzi epimastigotes of RF isolate which was isolated in Paraguay from case of chagas' disease (Mimori *et al.*, 1992) were used to prepare the soluble antigen. Epimastigotes were washed 3 times by centrifugation in phosphate buffered saline (PBS) and then resuspended in PBS containing 1% TritonX-100 (Wako Chemical Co., Osaka, Japan), 1mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St Louis, MO) and 10% glycerol (Wako). The suspension was sonicated 5 times for 30 sec periods, followed by centrifugation at 10,000 x g for 1h. The supernatant was used as antigen.

ELISA

This assay was performed with an ELISA kit as previously reported (Maldonado *et al.*, 1990). Antibody titers were determined as the highest dilution of the test serum which gave an optical density $\geqq 0.8$. Sera showing titers of over 1 : 20 were estimated to be antibody positive against *T. cruzi* antigen.

Gelatin-particles agglutination test (GPAT)

The GPAT was performed as previously described (Sato and Ryumon, 1990). Briefly, *T. cruzi* antigen (200 μg/ml) was conjugated to artificial gelatin particles (Fujirebio, Inc, Tokyo, Japan) treated with 10^{-5}M tannic acid. After conjugation of antigen, the gelatin particles were washed four times with 0.6% inactivated normal rabbit serum (NRS), then lyophilized and kept at 4°C until use. For estimation of agglutination titer, the lyophilized antigen-coated particles were resuspended to make a final 1% suspension in 0.6% NRS. One drop (25 μl) of the antigen-coated particles suspension was mixed in the U-bottomed wells with an equal volume of test serum in serial 2-fold dilutions. After settling at room temprature for 3h, agglutination patterns in the plates were read according to the results in the previous study (Sato and Ryumon, 1990). The antibody titer was determined as the highest serum dilution giving a positive agglutination pattern. Sera showing agglutination titers of over 1 : 16 were estimated to be antibody positive against *T. cruzi* antigen.

RESULTS

The results of GPAT were compared with those of ELISA using sera of patients who have *T. cruzi* in their blood. As shown in Table 1, all patients were antibody positive by GPAT assessment and showed titers ranging from 1:16 to 1:1024. On the other hand, two acute cases with Romana's sign were shown to be antibody negative by ELISA assessment with a cutoff titer of 1:10.

GPAT and ELISA titers were then examined for 10 patients who were parasite negative in their blood but were found having cardiomyopathy or megacolon based on physical examination. The results, shown in Table 2, demonstrated that all patients were antibody-positive in both GPAT and ELISA. Antibody titers using GPAT were generally higher than those with ELISA.

Following the acute phase, most patients remain serologically positive, but asymptomatic, with the absence of demonstrative parasites in either their blood or tissues (Andrade and Andrade, 1979). Sera from persons having neither *T. cruzi* in their blood nor any symptoms, but which were antibody positive by ELISA, were examined to further estimate the antibody titer using GPAT. As shown in Table 3, all sera were antibody positive with GPAT and titers were higher than those determined by ELISA in 8 out of 10 samples.

Finally, sera samples from healthy subjects that were antibody negative to *T. cruzi* by ELISA were assessed by GPAT (Table 4). Ten sera used in this study were also antibody negative to *T. cruzi* antigen by GPAT. Furthermore, the gelatin particles used as a

Table 1 Comparison of antibody titers by GPAT and ELISA of sera of *T. cruzi* positive patients

Case No.	Titer	
	GPAT	ELISA
*1	1:128	1:10
*2	1:64	1,20
*3	1:32	1:10
*4	1:128	1:20
**5	1:128	1:80
**6	1:1024	1:160
***7	1:1024	1:80
***8	1:16	1:40
***9	1:32	1:40
***10	1:64	1:320

*Acute infection with Romana's sign
**Chronic infection with cardiomyopathy
***Chronic infection without lesions

Table 2 Comparison of antibody titers by GPAT and ELISA of sera of patients with lesions but who are parasite negative

Case No.	Titer	
	GPAT	ELISA
*1	1:512	1:320
*2	1:256	1:80
*3	1:128	1:80
*4	1:512	1:160
*5	1:4096	1:320
*6	1:256	1:40
*7	1:32	1:320
*8	1:64	1:40
**9	1:128	1:640
**10	1:1024	1:160

*Cardiomyopathy, **Megacolon

Table 3 Comparison of antibody titers by GPAT and ELISA of sera of subjects without lesions or parasites but who showed a positive antibody response to *T. cruzi* antigen by ELISA

Case No.	Titer	
	GPAT	ELISA
1	1:256	1:160
2	1:64	1:160
3	1:256	1:40
4	1:2048	1:160
5	1:128	1:80
6	1:32	1:40
7	1:512	1:320
8	1:128	1:320
9	1:512	1:80
10	1:512	1:40

Table 4 Comparioson of antibody titers by GPAT and ELISA of sera from healthy subjects

Case No.	Titer	
	GPAT	ELISA
1	<1:16	<1:20
2	<1:16	<1:20
3	<1:16	<1:20
4	<1:16	<1:20
5	<1:16	<1:20
6	<1:16	<1:20
7	<1:16	<1:20
8	<1:16	<1:20
9	<1:16	<1:20
10	<1:16	<1:20

control did not react with any serum sample used in this experiment.

DISCUSSION

For diagnosis of Chagas' disease, either the *T. cruzi* parasite or a serological response to *T. cruzi* antigen must be obtained. Direct detection of *T. cruzi* in blood is possible during the acute phase but is difficult in patients with lower blood levels of the parasite. To overcome this problem, alternative procedures such as xenodiagnosis and hemoculture are used. Xenodiagnosis, however, is expensive, laborious, and limiting with respect to the length time to get results. In addition, the success rate is as low as 40% (Minter-Goedbloed *et al.*, 1978). In hemoculture, the blood sample is incubated in an apropriate medium for the growth of a potential parasite (Minter-Goedbloed, 1978) but a long cultivation period is needed and contamination can be a problem. A modern method using DNA probes allows for rapid and direct detection of the parasite in the blood (Gonzalez *et al.*, 1988). However, it is difficult to perform without specialized DNA probes and equipment.

In Chagas' disease, IgM antibody is first produced as a primary response and then IgG antibody appears 20-40 days after the onset of the acute phase of infection (Schmunis *et al.*, 1980). Many methods have been used to detect these antibodies, including complement fixation (Pereira *et al.*, 1980), hemagglutination (Camargo, 1971), indirect immunofluorescence (Cerisola *et al.*, 1970) and ELISA (Maldonado *et al.*, 1990). These techniques have various advantages and disadvantages based on requirements of special equipment, time required for testing and sensitivity.

GPAT was reported to be a simple and sensitive agglutination test for the screening of strongyloidiasis by Sato (1990). In this study, we confirmed the findings that GPAT is easy to perform and has a sensitivity comparable to ELISA and showed that this method could be used for serological diagnose of Chagas' disease. Furthermore, the antibody titer using GPAT was higher than that with ELISA in 76% of subjects who were shown to be antibody-positive to *T. cruzi* -antigen. GPAT also requires no specialized equipment or facilities and results can be obtained within about 3h. In addition, the lyophilized antigen-sensitized carrier particles can be stored at 4°C for a long period, at least a year or more, without deterioration of the antigen. Moreover, the serum samples can be used without prior absorption by the gelatin particles because these non-

sensitized particles did not react with the 40 samples used in these experiments. The coloured gelatin particles were also found to be convenient for reading the agglutination pattern. Therefore, GPAT should prove to be useful for serodiagnosis of this disease, both in the laboratories as well as in the field.

In Latin America, distributions of *T. cruzi* and *Leishmania* infection often overlap. *T. cruzi* antigen has been shown to cross react with sera from leishmaniasis patients (Chaffee, 1956; Gam and Neva, 1977; Chiller *et al.*, 1990). In view of this, when parasitological examination is negative and symptoms are not characteristic of recent *T. cruzi* infection but the serological test is positive, there may exist the possibility of leishmaniasis or double infection. We examined ten serum samples from subjects without parasites or lesions. These sera were antibody positive to *T. cruzi* - antigen by GPAT and ELISA. However, it is still difficult to conclude whether these subjects had previous *T. cruzi* and/or *Leishmania*-infections. In the future, we will have to provide the specific antigen from *T. cruzi* and develop the specific sero-diagnosis for Chagas' disease.

ACKNOWLEDGMENTS

We would like to thank Fujirebio Inc., Tokyo, Japan (Mr. S. Hanzawa and Mr. Y. Nagafuchi) for supplying the gelatin paticless. We also thank Drs. M.A. Cabello, A.R. Arias and R.M. Azorero, Instituto de Investigaciones en Ciencias de la Salud, Asuncion, Paraguay, for their help in providing serum samples from patients with Chagas' disease. This study was performed as part of a Paraguay/Japan medical cooperation project on Chagas' disease supported by the Japan International Cooperation Agency (JICA).

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Jpn. J. Trop. Med. Hyg., Vol. 22, No. 1, 1994, pp. 9-12

DAILY OBSERVATION OF ANTIBODY LEVELS AMONG DENGUE PATIENTS DETECTED BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Received November 11 1993/Accepted December 21 1993

Abstract: Serial serum specimens from forty eight dengue patients admitted to the hospital on day 2, day 3, or day 4 post onset were examined sequencially by enzyme-linked immunosorbent assay for laboratory diagnosis according to the criteria set by Innis *et al.*(3) Cumulative ELISA positive rates among forty secondary dengue infection patients were 95 % and 100 % at day 6 and day 7 post onset, respectively while the ELISA positive rates at day 3, day 4, and day 5 were 17.5 %, 37.5 %, and 75 %. Cumulative ELISA positive rates among eight primary dengue patients were 87.5 % and 100 % at day 6 and day 7 post onset, while the rates at day 4, and day 5 were 12.5 % and 50 %. Thus, in order to achieve better diagnostic efficiency according to the criteria, convalescent sera should be taken after the 6th day from the onset of the disease. Four out of 74 secondary infection patients, corresponding to 5 % of the patients, showed poor response of dengue-specific IgM antibody (less than 10 units) even when discharged, indicating that both IgG and IgM examinations are necessary in secondary dengue infection.

INTRODUCTION

Dengue/dengue hemorrhagic fever is epidemic in most of the countries of southeast Asia and is an important problem in public health.(2) Because most of the clinical symptoms of dengue infection are not specific to dengue, serological diagnosis is important for confirmation of the etiological agents.

Enzyme-linked immunosorbent assay (ELISA) for dengue was developed by several institutions, and measurement of anti-dengue IgM antibody has been proved to be useful as a rapid and sensitive diagnostic method for acute dengue infection.(1, 3, 4)

In Thailand, an ELISA system produced by Innis *et al.*(3) has been widely introduced and applied by HIN of Thailand for laboratory diagnosis. Although the system is very useful for rapid serological examination than and easier to use than hemaglutination inhibition test (HI), some of the test specimens did not show elevation of anti-dengue IgM and IgG antibodies greater than the cut-off level. We supposed that in such cases the convalescent sera were collected too early to demonstrate antibody elevation. For this paper, we collected serum and plasma specimens from dengue patients every day and observed the antibody levels in order to determine what day after the onset of the disease was most suitable for collecting the test sera for laboratory diagnosis by the ELISA.

MATERIALS AND METHODS

Serum specimen.

Sera were collected from patients at admission to and discharge from the Department of Pediatrics in Nakhornphanom Provincial Hospital, Thailand, in July 1992. Plasma fractions in hematocrit tubes were col-

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Table 1 ELISA positives according to the criteria by Innis *et al.*

	Day admitted post onset of the disease					
	2	3	4	5	6	Total
No. of Cases	18	22	31	10	1	82
No. of Positives	1	6	11	5	1	24
% Positives	5.5	27	35	50	100	29

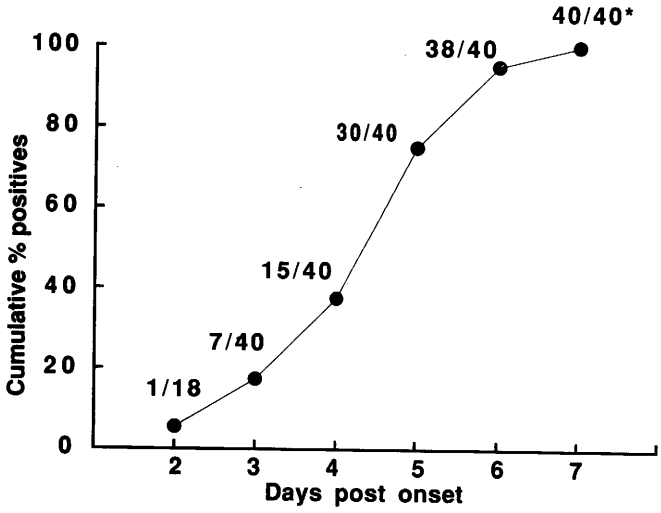

Figure 1 Cumulative ELISA positive rates among forty secondary dengue patients who were admitted on day 2 and day 3 post onset.
*: number of positive patient(s)/Total number of patents.

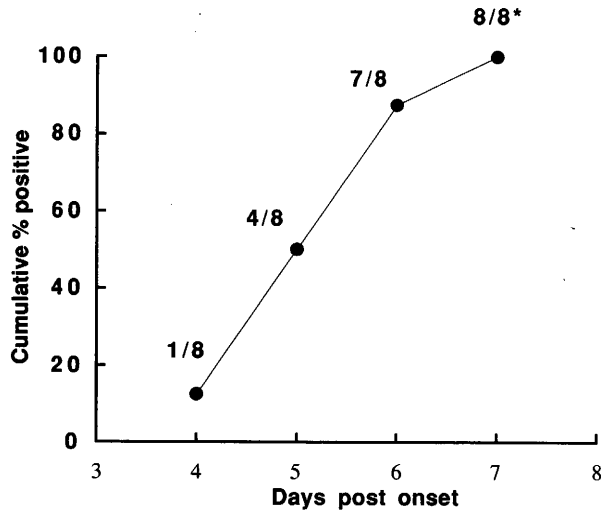

Figure 2 Cumulative ELISA positive rates among eight primary dengue patients.
*: number of positive patient(s)/Total number of patents.

lected every day after routine hematocrit examination. All serum and plasma specimens were kept at −20°C until use.

Enzyme linked immuno sorbent assay (ELISA).

ELISA was performed as described previously. (3) Titered standard positive sera of IgG and IgM were kindly provided by Dr. Innis, AFRIMs in Bangkok.

Criteria of ELISA.

1) Criteria 1.
Daily continuous increase of anti-dengue IgG or IgM antibody.

2) Criteria 2. (Advocated by Innis *et al.*)
The cut off titer for lgG was changed from 40 units to 100 units by Dr. Innis recently. (personal communication)
Negative: Titer of IgM<40 units, and IgG<100 units
Positive: Others
IgM≧40 units and IgG<100 units: Positive (Primary infection)
IgM<40 units and IgG≧100 units: Positive (Secondary infection)
IgM≧40 units and IgG≧100 units: Positive
IgM/IgG<1.8 (Secondary infection)
IgM/IgG≧1.8 (Primary infection)

RESULTS

1. ELISA positive rate of serial specimens.

We followed forty dengue patients diagnosed by criteria 1, who had been hospitalized with secondary dengue on day 2 or day 3 post onset of the disease. Figure 1 shows the cumulative ELISA positive rates from day 2 to day 7, according to criteria 2. ELISA positive rates were 17.5 %, 37.5 %, 75 %, 95 %, and 100 % at day 3, 4, 5, 6, and 7 post onset, respectively.

Figure 2 shows the cumulative ELISA positive rates among 8 patients diagnosed by criteria 1, who had been admitted to the hospital with primary dengue. The ELISA positive rates according to criteria 2 were 12.5 %, 50 %, 87.5 % and 100 % at day 4, 5, 6, and 7 post

Table 2 Anti dengue IgG and IgM units among low IgM responder

Day	Pt1		Pt2		Pt3		Pt4	
(p.o.)	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
3	5*	14	0	19	0	3	—	—
4	0	17	0	23	0	4	2	3
5	0	126	0	105	6	28	4	7
6	1	146	1	121	6	67	5	32
7	8	161	—	—	6	108	6	72
8	—	—	—	—			9	113

p.o.: post onset, Pt1-4: Patients number 1-4
*: units, —: not tested

onset, respectively. All of these patients were diagnosed by IgM-ELISA rather than IgG ELISA, because of the poor and late responce of anti-dengue IgG in dengue primary infections.

These results showed that in order to get more than 90 % effectiveness using the ELISA criteria 2, serum should be taken from the patients after the 6th day from onset.

2. ELISA positive rate at admission.

A total of 82 dengue patients, 74 secondary and 8 primary dengue, were admitted to the hospital during our project. Table 1 shows the ELISA positive cases and rates by criteria 2 on the day of admission. Those who were admitted to the hospital on day 2, 3, 4, and 5 from onset showed 5.5 %, 27 %, 35 % and 50 % ELISA positive rate on the day of admission respectively. These ELISA positive rates were quite compatible with the data in Figure 1. The over-all ELISA positive rate by criteria 2 at admission was 29 %.

3. Low IgM responder levels observed among secondary infection group.

It has been emphasized by several investigators that IgM ELISA is a very useful rapid diagnostic tool for primary and secondary dengue infection.(1, 3, 4)

However, among the 74 secondary dengue cases we observed in this research, four individuals showed very poor IgM responses. All of them showed no elevation of anti-dengue IgM antibody, less than 10 units, even at their discharge. Their IgG and IgM responses are shown in Table 2. These patients numbered about 5 % of the total.

DISCUSSION

It was demonstrated that at least 6 days were required from the onset of the disease for anti-dengue antibody level to rise a sufficient level to be diagnosed by the criteria of Innis *et al.* Therefore, when their ELISA system and criteria are used, serum specimens should be collected after the 6th day from onset in order to achieve better than 90 % diagnostic effectiveness.

The ELISA positive rate at admission that we observed was only 29 %, though Innis *et al.* reported that the IgM-ELISA sensitivity at admission was 78 % in their study. This discrepancy could be explained by the fact that many of the patients in our research area were hospitalized at an early phase of the infection, as was indicated in Table 1.

Examination on sequential serum specimens as in this paper, could provide a confident conclusion on serodiagnosis when daily increase of anti-dengue antibody is observed. Practically speaking, however, for routine laboratory diagnosis examination of a single serum specimen is important. Therefore, it is useful and important to determine criteria for ELISA serological analysis such as Innis *et al.* proposed.

Many investigators have emphasized the usefulness of IgM ELISA for dengue sero-examination even among the secondary infection group.(1, 3, 4) However, we found that 5 % of secondary infection group showed almost no dengue specific IgM antibody responce during their time in the hospital, though IgG antibody was markedly elevated. On the other hand, eight out of eight primary dengue patients were diagnosed by IgM ELISA alone, because of the low responce of anti-dengue IgG. These results suggest that both IgG and IgM ELISA are always necessary for the dengue Ig capture ELISA diagosis.

ACKNOWLEDGEMENTS

The authors appreciate the kind gift of positive serum form Dr. Innis. This work was supported by a Grant in Aid for International Collaberative Research (#

04041082) from the Ministry of Education, Science and Culture of Japan, and by the Japan International Collaborative Agency (JICA).

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Jpn. J. Trop. Med. Hyg., Vol. 22, No. 1, 1994, pp. 13-19

THE PREVALENCE OF MALARIA IN AN ENDEMIC AREA OF BANGLADESH

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Received December 9 1993/Accepted January 20 1994

Abstract: Eight hundred and nine patients with the symptom of fever at a rural health complex in southeastern Bangladesh were studied for the presence of malaria: 48.1% were malaria parasite-positive. Of these patients 71.5% had falciparum malaria and 28.5% had vivax malaria. The 5~9 years age group had the highest percentage of malarial parasite positivity (58.6%). Splenomegaly was found more frequently in children than in adults. By occupation, malaria was most prevalent among woodcutters who worked in forests. Forest dwellers in general had a significantly higher ($p<0.001$) malaria positivity rate than did those persons residing in non-forested areas. Indigenous tribal people had significantly lower ($p<0.05$) malaria-positivity than did Bengalee settlers. Illiteracy and low incomes, customary reasons for failure to practice prevention, were associated with higher prevalence of malaria.

INTRODUCTION

The incidence of malaria in Bangladesh is worsening. The development of drug reistant strains of *Plasmodium falciparum* (*P. falciparum*) as well as generally ineffective chemoprophylaxis may have contributed to this state of affairs (Waiz and Chakraborty, 1986; Waiz and Chakraborty 1990). Malaria is endemic in the districts of Bandarban, Rangamati, Khagrachari, Cox's Bazar, which are located in southeastern Bangladesh, and, in the border area of Mymensingh and Sylhet (Begum *et al.*, 1988). The malaria eradication program started in 1961 (Hossain *et al.*, 1984) faltered during the liberation war of 1971. By the recommendation of the World Health Organization (WHO), this malaria eradication program was changed in 1977 to a malaria control program, which was integrated into the primary health care system (Rahman, 1991).

Monitoring the incidence and prevalence of malaria may identify high risk groups and help to guide malaria control activity. Unfortunately, surveillance and reporting of malaria cases in most malaria endemic countries has been haphazard at best (Oaks *et al.*, 1991). The present study was aimed at obtaining an accurate picture of malaria situation in Kaptai, which is located in the Rangamati district of Bangladesh. Specific questions to addressed included (i) the distribution of different *Plasmodium* species; (ii) the relationship between types of malaria and the age, sex, occupation, race, level of education, and economic status of infected patients; and (iii) the parasite-positive rate and the ratio of *P. falciparum* and *P. vivax* among fever cases.

MATERIALS AND METHODS

Location

Kaptai Thana Health Complex was selected for this study. This area is hilly and forested and located in Rangamati district (Figure 1). The total population within the purview Kaptai Thana Health Complex is 52, 653. The populations are indigenous tribal and non-tribal Bengalee settlers.

Patients

All patients with the complaints of fever attending the out-patient clinic of the health complex were included in the study. Verbal consent was obtained from patients and/or parents of the patients. No patient was taking any chemoprophylaxis for malaria. Information concerning the age, sex, educational qualifications, total monthly family income, occupation, population category, housing conditions were also included in this study, which comprised 809 cases. There were 414 male and 395 females. Ages of the patients ranged from 0.33 to 82 years. Their median age was 19 years. However due to unavoidable circumstances, only the age and population

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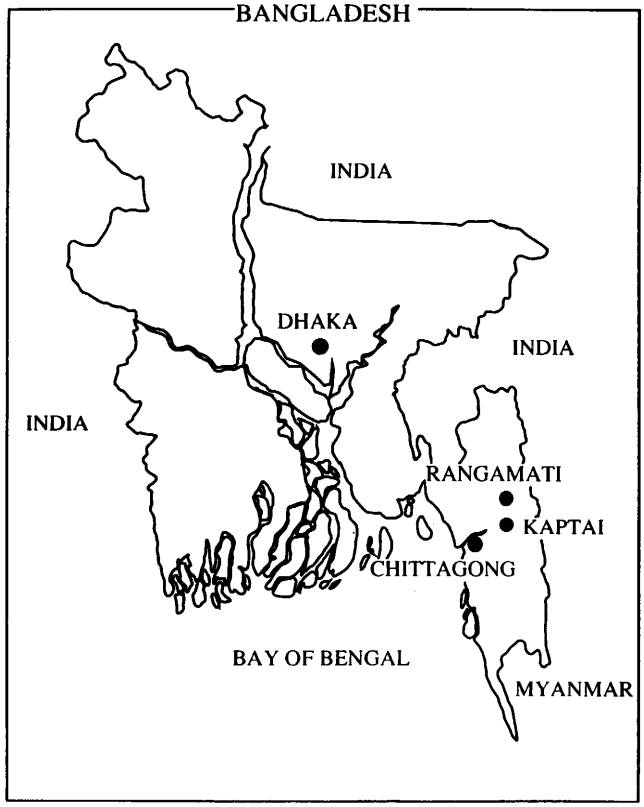

Figure 1 Location of the study place.

information could be obtained in 165 cases.

Diagnosis of malaria

Venous blood was taken from all patients with fever. A smear immediately was made and stained with 3% Giemsa stain. At least 100 fields were viewed extensively under an oil-immersion lens, by a technician and by one of the authors.

Detection of splenomegaly

Abdominal palpation was done by one of the investigators for the diagnosis of splenomegaly.

Statistical analysis

Statistical analysis was done by *Chi square test*

RESULTS

Table-1 shows the distribution of malaria patients by age. Malarial parasites were found in peripheral blood smears in 389 (48.1%) cases out of 809 cases. Among them, 278 (71.5%) were *P. falciparum* and 111 (28.5%) were *P. vivax*. The highest prevalence (58.6%) was found in the age group 5~9 years. The lowest (29.4%) was found in the age group 55 years and older.

In a total of 414 male cases with fever, 209 (50.5%) were malaria parasite (MP) positive; 137 (65.5%) cases were *P. falciparum* and 72 (34.5%) cases were *P. vivax*. In 395 female cases with fever, 180 (45.6%) were MP positive; 141 (78.3%) cases were *P. falciparum* and 39 (21.7%) cases were *P. vivax* (Table-2). Although

Table 1 *Distribution of malarial parasite positive cases by age.*

Age group (yrs)	No. of fever cases	No. of malaria parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
< 1	19	8 (100)	0	8 (100)	42.1
1 — 4	112	43 (75.4)	14 (24.6)	57 (100)	50.9
5 — 9	162	67 (70.5)	28 (29.5)	95 (100)	58.6
10—14	87	26 (61.9)	16 (38.1)	42 (100)	48.3
15—19	86	31 (72.1)	12 (27.9)	43 (100)	50
20—24	92	25 (69.4)	11 (30.6)	36 (100)	39.1
25—29	61	19 (76.0)	6 (24.0)	25 (100)	41
30—34	57	21 (72.4)	8 (27.6)	29 (100)	50.9
35—39	29	10 (66.7)	5 (33.3)	15 (100)	51.7
40—44	29	9 (75.0)	3 (25.0)	12 (100)	41.4
45—49	17	5 (62.5)	3 (37.5)	8 (100)	47.1
50—54	24	8 (88.9)	1 (11.1)	9 (100)	37.5
55<	34	6 (60.0)	4 (40.0)	10 (100)	29.4
Total	809	278 (71.5)	111 (28.5)	389 (100)	48.1

*MP: Malarial parasite, percentage of malarial parasite positive cases among fever cases. Parenthesis is the percentage.

Table 2 *Distribution of malarial parasite positive cases by sex.*

Sex	No. of fever cases	No. of malarial parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
Male	414	137 (65.6)	72 (34.5)	209 (100)	50.5
Female	395	141 (78.3)	39 (21.7)	180 (100)	45.6
Total	809	278 (71.5)	111 (28.5)	389 (100)	48.1

*MP: Malarial parasite, percentage of malarial parasite positive cases among fever cases. Parenthesis is the percentage.

Table 3 *Frequency of malarial parasite positive cases by living environment.*

Living environment	No. of fever cases	No. of malarial parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
Inside Forest	344	141 (68.8)	64 (31.2)	205 (100)	59.6
Outside Forest	300	84 (71.2)	34 (28.8)	118 (100)	39.3†
Total	644	225 (69.7)	98 (30.3)	323 (100)	50.2

*MP: Malarial parasite, percentage of malarial parasite positive cases among fever cases. †Outside forest vs. inside forest, $p<0.001$. Parenthesis is the percentage.

Table 4 *Distribution of malarial parasite positive cases by preventive measures.*

Preventive measures	No. of fever cases	No. of malarial parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
Practiced Mosquito net	217	65 (70.7)	27 (29.3)	92 (100)	42.4
Mosquito coil	1	0	0	0	0
Smoke	65	22 (59.5)	15 (40.5)	37 (100)	56.9
Multiple measures	66	6 (54.6)	5 (45.5)	11 (100)	16.7
Total	349	93 (66.4)	47 (33.6)	140 (100)	40.1
Not practiced	295	132 (72.1)	51 (27.9)	183 (100)	62†
Total	644	225 (69.7)	98 (30.3)	323 (100)	50.2

*MP: Malarial parasite, percentage of malarial parasite positive cases among fever cases.
†Preventive measures practiced vs. not practised, $p<0.001$.
Parenthesis is the percentage.

Table 5 *Distribution of malarial parasite positive cases by occupation.*

Occupation	No. of fever cases	No. of malarial parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
Cultivation	69	29 (65.9)	15 (34.1)	44 (100)	63.8
Woodcutter	28	14 (63.6)	8 (36.4)	22 (100)	78.6
Labour	30	13 (68.4)	6 (31.6)	19 (100)	63.3
Service	24	7 (70.0)	3 (30.0)	10 (100)	41.7
Business	20	4 (80.0)	1 (20.0)	5 (100)	25
Household Chores	156	39 (76.5)	12 (23.5)	51 (100)	32.7
Student	116	31 (58.5)	22 (41.5)	53 (100)	45.7
Non-Occupational (0-10 yrs Children)	165	79 (79.0)	21 (21.0)	100 (100)	60.6
Other Occupation	36	9 (47.4)	10 (52.6)	19 (100)	52.8
Total	644	225 (69.7)	98 (30.3)	323 (100)	50.2

*MP: Malarial parasite, percentage of malarial parasite positive cases among fever cases.
Parenthesis is the percentage.

malaria was found more in the male than the female patients, no statistical significance was found in this study.

Among 644 cases with fever, 344 cases lived within the forest and 300 cases lived outside the forest (Table -3). MP positive was found in 205 (59.6%) cases who lived within the forest, 141 (68.8%) and 64 (31.2%) cases were falciparum and vivax malaria respectively. MP positive were found in 118 (39.3%) cases who lived outside the forest, 84 (71.2%) cases and 34 (28.8%) cases were falciparum and vivax malaria respectively. The frequency of MP positive cases by living environment was statistically significant ($p<0.001$).

In a total of 323 MP positive cases 140 cases used mosquito preventive measures such as mosquito net, mosquito coil, smoke, and 183 cases did not use them (Table-4). There is significant difference ($p<0.001$) of MP positive cases between mosquito preventive measures practiced and those did not. In practiced cases falciparum and vivax malaria were 93 (66.4%) and 47 (33.6%) respectively. In non-practiced cases 132 (72.2%) were *P. falciparum* and 51 (27.9%) were *P. vivax*.

According to the occupation out of 69 cultivators, 28 woodcutters, 30 laborers, 24 service holders, 20 bussinessmen, 156 persons doing household chores, 116 students, 165 non-occupational and 36 other occupational groups of fever cases; 44 (63.8%), 22 (78.6%), 19 (63.3%), 10 (41.7%), 5 (25%), 51 (32.7%), 53 (45.7%), 100 (60.6%) and 19 (52.8%) cases were MP positive respectively (Table-5).

The most affected group was those with a monthly family income of Taka<1000 (Table-6); out of 48 fever cases, 33 (68.6%) were MP positive cases. In the group with an income of Taka 1001~2000; out of 297 febrile cases, 196 (66%) were MP positive. With family income of Taka 2001~3000; out of 205 febrile cases 72 (35.1%) were MP positive cases. In the income group with Taka 3001~4000; out of 72 febrile cases 17 (23.6%) cases and with Taka 4001~5000; out of 15 febrile cases 3 (20%) were MP positive cases. In the highest family income group earning Taka<5000, out of 7 fever cases 2 (28.8%) were MP positive cases.

Among febrile cases, 274 were illiterate, 180 patients had primary education, 36 had secondary education, 23 had higher secondary education, 4 had graduate

Table 6 *Distribution of malarial parasite positive cases by income.*

Monthly family income †	No. of fever cases	No. of malarial parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
<1000	48	21 (63.6)	12 (36.4)	33 (100)	68.8
1000—2000	297	140 (71.4)	56 (28.6)	196 (100)	66
2001—3000	205	51 (70.8)	21 (29.2)	72 (100)	35.1
3001—4000	72	9 (52.9)	8 (47.1)	17 (100)	23.6
4001—5000	15	3 (100)	0	3 (100)	20
>5000	7	1 (50.0)	1 (50.0)	2 (100)	28.6
Total	644	225 (69.7)	98 (30.3)	323 (100)	50.2

*MP: Malarial parasite, percentage of malaria parasite positive cases among fever cases. Parenthesis is the percentage. † Monthly family income expressed in Taka, 1US $=40.00 Taka (approximately).

Table 7 *Distribution of malarial parasite positive cases by educational qualifications.*

Educational Qualifications	No. of fever cases	No. of malarial parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
Illiterate	274	103 (68.2)	48 (31.8)	151 (100)	55.1
Primary	180	51 (63.8)	29 (36.3)	81 (100)	44.4
Secondary	36	7 (58.3)	5 (41.7)	12 (100)	33.3
Higher Secondary	23	6 (100)	0	6 (100)	26.1
Graduate	4	0	0	1 (100)	25
Infant & preschool	127	57 (78.1)	16 (21.9)	73 (100)	57.5
Total	644	225 (69.7)	98 (30.3)	323 (100)	50.2

*MP: Malarial parasite, percentage of malaria parasite positive cases among fever cases. Parenthesis is the percentage.

Table 8 *Distribution of malarial parasite positive cases by category of population.*

Population category	No. of fever cases	No. of malaria parasite positive cases			% of MP* positive cases
		P. falciparum	*P. vivax*	Total	
Tribal	56	13 (68.4)	6 (31.6)	19 (100)	33.9
Non-Tribal	753	265 (71.6)	105 (28.4)	370 (100)	49.1
Total	809	278 (71.5)	111 (28.5)	389 (100)	48.1

*MP: Malarial parasite, percentage of malaria parasite positive cases among fever cases.
Parenthesis is the percentage.
Incidence of malaria, tribal vs. nontribal, $p<0.05$.

Table 9 *Distribution of enlarged spleen by malarial parasite positive cases.*

Population	No. of fever cases	No. of enlarged spleen cases	No. of malaria parasite positive cases with enlarged spleen		
			P. falciparum	*P.. vivax*	Total
Children (0-10 yrs.)	244 (100)	57 (23.4)	33 (13.5)	11 (4.5)	44 (18.0)
Adults	400 (100)	46 (11.5)	27 (6.8)	11 (2.8)	38 (9.5)
Total	644 (100)	103 (16)	60 (9.3)	22 (3.4)	82 (12.7)

*MP: Malarial parasite, percentage of malarial parasite positive cases among fever cases.
Parenthesis is the percentage.

level education and 127 cases were infant and preschool children (children before the age to enter school); among them 151 (55.1%), 80 (44.4%), 12 (33.3%), 6 (26.1%), 1 (25%) and 73 (57.5%) cases were MP positive respectively (Table-7).

Out of 56 fever cases among the tribals, 19 (33.9%) cases were MP positive and out of 753 fever cases among the non-tribals, 370 (49.1%) cases were MP positive (Table-8). There is statistically significant ($p<0.05$) difference between the occurrence of malaria between the tribals and non-tribals. In the tribals 13 (68.4%) and 6 (31.6%) cases were *P. falciparum* and *P. vivax* respectively. In the non-tribals 265 (71.6%) and 105 (28.4%) cases were *P. falciparum* and *P. vivax* respectively. By analyzing 44 tribal cases with fever we found that out of 12 cases residing outside the forest, 3 cases were MP positive and out of 32 cases residing within the forest, 8 cases were MP positive. Interestingly, in both groups 25% were MP positive.

In a total 644 cases with fever splenomegaly was found in 103 (16%) cases (Table-9). Among children 57 (23.4%) cases had splenomegaly, 44 (18.0%) were MP positive and 13 (5.3%) were MP negative. Among the MP positive cases 33 (13.5%) and 11 (4.5%) had *P. falciparum* and *P. vivax* respectively. Splenomegaly was found in 46 (11.5%) adult cases, 38 (9.5%) were MP positive and 8 (2.0%) were MP negative. Among the MP positive cases, 27 (6.8%) and 11 (2.8%) were associated with *P. falciparum* and *P. vivax* respectively.

DISCUSSION

The present study has been designed to asses the cross sectional picture of distribution of different *Plasmodium* species in an endemic area of Bangladesh. This will virtually reflect the factors associated with the trends of malaria infection. The study was conducted in a forested hilly area. The communications from the residence of the health complex played an important role on patients' availability. Only those who had easy approach to the health complex attended the out-patient clinic for seeking treatment. The rest of the population were naturally dropped out from the study population.

A steadily increasing trend of malaria is being observed in Bangladesh. The slide positive rate (SPR) in Bangladesh was 2.4% and the *P. falciparum* infection was 20.2% in 1978. Whereas in 1982 the SPR reduced to 1.6% and the percentage of *P. falciparum* increased to 40.8%; in 1987 SPR further reduced to 1.2% and *P. falciparum* increased to 57.1% (Begum *et al.*, 1988). However, in 1988 the SPR became 1.3% and *P. falciparum* 61.4%: in 1989 SPR increased to 1.7% and *P. falciparum* 66.3%; and in 1990 SPR more increased to 2.2% and *P. falciparum* was 63.3% (World malaria situation in 1990, 1992). In the present study the parasite rate was 48.1% and, the *P. falciparum* and *P. vivax* proportion were 71.5% and 28.5% respectively. The proportion of *P. falciparum* in endemic area of Bangladesh varied

between 91~100% (Waiz *et al.*, 1989). The study was done in April and at that time the endemicity of malaria is usually low. There are two peaks of malaria incidence in the place of study. One takes place during the pre-monsoon. i.e. in June-July and the other in the post -monsoon i.e. in November-December (Rahman, 1991). As a consequence the distribution of different species of MP in the present study was found to be much lower than expected, although it still presents the endemic picture. In this study among 389 cases of malaria, only three were mixed infection by *P. falciparum* and *P. vivax*. In the results we included the mixed infections under *P. falciparum*. We did not find *P. malaria* infection among the patients (Begum *et al.*, 1988).

The incidence of malaria among different age groups varied on the levels of endemicity. It is believed that in Africa malaria is mainly a childhood disease, whereas in many places of Asia and South America it is a disease of young adult (Oaks *et al.*, 1991). In hyper/ holoendemic areas, malaria incidence is mostly confined to young children. On the other hand, in the zone of epidemic malaria, the disease is more or less distributed among different age groups during the epidemicity (Rahman, 1991). The prevelence of malaria increased gradually with age and the peak was found in the age group of 5~9 years. Then it decreased with the lowest in the 55+ age group. As regards occupation, the non -occupational group that is the children had the high prevalence of malaria and we found that <1 years group 42.1% fever cases were MP positive and all were *P. falciparum*. Our observation is consistent with the comment that there is no evidence for protection against malaria infection during the first months of life (Greenwood, 1991). According to occupation the most affected group was the woodcutters and next the laborers and cultivators had the similar high in prevalence because they are mostly exposed to the forest for their occupation. We also found that MP positive rate were significantly high in those living inside the forest. Apparently due to less exposure to the forest, bussinessmen, service holders and the persons doing house hold chores had less malaria. The prevalence of malaria in male were more than the female though statistical significance was not found. This higher prevalence of male may be due to more exposure to mosquitoes due to work related conditions.

The border areas and the continuous foot-hill areas in the northern, easterm and south-eastern foot-hills including Bandarban, Khagrachari, Rangamati *An. minumus* and *An. dirus* are the responsible vectors (Begum *et al.*, 1988). Fruitless vector control in forest areas is one of the reasons for higher MP positive rate in people living in the forest areas (Oaks *et al.*, 1991). As expected, our study showed that there was significantly less malaria those used vector prevention measures. Due to illiteracy and economic problems many people could not take preventive measures. Income and wealth clearly affect the severity of the malaria problem (Oaks *et al.*, 1991). Researchers have estimated that each case of malaria causes between 5 and 20 days of disability (Van, 1916; Russel and Menon, 1942; Malik, 1966; Conly 1975). In a developing country like Bangladesh this is a severe problem.

In malaria endemic area due to repeated experience of dense parasitemias the spleen progressively enlarge and in holo- or hyperendemis areas most of the children, but relatively few adults, have palpable splenomegaly due to malaria alone (Crane, 1991). In our study significantly high percentage of children with MP positive smear had splenomegaly compared to the adults. All cases with splenomegaly were Bengali settlers. No cases of splenomegaly was found among the indigenous tribal patients.

A lower incidence of malaria was found among the indigenous tribal people than the Bengalee settlers. Among the Bengalee settlers those were residing within the forest had a greater prevalence of malaria than those living outside the forest. This indicates Bengalee settlers has higher susceptibility than the tribal peoples. A similar result was found in Pakistan. There was higher incidence of malaria among Afgan refugees than among the indigenous population (Suleman, 1988). Indians of the Amazon, the pigmies of Central Africa, and aborigines of the Malaysians jungle, traditionally have been unaffected by malaria (Oaks *et al.*, 1991). Due to economic reasons there has been malaria-related morbidity and considerable mortality among the newcomers and the reintroduction or increase of malaria in indigenous population. From this study we can find that the forest-malaria condition in Bangladesh is different, the settlers are more infected than the indigenous population. However, many of these indigenous population have been residing and working within the forest. It deserve to mention here again that no case of splenomegaly was found in the tribal patients with malaria. There are evidences on the effect of HLA variation (Hill *et al.*, 1991) and ABO blood group (Hill, 1992) on the susceptibility to malaria. The factors which made the indigenous populations less susceptible to malaria in this area of Bangladesh certainly deserve further investigations.

Malaria treatment in this area is done to let the

patient survive the current acute episode without nescessarily eradicating the parasitemia (Oaks *et al.*, 1991). We think this type of treatment is not suitable to releive the morbidity and mortality. Because the source of infection will be more, rapid diagnosis and treatment must be done in these areas, because life-threatening disease can develop within hours. Rapid diagnosis and standard treatment must be nescessary for these people.

ACKNOWLEDGMENT

We thank Prof. Masamichi Aikawa, Case Western Reserve University, for his critical comments and review of the manuscript. We also thank Miss Momoko Matsuda for preparing the manuscript.

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Jpn. J. Trop. Med. Hyg., Vol. 22, No. 1, 1994, pp. 21-71

Symposium I Prophylaxis and control for Infectious Diseases in the Tropics

S I-1

DEVELOPMENT OF SHIGELLOSIS VACCINE: CURRENT STATUS AND PROBLEMS TO BE SOLVED

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S I-2

THE PRESENT AND FUTURE CONDITIONS OF TREATMENT AND PREVENTION OF LEPROSY

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According to the WHO, the population of active leprosy patients in the world is estimated at 5.51 million, and about 4 million people are supposed to be physically disabled because of leprosy. Between 600 thousand and 800 thousand new patients are discovered each year. Leprosy still forms a serious problem concerning the communicable diseases in the developing countries in the Tropics.

Multidrug therapy (MDT), which was introduced by the WHO in the early 1980's, is widely accepted in the world because it not only proved to be very effective as a therapy compared to the dapsone monotherapy, but also enables an excellent patient management system. It is said that several millions of patients benefited from this therapy.

The forte of the MDT is that it can significantly suppress the emergence of resistant bacteria by combined use of Rifampicine, strong bactericidal antibiotics, and chemotherapeutic drugs such as Lamprene and dapsone. The duration of chemotherapy is six months for paucibacillary form, indeterminate and tuberculoid leprosy, and two years for multibacillary form, lepromatous and borderline leprosy. The number of registered patients and prevalence in endemic countries have been markedly reduced as the patients were released from the registration after completing the MDT.

The MDT is also thought to be an effective measure against the source of infection since it rapidly reduces the infectivity of the bacteria. In 1991 the WHO General Assembly declared its hope for attaining "the global elimination of leprosy as a public health problem by the year 2000." This optimistic prospect, however, does not seem to be realistic, as the number of new patients has not dropped, contrary to our expectations.

Studies on leprosy vaccine was progressed rapidly in the 1970's. Field studies of the first generation vaccine, BCG vaccine supplemented with killed leprosy bacilli, was conducted in Venezuela and Malawi. The result of Venezuela trial proved that the effect was no greater than BCG alone. The second generation vaccine is still at laboratory phase, and the prospects of practical use still appear to be poor.

In 1991, our group conducted an epidemiological survey on the rate of infection and distribution of leprosy bacilli among inhabitants in South Sulawesi, Indonesia, a known highly endemic area, using serological technique and PCR. The result showed that the infectivity of leprosy bacilli is rather strong, for at least one third of the inhabitants were infected by leprosy bacilli, and the distribution of leprosy bacilli on the surface of nasal mucosa was as high as 8%. It is known that onset of the disease can be prevented by chemoprophylaxis, but we are yet to see the development of new techniques for early diagnosis of high-risk contacts and chemoprophylactic system with reasonable cost performance given the low incidence as 0.05% at most.

Increasing coverage of the MDT and new chemotherapeutics have made leprosy treatment much easier than before. The global eradication of leprosy, however, is not yet feasible as the development of early diagnosis techniques and practical tools for prevention remains to be achieved.

S I-3

DEVELOPMENT OF ADDITIVES THAT CONFER HEAT-STABILITY TO ORAL POLIO VACCINES

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Poliomyelitis is caused by poliovirus, a human enterovirus that belongs to the *Picornaviridae* family. The poliovirion is an icosahedral nonenveloped particle that is composed of 60 copies each of four capsid proteins VP1, 2, 3, and 4, and a single-stranded RNA genome. Chemical and three-dimensional structures of the poliovirion have been elucidated. Depressions, called canyons, have been observed on the surface of the virion, and have been suggested to be attachment sites for specific cellular receptor. There is a hydrophobic pocket on each canyon floor.

Poliovirus infection is initiated by binding of the virus to a specific cell surface molecule that serves as poliovirus receptor (PVR). Interaction between poliovirus and PVR destabilizes the virion particle. Indeed, the binding leads to the formation of "A-particles" of 135S that do not contain the capsid protein VP4. These "A-particles" are considered to be intermediates during the virus uncoating process, and are easily converted to 80S particles that do not have the genomic RNA as well as the capsid protein VP4. Thus, PVR appears to have a dual function, that is, destabilization and binding of the virus.

To control poliomyelitis, attenuated poliovirus strains of all three serotypes, 1, 2, and 3 have been developed and effectively used as oral polio vaccines (OPV), [Sabin 1 (serotype 1), Sabin 2 (serotype 2), and Sabin 3 (serotype 3)]. These OPVs are being employed for the global eradication of poliomyelitis by the year 2000. However, the Sabin-derived OPVs have serious deficiencies for this purpose. Heat-sensitive nature associated with OPV is one of these deficiencies, since the cold-chain for OPV transportation is not perfectly established in the world. Most heat-sensitive is the Sabin 3 strain of type 3 poliovirus. Thus, it is desirable to develop additives that are able to make OPV, especially the Sabin 3 strain, heat-stable.

The structure of heat-inactivated OPV resemble that of 80S particles. In fact, heat-inactivated OPV do not have the capsid protein VP4 and the genomic RNA like as 80S particles. Thus, specific inhibitors of the viral uncoating process may be stabilizers of the virus at elevated temperatures. Two strategies have been pursued to develop such stabilizers. One is to make mutant PVRs that do not mediate the viral uncoating but bind to poliovirus. Such PVRs may be able to block the canyon structure, resulting in stabilization of the virus. The other is to find WIN compound derivatives that are able to prevent distortion of the hydrophobic pocket, since this distortion is considered to be essential for the viral uncoating process.

To examine the first strategy, we have prepared a cDNA encoding human PVR (hPVR). Molecular genetic analysis of hPVR revealed that the N-terminal immunoglobulin-like domain (domain 1) is essential for poliovirus binding and infection to cells. To identify amino acids involved in interaction with virus, we constructed a number of cDNAs encoding mutant hPVRs whose domain 1 were partially derived from mouse PVR homolog (mPVR), that does not serve as a binding site for poliovirus. Poliovirus binding and infection assays were performed on mouse L cells that express these chimera cDNAs. Consequently, five mutants involving amino acids Gly73, Ser74, Gln82, Leu99-Glu102, and Gln130-Ser132 were thought to contain possible hPVR contact residues for poliovirus. However it was failed to find mutant PVRs that did not mediate the infection but bound to poliovirus. Thus poliovirus binding activity appears to be always associated with functional PVR activity.

As for the other strategy, we synthesized WIN51711 (A compound) and four its derivatives (B-E compounds), and tested them for protection capacity of the Sabin strains at 45°C. After incubation at 45°C for 24 hours, titer of the Sabin 3 strain was decreased to 10^{-3}, whereas those of the Sabin 1 and 2 strains to $10^{0.5}$. Of five WIN compounds, five to ten μg/ml of compound E showed a stabilizing activity on the Sabin 3 strains. In the presence of compound E at indicated concentration, virus titer of the Sabin 3 was reduced only to $10^{0.5}$ at 45° C for 24 hours. The compound E did not show stabilizing activity on the Sabin 1 and 2 strains under the same condition. Thus it was succeeded to develop an additive to stabilize the Sabin 3 activity at an elevated tempera-

ture of 45°C. Since viral uncoating mediated by hPVR was blocked by compound E, it is likely that the compound E enter into the hydrophobic pocket on a floor of the canyon.

S I - 4

DEVELOPMENT OF MALARIA VACCINE: CURRENT STATUS AND PROBLEMS TO BE SOLVED

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Combat against malaria has centered around vector control and chemoprophylaxis. However, *Anophiline* resistance to various insecticides and wide spread of *Plasmodium* resistance to currently available antimalarial drugs have caused serious problems on malaria control programs in many endemic areas. As an alternative measure to control malaria, vaccines are urgently needed. Here, current status of development of malaria vaccine against *P. falciparum* is described.

The following stages are the target of malaria vaccine: sporozoites, infected hepatocytes, merozoites, infected red cells, and sexual stages. (1) An immunodominant protein called circumsporozoite (CS) protein covers the entire surface of the sporozoite. The CS protein contains 40 repeats of tetrapeptide NANP at the central part. Experimental immunization of X-irradiated sporozoites, which induces protective immunity in humans has shown that antibodies produced against sporozoites are almost directed toward the NANP repeats. Anti-repeat antibodies inhibit the sporozoite entry into a hepatocyte in culture and neutralize the sporozoite infectivity. These findings stimulated clinical trials of subunit vaccines based on the NANP repeats. However, the CS vaccines turned out to be less immunogenic and provoked poor protection against sporozoite challenge. To improve the CS subunit vaccine, universal T cell epitopes have been incorporated to overcome genetically restricted immune response. Nevertheless, antibodies against the NANP repeats alone are insufficinet to induce protective immunity. (2) Liver cells harboring parasites can be destroyed by cytotoxic T lymphocytes (CTL) after immunization with X-irradiated sporozoites. It is presumed that CTL epitopes derived from intrahepatocytic parasites are presented on the hepatocyte surface with class I MHC and recognized by CTL. The Cs protein contains only one CTL epitopes with polymorphic sequences. Therefore, further identification of candidate antigens at the exoerythrocytic stage is necessary. (3) Merozoites have antigens which induce protective immunity in animal models at the surface (MSP1, MSP2) and in the rhoptries (RAP). MSP1 is a strong candidate: anti-MSP1 antibodies inhibit merozoite entry into a red cell in culture and immunization with MSP1 or MSP1-derived polypeptides protects experimental monkeys from challenge infections. Although MSP1 exhibits antigen diversity, sequence variation is not polymorphic but dimorphic. Thus, vaccines based on MSP1 seems to be feasible. Vaccine aimed at blood stage antigens will reduce the morbidity of malaria and eventually induce clinical immunity against natural infection. (4) Among antigens expressed at the surface of infected red cells, PfEMP1 appears to mediate adherence of infected cells to the endothelial cells. Vaccines based on this antigen will prevent cerebral malaria. However, imunity to antigens epressed at the surface of infected red cells is often specific to parasite isolates, probaby due to antigenic variation and antigen diversity. (5) Antigens expressed at the surface of sexual stage parasites are the target of transmission blocking immunity. When antibodies against Pfs230, Pfs48/45 (gametocytes and gametes) or Pfs25 (zygote and ookinete) are taken up by the mosquito vector, they disturb fertilization of gametes and/or subsequent development. Meanwhile, a cocktail vaccine SPf66, a synthetic polymer consisting of two NANP and oligopeptides derived from three merozoite antigens including MSP1, has been designed by Patarroyo's group. Following Phase I clinical tests, field trials have recently been conducted in an endemic ares of malaria in Colombia. Significant protection in SPf66-immunized groups has been observed in a follow up period.

Several vaccines tested to date in humans are not fully satisfactory. Development of effective subunit vaccines requires a method for long lasting presentation of epitopes, development of safe, effective adjuvants and

strategies to manage antigenic variation and antigen diversity displayed by certain antigens. In addition, further identification of relevant epitopes and a method of immunization that induces an optimal immune response will be necessary.

S I-5

DEVELOPMENT OF AN ANTHELMINTIC IVERMECTIN DERIVED FROM A MICROORGANISM

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Ivermectin (Mectizan) is attracting attention as a curing and preventive medicine for a serious tropical disease, onchocerca infectious disease, presuming that there are 18 millions of infectants, and 340 thousands of these have become already blind. The circumstances of discovery, biosynthesis and epidemic study including our own investigations for the compound will be discussed.

Avermectin is a secondary metabolite isolated from culture broth of *Streptomyces avermitilis* as a compound possessing antiparasitic activity in a series of collaborative studies with Merck Co., Inc. in U.S.A. that started by the speaker's offering.

It was found at first that avermectin had antiparasitic activity in an infecting system of *Nematospiroides dubius* in mice, after that the compound was showed to possess also antitick and insecticidal as well as antinematoda activities. Avermectin consists of several kinds of components, but dihydro derivative of avermectin B1a has the strongest activity, and so has been widely used as the drug for animals. At that time when the utilization of ivermectin began as an antibiotic for livestock, the efficacy of the compound on onchocerciasis or river blindness was reported. Ivermectin decreased remarkably microfilaria of *Onchocerca volvulus* in skin by orally administration at 150 mg/kg. According to epidemic studies until now, it was clarified that the administration of only one tablet (6 mg, 150 μg/kg) a year of ivermectin (Mectizan®) by oral shows striking effect on the worm infection and prevents newly infection. About 15% of the patients taken the drug complained of by-effects such as muscleache, fever and chill, however, most of these side effects are considered to be due to the immune reaction by the corpses of microfilaria. At present the exterminating plan against the disease is performing by using ivermectin at each place under supervising by WHO.

Ivermectin is considered to act as a GABA agonist by binding to synaptic membrane-specific binding protein which exists in the worm and, as a consequence, inhibits neurotransmitter.

We have been working on the selective production of avermectins and new avermectin analogues by gene manipulation of *Streptomyces avermitilis*. One of expected recombinant strain K2038 obtained by genetic recombination between two mutants of *S. avermitilis* K2034 and K2021 produced two kinds of effective components of avermectins B1a and B2a. However, the strain still produced useless and toxic compound oligomycin, too, which is a specific inhibitor for oxidative phosphorylation system in mammalian cells. We planned to isolate oligomycin-nonproducing mutants by transposon-induced mutagenesis. Out of 2,400 clones containing transpositions, two oligomycin-nonproducing mutants transposed by Tn*4560*, a derivative of the transposon Tn*4556* in *S. fradiae*, in the gene for oligomycin biosynthesis were isolated from wild type strain. Furthermore, we could transfer the oligomycin-nonproducing phenotype to the selective producer K2038 by gene transreplacement technique. The resulting transreplacement clones produced only two components of avermectins B1a and B2a but not oligomycin because the gene cluster for oligomycin biosynthesis was disrupted by insertion of Tn*4560*.

Symposium II Diarrhea in the Tropics

S II-1

THE COURSE OF 7TH CHOLERA PANDEMIC AND ITS PRESENT SITUATION

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Cholera is a disease due to the infection of cholera vibrios and characterized by a profuse amount of watery diarrhea. If the diarrhea start in the morning and if the patients are left as they are, the patients will die of dehydration in the evening. Of course, the proper treatment completely save the patients, but there is no insurance that the patients can get the proper treatment wherever they are.

Cholera has been an endemic disease in Bengal district of Indian subcontinent. It had occasionally produced the pandemics in 1800s. The present pandemic of El Tor cholera originated from Sulawesi island, Indonesia. El Tor cholera was recognized to be an endemic disease in Sulawesi before 1960, and the disease started to spread out for the other area in 1961. Although more than 30 years has passed since then, the epidemic has no signs to cease. Far from that, number of patients and infected area are still increasing.

El Tor cholera came out of Sulawesi in 1961 and the epidemic appeared in Java, Sumatra, Kalimantan, Philippines and New Guinea up to 1962. The cholera invaded into Asian continent in 1963 and it reached Bengal district, in 1964, where classical cholera has traditionally been endemic. Therefore, 2 kinds of cholera coexisted there for a while. The epidemic potential of the invader was superior to the native cholera. Classical cholera cases gradually decreased and finally had disappeared by the year 1973. During these period, El Tor cholera was further spreading to the Middle East countries, and occasionally small epidemics occurred even in some European countries.

And almost all African countries fell into epidemic area by the year 1972.

The invasion of cholera into American continent was being very worried, but no cholera case was reported in the American continent until 1991 except a small number of cases with autochtonous cholera in the United States of America. In the period between 1973 and 1991, cholera map in the world was relatively stable but the Pacific Islands were newly infected. In January 1991, large outbreak of cholera suddenly appeared in Peru and was rapidly spreading to the neighboring countries. Number of cholera cases reported was about 400,000 within the year and the case fatality rate was about 1%.

It looks far from the end of 7th cholera pandemic. Nevertheless "New cholera" due to *Vibrio cholerae* O139 emerged in October 1992. The new cholera appeared in India and is rapidly spreading to the neighboring countries. It is said "Before ending 7th cholera pandemic, the 8th pandemic has started."

During the past three decades, the research on cholera dramatically advanced not only in bacteriology of *Vibrio cholerae* O1 but also in clinical, pathophysiological and epidemiological fields. Nevertheless, the infected area and the number of cholera patients have been intermittently increased to date. However, the fruits of study have been applied in the other diarrheal diseases, and the mortality of diarrheal disease in general has markedly decreased in the past 10 years.

S II-2

PATTERN OF DISSEMINATION OF *VIBRIO CHOLERAE* O139 BENGAL IN INDIA AND COMPARISON BETWEEN THE PHENOTYPIC AND GENOTYPIC TRAITS OF SEROGROUPS O1 AND O139

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The appearance of a totally new toxigenic serogroup of *V. cholerae* recently assigned to the serogroup O139 with the synonym Bengal which possesses epidemic and pandemic potential is an unparalleled event in the history of the disease cholera. What were the set of circumstances which triggered this event are unknown for the present. In this report, we present the chronology of events in the emergence of the O139 serogroup, the temporal sequence of its spread in India and a comparative account on the traits of the O139 serogroup as compared to the O1 serogroup.

During the period from November 1992 to July 1993, 972 strains of *V. cholerae* tentatively designated as non -O1 were submitted to the National Institute of Cholera and Enteric Dieases from 28 places in India for confirmation of identification. Of these 876 90.1%) were confirmed to belong to the O139 serogroup using specific antiserum, 56 (5.8%) belonged to the O1 serogroup and 40 (4.1%) of the strains did not agglutinate with either the O1 or O139 antisera. Majority (64.8%) of the strains of *V. cholerae* confirmed to belong to the O139 serogroup was submitted from the state of Tamilnadu (southern India) followed by Maharashtra (20.3%). As of date, *V. cholerae* O139 has been isolated from 13 states and an Union Territory (Delhi) in India. Within a span of 10 months starting from October 1992, most of the cholera endemic areas in India have been invaded by the O139 serogroup. The pattern of entry of O139 *V. cholerae* and the propensity of replace the existing eltor O1 serogroup of *V. cholerae* in some places is reminiscent to the entry and dispersal of the eltor *V. cholerae* O1 in India almost three decades ago.

Serological characterization revealed that none of the six representative strains of *V. cholerae* O139 from India (5 strains) and from Bangladesh (1 strain) were agglutinated by polyvalent O1 antiserum, by monoclonal antibodies prepared against factors A, B and C of *V. cholerae* O1 or by antisera prepared against the 137 (O2 to O138) non-O1 serogroups. While dissimilar in the somatic antigen composition, our studies on the composite traits reveals that there are several similarities between the O139 and O1 serogroups. The most prominent similarity is that cholera toxin (CT) produced by the O139 serogroup is identical to that of prototype CT produced by the eltor O1 serogroup. This was evident since polymerase chain reaction with two sets of highly specific and sensitive primers amplified the CT gene from all the strains of O139 examined and the nucleotide sequence of the CT gene cloned from a strain of O139 was identical to that of O1 eltor. Additionally, all the strains also hybridized with DNA probes specific for CT and for the zonula occludens toxin but did not hybridize with a DNA probe specific for the *nag-st* gene. Further, with the exception of susceptibility to 10 and 150 μg of vibriostatic agent, all other biochemical and physiological tests conducted on 165 strains of O139 originating from India and Bangladesh evoked universally positive or negative responses and were similar to that exhibited by the O1 serogroup of *V. cholerae*. Like the O1 serogroup, O139 belonged to Heiberg group 1. However, pulsed-field gel electrophoresis of *Sma*I-digested genomic DNAs showed that strains belonging to the serogroup O1 of *V. cholerae* showed different restriction fragment length polymorphism (RFLP) profiles as compared to those belonging to the serogroup O139. The RFLP pattern exhibited by O139 strains of *V. cholerae* isolated from India, Bangladesh and Thailand were indistinguishable proving the pandemic spread of an identical clone.

S II-3

ESCHERICHIA COLI DIARRHEAS IN TROPICAL AREAS

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Escherichia coli is one of the predominant species forming normal flora of the intestine. Most strains in this species are non-pathogenic, but some have acquired a diarrheagenic disease-producing ability. A group of *E. coli* causing such diarrhea is comprehensively referred to as diarrheagenic *E. coli* (or pathogenic *E. coli* or enteropathogenic *E. coli* in a broader definition). Currently, there are five categories of diarrheagenic *E. coli*. These include: (1) Enteropathogenic *E. coli* (EPEC), (2) Enteroinvasive *E. coli* (EIEC), (3) Enterotoxigenic *E. coli* (ETEC), (4) Enterohemorrhagic *E. coli* (EHEC or VTEC) and (5) Enteroaggregative *E. coli* (EAggEC).

These diarrheagenic strains are not distinguishable from those of normal flora in terms of morphology, biochemical reaction or culture properties, but they produce pathogenic factors, such as toxins, invasive factors and colonization factors, which the normal strains do not. Thus various detection methods developed for diarrheagenic *E. coli* are directed to the virulence factors. Biological methods are important especially for examining uncharacterized virulence factors, but they require animals, tissue cultures and intensive labors and time. Immunological methods, if specific antiserea become available, are usually simpler and more practical. DNA hybridization and PCR techniques are introduced rather recently in this field and seems to be very useful. These techniques are successfully applied for detections of diarrheagenic *E. coli* virulence factors such as VT1, VT2, LT, ST, IpaB (EIEC) and CFA/I, II, III and IV.

These detection methods were applied in survey studies on *E. coli* diarrheas in tropical areas. In Kenya (a JICA project), diarrheagenic *E. coli* was found to be a most prevalent cause among diarrheas at Malindi (a seaside village in Kenya). We are also surveying traveller's diarrheas returning from tropical countries. From these studies enterotoxigenic *E. coli* was found to be major cause of traveller's diarrhea and other types of diarrheagenic *E. coli* (EPEC, EHEC, and EIEC) were rare.

S II-4

COLONIZATION OF BACTERIAL ENTEROPATHOGENS ON THE INTESTINAL MUCOSA

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When bacterial enteropathogens are orally administered to volunteers, a high level of cell number is observed in stools for a prolonged period. This phenomenon is thought, generally, to be bacterial colonization. Bacterial factors directly associated with this phenomenon are called colonization factor (or adhesin). Colonization factors are in many cases bacterial surface pili or outer membrane proteins. Colonization represents the first step of bacterial infection and in some cases it may be an infectious mechanism to evade the host's local mucosal immunity. Eventually there exist colonization mechanisms unique to each bacterial infection. This presentation is concerned with the interactions between bacterial enteropathogens and human intestinal mucosa, focusing on the colonization mechanism of (e.g.) enteropathogenic *Eschercihia coli* (EPEC) that has been well understood with rapid accumulation of new knowledges.

EPEC and related E. coli. Clinically, EPEC illness is characterized by diarrhea with prominent amounts of mucus but without gross blood, vomiting, malaise, and fever. EPEC illness tends to persist for >2 weeks and to be clinically more severe than other diarrheal cases such as rotavirus or enterotoxigenic *E. coli* (ETEC) infections in infants. Historically, *E. coli* of some

unique O:H serotypes have been recognized as the causative agents. In 1979, Cravioto *et al.* found that some EPEC strains adhere to tissue culture cells, and later, Scaletsky *et al.* (1984) demonstrated a unique adherence pattern of EPEC, called localized adherence (LA). Levine (1987) has suggested to refer to EPEC of this unique adherence pattern as class I EPEC and distinguish from other EPEC, on the basis of their data that class I EPEC actually causes diarrhea in volunteers. Since this, class I EPEC has been a major taret in EPEC study. Class I EPEC has an adherence-mediating plasmid called EAF. The EAF plasmid carries genes for hemagglutinin (MRHA) and pili (BFP) that are responsible for EPEC autoagglutination as well as EPEC adherence to tissue culture cells (and intestinal mucosa). The receptor has been shown to be asialoGM1. Attached EPEC, then, causes cytoskeletal lesions at the site of bacterial attachment with an accumulation of filamentous actin beneath areas of close bacterial attachment. This lesion, called "attaching and effacing", is mediated through expression of the EPEC chromosomal gene, *eae*. The *eae* gene product has been shown to be 94-kda outer membrane protein (called intimin). In addition to actin, myosin, α-actinin, talin, and ezrin have been demonstrated at the accumulation site. In the infected cells, phosphorylation of proteins takes place and intracellular Ca^{2+} concentration increases. Class I EPEC adheres to human small intestinal mucosa similar to the cases on tissue culture cells. At the EPEC infection site (on the villi), the microvilli of the absorptive cells are markedly elongated, and lock the attached EPEC (by wrapping the EPEC with the elongated microvilli). EPEC that is able to tightly attach to the surface of the villi, however, will not adhere to M cells (antigen-sampling cells) of the Peyer's patches, as to evade antigen-sampling by M cells on the mucosa. Such evading from antigen sampling may lead to escape from an intestinal local immune system and eventurally may cause chronic infections on the mucosa.

As has been done with EPEC, on the basis of adherence patterns on tissue culture cells, some new categories of diarrhea-associated *E. coli* have also been recognized.

Distribution patterns of M cells in infants and adults. M cells are present in the epithelium of the Peyer's patches as clusters whirling around the cript (the central hole). This unique pattern of M cell distribution ("eddy structure") is discussed in terms of bacterial infections and intestinal immunity.

S II-5

MODE OF ACTIONS OF ENTEROTOXINS PRODUCED BY ESCHERICHIA COLI

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Bacterial diarrheal diseases are very important problem for human health, and many people, especially infants and children, die every year from diarrheal diseases, particularly in developing countries. Among *E. coli.* that cause acute diarrhea, at least five kinds of *E. coli* (enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli.* [ETEC], enteroinvasive *E. coli* [EIEC], enterohemorrhagic *E. coli.* [EHEC], and enteroaggregative *E. coli.* [EAggEC]) have been clearly identified as diarrheal pathogens. Most ETEC produce a heat-labile enterotoxin (LT) and sometimes also elaborate a distinguishable heat-stable enterotoxin (ST). It has been found to be superfically similar to LT and cholera toxin (CT). ST is distinguished from LT because of its resistance to boiling at 100°C for 30 min. Two different kinds of ST have been described. One is STa that is methanol soluble and active in suckling mice and piglets. The other is STb that has been characterized with regard to be methanol insoluble. These ST's are characterized as a low-molecular weight enterotoxin. STa activates the membrane bound form of intestinal guanylate cyclase and mediates a secretory response by increasing the concentration of cyclic GMP (cGMP) within intestinal epithelial cells. Two genetically distinct STa's, that is STp and STh, have been reported and their primary structures with 19 and 18 amino acid residues. Production of STa is mediated by transmissible plasmid. Similar heat-stable enterotoxins are produced by several Gram-negative enteric bacteria such as *Yersinia enterocolitica, V. cholera* non-O1,

Vibrio mimicus, and *Citrobacter freundii.* All heat-stable enterotoxins share the highly homologous sequence of 13 amino acid residues in the cases of STh and STp.

The initial step in the biological action of STa is its interaction with specific high-affinity receptors. The binding of STa to the cell membranes of intestinal epithelial cells of rabbits and rats through these receptors stimulates membrane-bound guanylate cyclase in the cells, leading to an increase in the intracellular concentration of cGMP, followed by activation of cGMP -dependent protein kinase, and culminating in the final biological reaction, inhibition of absorptions of Na^+ and Cl^- in the villi and stimulation of Cl^- secretion in the crypts. Recently, a guanylate cyclase-coupled STa receptor has been reported by several investigators. Schulz *et al.*. determined a novel genetic sequence encoding the STa receptor protein proving it to be a new form of guanylate cyclase (GC-C), of which extracellular region completely differed from those of two guanylate cyclases (GC-A and GC-B) of atrial natriuretic peptide (ANP) receptors. We also have confirmed and characterized the binding capability of GC-C for STa and the marked activation of GC-C by STa using a cloned cDNA of pig GC-C and expressing it on the mammalian cells. Photoaffinity labeling with ^{125}I-ANB -STh[5-18] resulted in a radiolabeled protein with molecular weight of 140,000, suggesting this protein as a glycoprotein. The general features of GC-C are the same as those of GC-A and GC-B. GC-C has a single transmembrane region which divides it in about one half. Intracellular region of GC-C contains a guanylate cyclase catalytic domain and a protein kinase-like domain. Apparent similarity between GC-C and other membrane-bound guanylate cyclases, GC-A and GC-B, has been identified in intracellular regions, while no similarity is detected in their extracellular regions. The protein kinase-like domain of GC-A normally represses the guanylate cyclase activity of GC-A. Binding of ANP to GC-A at extracellular region releases this inhibition of its guanylate cyclase activity. As to activation of intestinal guanylate cyclase by STa, a protein kinase C activator, phorbol dibutyrate, acted synergistically with STa to elevate cGMP in T84 human colon carcinoma cell line. In predicted amino acid sequence of GC-C, there are the consensus phosphorylation sites for protein kinase C, but as yet no direct proof of the phosphorylation of GC-C by protein kinase C.

There is an additional recent report of considerable interest with respect to the function of protein kinase -like domain on activation of guanylate cyclase catalytic domain in the ANP-receptor guanylate cyclases. By examination with chimeric guanylate cyclase made by replacing the protein kinase-like domain of GC-A with the protein kinase-ike damain of GC-C, the protein kinase-like domain of GC-C were not able to regulate the guanylate cyclase activity as those of GC-A and GC -B did. It is very likely that the regulation of guanylate cyclase catalytic domain of GC-C is different from those of GC-A and GC-B.

It is very noteworthy that molecular mode of action of Vero toxin produced by EHEC has been extensively studied. VT inhibits protein synthesis in eukaryotic cells by inactivating 60S ribosomal subunits. VT exhibits an RNA N-glycosidase activity and cleaves the N-glycosidic bond of the adenosine residue at position 4324 from the 5' terminus of the 28S ribosomal RNA of 60S ribosomal subunit resulting in inhibition of its binding to EF-1-dependent aminoacyl-tRNA.

A-1

General Presentation

FEBRILE JAPANESE PATIENTS RETURNED FROM TROPICAL OR SUBTROPICAL AREAS

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From April 1, 1987 to July 31, 1993, 46 patients returned from tropical or subtropical areas were admitted to the Department of Infectious Diseases, Tokyo Metropolitan Bokutoh General Hospital, because of fever, and the diagnosis were unknown on their admission. The final diagnosis of them were as follows: 5 patients were falciparum malaria, 4 vivax malaria, 6 typhoid fever, 6 dengue fever, 5 bacillary dysentery, 4 salmonella enterocolitis, 2 paratyphoid fever, 1 campylobacter enterocolitis, typhoid fever with bacillary dysentery, bacillary dysentery with salmonella enterocolitis, amebic liver abscess, aseptic meningitis, pneumonia, tonsilitis and suspected case of infectious enterocolitis. The diagnosis of other 6 patients were not able to make.

Mean WBC count of falciparum malaria patients on admission was 6,000/mm^3, vivax malaria patients was 5,200/mm^3, typhoid fever patients was 4,900/mm^3, dengue fever patients was 3,200/mm^3 and bacillary dysentery patients was 9,800/mm^3. Mean platelets count of falciparum malaria patients on admission was 9.1×10^4/mm^3, vivax malaria patients was 9.7×10^4/mm^3, typhoid fever patients was 16.5×10^4/mm^3, dengue fever patients was 15.3×10^4/mm^3 and bacillary dysentery patients was 25.9×10^4/mm^3. Meal LDH level of falciparum malaria patients on admission was 757 IU/L, vivax malaria patients was 380 IU/L, typhoid fever patients was 785 IU/L, dengue fever patients was 622 IU/L and bacillary dysentery patients was 343 IU/L.

Malaria patients were treated by the administration of anti-malaria drugs, typhoid fever and bacillary dysentery patients were treated by the administration of antibiotics. Dengue fever was treated only by supportive therapy.

The diagnosis of malaria was confirmed by demonstration of plasmodia in peripheral blood film. The diagnosis of typhoid fever and bacillary dysentery were made by the demonstration of the causative organisims in peripheral blood and stool, respectively. The diagnosis of dengue fever was made by positive serologic test.

A-2

MALARIA CASES AT TOKYO METROPOLITAN KOMAGOME HOSPITAL, 1975-1992

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A total of 109 laboratory-confirmed malaria cases which were seen at the Department of Infectious Diseases of Tokyo Metropolitan Komagome Hospital during the period of 1975-1992 were analyzed. These comprised 67 *Plasmodium vivax* (Pv), 33 *P. falciparum* (Pf), one *P. ovale* (Po), two *P. malariae* (Pm), three mixed (two Pf+Pv and one Pf+Pm) and three unidentified or unknown cases. Malaria cases have been increasing in the last decade. The major proportion of these patients were in their 20s and 30s. There were 101 males and eight females, of which 78 were Japanese and 31 were non-Japanese patients. All were imported cases. Most of the Pv cases were contracted in Southeast Asia and the Indian subcontinent, with 10 cases being contracted

in Africa. Pf cases were reported to have been contracted in Africa but acquired in Southeast Asia in nine cases, mostly in Thailand. Po and Pm were acquired in Africa.

The days from onset of fever to start of effective chemotherapy ranged from zero (therapy started on the same day of onset of fever) to 20 days or more in the Pv cases; in contrast, the days were eight or less in most cases with Pf. Severe and complicated cases were seen in one of the Pv and four of the Pf cases. One patient with Pf died.

Identification of the protozoa was performed microscopically on Giemsa-stained blood smears in most cases (108/109), and serum antibody was determined in 61 cases. Blood cultures were also carried out to demonstrate *Plasmodium* for a small number of patients. Out of the six patients who were seronegative for *Plasmodium* at their first visit to our hospital, most patients (5/6) were seroconverted by day seven and the rest by day 11. One patient with primary Pv infection seroconverted by day 8.

Chloroquine or Fansidar was used in the treatment of most cases of Pv infection. The temperature dropped to normal by day 3 of treatment with chloroquine; in contrast, fever went down by day 4 or later with Fansidar. Fansidar was also used for the treatment of 18 cases with Pf. The decrease in fever was recorded to be at day 4 or later of treatment in this group. In the two patients administered halofantrine, the fever declined on day 5 of medication. Miscellaneous drugs including quinine, Fansidar, mefloquine, qinghaosu or tetracyclines were used mostly in combination for the treatment of Pf.

A-3

A CASE OF FALCIPARUM MALARIA RESISTANT TO PYRIMETHAMINE AND SULFADOXINE

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Chloroquine resistant falciparum malaria is prevalent in many endemic countries including Kenya. Moreover, falciparum malaria resistant to pyrimethamine and sulfadoxine is increasing recently. A few cases of it are reported in Japan yet. We report a 20 years old male patient with falciparum malaria resistance to pyrimethamine and sulfadoxine. He traveled around Kenya for a month without taking prophylaxis for malaria. He had high fever with chill and trepidation every other day from the last four days of the trip. He came back to Japan on 24th February, 1993, and was bought to the Fujisawa Shimin Hospital the next day. Slight hepatomegaly was revealed, but the remainder of the physical examination was normal, including neurologic findings. He was thrombocytopenic (platelet count, $5\times10^4/mm^3$) and low serum cholesterol (101 mg/ml), but no anemic. The thin blood smear showed that 1.1% of the red blood cells (RBC) were parasitized with ring-form trophozoites of *Plasmodium falciparum*. After admission 50 mg of pyrimethamine and 1500 mg of sulfadoxine (Fansidar 2 tablets) was administered at first. But the patient vomited after an hour. (The serum concentration at 10 hour after administration was pyrimethamine 249.4 ng/ml, sulfadoxine 105.2 μg/ml. This is as about the same as serum concentration after taking an adult dose (pyrimethamine 50 mg+sulfadoxine 1000 mg).) Because paroxymal fever attack lasted, chroloquine was given to him 1500 mg as a total amount on the third and fourth days. However, it did not suppress the parasitemia and the ratio of infected RBC was elevated to 11% on the fourth day after admission. A loading dose of quinine was intravenously given immediately and subsequent maintenance was infused 3200 mg as a total amount for three days. Body temperature dropped to normal and the ratio of infected RBC fell to 0.04%. At 12 hours after the last injection of quinine 1500 mg of mefloquine was given orally. Infected RBS disappeared on the eighth day. However, total serum bilirubin raised to 5.5 mg/dl, and blood hemoglobin reduced to 9.2 g/dl. Hemolytic anemia continued for over three weeks. He was discharged on foot on 23th March, 1993. Although the patient needed more than a

week since onset to receive effective medicine, he could recover without severe and complicated malaria, i.e., acute renal failure, pulmonary edema and cerebral malaria.

A-4

THREE CASES OF CUTANEOUS LEISHMANIASIS WITH ATIPICAL CUTANEOUS LESIONS IN ECUADOR

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Leishmaniasis, a zoonotic disease, is endemic in many tropical and subtropical regions in the Old and New World, and is classified as one of the six most important tropical diseases of the World Health Organization. There is no endemic in Japan, but only several cases infected in Africa, South America and Middle East are reported so far. We investigated almost two hundreds cases of cutaneous leishmaniasis in Ecuador from 1988 to 1992. Most popular cutaneous change was an ulcer in patients with cutaneous and mucocutaneous leishmaniasis. The ulcer had a well demarcated border and indurated bank. Base of the ulcer was covered with soft granulomatous tissues. However, red papules, nodules and erythematous plaques were also frequently seen. These cutaneous changes were slightly different in each endemic areas. Several cases with spontaneous healing were seen. Three cases of cutaneous leishmaniasis with atypical cutaneous lesions were presented in this study. Case one, a 7-year-old girl, showed mucocutaneous type of leishmaniasis on the nose and face since three years earlier. The patient had lived in the province of Esmeraldas. Two years earlier, erythematous and verrucous plaques had gradually expanded over the lower body, especially on the buttock, thighs and foot. Hyperkeratosis, parakeratosis and pseudocarcinomatous hyperplasia were seen in the epidermis. Histiocytic granuloma was present in the upper and middle dermis. Amastigotes were scattered in the granuloma. Clinically, we needed for differentiating the disease such as cutaneous tuberculosis or chromomycosis. The *leishmania* species could not be identified because of failure of the culture. Case two, a 19-year-old boy, had red papules on the face, trunk and extremities since 8 years earlier. His eruptions had gradually spreaded over the entire body. The papules slowly evolved into infiltrated plaques and nodules without any ulceration. The *leishmania* amastigotes were confirmed by a smear taken from the lesion. Zymodeme and Schizodeme analysis revealed they were *Leishmania mexicana*. This patient was diagnosed following diffuse cutaneous leishmaniasis. Case three, a 13-year-old boy noticed a small ulcer on his right back of the hand. The patient noticed two subcutaneous indurations on his right forearm, and appeared gradually reddish nodules. A small finger tip sized lymphnode was palpable in his right axilla. Leishman ia amastigotes were seen in the smear. The patient was diagnosed as sporotrichoid type of cutaneous leishmaniasis. These lesions were improved by the treatment of topical paromomycin ointment.

A-5

DENGUE HAEMORRHAGIC FEVER DEVELOPING IN A JAPANESE WOMAN

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A 33-year-old Japanese female developed high fever up to 40.5°C associated with shivering chills on August 23, 1991 (day 1) after her travel to Thailand from July 31 to August 21, 1991. Fever continued there-

after, and she had dull retroorbital pain, headache, generalized muscle pain, joint pain, profuse sweating and general fatigue. She had no diarrhea. Numerous rubella-like skin rashes appeared on her back on August 26 (day 4) which rapidly spread over her entire body surface on the following day accompanied by an itchy sensation. She was nauseated and vomited on this day. Her fever subsided on day 6, and as the patient presented with clinical manifestations of nasal, gum and genital bleedings, she was hospitalized in the Department of Infectious Diseases of Tokyo Metropolitan Komagome Hospital on day 8. The patient had no previous history of travelling abroad and had no evidence of dengue or dengue-like illness. She had never been vaccinated for Japanese encephalitis. She had no particular family history.

On admission, the patient was an alert, well-nurished female with a temperature of 37.5°C and blood pressure of 92/44. Numerous petechial skin rashes were visible over her entire body surface especially on the extremities. Petechial rashes were also seen on the mucosa of her soft palate. Neither anemia, jaundice nor lymphadenomegaly was noted. No abnormal findings were demonstrated in the chest and abdomen on physical examination. Pitting edema was noted on the lower extremities. Laboratory data included: WBCs 2,900/μl (Meta 5, Stab 1, Segs 28, Eo 4, Lymph 34, Atypical lymph 22, Mono (6%), Hb 11.9 g/dl, platelets 5.8×10^4/μl, Prothrombin 100%, Creatinine 0.6 mg/dl, BUN 5 mg/dl, AST 135 IU/l, ALT 69 IU/l, LDH 583 IU/l, CRP 0 mg/dl, positive tourniquet test.

With the clinical diagnosis of dengue haemorrhagic fever (DHF) (Grade II: WHO criteria) no specific medication was given. Nasal and gum bleedings were not reported after admission and the clinical course was favourable thereafter. She was discharged on day 11. Laboratory data including WBCs, platelets, and liver enzymes were normal on day 19. Serum antibody titer to dengue virus II (HI) was raised from 320× (day 8) to 2,560× (days 11 and 19) confirming this case to be a dengue virus infection.

A-6

HEALTH PROBLEM OF JAPANESE RESIDENTS IN DEVELOPING COUNTRIES

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To know the health problem of Japanese residents in developing countries, we conducted a survey with questionnaire by mail. The subjects were 67 Japanese associations in developing countries and 517 medical offices in large companies around Tokyo.

In the survey of Japanese associations, questionnaire was included health complaint of Japanese residents in the association. Thirty eight associations (East and South East Asia:9, South Asia:7, West Asia:8, Africa:3, Latin America:11) were involved in this analysis. The percentages of the associations, which replied to make a complaint about climate, mental condition, sanitary condition and medical service system as a health problem, were 92%, 87%, 50% and 76%, respectively. By regional groups, the number of the complaint was numerous in South and West Asia.

In the survey of medical offices in large companies, questionnaire included health problem and counterplan of their employees in developing countries. 159 companies were involved in this analysis. As health problem, hot and humid climate, stress, poor sanitary condition and immature medical service system were pointed out by most of the companies. Therefore, it is likely that medical offices had been aware of the health complaint of residents in developing countries. Although these medical offices recognized that health education before departure and health consultation throughout the overseas mission were the best way to settle these problems, their present counterplan was mainly regular health check in Japan, because of limited health informations in these countries. It is necessary for specialist in tropical medicine to furnish health informations of developing countries in order to protect Japanese residents from unhealthy conditions.

A-7

PREVALENCE OF THE POLYMICROBIAL ETIOLOGY IN SEX-TRANSMITTED DISEASES AMONG JAPANESE TRAVELERS

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The present study was carried out to determine the frequency of the polymicrobial STD, including *Neisseria gonorrhoeae, Clamydia trachomatis* and *Treponema pallidum* among travelers (63 males and 21 females) visiting foreign countries for a short period (within 3 months). Anti-*N. gonorrhoeae* IgM antibody titers were determined by ELISA using G-Ag 6.8 as an antigen, anti-*T. pallidum* IgM antibody titers by TPHA test using a commercial kit and anti-*C. trachomatis* IgM antibody titers by indirect-micro IF test using a commercial kit. Cut-off titers of these tests were 32 for both the anti-*N. gonorrhoeae* and anti-*C. trachomatis* antibodies, and 8 for anti-*T. pallidum* antibody. Out of 84 subjects, 23 males were positive with anti-*N. gonorrhoeae* antibody or anti-*C. trachomatis* antibody, and 16 females were positive with either antibody. Among these seropositive subjects, 9 males and 11 females were positive with both antibodies. Among 84 subjects, only one traveler was positive with anti-*T. pallidum* antibody. Out of 20 subjects, being seropositive with *N. gonorrhoeae* and *C. trachomatis,* serological test were done with 4 males and 2 females after chemotherapy. All of these subjects became seronegative with anti-*N. gonorrhoeae* antibody, while 2 males and one female were still positive with anti-*C. trachomatis* antibody. Most of male subjects, positive with the gonococcal and chlamydial antibodies, traveled for the South East Asian counties, while the double-seropositive female stayed at countries in the North America and Europe. These results may indicate that double infections caused by *N. gonorrhoeae* and *C. trachomatis* are more prevalent among Japanese travelers in the South East Asia than in the North America and Europe.

A-8

HEPATO-GASTROINTESTINAL DISEASES IN DOMINICAN REPUBLIC (CARIBBEAN)

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Oita Medical University

Rearch and clinical project of digestive disease in the Dominican Republic which was financially supported by JICA, was performed by Oita Medical University. We presented the prevalence of digestive diseases in the Dominican Republic for the period from July 1991 to June 1992.

【Stool exam. 2012 samples】 parasites were found in 31%. Entamaeba coli 15%, Giardia lamblia 7.0%, Ascaris lumbricoides 4.0%, Trichocephalus trichuris 1.4%, Strongyloides stercoralis 0.9%, Entamaeba histolytica etc. 【Sonography 2106 cases】 fatty liver 6.3%, liver cirrhosis 5.9%, stone of gallbladder 8.7%. 【Upper GIF 1436 cases】 gastric ulcer 10.6%, Gastric ca. 2.2%, duodenal ulcer 15.4%, esophagitis 2.8%, esophageal stenosis, achalasia, etc. 【Colon fiber 247 cases 】 colon ca. 6.9%. 【Serological exam.】 HBsAg positive 4.5% (71/1568), HIV antibody positive 1.5% (70/4885).

In conclusion, based on our findings the majar disease of digestive tract in the Dominican Republic for the investigated period was infectious colitis. It was suggested that parasites may play an important role in infectious disease of digestive tract. In addition, a high frequency of gastro-duodenal ulcer, esophageal disease (esophagitis, achalasia), colon ca., fatty liver, or stone of gallbladder was observed. Finally a relation between the digestive diseases and food was suggested.

This work was cooperated by K. SUGAWARA, T. NAKANO, Y. MAGARI, Y. YAMADA, Y. FUJITA and A.S. HIDALGO.

A-9

ANALYSIS OF SEROPOSITIVITIES TO TRYPANOSOMA CRUZI AND ELECTROCARDIOGRAM ABNORMALITIES IN AN ENDEMIC AREA OF CHAGAS DISEASE

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In the Project of Research for Control of Tropical Diseases in Guatemala, Chagas disease is one of the most important targets. In endemic areas, we locate a pilot town, Ixuatan (The population is about 2700,1290m above the sea). The inhabitants who are more than 10 years old, are examined about the following data; questionaire, physical examinations, electrocardiograms (ECGs) and serologic tests; indirect hemagglutination (IHA) for the detection of anti-Trypanosoma cruzi antibodies. ECGs are analyzed according to the modified Minnesota Code. IHA titres more than 1:32 are interpreted as positive.

In 650 inhabitants, seropositive individuals progressively increase with age. The frequency of Ventricular Conduction Defects is 14.3% among seropositives and 2.3% among seronegatives. The frequency of Arrythmias is 13.1% among seropositives and 3.7% among seropositives. These results show that Conduction System is mainly involved.

A-10

EPIDEMIOLOGICAL AND ANTHROPOLOGICAL STUDY IN AN ENDEMIC AREA OF CHAGAS DISEASE IN GUATEMALA -A PRELIMINARY QUESTIONNAIRE SURVEY TO HEADS OF HOUSEHOLDS AND WIVES

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The present study was carried out to identify what kinds of sociocultural determinants are important in the transmission of Chagas disease in an endemic area, Santa Maria Ixhuatan in Guatemala.

We compared seropositives and negatives to determine if there is any difference in sociocultural factors. The subjects were heads of households or wives of villagers who received serodiagnosis by IHA and responded to a questionnaire. The total number of households was 564, and 218 received serodiagnosis and answered the questions.

There was no correlation between cash income and infection. The educational level did not correlate to the infection, either. Although there are several references which emphasize that the construction materials of houses are a risk factor of house infestation, we could not see any correlation between the materials and infection.

The relationship between having a latrine and infection was significant. We found a relevant correlation

between defecation and infection, also. If they defecate outside the home, the possibility of infection is high. Bathing also related to infection. The more females take bath, the higher the possibility for getting infected.

Interestingly, the prevalence of infection differed by the local name of the vector that people use in the village. They have 8 different names for the insect, but most of the people using the name 'Picudo' showed a higher prevalence. There is a possibility that some names have concepts that provide people special attitudes toward the insects. The reaction of the inhabitants against the vector could be different if they used a different name for the insects.

From these results, we conclude that sanitary conditions or hygienic behavior is one of the key factors of getting infected in our study area. Also, the attitude toward Triatoma, expressed by its local names, may be an important factor. Different from the others endemic areas, economical factors, educational levels and house construction materials seem not to be important.

A-11

ANDEAN LEISHMANIASIS IN ECUADOR

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Andean leishmaniasis in Ecuador has been reported for the first time in 1986 at a small town (Paute) of the Department of Azuay, southern part of the country near Peruvian forntier (Hashiguchi *et al.*, 1986). Clinically the disease is very similar to the Peruvian uta. However, it was recently found that the causative agent and vectors of the Ecuadorian form are completely different from those of the Peruvian one (Hashiguchi *et al.*, 1991); *Leishmania mexicana* or *Le. major*-like and *Lutzomyia ayacuchensis* in the former, while *Le. peruviana* and *Lu. peruensis* or *Lu. verrucarum* in the latter. After the discovery of this Andean form of leishmaniasis in Ecuador, special attention has been paid to its distribution in the Andean regions. We therefore made further epidemiological studies of Andean leishmaniasis in the country, in addition to data collection in hospitals and health centers. Autochthonous Andean leishmaniasis was found in two regions (lower- and mid-Andes) of Ecuador, Huigra, 1,300-1,500 meters above sea level and Alausi, 2,300-2,500 meters above sea level, Department of Chimborazo. In the present examination subjects with active lesions positive for *Leishmania* parasites were two (3- and 5-year old) in numbers at Alausi, and six (0- to 2-year old) at Huigra, during the periods from July to September 1991, January to February 1992, July to September 1992 and January to February 1993. They had not visited other leishmaniasis-endemic areas such as the other Andean endemic area (Paute), the Pacific coastal and Amazonian region. Leishmanin skin test revealed positive in 23 (18.9%) out of 122 subjects at Alausi and 44 (66.7%) out of 66 subjects at Huigra. Sandflies were also collected and dissected in order to find natural infection with *Leishmania*. The following flies positive for the parasite were observed: at Alausi *Lu. ayacuchensis*, five (4.3%) out of 116, *Lu. hartmanni*, nil out of 16; at Huigra *Lu. ayacuchensis*, three (0.2%) out of 1,377, *Lu. nevesi*, nil out of 26, *Lu. hartmanni*, nil out of seven, *Lu. gomezi*, nil out of one. None of the wild animals (20 *Rattus rattus* and 2 *Sciurus granatensis*) captured at Huigra were positive for *Leishmania* in culture and/or hamster inoculation. The *Leishmania* isolates from patients and sandflies were characterized by karyotype analysis using pulsed-field gel electrophoresis, and they were identified as *Le. mexicana* (Katakura *et al.*, unpublished data).

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A-12

SEROEPIDEMIOLOGY OF HEPATITIS, HTLV-I/III INFECTION, AND SYPHILIS IN AN AMAZONIAN INDIAN COLONY

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Seroepidemiological studies on hepatitis, HTLV-I/III, and syphilis were conducted on Amazonian Indians in a colony located along the Xingu river, about 800km south-west of Belém. The inhabitants are of the Amerindian tribe of Kaiapós with a population of 912 people. Blood samples were collected with informed consent from 20 donors, 8-75 years of age. All 20 sera were positive for Hepatitis A antibody, but none showed HBs antigen. Fifteen people were positive for HBs-Ab, 16 for HBc-Ab, none for HC-Ab. Eleven were HTLV-I Ab positive, but none was HTLV-III Ab positive. With regard to syphilis, 7 were TPHA positive and 4 were RPR positive. These findings reflected their way of life: isolation from the rest of the people culturally, dependence on agriculture for subsistence and a relatively relaxed restriction on pre-marital sexual practice. This high prevalence of HTLV-I appears to substantiate the paleo-anthropologic point of view that they are the descendants of Asiatic immigrants who, having passed through Beringian, North and Central America, immigrated into South America more than 10,000 years ago.

A-13

LEISHMANIA MAJOR-LIKE PARASITE AS A CAUSATIVE AGENT OF CUTANEOUS LEISHMANIASIS IN PARAGUAY

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Leishmania mexicana and *L. braziliensis* complexes are the causative agents for the cutaneous and mucocutaneous leishmaniasis, respectively, in the New World. Recently, a newly isolated parasite, which shows zymodeme (isoenzyme pattern) profiles similar to those of *L. major* in the Old World, is identified as *L. major* -like in Brazil and Venezuela (Moment *et al.*, 1985), and Ecuador (Hashiguchi *et al.*, 1991). In our previous report, restriction fragment length patterns from the kinetoplast DNA (schizodeme) analyses of the two *Leishmania* isolates from Paraguayan patients with cutaneous leishmaniasis exhibited different schizodeme profiles from those of *L. braziliensis* and *L. mexicana amazonensis,* leaving these isolates unidentified (Yamasaki *et al.*, 1990) to date.

In the present study, we have further characterized

these Paraguayan isolates by schizodeme and zymodeme analyses. Two isolates obtained from leg skin lesions of patients at geographically different localities in Paraguay shared identical schizodeme profiles using three restriction enzymes (*Msp* I, *Hae* III, and *Taq* I). Namely, *Msp* I digestion of the kDNA resulted in 2 major fragments, 1000 and 800 bp, *Taq* I yielded 3 major fragments of 770, 360, and 350 bp, and 2 major fragments at around 210-220 bp were obtained by *Hae* III digestion. These results indicate schizodeme profiles distinctly different from those of the seven reference strains used and indicate that the schizodeme patterns are equivalent to those of *L. major*-like parasite from Ecuador.

These observations were also confirmed by zymodeme analysis using 14 enzymes (alanine aminotransferase, aspartate aminotransferase, enolase, fumarate hydratase, glucose phosphate isomerase, glucose-6-phosphate dehydrogenase, malate dehydrogenase, malic enzyme, mannosephosphate isomerase, nucleoside phosphorylase, peptidase-D, 6-phosphogluconate dehydrogenase, phosphoglucomutase, pyruvate kinase). The present study revealed the much wider distribution of *L. major*-like parasite in South America, and thus attention should be given for the identification and diagnosis of American cutaneous leishmaniasis.

A-14

OUTBREAK OF FALCIPARUM MALARIA AMONG A CARAVAN IN WEST AFRICA

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A group of 4 men and 11 women participated in a 20,000 km tour of West Africa, starting from Bamako, Mali on 4 March, 1993 to Burkina Faso, Ghana and to Ivory Coast for 3 weeks, and a man and 3 females caught falciparum malaria. The tour was sponsored by a British tour company Guerba. All tour members stayed in tents at night throughout the trip and were bitten by mosquitoes frequently. They took proguanil, 100mg daily during the trip, but all 4 patients who became ill had stopped taking the drug upon leaving Africa on 24. Falciparum malaria developed in 4 days from 2 to 5 of April. The incubation period of falciparum malaria is 12 days in an average, with a median of 13 and a range of 8-14 days in our experience in the single night exposure in Sri Lanka. Therefore, the dates of infection were 20 to 23 in March, when the group was in a sea coast village of Ghana. Three of the 4 patients stayed some time at a restaurant on 22 in the village where they said they were most heavily bitten by mosquitoes during the trip. Two patients were cured by chloroquine, one by meflouine and 1 by mefloquine followed by quinine. The statistical analysis revealed following figures: infection rate $p=0.267$, 95 % confidence interval; 0.491, 0.044. Time required for 14/15 members develop malaria; 9 days if they stay in the Occidental coast village of Ghana continuously.

A-15

RESEARCH ON CORRELATION BETWEEN ENTEROPATHOGENS AND CLINICAL SEVERITY OF CHILDHOOD DIARRHOEA IN GHANA

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Two hundreds and twenty five Ghanaian children under five years of age with acute diarrhoea and 64 without diarrhoea were examined for their clinical severity and enteropathogens in their faeces from September to November, 1992. The children with diarrhoea were allocated to two groups according to the criteria for clinical severity of the disease. Those without diarrhoea were also recruited as the controls. Among 225 children with diarrhoea, 157 (69.8 %) were diagnosed as having mild disease, while 68 (30.2 %) were regarded as severe disease. Any enteric pathogen was found in 108 (68.8 %) in mild disease, 51 (75.0 %) in severe disease and 30 (46.9 %) in controls. Rotavirus was detected in diarrhoea patients significantly more than in controls ($p<0.05$). Shigella, Salmonella, Campylobacter, Giardia lamblia and Entamoeba histolytica were likely to be found more in diarrhoea patients as compared to controls. More mixed infections associated with rotavirus and some bacteria were observed in severe disease than mild disease ($p<0.02$). Rotavirus, Salmonella, Campylobacter, Entamoeba histolytica were likely to be found at higher rates in severe disease though the significant differences were not noted between the two groups with diarrhoea. Enterotoxigenic and enteropathogenic Escherichia coli, Shigella and Giardia lamblia were similarily detected in the two groups. The result proved that rotavirus infection or mixed infection including the virus and bacteria is the important factor influencing the clinical severity of acute diarrhoea in Ghanaian children.

A-16

THE PREVALENT DISEASES AND SEASONALITY IN THE PAEDIATRIC WARD OF QUEEN ELIZABETH CENTRAL HOSPITAL IN MALAWI, CENTRAL AFRICA

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Queen Elizabeth Central Hospital (QECH) is one of two central referral hospitals in Malawi and serves the southern regions of the country. The precise data of leading diseases and seasonality in the pediatric ward of QECH was unknown. I had an opportunity to collaborate with my colleagues on collecting the data in the ward. Being collected firsthand by pediatricians, this data is very reliable.

We made final diagnoses for 13,630 children who were admitted to the pediatric ward of QECH from March 1990 to February 1991. As leading diseases, malaria, respiratory disease, gastroenteritis, anemia, malnutrition, cerebral malaria, measles, and meningitis were chosen for data collection and the final diagnosis was made by three pediatricians.

There were 13,630 children admissions to the pediatric ward and 1,419 of them died a year, which means that monthly, an average of 1,139 children were admitted and 118 of them died. Malaria was the most frequently seen (3,044 cases/year), followed by respiratory infection (1,689), gastroenteritis (1,506), anemia (1,087), cerebral malaria (650), and malnutrition (641). The leading causes of death were malnutrition (322 cases), gastroenteritis (178), respiratory infection (150), anemia (142), malaria (128), cerebral malaria (81), measles (58), and meningitis (46). Mortality rate of each disease was as follows: malnutrition 50.2% (322/641), meningitis 30.7% (46/150), anemia 13% (142/1087), cerebral malaria 12.5% (81/650), gastroenteritis 11.8% (178/1506), and measles 10.1% (58/574).

Seasonality was seen in malaria, gastroenteritis, anemia, and malnutrition. Such epidemics started in December about one month after the beginning of the rainy season. The peak was during January to March.

Case fatality rate of cerebral malaria was 12.5%, though malaria was only 4.2%, suggesting that treatment for malaria was well done at our department to prevent from progressing to cerebral malaria. Only 641 malnutrition children were admitted, but more than half of them (50.2%) died. In addition, malnutrition was complicated with diarrhea, respiratory infection, malaria, and measles, so this is the most dangerous disease in Malawi and should take top priority when assistance is considered. Also the mortality rate of measles, 10.1% suggests that the prognosis of measles with malnutrition is very poor. By showing that disease outbreaks peaked simultaneously the data clearly demonstrates the interrelated nature of the diseases which increased during the rainy season. Lastly, AIDS infants born to HIV positive mothers were increasing, and we can predict the increase of AIDS children infected from blood transfusion in the near future.

A-17

ISOLATION OF *ACANTHAMOEBA* FROM SOIL IN ZAMBIA AND THE RELATIONSHIP BETWEEN CYST'S MORPHOLOGY AND CULTURAL TEMPERATURE

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In an effort to elucidate background features of amoeba keratitis, we isolated Zambia, Zimbabwe and South Africa.

The overall rate of detection was high (86.9%); by country it was 86.3% in Zambia, 85.7% in Zimbabwe and 100% in South Africa.

Of the total of 46 isolates of *Acanthamoeba* in Zambia, group II accounted for 95.6%, group I and group III for 2.2% respectively.

Cysts of 3 groups from Zambia were excysted at 30°C on 2 or 3 days. These trophozoites were incubated to encyst at 2 cultural temperatures (15°C, 37°C). After 3 weeks, diameter (Ø) and ray number (R) of these cysts (65 examples) were compared with those of cysts cultured at 30°C. In the group I and group III, the morphological changes were not observed on any experimental temperatures. On the other hand, in the group II, both of Ø and R of the cyst changed significantly only at 37°C, but did not change at 15°C.

A-18

HIV INFECTION AND TUBERCULOSIS IN UGANDA

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We developed a pilot study associated with the project titled "Treatment and prophylaxis for AIDS -related infections in Kampala, Uganda" to investigate the distribution of the immune-status and the concentration of p24 antigen and TNF-α in HIV-seropositive patients with or without new pulmonary tuberculosis. This study was designed on the hypothesis that tuberculosis accelerates the production of TNF-α from T/B lymphocytes and macrophages and it also accelerates the reproduction of HIV and CD4 positive lymphocyte depletion. The concentration of p24 is a reflection of HIV reproduction in the blood. We investigated totally 51 persons, they were 20 HIV(+)ve and TB(+) patients, 9 HIV(−)ve and TB(+)ve patients, 9 HIV

(+) carriers and 13 normal volunteers. They were 28 men and 23 women and the mean age was 29. The mean number of CD4(+) lymphocytes was 297/μl in the HIV (+) and TB(+) patient group and there was a significant difference between this group and the normal volunteer (mean CD4(+)ve cell number: 844/μl) and HIV(−)ve and TB(+)ve patient group (mean CD4(+) cell number: 833/μl). The mean CD4/CD8 ratio of HIV (+)ve patients was 0.64 and was also significantly low compared to the normal volunteers. But there was no significant difference between the HIV(+)ve/TB(+) ve patients and HIV(+)/TB(−)ve people. The level of p24 antigen and the TNF-α in the blood were not significantly different between each group. On the clinical point of view, there was a tendency that the HIV (+)ve/TB(+)ve patients have infiltrative shadows and few cavity in plain chest roentogenographs.

The determination of p24 antigen was not suitable for the evaluation of viral replication in vivo because the HIV antigen makes complex with the anti-HIV antibody. The TNF-α of HIV(+)ve/TB(+)ve patients was not higher than any other groups probably because the patients were not so advanced cases.

A-19

LOW DENSITY MICROFILARAEMIA OF PERIODIC BANCROFTIAN FILARIASIS IN KENYA

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Although the studies have been carried out in some endemic areas of subperiodic bancroftian filariasis, a role of low density microfilaraemia (LDM) carriers in transmission of bancroftian filariasis remained to be studied. We have been studying on an epidemiology and control of bancroftian filariasis in Kenya since 1990. The present paper analyses results of night blood survey done in 1991 and 1992 and reports the LDM of *W. bancrofti* (periodic form) infection in Kenya.

Night blood survey was done between 20:00 and 23:00. One ml of venous blood was examined for microfilariae by nuclepore filter method. A total of 1,733 subjects was examined in three villages, Lutsangani, Gandini and Dzivani, Kwale Coast Province, Kenya. A LDM was defined as 20 mf/ml or under (Kimura *et al.*, 1985).

In these villages the mf prevalence was 22.6, 11.9 and 12.5 % respectively. The ratio of LDM to the mf-positive subjects was 19.7, 27.8 and 46.5 % respectively and the median mf density (MFD50) was 101.5, 92.7 and 30.0 respectively. The proportion of LDM in villages increases as the MFD50 of villages decreases.

34.4 % of females with microfilaraemia showed LDM and 23.9 % of males with microfilaraemia showed LDM. The higher rate of LDM in females is probably due to the fact that MFD50 in females (53.2) was lower than that of males (83.3). The LDM rate varied with age of patients.

In the endemic area of subperiodic *W. bancrofti* infection, younger patients showed high prevalence of LDM and no difference was obtained in prevalence of LDM between males and females.

The present study suggests that there is the difference in prevalence of LDM between periodic and subperiodic *W. bancrofti* infection.

A-20

CHARACTERISTIC DISTRIBUTION OF PEDIATRIC MALIGNANT SOLID TUMORS IN WESTERN KENYA

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We performed the ethnogeopathological study on 576 cases of pediatric malignant solid tumors in western Kenya for the period of 13 years between 1979 and 1991. The most common tumor was Burkitt's lymphoma (194 cases, 33.7%), followed by non-Hodgkin lymphoma (133, 23.1%), retinoblastoma (62, 10.8%), Kaposi's sarcoma (30, 5.2%), nephroblastoma (27, 4.7%), fibrosarcoma (21, 3.6%), Hodgkin disease (18, 3.1%), squamous cell carcinoma of the skin (17, 3.0%), osteosarcoma (15, 2.6%) and others. The high incidences of lymphoreticular malignancies, such as Burkitt's lymphoma, non-Hodgkin lymphoma and Hodgkin disease were found in the tropical savannah areas along Lake Victoria (Lymphoma belt) and among the inhabitants there. Other malignancies, such as Kaposi's sarcoma, fibrosarcoma and retinoblastoma also showed characteristic ethnogeographical distributions. Especially, Kaposi's sarcoma and Burkitt's lymphoma showed very similar pattern of distribution. As a preliminary study, using PCR method, we detected Epstein-Barr virus genome in lymph node type Kaposi's sarcoma as well as Burkitt's lymphoma and we are proceeding to the etiological study on pathogenic relations among Kaposi's sarcoma, Hodgkin's disease and Burkitt's lymphoma.

A-21

SEROEPIDEMIOLOGICAL INVESTIGATION OF VIRAL HEPATITIS AND SCHISTOSOMIASIS JAPONICA IN JIANGSU, CHINA

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The Yangtse River region of China is well-known for endemic area of viral hepatitis and schistosomiasis japonica. These two different infections are believed to induce the hepatic cirrhoss and carcinoma. Blood samples were collected from residents in two villages of Yangzhong County and hospitalized patients in the affiliated hospital of Nanjing Medical College. Serological examinations were carried out for viral hepatitis and schistosomiasis. Out of 385 sera from two villages, 68 (17.7%) were positive for HBsAg by reversed passive hemagglutination (RPHA), 70 (18.2%) were positive for HBsAb by passive hemagglutination (PHA), 277 (71.9%) were positive for HBcAb by hemagglutination inhibition (HI) and 89 (23.1%) were negative for three assays. Out of 103 sera from hospitalized patients, 37 (35.9%) were positive for HBsAg, 12 (11.7%) were positive for HBsAb, 87 (79.6%) were positive for HBcAb and 15 (14.6%) were negative. For HCV detection, 3 out of 385 were positive by particle agglutination (PA) and all were negative by enzyme immunoassay (EIA). Out of 103 sera from hospitalized patients, 2 were positive by PA and one of them was also positive by EIA.

The treatment for schistosomiasis and snail control has been carried out in this investigated area 17 years ago. Therefore, ELISA using Schistosoma egg antigen (SEA) was used for the screening of schistosomiasis. Out of 385 sera, 52 were titer 1:200, 15 were 1:400, 3 were 1:800 and 1 was more than 1:1,600. Double diffusion test was done to 19 cases showed> 1:400 by ELISA. Precipitating bands against SEA were observed in 6 cases. Out of 103 sera from hospitalized patients, 4 were

titer 1:200 and 1 was 1:400.

A-22

IDENTIFICATION OF HIGH RISK PEOPLE OF FALCIPARUM MALARIA IN RURAL CAMBODIA

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Purpose; About 20% of 9-million Cambodian people are supposed to be suffering from falciparum malaria, and its control is urgently needed. We diagnosed and treated malaria from out-patients of the district hospital in Phnom Srouch District, Kompong Speu Province. However, civil war and lack of infrastructure and man-power made difficult an entomological and epidemiological research to evaluate endemicity and high risk people in the region.

Purpose of our survey is 1) to investigate high risk people of falciparum malaria, and 2) to confirm priority to supply impregnated bednets.

Methods; Blood films of patients suspicious to malaria were double-checked by experienced laboratory technicians or medical doctors. Age, sex and living/working areas were asked to the malaria patients, and they were classified according to climate conditions.

Results; During October 1992 to June 1993, 1,259 (20.9%) out-patients were diagnosed as malaria. In the rainy season, the malaria patient rate was 25.5% and the sex ratio was 2.39. Peak of the patient age lay in 21 to 30 years old. In a dry season, the patient rate reduced 16.7%, the sex ratio being 1.71 and the peak being under 5 years old.

In our questionnaire 14% of the under 5 and 93% of the 20s acknowledged episodes of entering forest areas.

Conclusions/implications; The high risk people were different according to the season, and they were male in the 20s in the dry season, and changed into under 5 years old in the dry season. Young men whose occupation was woodcutter, charcoal maker or soldier were supposed to mosquito bites in the forest particularly in the rainy season, whereas many of young children were probably infected at home in the dry season. Therefore, we decided to supply the impregnated bednets firstly to families in the field areas which had children under 5 years old.

A-23

CAUSATIVE ORGANISMS AND CHEMOTHERAPY ON RESPIRATORY INFECTIONS IN MAE SOT AREA, THAILAND

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The aim of our presenting study is to clear the causative agents in community-acquired respiratory tract infections, and to provide the principle of antibacterial chemotherapy. The investigations of adult

outpatients were performed in Mae Sot General Hospital in Thailand through one month examination over the three years, from 1989 to 1992. In each studies, Gram-stainning of sputum smear, quantitative sputum culture, and nasopharyngeal culture were done for identification of respiratory pathogens. For the estimation of the antibiotic sensitivity pattern, minimal inhibitory concentrations (MICs) of each pathogens to various antimicrobial agents were determined.

From the results of bacterial culture, it was clear that major causative organisms of community-acquired respiratory infections (mainly in acute bronchitis, acute exacervation of chronic bronchitis, acute nasopharyngitis and pneumonia) were *Haemophilus influenzae* (*H. influenzae*), *Streptococcus pneumoniae* (*S. pneumoniae*) and *Moraxella catarrhalis* (*M. catarrhalis*), and this results were the same as those in Nagasaki, Japan. The analysis of antimicrobial sensitivity pattern of those pathogens revealed that *S. pneumoniae* was resistant to tetracycline, gentamicin, and norfloxacin. And *M. catarrhalis* showed high resistance against penicillins, 1st and 2nd generation cephems (penicillin G, amoxicillin, piperacillin, cephazolin, and cephotiam) and also against tetracycline. In addition, ampicillin combined with clavulanic acid (β-lactamase inhibitor) was very active to *M. catarrhalis*. These data strongly suggested that *B. catarrhalis* might have acquired pathogenesis partly by the production of β-lactamase in Thailand as seen in Japan.

For successful chemotherapy, penicillins are active against *H. influenzae* and *S. pneumoniae,* but it shoud be emphasized that most of β-lactams are not active to *M. catarrhalis*. In addition, tetracycline is not expected for the treatment to *S. pneumoniae* and *M. catarrhalis* infections. On the other hand, recent developed new quinolones are effective to the infections caused by both *H. influenzae* and *M. catarrhalis*.

The examination of nasopharyngeal flora in children revealed that *H. influenzae* and *S. pneumoniae* were highly isolated even from the healthy children who were free from respiratory symptoms. Nevertheless, follow-up study of those children on the incidence of following respiratory infections will make it clear how we should manage them especially in order to prevent the severe infections.

A-24

ISOLATION OF BACTERIA FROM THE HUMAN FECAL SAMPLES IN THE RURAL AREAS OF NORTH EAST THAILAND

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Bacteria were isolated from the fecal samples of villagers, hospital patients and diarrheal patients in North East Thailand. Specimens were collected from 180 persons. After aringing in transport medium to Japan, enteric bacteria and the resemblance organisms were isolated. Number of specimens, collected from the children of under 10 years old, patients of Khon Kaen University Hospital and patients of Children Hospital were 84, 43 and 53, respectively. *Salmonella* sp. was inoculated into Rappaport broth and then streaked onto DHL agar plate. For the *Vibrio* sp. the enrichment and selective media were alkali peptone water and TCBS agar plate respectively. *E. coli* and *Pseudomonas* sp. which showed characteristic colonies on DHL agar plate predominantly were detected. Twenty-six (31%) of 84 feces samples from the children of the villages posses the enteropathogenic bacteria. Of these, only 5 cases showed the signs and symptoms of diarrhea. It seemed that the inapparent infection rate was high. Though *Salmonella* sp. were isolated high rate from the village children, no *Salmonella* sp. was isolated from the faeces of diarrheal patients in the children hospital. The inapparent infection of *Vibrio* sp. were higher in the village children rather than the patients in both hospitals. The fact that *Vibrio* sp. could isolated in Khon Kaen province which is far from sea is interesting because *Vibrio* sp. is usually profusely available near the sea region. The higher sodium chloride concentration of the soil in Khon Kaen may be one of the causative factors for the survival of *Vibrio* sp. Inapparent infec-

tion of *Aeromonas* sp. was higher in all groups. A lot of enteropathogenic *E. coli* were isolated from the patients of Khon Kaen University Hospital. As the natural water of North East Thailand which is used as the drinking water is highly contaminated with *Aeromonas* sp. so this organism was occassionally isolated from their feces. An investigation was done for the heat labile, heat stable, cholera toxin and vero toxin in different bacteria, resulted almost the absence of these toxins, excepting the *Aeromonas* sp. which showed Vero cell toxicity. The cell free culture supernatant of *Aeromonas* sp. was neutralisable by rabbit antiserum of vero toxin producing *E. coli* 0157:H7.

A-25

A STUDY FOR TRIAL OF MAKING AGAR PLATE METHOD AS APPROPRIATE TECHNOLOGY FOR DEVELOPING COUNTRIES, IN NORTHEAST THAILAND; ARE NUTRIENT AGAR AND STERILIZATION REALLY NECESSARY?

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Recently, the agar plate method has been shown to be successful in detecting *Strongyloides stercoralis* in fecal materials, while traditional methods have been reported to be unreliable. We have been reporting that the agar plate method is very efficient, based on results of our previous studies in Chiang Mai Province, Northern Thailand, and Cambodia, not only for its reliability but also for its field applicability in developing countries. Based on this characteristic of the method, we have been studing about improvement of procedure as an appropriate technology for developing countries, so that the usage of the agar plate method would be globally advantageous in the diagnosis of strongyloidiasis that is broadly prevalent in tropical area in the world.

Cutting down the expenses of the method is essential for appropriate technology. We wondered wether the nutrient agar and autoclaving that we used in present procedure is necessary or not, for further cutting down the expenses. In this study, we made a comperative study between procedure using edible agar or without autoclaving, and the conventional one, in Khon Kaen, Northeast Thailand.

Twenty-five samples from adult farmers in Khon Kaen Province were used for the study. Five groups of the plates, i.e.; A) Control group, using nutrient agar for bacteriological examination which costs 7.5 Yen per plate and with autoclaving agar and petri dishes, B) Using edible agar which was bought at a market in Khon Kaen City and which costs 0.75 Yen per plate, and with autoclaved agar and petri dishes, C) Using nutrient agar, with non-autoclaved agar (just boiled) and autoclaved dishes, D) Using edible agar, with non-autoclaved agar and autoclaved dishes, E) Using edible agar, with non-autoclaved agar and dishes; were compered for this study. Each sample was distributed to 5 groups of plates and cultured following our improved agar plate method[1].

In the result of control group, *Strongyloides* was detected in 11 cases out of a total of 25 samples (44%), and hookworm in 6 (24%). Seventeen cases were track-positive and 8 were negative. *Strongyloides*-positive rate was very high, so prevalence rate was suggested to be high also in this area.

In the result of a comperative study depending on result of track, 22 cases out of 25 showed same result in all 5 groups. In 2 cases, only group E was negative, and in 1 cases, only group C was positive.

We concluded that nutrient agar and sterilization were not necessary for the procedure. After this improvement, the agar plate method demands less than 1 Yen per sample and it is not in need of electricity supply any more.

We have been reporting that since prevalence rates of *Strongyloides* in our previous study in Northern Thailand and Cambodia were much higher than previous surveys performed without the agar plate method, it is suggested that global prevalence of *Strongyloides* infection is much higher than the present estimation. The result of this study made that clearer.

[1]Am. J. Trop. Med. Hyg., 45(4), 1991, 518-521.

A-26

THE PREVALENCE OF MALARIA IN AN ENDEMIC AREA OF BANGLADESH

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Malaria is endemic in the South-East part of Bangladesh. The present study was done in Kaptai Thana Health Complex in Rangamati district situated in the endemic area. The area is hilly with forest. The population are tribal and nontribal Bengalee settler. The tribal people are living in this area for long time and Bengalee settlers migrated here from other part of Bangladesh for opportunities of land and other bussiness. The total population under the health complex is 52,653. The study was done in April 1993. A total 809 patients were included in the present study. Venous blood were taken from all patients, attending this health complex with the complain of fever. Blood smear were made and after Giemsa stain at least 100 fields were observed under the oil-immersion lens for the diagnosis of malaria. The parasite rate was 48.1% and the rate of *Plasmodium falciparum* and *Plasmodium vivax* were 70.7% and 28.5% respectively. *P. falciparum* and *P. vivax* mixed infections were 0.8%. The highest incidence (58.6%) of malaria was found in the 5~9 years old age group. By occupation, those were more exposed to the forest, the incidence of malaria was high among them. Those residing within the forest had also significantly ($p<0.001$) higher incidence of malarial parasite positive rate compared to those residing outside the forest. Illiteracy and low income were associated with higher incidence of malaria. Splenomegaly was found more in the children than in adults. Interestingly splenomegaly was not found in tribal people with or without malaria. It is noteworthy that the incidence of malaria was significantly ($p<0.05$) less among the tribal people than the Bengalee settlers. Further studies deserve importance to find out the reason of less suceptibility of malaria in the tribal people.

A-27

EPIDEMIOLOGICAL SURVEY OF MALARIA IN KANAYNAYON AND IRANKABARAN, AETA, PHILIPPINES

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In June 1991, in the Philippines, many people lost their houses and fields because of the calamity of Mt. Pinatubo's eruption. They took refuge in the evacuation centers. At two of those centers, Kanaynayon and Irankabaran, our non-governmental organization (NGO) group called "Sharing Japan" participated in the community medicine program initiated by a Philippine medical doctor, Dr. Emma D. Palazo. In this activity, we recognized that the eradication of malaria was one of the most important issues in these areas. So we have decided to conduct the epidemiological survey of malaria in both evacuation centers as the first step.

Kanaynayon was initially inhabited by the refugees from Mt. Pinatubo with the support of one NGO group and now people are beginning to be able to live independently. Irankabaran was established by the Philippine governmental project. The great difference from Kanaynayon is that it is over populated with gathering

houses and scanty farming fields. Now people in this area need some supports from others. Usually, a medical doctor visits the evacuation center and open a clinic once a month.

Firstly, we selected patients suspected of malaria and checked their blood on March 1993. In Kanaynayon, the infection rate of malaria parasites was 39% (14/36) and the majority species was *Plasmodium falciparum* (57%). But, in Irankabaran only one patient (1/7) was found. And secondly, we checked the blood of all people who wished malarial examination. The infection rates were high (35%, 21/60) in Kanaynayon and low (3%, 1/29) in Irankabaran. And many 1- or 2-year-old children in Kanaynayon been infected with malaria parasites (50%, 5/10).

In these two areas, although temperature and natural environment are almost the same, there was a difference in the infection rates of malaria parasites. Therefore, we suspect people in Kanaynayon had been already infected with malaria parasites before they moved there in 1991. However, there were many 1- or 2-year-old child patients who were infected after migration, indicating that in Kanaynayon there were not only malarial pathogen but also route for infection.

In Kanaynayon, we think that distribution of mosquito net infiltrated with insecticides is the most effective and realistic way for the control of malaria in the current situation. In Irankabaran, although patients of malaria are few, we must consider the possibility that it will be prevalent in the near future.

A-28

INFECTION AND DIARRHEA CAUSED BY *CRYPTOSPORIDIUM* IN SURUBAYA, INDONESIA

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During a one year study in a hospital, *Cryptosporidium* oocysts were detected in 26(2.8%) of 917 diarrhea cases and 15(1.4%) of 1043 nondiarrheic individuals. The frequency of detection of the parasite was higher in the 0-10 year age group; no sex-specific difference was discernible. The detection rate of the parasite was higher in rainy season. Stool samples were also collected from children below 10 years old in five areas in Surabaya and examined for the presence of the parasite. Forty-seven(2.3%) of 2086 children in rainy season and one(0.06%) of 1780 children in dry season were positive for the parasite. Water samples were collected from seven rivers in Surabaya and examined. The parasites were found in two rivers in rainy season and one river in dry season. Stool samples from cats were also examined, and 13 of 296 cats(4.4%) were positive in rainy season but there was no positive among 236 cats in dry season. The prevalence of the parasite among children and cats in one particular area were higher, where river water was positive for the parasite and higher frequency of flood(67%) was found by questionaire. Contaminated water seems to be playing a significant role in transmission of *Cryptosporidium* in Surabaya.

A-29

SURVEYS ON THE INTESTINAL PARASITIC INFECTIONS IN LIKUPANG AND CAMPALAGIAN, SULAWESI AND KAO, HALMAHERA, MALUKU, INDONESIA

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On a Grant under the International Scientific Research Program, the Ministry of Education, Science and Culture Japan (Research Grant No. 03041065), this research took place during June-July, 1991-1993, at the villages of Campalagian, Lindu and Likupang, South Sulawesi and the villages of Kao, Halmahera, Maluku, Indonesia. We controlled for about 300-500 population of their life-style by questionnaire and examinated the parasites in feces.

The data from examination of internal parasite in feces, at the village of Lindu that found the Oriental blood fluke, *Schistosoma japonicum,* one kind of flukes, but tapeworms have not seen. For soil-transmitted nematodes have a high-rate infection, that were comparing as following, ascariasis were 4-60 (40) % in 2-14 years old, more than 15 years old were 0-53 (17) % and a high-rate for the children. Also many trichuriasis were shown. *Trichuris trichiura* in 2-14 years old were 0-18 (51) %, more than 15 years old were 0-61 (46) %, but no difference-age for infection rate. Hookworms were 22-93 (59) % in 2-24 years old, 50-100 (84) % for more than 15 years old that have a high-rate in adult, also converse with ascariasis. Infected per cutaneously of *Necator americanus* were the most greater part of hookworms. For *Strongyloides stercoralis* have a low -rate of that found 0-9 (3) %, and *Enterobius vermicularis* were involved low too.

Entamoeba histolytica were 0-20 (8) %, *Entamoeba coli* were 1-43 (19) %, and *Giardia lamblia* were 0-14 (4) %, severally.

At the endemic cholera areas, feces strewed in the field but without their habit, especially the children and farmers, with the simple toilet when it is rainy that stool will overflow on the ground and contaminate to their milieu. More than a half of them with bare foot and sandals, easily to catch the infection percutaneous. Commonly, they expect that the spread of in need of the health-information.

A-30

STUDIES ON EPIDEMIOLOGY OF MALARIA AND NUTRITIONAL STATE IN GUADALCANAL, THE SOLOMON ISLANDS

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We conducted the epidemiological studies of malaria in some villages situated in the north coast of Guadalcanal, the Solomon Islands. The study was done in 292 habitants, 69 (<6 years), 92 (<12 yrs), 30 (<16 yrs) and 98 (>17 yrs). A blood sample was collected from the finger-tip for thick and thin smears, hemoglo-

bin (Hb) concentrations and blood glucose levels. The slide positive rate by Giemsa staining was 51.7% and the rate of less than or over 20 years old were 59.2% and 32.1%, respectively. The rate of P. falciparum/P. vivax were 75.4%/31.1%, and 11 cases showed coinfection, 20 cases had gametocytes. Abdominal palpation showed high incidence of splenomegaly (69.5%), especially children (<8 yrs; 86.3%). Eighty-seven (57.6%) for slide positive and 76 (53.9%) for slide negative cases were anemic (Hb<12.0g/dl). Physical examination was revealed that low height and underweight in boys and girls (<16 yrs). About a quarter of them were below the -2SD range for both height and weight applied mean growth chart of Japanese boys and girls. We estimated somatomedin C as a nutritional indicator, which show low level in malnourished patients. About 47.5% and 47.9% cases were below the normal range in positive and negative slide cases, respectively. These data suggested that malaria affected not only growth and development for child but also nutritional state in high endemic area such as the Solomon islands.

A-31

EPIDEMIOLOGY AND CONTROL TRIAL OF MALARIA IN SOME VILLAGES OF GUADALCANAL, THE SOLOMON ISLANDS

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Epidemilogical survey studies were conducted in several community villages of Northern Guadalcanal island, the Solomon Islands in 1993. Chemotherapeutic control trial was attempted at the same time of examination with a combination of not only treatment dose of chloroquine but also single dose (45mg/kg) of primaquine.

Mobile malaria unit was composed of a driver, health staff and experts with equipments including a portable generator. Blood examination was performed on thick and thin blood smear not only by Acridine Orange fluorescent staining but also Giemsa staining. Fluorescent staining using ordinary light microscope equipped with two interference filters made it possible to detect and treat high density parasite carriers on the spot of examination. Portable ultrasonic machine was used to examine splenomegaly.

In February, a total of 289 was examined and 35% of them was infected with malaria. The rate of *P. falciparum* over *P. vivax* was 2.6. Children under 6 was the highest group with parasite rate being 47.3%; 6-12; 43% and over 17 was 14%. Gametocyte carrier was detected mainly under 16 and children under 4 was the highest group. Spleen rate of children under 9 was 77% showing holoendemic malaria transmission and the rate was highest in children of 4-6 years being over 85%. Ultrasonic detection of splenomegaly was useful and feasible with a generator. G6PD deficiency was examined by the method of Fujii and 11% was deficient. Duffy negative and Gerbich negative was not found among 140 subjects. Among 140 samples, HBs Ag positive was high as 29% and only 3 was HCV antibody positive.

In August, a total of 275 was examined and parasite positive rate changed from the starting 46% at February to 44% in villages treated with chloroqine and from 61% to 38% in villages treated with chloroquine and primaquine.

People especially children living in community villages of Guadalcanal are highly infected with malaria. Drug distribution at clinic on PCD (passive case detection) is not enough for malaria control and active intervention with mobile malaria unit as a primary health care activity would be necessary.

A-32

DNA DIAGNOSIS OF MALARIA IN THE SOLOMON ISLANDS

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We have developed a colorimetric assay "microtiter plate hybridization (MPH)" to detect amplified nucleic acids of malaria parasites on a microtiter well. This assay system allows us to detect and identify the four species of human malaria parasites. A pair of oligonucleotide primers were designed for the amplification of the conserved region of the gene coding for the 18S small subunit ribosomal RNA. Species-specific oligonucleotide probes were also designed and immobilized on microtiter wells. The target sequence of malaria parasites in human blood was amplified by the polymerase chain reaction (PCR) and the PCR-amplified product was captured by the species-specific probe on the microtiter well. The biotin-streptavidin system was used to detect the captured materials. Positive samples gave yellow color by the chromogenic reaction.

Plasmodium falciparum-specific and *P. vivax*-specific microplates were tested in Guadalcanal, Solomon Islands, in January and February, 1993. Blood samples (10 μl each) were obtained from 130 asymptomatic donors by finger puncture and subjected to the microplate hybridization assay. Thick and thin blood smears were also prepared for microscopic examinations. Thick blood smears were stained with Giemsa and examined by the local microscopists. Thin blood smears were stained with acridine orange (AO) and examined by Dr. F. Kawamoto.

Among the 130 blood samples tested, 30 (23%) were positive by *P. falciparum*-specific microplate, 28 (22%) were positive by *P. vivax*-specific microplate, and 8 (6%) were positive by both of them. These results of MPH were similar to those of AO microscopy. However, there was considerable disagreement of results between thick smear-microscopy and MPH or AO microscopy. Thick smear-microscopy gave only 78 (60%) compatible results with MPH. There were 10 samples whose results by MPH and AO microscopy differed, as far as *P. falciparum* infection and *P. vivax* infection were concerned. Three cases were positive by MPH but negative by AO microscopy. We think the detection sensitivity of MPH was higher than that of AO microscopy. Five samples were *P. vivax*-positive by AO microscopy, but negative by *P. vivax*-specific MPH. We think the parasites identified as *P. vivax* in these 5 cases might be a new malarial species "*P. vivax*-like". In two cases, *P. falciparum*-specific MPH gave negative results, while gametocytes of *P. falciparum* were detected by AO microscopy. These discrepancies were possibly due to the fragility of the red blood cells infected with gametocytes.

B-1

INDUCTION OF CEREBROSPINAL FLUID EOSINOPHILIA IN RATS BY INTRAVENTRICULAR INJECTION OF *ANGIOSTRONGYLUS CANTONENSIS* EGG ANTIGEN

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Nonpermissive hosts (mice and guinea pigs) including humans, infected with *Angiostrongylus cantonensis* (Ac), provoke marked eosinophilia in cerebrospinal fluid (CSF) during the time (at days 10-20 postinfection) when young adult worms (YA) are parasitizing in the brain. Contrarily, permissive rat host fails to provoke CSF eosinophila.

In order to determine whether CSF eosinophilia can

be experimentally induced in rats, intraventricular injection of various antigens (Ag) was performed in inbred male WKAH/HKm rats with peripheral eosinophilia due to the previous infection with either *Mesocestoides corti* (Mc) or Ac. Rats infected with Ac for 40-43 days (Ac-infected rats) or with Mc for 20-23 days (Mc -infected rats), and normal control rats were used as recipients for intraventricular Ag injection. Under nembutal anesthesia, rats were placed on a stereotaxic apparatus for brain, and injected with Ags (15μg protein/50μl) into the lateral ventricle using a microsyringe. Ac-egg-Ag was prepared by homogenizing genital organs removed from female Ac adult worms with PBS, followed by dialysis. YA-and Mc-Ags were similarly prepared from day 17-intracranial worms harvested from Ac-infected rats, and from tetrathyridia collected from the peritoneal cavity of Mc-infected mice. PBS injected rats served as controls. Eosinophil accumulation in CSF was assessed on days 7-8 after Ag injection. Peripheral blood eosinophil counts were 4.5 and 11.4 times higher in Ac-infected and Mc-infected rats respectively, than those in normal control rats. Ac-infected and then egg-Ag injected rats provoked significantly high CSF eosinophil accumulation (14.9%), when compared with Ac-infected plus PBS- (2.2%) or YA -Ag- (3.2%) injected rats ($p<0.01$). By contrast, Mc -infected rats failed to induce CSF eosinophilia, even when they were injected with either egg- (3.3%) or Mc -Ags (3.9%). These data suggest that previous Ac infection (pre-existence of worms in the brain) is a prerequisite for CSF eosinophil accumulation following egg-Ag inoculation. An additional experiment was thus conducted on rats with peripheral eosinophilia due to surgical transfers of day 24-25 YA worms (10 male and 16 female worms) into pulmonary arteries. Prior to Ag injection, peripheral eosinophil count of the recipient rats was 6.5 times higher than that of normal control rats. Nevertheless, no eosinophil accumulation in CSF could be noted in all rat groups injected with YA- (0.9%) and egg- (2.8%) Ags, or with PBS (1.4%). In conclusion, the induction of CSF eosinophilia in rats requires previous meningeal stimulation due to Ac infection and subsequent specific egg-Ag inoculation.

B-2

HUMAN AND MURINE LYMPHOCYTE ACTIVATION ELICITED BY *DIROFIRALIA IMMITIS*

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The hypergammagloblinemia following to infections of *Dirofiralia immitis* is observed frequently in dogs. So we have attempted to isolate B cell activators and lymphokine producing substances from somatic components of *D. immitis*. Adult *D. immitis* worms extracted from the infected dogs were stored at −20°C due to use as materials for partial purification. *D. immitis* were extracted with PBS after defatting the worms with acetone and fractioned on a column of Sephacryl S-200 to 5 fractions. Each fraction was dialyzed in PBS and mesured immune activities. Human lymphocytes (2×10^6/ml) were cultured 7 days with isolated fractions in order of A, B, C, D, E in RPMI 1640 medium containing 10% fetal bovine serum. Fractions A, B, C, and D were strongly cytotoxic to human lymphocytes. Only fraction E elicited an increase in the number of polyclonal IgM and IgG antibody-forming cells determined by protein A plaque method. Only fraction E also augmented the production of polyclonal IgM and IgG antibody in murine spleen cells. Therefore, fraction E, low M.W. somatic component of *D. immitis*, was found to induce polyclonal B cell activation. It is well known that parasitic infections induce an increase of IL-4 secretion from T helper cell (Th2). Therefore, we examined IL-4 production in human lymphocytes by somatic components of *D. immitis* according to ELISPOT assay. Fraction D shows a tendency to lead to an increase of IFN-γ production from human lymphocytes, whereas fraction E increases IL-4 production. These results indicate that high M.W. fractions of somatic components of *D. immitis* show cytotoxicity and immunosuppression, but fraction E leads to augmentation of polyclonal antibody production and increases IL-4 and IFN-γ production.

B-3

TRIALS OF INFECTION RESISTANCE AGAINST *BRUGIA PAHANGI* ON MICE

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We investigated cellular immune response to *Brugia pahangi* using immune modulators. Indomethacin suppresses prostaglandin E_2, and Natural Killer and Lymphokine-activated Killer are activated. So we tried to detect the effects of indomethacin administration on the susceptibility to a primary *B. pahangi* infection in male BALB/c mice. Indomethacin therapy could activate killer cells in situ and interfere the peritoneal infection with larvae of *B. pahangi*. On day 14 of inoculation, male mice treated with oral indomethacin showed stronger resistance than vehicle-treated controls.

Brucella abortus activates Th1, and does killer T cell. Staphylococcal enterotoxin B also activates Killer T cell. Inactivated *Brucella abortus* or Staphylococcal enterotoxin B were administrated intraperitoneally on day 0 of inocuration. And on day 7 of inoculation, no effects to induce a resistance were not detected. The results suggest that immune modulators may not work to induce a resistance against *B. pahangi* at early L3 stage.

Nakanishi *et al.* have reported that carbon particles interefere macrophages being effectors against *B. pahangi* and the macrophage blockade effect decreases after day 5.

These results suggest that the time when L3 exuviates into L4 may be critical to induce resistance against *B. pahangi*.

B-4

GROWTH INHIBITION OF CULTURED *PLASMODIUM FALCIPARUM* BY IMMUNE SERA FROM TANZANIA

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Residents in hyperendemic areas of malaria acquire increasing protective immunity against *Plasmodium* infection with age. In order to examine humoral growth-inhibiitory factors to intraerythrocytic stages of *Plasmodium falciparum* (*P. f.*), serum samples were collected from patients in Muhimbili hospital, Dar es Saraam, Tanzania, from a Dar es Saraam resident who had lived in a non-endemic area, Japan for six months prior to the present work and from a Japanese who had contracted *P. f.* malaria in Mozambique. The results showed that the inhibition capacity of an immune serum is not necessarily correlated to its antibody titer examined by ELISA of IFA. There is another factor(s) in the low molecular fraction (LMF) that is capable of inhibiting the intraerythrocytic growth of *P. f.* parasites and frequently exhibits more potency than the immunoglobulin rich high molecular fraction (HMF). The two fractions were separated from each other by using centriprep-30 concentrator (Amicon, USA), i. e., a filtered fraction (LMF) and a concentrated fraction (HMF). This factor with a molecular weight of less than 30,000 is present in low concentration, induces morphological changes of intraerythrocytic parasites and does not require the presence of immunoglobulins for its action. It could be demonstrated even in the serum of the immune individual more than six months after he had left the endemic area. All the examined sera exhibited variable inhibition activity in their LMFs accompanied by variable antibody titers, suggesting a possible parallel mechanism to specific antibodies in the elimination of asexual blood forms. Although the factor seems to play an important role in clinical immunity, further studies are required to make it clear.

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B-5

EFFECTS OF IMMUNOSUPPRESSION ON THE GROWTH OF MALARIAL PARASITE

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Relation between infection of malarial parasite and immunossupressant was studied. Mice infected with *P. berghei* was tried to cure by immunosuppressive agents deoxispergualin (DSG) and methotrexate (MTX). Some mice were started to administrate these drugs as same day as inoculation, other mice were started the drugs treatment after confirmed the appearance of the parasite in mice blood. Both DSG and MTX prevented mice from theinfection of *P. berghei* into the RBC of mice. However, DSG could cured the mice that % parasitemia became nearly 10 %, MTX could not cured because of the side effects even though, the % parasitemia was decreased by MTX treatment.

Immature erythrocyts number and amount of hemoglobin per dl were decreased by these chemicals. The mechanism of *P. berghei* infection that the protozoa can fusion only young immature erythrocyte was hypothesized.

B-6

COMPARISON OF SWEAT-FUNCTION BETWEEN JAPANESE AND THAI SUBJECTS

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Recently, we have reported the longer latent period for sweating, smaller local sweat volume and slightly smaller rise in core temperature in Thai subjects during heat load compared with Japanese subjects. Furthermore, sweat volume on the abdomen was larger than that on the chest in 9 out of 10 Thai subjects. On the contrary, sweat volume on the chest was larger than that on the abdomen in 7 out of 10 Japanese. In the present study, therefore, such regional discrepancy of sweating between Japanese and Thai subjects were further examined.

Mean annual air temperature is reported to be 16.6° C in Nagasaki and 25.9°C in Chiang Mai. Sixty sweat tests in 28 Japanese lived in Nagasaki (22 male and 6 female) were carried out with lower leg immersion into hot water, sauna or exercise load, and 30 sweat tests in 20 Thai subjects lived in Chaing Mai (10 male and 10 female) with lower leg immersion heat load were carried out. Local sweat rates on the chest and abdomen were recorded by using of capacitance hygrometer -sweat capsule method. Sweat volume on the abdomen was larger than that on the chest in 26 out of 30 tests in Thai subjects. On the contrary, sweat volume on the chest was larger than that on the abdomen in 55 out of 60 tests in Japanese subjects. Regional discrepancy in sweat rate between Japanese and Thai subjects was clearly confirmed in this study. It is supposed that the smaller sweat volume in Thai subjects is attributed to the habituation phenomenon in sweating response, especially, suppression of ineffective sweat dripped from the skin. In order to clarify the physiological significance of the regional discrepancy of sweating, further study will be done from the following view points; that's body heat content, fat thickness on the abdomen, numbers of active sweat glands, ratio of effective sweat to ineffective sweat, innervation of sweat glands, influence of clothing material on the abdomen, and skin pressure response on the abdomen.

B-7

EFFECT OF ENVIRONMENTAL FACTORS ON AN INCIDENCE OF ENCEPHALOMYOCALDITIS VIRUS INDUCED DIABETES IN MICE

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It is well known that encephalomyocarditis(EMC) virus inoculation induces diabetes in mice. It was also reported that there is some differences in the incidence of symptoms among strains or sexes of mice. But, the cause of the difference has not been analyzed yet. We think that the differences of the incidence might be related to rearing conditions of mice. We made a comparative study on the incidence of EMC virus induced diabetes of mice between two different rearing conditions.

Seven-week-old DBA/2N male mice were purchased and were reared under sterile(SPF) or non-sterile(conventional) conditions. Both groups of mice were inoculated intraperitoneally with 10^4 $TCID_{50}$/0.1ml or 10^5 $TCID_{50}$/0.1ml of EMC virus M variant solution. Control groups were inoculated with medium only. Food and water intakes, body weight and blood sugar were measured. And glucosuria were detected during four weeks after inoculations.

All survival SPF mice inoculated with both doses of EMC virus M variant had rapid increases of food and water intakes, decreases of body weights, high blood sugar levels and appearances of glucosuria. Those were typical symptoms of diabetes. On the contrary, only two conventional mice inoculated with higher dose of EMC virus showed signs of diabetes.

These results suggested that the rearing condition of mice would affect the incidence of diabetes induced EMC virus and we have to pay attention to standerdize the rearing condition of laboratory animals when we make a comparative study on sensitivity to parasites among some species of hosts.

B-8

THE REPRODUCTIVE CYCLE OF THE MALE HABU, *TRIMERESURUS FLAVOVIRIDIS*

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The reproductive cycle of the male habu, *Trimeresurus flavoviridis* was studied. Spermiogenesis was observed from late July to November, when serum testosterone increased and the size of the testes also enlarged. However spermatogenesis and the testosterone level did not correspond during winter and early spring. Mating occurred in late April when the testicular size was minimum. Eosinophilic granules of the sexual segment of the kidney disappeared about the time of mating. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were recognized in the hypophysis by immunohistological staining from June to November, and serum LH and FSH increased in June preceding the elevation of serum testosterone. Other pituitary hormones, growth hormone, adreno-corticotropic hormone, and prolactin were also detected in the hypophysis at the same time as gonadotropin.

In order to reduce the number of habus and habu-bite, we proposed a procedure that the opposite sexual habus should be used as the decoy for the short period of mating in April.

B-9

STUDIES ON THE PREPARATION OF RABBIT ANTIVENOM AGAINST THE VENOM OF HYDROPHIS CYANOCINCTUS

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Although the bite by seasnake is not common in Japan, a case of the bite by Hydrophis cyanocinctus has been reported recently in Okinawa (higa *et al.*, 1990). It is also reported that the same species of seasnakes are common in Hong Kong, and Beihai, China (awai *et al.*, 1978, 1992). This study concerns the preparation of H. cyanocinctus antivenom for emergency use of seasnake bite.

H. cyanocinctus venom was supplied from Guangxi Medical College, China. Rabbit were injected repeatedly with formolized venom of Hydrophis cyanocinctus with Freund's adjuvant. The antivenom collected from immunized rabbits was fractionated by sodium sulphate to obtain γ-globulin. One vial contained 10 ml of purified antivenom (2% protein) of which one ml of the antivenom neutralized 153 μg (14.3 MLDs) of H. cyanocinctus venom tested in mice, 92 μg (10.4 MLDs) of Lapemis hardwickii, and 21.5 μg (7.2 MLDs) of Enhydrina schistosa venom.

The precipitin lines between the antivnom of H. cyanocinctus and four venoms' of H. cyanocinctus, Lapmis hadwickii, E. schistosa and H. melanocephalus fused together, but not fused with the venom of L. semifasciata. From the results, it is suggested that H. cyanocinctus antivenom is effective not only for treatment of the bite by homologous species but also those of heterologous species of L. hardwickii and E. schistosa. The results indicate the presence of certain common antigens in the venoms of four of the five seasnake species studied, too.

B-10

ON THE PATHOGENICITY OF *ENTAMOEBA HISTOLYTICA* ISOLATED FROM MYANMAR PATIENTS

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Human infections with *Entamoeba histolytica* are widely distributed throughout the world. Of these, only around 10% develop symptoms that can include life-threatening visceral lesions; the others remain asymptomatic carriers who pass cysts in the stool. Brumpt (1925) presupposed the existence of two morphologically similar but biologically different species: *E. disenteriae,* inducing disease and *E. dispar,* nonvirulent for man. After works, to explain this enigma, most investigators have studied the antigenic, isoenzymatic, and molecular biological differences between the two amoebae. Recently, Diamond & Clark (1993) reconsidered Brumpt's hypothesis in light of recent biochemical, immunological, and genetic findings and proposed that *E. histolytica* indeed represents two species; the invasive organism retains the name *E. histolytica* Schaudinn, 1903, and *E. dispar* Brumpt, 1925, for the noninvasive. However, some isolates from symptomatic patients were found to have non-pathogenic characteristics, as well as the reverse.

Amoebic trophozoites were isolated, by stool culture in Robinson's medium, from two Myanmar patients, a 42 year-old woman with bloody mucoid stools and a 62 year-old man with bloody diarrhea. We sought to

determine the pathogenicity of the two isolates by antigenic analysis with monoclonal antibodies, and by isoenzymatic patterns. Because neither isolate reacted with the pathogen-specific monoclonal antibodies, and because both strains exhibited the nonpathogenic isozyme, zymodeme-I, we classified the two isolates as nonpathogenic, although both were recovered from symptomatic individuals. Following anti-amoebic treatment, the patients' symptoms disappeared.

If *E. dispar* is indeed nonpathogenic and does not give rise to symptoms, then the patients must have been infected with both species of *Entamoeba*. It therefore seems likely that our stool-culturing procedure selectively favored the growth of *E. dispar*, to the detriment of *E. histolytica*.

B-11

CHANGES OF HOST MUSCLE CELLS AFTER *TRICHINELLA SPIRALIS* INFECTION IN MOUSE (PART II)

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The infective form of *Trichinella spiralis* lives in the specialized cell named nurse cell, which developes after the newborn larva penetration into a striated muscle cell of the host. Transformation of the muscle cell to nurse cell seems to be unique in terms of cell differentiation, and is expected to afford an excellent experimental system to dissect molecular mechanisms of cell differentiation. To take advantages of this system we launched a project to disclose whole sequence of events that occurr during transformation of muscle cell to nurse cell. This contribution deals with morphogenesis of nurse cells. In the very early phase of cystogenesis, the nurse cell began to lose preexisting myofibrils and mitochondria without accomypanying morphological changes in nucleus. Association of mitochondria with lysozomes suggested lysozomes are responsible for degradation of mitochondria. Such morphological alterations occur simultaneously in the whole cell. In the next stage the cytoplasma was characterized by flattened endoplasmic reticulum and structure-less cytoplasmic ground substance. Then cytoplasma began to be filled with prominent vacuoles and mitochondria probably newly synthesized. By that time nucleus of nurse cell obtained its characteristic features, that is, enlarged size, euchromatic appearance and prominent nucleolus. Mature nurse cell had a cyst wall composed of inner and outer portions, the former was formed by products of fibroblasts in the late phase of cystogenesis, and the latter was formed by secreory prodocts of nurse cell which occurred from the ealy phase. During the cystogenesis no morphological evidence was observed that suggests cell death due to the infection. Thus infected muscle cells, to become nature nurse cell, seemed to perform extensive reconstruction of cell organelles including nucleus, mitochondria and myofibrils.

B-12

ISOLATION AND CHARACTERIZATION OF AN EGGSHELL PRECURSOR PROTEIN OF *SCHISTOSOMA JAPOSICUM*

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We have identified a 34kDa major female-specific protein of *S. japonicum* by fluorography of SDS-PAGE using pulse incorporation of ^{14}C-tyrosine, and reported reasons for considering that the 34kDa protein is an eggshell precursor (Int. J. Parasitol. Vol. 21: p225, 1991, ibid., Vol. 22: p589, 1992). It has been suggested that the eggshell formation in trematodes is a quinone tanning or a sclerotization process where the tyrosine residures on

precursor proteins are oxidized to quinones via the formation of dihydroxyphenylalanine(DOPA). Recently, Wait & Rice-Ficht have characterized the presclerotized eggshell proteins of *F. hepatica,* which are unique in containing rather high levels of DOPA, and they have isolated three eggshell precursor proteins using DOPA -rich residue as a marker source(1987, 1989). However, in the case of the 34kDa protein from *S. japonicum* it is not clear whether the tyrosine residues on this protein are oxidized to DOPA or not.

The 34kDa protein was isolated by means of the electro-elusion from the gel of acidic polyacrylamide gel electrophoresis. The DOPA containing proteins were stained with the nitroblue tetrazolium /glycine regagent by Paz *et al.*(J. Biol. Chem. Vol. 266, p689, 1991). In this report we show that the 34kDa protein could not be characterized as a DOPA-containing protein. It is also demonstrated that the 34kDa protein acts as a substrate for mashronm phenol oxydase that catalyzed the oxydation from tyrosine to DOPA.

B-13

SCHISTOSOMA JAPONICUM : CLONING OF THE GENE ENCODING A 34 KDA EGGSHELL PRECURSOR PROTEIN

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We have identified a 34 kDa female-specific protein of *S. japonicum* as a putative eggshell precursor (Kawanaka, 1991). In order to isolate the eggshell precursor protein gene, mature female cDNA library constructed in lambda gt11 was screened with a rabbit antiserum raised against the 34 kDa female-specific protein. Forty immunopositive clones were isolated from $3x10^5$ recombinants. These positive clones were separated into groups by restriction enzyme mapping and cross-hybridization analysis of their corresponding inserts. By nucleotide sequence analysis of representatives of each group, one clone, designating Sj23, containing 246-bp insert, was found to have significant homology to the N-terminal coding region (Zurita *et al.*, 1987) and the central area of the coding region (Rice-Fichit *et al.*, 1992) of the eggshell precursor protein genes of *Fasciola hepatica*. The predicted amino acid sequence from Sj23 also revealed significant homology to the eggshell precursor proteins of *F. hepatica*. This indicates that Sj23 is a cDNA clone encoding part of the eggshell precursor protein of *S. japonicum*.

To date, two different genes, p14 and p48, have been reported in *Schistosoma mansoni*. No significant sequence homology at the nucleotide level was observed among p14, q48 and Sj23.

B-14

PURIFICATION AND CHARACTERIZATION OF PURINE NUCLEOSIDE PHOSPHOTRANSFERASE FROM *LEISHMANIA TROPICA*

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Leishmania tropica, parasitic protozoa causing cutaneous leishmaniasis, is incapable of synthesizing purines *de novo* and is thus dependent on the host for the source of performed purines. This qualitive difference in the enzymes of purine salvage and interconversion pathways between parasite and host suggests a rational approach toward the design of selectivly toxic agents against the parasite.

We, therefore, purified purine nucleoside phosphotransferase which is unique purine salvage enzyme of parasite to design nucleoside analogs having selective toxicity against parasite and to define mechanism of

metabolism of toxic nucleoside analogs such as carbocyclicinosine (C-Ino), 3'-fluoroinosine (3'-Fl) and 3'-deoxyinosine (3'-dl) from *L. tropica*.

L. tropica cells of middle log phase (2×10^6 cells/ml) were harvested and suspended in phosphate buffered saline (PBS, pH 7.4). The cell suspension was disrupted by ultrasonicator and then centrifuged at 100,000 × g for 60 min. Total enzyme activity of the supernatant (soluble enzyme) was about 35 % of cell suspension. On the other hand, the cell pellet was resuspended in 100 mM Hepes buffer (pH 7.4) containing 5 mM $MgSO_4$, 1 mM dithiotreitol, 1 mM 5-phosphorylribose 1-pyrophosphate and 5 % n-octyl β-D-glucopyranoside and homogenized. The homogenate was incubated in ice bath for 60 min and centrifuged at 100,000 × g for 60 min. Total enzyme activity of the supernatant (membrane enzyme) was about 65 % of cell suspension. From these reqults, we found that purine nucleoside phosphotransferase exist in both membrane and cytoplasm and total activity and specific activity of membrane enzyme were higher than those of soluble enzyme. Both enzymes were partially purified by DEAE-Sepharose CL-6B anion exchange column chromatography, Hydroxylapatite column chromatography and Affi-Gel heparin affinity column chromatography and showed same characteristics in this procedure. Molecular weight of both enzymes is same as 110 kDa by gel filtration of Sephacryl S-300 HR. The Km value of the membrane enzyme for inosine (control), C-Ino, 3'-Fl and 3'-dl were 6.22×10^{-6}, 6.99×10^{-6}, 7.03×10^{-6} and 7.29×10^{-6}, respectively. The Vmax of the membrane enzyme for inosine (control), C-Ino, 3'-Fl and 3'-dl were 7.38×10^{-2}, 9.20×10^{-2}, 9.00×10^{-2} and 8.11×10^{-2}, respectively. These results suggest that there are little differences between inosine and three nucleoside analogs in Km value and Vmax.

B-15

MOLECULAR MECHANISMS OF DRUG RESISTANCE IN *LEISHMANIA*: ORNITHINE DECARBOXYLASE GENE AMPLIFICATION IN APLHA-DIFLUOROMETHYORNITHINE RESISTANT *LEISHMANIA*

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Understanding of molecular mechanisms of drug resistance in *Leishmania* is of great advantage to make a rational design for chemotherapy of leishmaniasis. Gene amplification is widespread phenomenon in experimental drug-resistant *Leishmania,* occurring in many different species in response to a variety of compounds. Previously, we reported amplification of the P-glycoprotein gene in arsenite-resistant *L. amazonensis,* and amplification of *N*-acetylglucosamine-1-phosphate transferase gene in several *Leishmania* species resistant to tunicamycin. In the present study, we have selected variants of *L. donovani* promastigotes resistant to alpha-difluoromethylornithine (DFMO), an enzyme-catalyzed irreversible inhibitor of ornithine decarboxylase (ODC), by increasing in the drug concentrations up to 30 mM in culture. The resultant DFMO-resistant variants were analyzed by pulsed-field gel electrophoresis and Southern blot hybridization. Amplification of subchromosomal DNAs of 230 kb in size was observed in variants resistant to 2 mM DFMO (DF2 line). The amplified DNAs hybridized with the $(TTAGGG)_n$ telomere DNA probe, indicating that structure of the amplicon was linear. Variants resistant to 30 mM DFMO (DF30 line) contained amplified extrachromosomal circular DNAs of about 40 kb in size, but not linear DNAs. The leishmanial ODC gene probe hybridized to both amplified linear and circular DNAs. The same probe also hybridized to two different chromosomes of 700 and 800 kb. However, no difference was observed in terms of hybridization intensity between wild-type and resistant cells, indicating no chromosomal amplification of the ODC gene in the DFMO-resistant lines. In the DF2 line, the amplified linear DNAs disappeared during *in vitro* passages of the parasites, while the ODC gene was newly amplified as extrachromosomal circular DNA molecules. In contrast, the amplified circular DNAs in the DF30 line was stably maintained. These results indcate that the ODC gene amplification occurs initially as extrachromosomal linear form and subsequently takes place as extrachromosomal circular form under the drug pressure of DFMO, suggesting involvement of several molecular

mechanisms in the ODC gene amplification in *Leishmania.*

B-16

AMEBIC ANTIGEN FOR GEL DIFFUSION PRECIPITIN TEST, RECOGNIZED BY SERA OF PATIENTS OF CHRONIC AMEBIC COLITIS

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Infection of *Entamoeba histolytica* was classified into three groups.

acute amebiasis abrupt onset with severe symptoms (abdominal pain, watery diarrhea, fever up *etc.*)

chronic amebiasis gradual onset of illness, presence of signs which indicate massive tissue destruction (bloody stool, colonic ulcer, abscess *etc.*), absence of severe symptoms

cyst carriers prolonged asymptomatic cyst carriage, absence of signs and/or symptoms

We have reported the R-band in modified G.D.P.T. (gel diffusion precipitin test) is characteristic to chronic amebic colitis. (The 32nd Annual meeting). We purified the antigen responsible for R-band and named it CRAR (chronic amebiasis related antigen). The molecular weight of CRAR antigen was 30kD (SDS-PAGE).

A micro-plate was sensitized with the CRAR antigen, and sera of various types of amebiasis patients were subjected to the ELISA. Anti-CRAR antibody was detected in the sera of chronic amebic colitis, but not in those of cyst passers and acute cases. It showed a contrast to anti-PHEX (phenol extract of amoebae) antibody.

B-17

ACTIVE SITE OF COMPLEX II IN THE MITOCHONDRIA FROM *PLASMODIUM*

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During the past five years, evidence has accumulated to suggest an active role of the intraerythrocytic *Plasmodium* mitochondria. A major role for mitochondrial electron transport is likely to be in serving dihydroorotate dehydrogenase, a mitochondrial membrane bound enzyme involved in the essential *de novo* biosynthesis of pyrimidines. Biochemical analysis of the purified mitochondria also showed a occurrence of the NADH-fumarate oxidoreductase system which is an anaerobic respiratory chain found in many parasitic animals such as *Ascaris suum.* In these electron transport systems from dihydroorotate and NADH, complex II functions as terminal oxidase and catalyzes the reduction of fumarate to succinate (fumarate reductase: FRD) which is reverse reaction of succinate-ubiquinone oxidoreductase (SDH) in the aerobic respiratory chain of mammalian mitochondria.

Mitochondrial complex II is located in the inner mitochondrial membranes and is generally composed of four polypeptides. The largest flavoprotein subunit (Fp) with a molecular weight of about 70 kDa contains covalently bound flavin as a prosthetic group and forms a catalytic portion of the enzyme complex together with ironsulfur subunit (Ip). A genomic DNA coding for a portion of the Fp subunit which contains the active site was cloned and sequenced by PCR using homology probing with mixed primers. The deduced amino acids sequence for the *Plasmodium* Fp subunit showed higher homology to those of SDH from other organisms than to those from FRD of anaerobic bacteria, even though the *Plasmodium* enzyme functions as a fumarate reductase. The active site of the enzyme contained invariant his-

tidine and arginine residues previously observed in Fp subunits from other organisms.

B-18

NEF BINDS TO TL_2

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The nef gene encodes the proteins of relative molecular weight are 27 and 25 kilodaltons. The formar product Nef is the major one and attached to the cellular menbrane through its myristoylation site.

The Nef plays an important role for efficient viral replication and developing AIDS in Simian Immunodeficiency viruses (SIV) infected rhesus monkeys. However, there have been tremendously controversial in vitro findindigs revelant to a role of Nef in both Human Immunodeficiency Virus (HIV) and SIV infected cells. A novel serineesterase Tryptase TL_2 (TL_2) has been revealed to specifically interact with envelop glycoprotein gp120 of HIV-1 IIIB strain. Here we have shown that Glutathione S-transferase-NL43 fusion protein of which the expression product of the nef gene derived from HIV-1 NL43 strain fused with Glutathione-S-transferase expressed in E. coli and then purified with Glutathione-Sephadex beads, binds tightly to TL_2 in vitro. We further studied the specificity of this binding between NL43 and TL_2 by cold competition, dose-dependency, and NL43 Nef deletion mutants-interactions. Although the biological meaning of this interaction is still to be determined, the binding of NL43 Nef to TL_2 may provide the clue to elucidate the function of Nef.

B-19

STRUCTURE AND FUNCTION OF RECOMBINANT PIG RECEPTOR (STaR) FOR A *ESCHERICHIA COLD* HEAT STABLE ENTEROTOXIN (STa) I: cDNA CLONING, FUNCTIONAL EXPRESSION, AND CHARACTERIZATION

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Heat-stable enterotoxin (STa) produced by *Escherichia coli* causes fluid secretion from intestine by binding to and activating a membrane bound guanylate cyclase. We examined the cloning and expression of a cDNA encoding the pig receptor for STa. The synthesized oligonucleotides corresponding to two regions of the rat STa receptor (GC-C) were used to amplify rat cDNA (1.7kb) that represents the 5' end by polymerase chain reaction. This probe was used to screen a pig intestinal cDNA library from the 10-week-pig. We isolated cDNA clones encoded pig STa receptor (GC-C) and determined 3773 base pairs following the first ATG. Cleavage of the 23 residue signal peptide would result in a mature 1050 residue protein (approximately 121kDa). Expression of pig STa receptor (GC-C) in CHO cells resulted in specific binding STa and gualylate cyclase activity. Photoaffinity-labeling of ^{125}I-ANB-STh(5-19) to expressing cells resulted in the labeling of proteins with molecular weights of 135-150 kDa. The stimulatory capacity of various STa analogs on guanylate cyclase in expressing cells was good correlated to their enterotoxicities on experimental animals.

B-20

STRUCTURE AND FUNCTION OF RECOMBINANT PIG RECEPTOR (STaR) FOR A *ESCHERLIHIA COLI.* HEAT-STABLE ENTEROTOXIN (STa) II: BINDING OF STa TO STaR UNDER AN ACIDIC CONDITION

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Heat-stable enterotoxins (STa) produced by *Escherichia coli* are known to cause an acute diarrhea in humans and domestic animals. The toxins bind to a receptor protein (STaR) on the brush border membrane of intestinal cells, stimulate guanylyl cyclase, and increase the concentration of cGMP in the cells. Recently, an unique membrane bound guanylyl cyclase (GC) was found to be STaR.

We isolated a cDNA of GC coding for STaR from pig intestine to investigate the structure and function of STaR. The cDNA of STaR was inserted mammalian expression vector (pCG), and STaR was expressed in the cell surface on transfection of 293T cells with pCG -STaR. Expression of STaR resulted in its specific binding to STa on transfected cells. Guarino *et al.* previously pointed out that ^{125}I-STa binding to intact T84 colon cells increased as the pH was lowered from neutrality to pH 4.5. In this study, therefore we have focused on the interesting phenomenon of binding of STa to STaR under an acidic condition (pH 4.5) and examined the STa binding to STaR overexpressing cells under various pH conditions.

Binding of ^{125}I-STa to STaR overexpressing 293T cells were mesured as a function of pH. From pH 8.5 to pH 5.5, binding of ^{125}I-STa to STaR were equal. At pH 4.5 and 3.5, the amount of ^{125}I-STa binding to STaR increased about three times more than the binding at neutral pH.

We examined whether higher binding of ^{125}I-STa to STaR under low pH conditions was due to the change of the binding affinity between ^{125}I-STa and STaR by scatchard analysis at pH 7.5 and pH 4.5. This result showed the similar affinity constant at both pH 7.5 and pH 4.5, but the binding site on the cells increased 5-fold at pH 4.5 compared to that at pH 7.5. Using deletion mutants of STaR, it was found that intracellular region of STaR was significance of the binding of ^{125}I-STa to STaR under an acidic conditions.

B-21

GENETICAL ANALYSIS OF *VIBRIO CHOLERAE* 0139 STRAINS ISOLATED FROM PATIENTS WITH CHOLERA-LIKE DISEASES IN INDIA, BANGLADESH AND THAILAND

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In October 1992, an outbreak of cholera-like infection occurred in the city of Madras located in southern India (1). The causative agent of this outbreak belonged to the non-01 serogroup of *Vibrio cholerae.* In subsequent months, similar strains of *V. cholerae* were associated with large outbreaks in Bangladesh and in varius parts of India. Serological studies on 223 strains from different parts of India and from Bangladesh revealed that the strains were identical and were therefore assigned to a new serogroup 0139 with the synonym Bengal (2).

In recent years, pulsed-field gel electrophoresis (PFGE) has been used to successfully detect genetic polymorphism among various bacteria (3, 4). In this study, we applied the PFGE tecinique to examine the restriction fragment length polymorphism (RFLP) pat-

terns of several strains of *V. cholerae* 0139 isolated from different parts of India, from Bangladesh and from Thailand and to compare the patterns with the different biotypes of the 01 serogroup of *V. cholerae*. The pattern exhibited by *Sma*I-digested genomic DNAs from the *V. cholerae* 0139 Bengal was identical despite the fact that these strains were from widely separated geographical areas. Distinct restriction fragment length polymorphism of DNA was observed between strains belonging to the 0139 and 01 serogroups of both classical and eltor biotypes. From this study, it is concluded that strains belonging to the 0139 serogroup are clonal in origin and are different from the 01 serogroup of *V. cholerae*.

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2) Shimada, T. *et al.* (1993) Outbreak of *Vibrio cholerae* non-01 in India and Bangladesh. Lancet 341, 1347.

3) Anderson, D.J. *et al.* (1991) DNA fingerprinting by pulsed field gel electrophoresis and ribotyping to distinguish *Pseudomonas cepacia* isolates from a nosocomial outbreak. J. Clin. Microbiol. 29, 648-649.

4) Ott, M. *et al.* (1991) Pulsed field electrophoresis of genomic restriction fragments for the detection of noscomial *Legionella pneumophila* serogroup 1. Diagn. Microbiol. Infect. Dis. 12, 295-302.

B-22

APPLICATION OF ULTRASOUND FOR MALARIA EPIDEPIOLOGY AND CONTROL

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Hackett's spleen rate is the proportion of children (aged 2 to 9 years) in a community who have enlarged spleens. The spleen rate (SR) is a useful index of malaria endemicity. Palpitation has long been used to measure spleen size and the proportion of individuals, espeacially children, with palpable splenomegaly is often estimated before control measures are introduced. However, palpitation is subjective, it needs considerable experience and skill, and the SR is not useful for the monitoring of responses for the control measures, for example, mass chemotherapy.

In 1991, we practiced ultrasonographic (US) examinations for the clinic patients with symptoms or sigms of malaria such as fever and chills in the Solomon Islands. In the study, we could measure even a low to mild degree of spleen enlargement by US and most of the under 15 with malaria were detected.

In January and August 1993, we carried out cross-sectional US examinations for the inhabitants in 2 malaria endemic villages (Talaura and Tadhimboko) in northeastern Guadalcanal of the Solomon Islands. The aims were to try out a simple portable US machine in some malaria endemic communities and to estimate application of US examinations for malaria epidemiology and control.

About 400 inhabitants were examined by US in each study. The US equipment weighed only 6 kg (Yokogawa type ULT-50) had a 'Polaroid, system. Splenomegaly was detected by the two methods. One is the method by Dittrich *et al.* to measure the lengths and the depths of the spleen by subcostal and intracostal scans and calculate the volume of the spleen, and the other is the method by Rosenberg *et al.* to measure the splenic length in the splenic hilus and detect spleen enlargement. Thick and thin blood films were examined by acridine orange stainning system which was designed by Dr. Kawamoto and Giemsa stainning to detect malaria parasite and get parasitaemiae, gametocyte

densities and parasite rate (PR).

In January, we found the difference of utilizing primary health care systems for malaria infection between Talaura and Tadhimboko. The mean times of malarious symptoms of the inhabitants for a year was 2.42±1.85 in Talaura and 2.21±1.67 in Tadhimboko. And we could not find significant difference between them. Most of the inhabitants had had chloroquine treatment in Tadhimboko, however in Talaura, about 20 % of the inhabitants had never had chloroquine treatment for a year. In Talaura, the PR was 60.1 % and the SR by Hackett was 76.1 %. However, in Tadhimboko, the PR was 45.0 % and the SR by Hackett was 12.0 %, and we could find significant difference between them. But each of the SRs by US was similar to each PR.

After the selective mass chemotherapy with chloroquine and primaquine, in August 1993, the PR was reduced to 43.3 % in Talaura and the SR by Hackett was rapidly reduced to 23.6 %. However, the SR by US was 64.2 % and it was similar to the PR. In Tadhimboko, after the selective mass chemotherapy with chloroquine, the SR by US; 59.1 % was similar to the PR; 47.5 %.

Asymptomatic gametocyte carriers take important roles in transmission of malaria parasites. And most of them are less than 15 years old. In our study, about 90 % of asymtomatic gametocyte carriers were detected to have splenomegaly by US among malaria infected children. And about 85 % of all the asymtomatic gametocyte carriers were easily detected by combination of US examinations and AO stainning syetem.

Since US is simple, quick and non-invasive, and since it can measure even a low to mild degree of spleen enlargement, it should be useful in assesing SRs and in monitoring of efficacy of the interventions, for example, mass selective chemotherapy.

B-23

COMPARISON OF THE DIAGNOSTIC RESULTS BETWEEN AO-THIN AND GIEMSA'S-THICK METHODS

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An acridine orange staining method using an interference filter system (AO/IFS) was used for the detection and identification of malaria in thin smears obtained from outpatients and volunteers at Ifakara, Tanzania, the Lombok island, Indonesia, and the Solomon islands, and their diagnostic results were compared with those by the ordinary Giemsa's thick smear (GTS) method. At the Solomon islands, we also compared three diagnostic methods, GTS, AO/IFS, and a new PCR technique using a microplate hybridization method with two specific probes for *P. falciparum* and *P. vivax*.

Out of 93 malaria positives obtained at Ifakara, 10 cases with low parasitemias were misdiagnosed as negative in GTS by the local examiners. In addition, more than 10 cases of *P. malariae* and 2 cases of *P. ovale* infections were overlooked by GTS. Particularly, GTS failed to detect the presence of schizonts of *P. malariae* in all positive cases.

Of 201 smears obtained at the Lombok island, 10 cases (Pf 8; Pv 2) were identified as malaria positive by GTS. AO/IFS confirmed 9 cases of them, except one case of a very low parasitemia with *P. vivax*. Among 8 cases of *P. falciparum* infections, however, two cases were found to be mixed infection with *P. malariae* and *P. vivax*, respectively.

In the comparison of three diagnostic methods at the Guadalcanal island, AO/IFS identified a total of 67 cases as malaria positive, while PCR detected 66 cases, and both results of species identification were similar. Of 67 positives, however, AO/IFS revealed the presence oh *P. malariae* in a total of 7 cases (Pm 1; Pf/Pm 1; Pv/Pm 5). On the other hands, GTS by the local microscopists included many false-negatives (AO or PCR positive; 20 among 61) and false-positives (AO and PCR negative; 12 cases among 67), and failed to detect all of *P. malariae* infections. Furthermore, only 27 cases were accurately diagnosed by the local examiners.

From these results, it was concluded that AO/IFS is much easier than GTS for detection and identification of *Plasmodium* species. In addition, our findings by AO/IFS on the presence of *P. malariae* in the Lombok island and the Guadalcanal island are the first reports, suggesting that *P. malariae* may distribute much more widely than we think. A combination of the AO/IFS and the new PCR methods might be a useful technique for detection and identification of malaria in the field.

B-24

A DESIGN OF RAPID SERODIAGNOSTIC METHOD FOR CHAGAS' DISEASE AND A PRACTICAL FIELD TEST IN GUATEMALA

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Serological techniques for medical examination are generally carried out in laboratories where many facilities and instruments are available to conveniently examine and analyze any materials. However, endemic areas are far from research laboratories located in the cities. Blood samples for serodiagnosis are, therefore, always collected from every individual and transported to the city where they are examined later. Until recently, any serodiagnosis kits for Chagas' disease needed blood sample not less than 10μl from each individual. When a seropositive case is detected by any kit in a laboratory, more blood from the seropositive individual must be collected so as to separate the parasites from the blood. If there is a technique that can instantly diagnose seropositiveness with a drop of blood from an individual's finger in the field, more blood samples can be taken from seropositive persons. Thus, immediate cultivation of these samples for parasitological diagnosis, known as hemoculture, on the very day of the examination in the field is possible.

We have just developed a simple, rapid serodiagnostic method using polystyrene particles (latex) adsorbed with the crude antigen of *Trypanosoma cruzi* epimastigotes. We applied this method to Chagas' disease serodiagnosis in Guatemala to confirm the capability of this technique in the field and to find seropositive inhabitants there.

Eight seropositive cases of 98 inhabitants were detected and examined by this technique in the field. More than 10ml of blood were thaken from the vein of a seropositive individual to cultivate the sample. Up to date there was a positive result in seven hemocultures.

These seropositive sera should be further examined by other immunological methods. Moreover seropositive persons are advised to see a doctor and extensive clinical examination, eg., ECG, chest X-ray, and/or ecodopplercardiography, is highly recommended.

We hope that this method is useful in epidemiological and parasitological studies of Chagas' disease in the future. This work was supported by Japan International Cooperation Agency.

B-25

IMPROVEMENT OF QUANTITATIVE DETECTION FOR HELMINTH EGGS WITH FILTRATION TECHNIQUE

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The Kato-Katz technique is being widely used for parasitological stool examination in many tropical countries under the WHO's guidance. This semiquantitative technique, however, has such defects as 1) small fluke eggs and thin shelled eggs may easily be missed, 2) specimens can not be kept for many hours due to drying soon.

The filtration technique was introduced to improve these points for stool examination. This technique utilizes micropore membrane with pore size of 14μ for filtration of helminth eggs after 1) sieving stool through 100 mesh nylon screen, 2) weighing 0.1-0.2g of stool precisely and 3) emulsifying in 0.1N NaOH solution. Egg count is possible for long period and immediately after mounting eggs together with micropore membrane with polyvinyl alcohol solution. By using this filtration technique, labor and time can be adjusted for mass processing of fecal materials. Comparative study was carried out between these two techniques using same stool materials at Pak Hinboun Village along Mekong River, Lao P.D.R.

The total infection rate with 5 helminths (*Ascaris lumbricoides* (*AS*), *Ancylostoma* sp. (*AN*), *Trichuris trichiura* (*TR*), *Opisthorchis viverrini* (*OV*) and *Taenia* sp. (*TN*)) was determined as high as 92.2% (59/64) by the filtration but only 57.8% (37/64) by Kato-Katz techniques. Detection efficiency was determined to be far higher by the filtration than by Kato-Katz techniques for 4 species (46.9 vs. 32.8% for *AS*, 51.6 vs. 20.3% for *AN*, 42.2 vs. 21.8% for *TR*, 79.7 vs. 12.5% for *OV*). In particular, detection efficiency is significantly higher in *OV* and *AN*, being 6.4 and 2.5 times higher by the filtration technique.

Egg count was possible only by the filtration technique because the specimens dried before counting eggs in Kato-Katz technique, and a general trend was observed that 1) the infection rate decreased with age for *AS* and *AN* while reversed for *OV*, and 2) egg density in terms of EPG also decreased with age for *AS* and *AN* while reversed for *OV* species.

These results suggest that the filtration technique is recommended in preference to Kato-Katz technique, especially for reliable detection for *Opisthorchis* and *Ancylostoma*.

B-26

DETECTION OF *PNEUMOCYSTIS CARINII* BY USING POLYMERASE CHAIN REACTION

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Polymerase chain reaction (PCR) method was applied to detect the genom of *P. carinii*. Two pairs of oligonucleotide primers were used in this study. For initial round of the PCR, primers described by Kitada *et al.* (1991) which amplify 120 base pairs of 5S rRNA sequences of *P. carinii* were used. For the second round of PCR, primers which amplify an internal fragment of 91 base pairs were used. Various clinical specimens (BAL, sputa, lung tissue) collected from immunosuppressed patients were investigated with PCR system. DNA was extracted by phenol-chloroform after proteinase K digestion. Results were compaired with Methenamine silver staining. PCR were able to detect *P. carinii* in clinical samples that were found negative

by silver stains. Application of PCR-based detection system showed allow very increases in sensitivity and automation. The results reveals that PCR system is useful for diagnosis of *P. carinii* pneumonia.

Using the same specimens, HHV-6, one of the agents of opportunistic infection were also investigated by PCR method. HHV-6 DNA were detected frequently in these specimens.

B-27

LABORATORY DIAGNOSES OF PATIENTS WITH SEQUENTIAL FLAVIVIRUS INFECTIONS

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Flavivirus comprises more than 60 different viruses. Some of them share common antigens. Therefore, sequential flavivirus infection causes complicated immune responses.

In Okinawa, 3 cases of Japanese encephalitis (JE) were confirmed by neutralization (N) test in 1991. All patient had previously been administered with yellow fever (YF) live vaccine. A significant increase ($\geqq 4^x$) of N titer to YF virus was observed in all patients. Since no YF virus exists in Okinawa, increases of N titers to YF virus were considered to be due to the results of cross-reactions after JE virus infection. This cross-reaction in N test was observed not only on IgG class antibody but also on IgM class antibody.

In order to confirm such cross-reaction in sequential flavivirus infection on another combinations of flaviviruses, serum specimens from patients with clinical diagnosis of JE (13 cases) or dengue (DEN) hemorrhagic fever (DHF; 39 cases) were collected in their acute and convalescent phases in Chiang Mai University Hospital in 1991. All specimens were examined by N test on JE and DEN viruses. The acute phase sera from DHF patients were examined for virus isolation and reverse transcreiptase polymerase chain reaction (RT-PCR).

Eight of 13 JE patients were diagnosed as JE with significant rises of N titer to JE but not to DEN viruses. Of 2 cases which showed significant rises of N titer to both JE and DEN viruses, 1 case was diagnosed as JE with marked increase of N titer to JE virus, and another case was judged as inconclusive. Two cases were diagnosed as JE, since their N titers to JE virus were much higher than those to DEN viruses, although they did not show significant rises of N titers to both JE and DEN viruses. One case was diagnosed as dengue encephalopathy by remarkable rises of N titers to DEN viruses but not to JE virus, together with successful isolation of DEN-2 virus from acute phase sera.

Of the 39 clinical DHF cases, 15 cases showed significant rises of N titer to both DEN and JE viruses, although 15 cases were diagnosed as DHF by significant rises of N titer to DEN viruses but not to JE. Of the first group and remaining 9 cases which did not showed significant rises of N titer to DEN viruses, 10 cases were regarded as DHF by comparing N titers to DEN viruses with that to JE virus. Eleven cases could be diagnosed as DHF only by successful RT-PCR and (or) virus isolation.

B-28

DISSEMINATED PENICILLIUM MARNEFFEI INFECTION IN PERSONS INFECTED WITH HUMAN IMMUNODEFECIENCY VIRUS

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Penicillium marneffei is a dimorphic fungus distributing Southeast Asia and southern part of China, which was rare cause of the infections in human being. However, as HIV carrier increases in Thailand, disseminated penicilliosis *marneffei* has also increased and becomes one of major complications of HIV infection in Thailand. We report here on the clinical status of 10 cases with the infection.

Chief complaints of the patients were prolonged fever in all cases (mean 1.6 months), skin eruption in 9, weight loss in 7, and cough in 5. All patients were male and anti HIV Ab positive. On admission, they had high fever in 9 cases, hepatomegaly in 7, splenomegaly in 5, and generalized lymphoadenopathy in 3. Laboratory data showed anemia (Hb: mean 8.37 g/dl), relative lymphocytepenia (1,028.2/mm^3). Blood culture was *Penicillium marneffei* positive in all cases.

The skin lesion was generalized papules with central necrotic umbilication. Touch smear with Wright's stain of the lesion showed many yeast cells with clear central septation. Chest roentogenography revealed abnormal finding in one patient, showing multiple consolidations with diffuse reticulonodular shadow.

B-29

CLINICAL STUDY ON IVERMECTIN AGAINST 125 STRONGYLOIDIASIS PATIENTS

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We treated 125 patients with strongyloidiasis (78 males and 47 females) by 2 oral doses of ivermectin (6 mg) at 2-week interval, and obtained the following results:

1. Eradication rate after treatment was 86.4% (108 of 125 patients), responsively. Out of the 17 patients were resistant (non-responsive) to treatment, 8 patients received further course of ivermectin and all *Strongyloides stercoralis* in their feces were eladicated.

2. Side effects were observed in 7.2% of the patients after the first dose treatment and in 3.2% after the second dose. But all symptoms were mild and self-limited. Although liver disfunction developed in 13.6% of the patients, no symptoms occured and no special treatment was required.

3. Positive rate of anti-HTLV-I antibody in the resistant group was significantly higher (80.0%) than in the eradicated group (29.2%) and in stool-negative group (0%).

4. Although eosinophils before treatment in the eradica-ted group was significantly higher than that of controls, there was no significant difference between resistant group and controls. IgE levels in the resistant group was significantly lower than in the eradicated group.

We would like to conclude that IVM is the best drug for treatment of the patients with *Strongyloides stercoralis* not only from this results but also our previous reports which had investigated the clinical efficacy about thiabendazole, mebendazole and albendazole.

B-30

PARAGONIMUS OHIRAI: IN VIVO AND *IN VITRO* EXPERIMENTS ON DRUG-INDUCED CELLULAR CHANGES IN THE GUT EPITHELIUM AFTER TREATMENT WITH PRAZIQUANTEL AND BITHIONOL

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Praziquantel (PZQ) is an anthelmintic agent with high activity against a broad spectrum of schistosomes and some cestodes. *In vitro* and *in vivo* studies on the effects of PZQ have shown that it causes contraction of musculature, a rapid and extensive vacuolation of tegument, and destruction of female reproductive organs as a result of altering Ca^{2+} flux. Bithionol (BT) is known to inhibit activity of some enzymes in the tricarboxylic acid cycle, resulting in the destruction of morphological integrity of tegument, subtegumental cells and parenchymal cells. Scant attention has been paid to ultrastructural damage or changes in the gut epithelial cells of digeneans after treatment with anthelmintics. The present study was carried out to investigate changes in gut epithelial cells of the adult lung fluke, *Paragonimus ohirai,* affected by PZQ and BT *in vivo* and *in vitro.*

For *in vitro* study, worms were incubated in the medium NCTC 135 containing 3×10^{-4}M PZQ or 10^{-4}M BT at 37°C for 1, 3, 8 and 20 h. For *in vivo* study, PZQ was administered orally twice to adult rats at the dose level of 500mg/kg body weight/day before examination. BT was administered orally 3 times every other day to adult rats as an aqueous solution at the dose level of 200 mg/kg body weight/day before examination.

The effects of PZQ: *In vitro,* the gut epithelium showed an increase in numbers of autophagic vacuoles, most of which included mitochondria and/or lipid droplets and/or whorls of endoplasmic reticulum. Multivesicular bodies appeared and the number of Golgi complexes increased. *In vivo,* myelin-like membranous bodies were observed. Autophagic vacuoles including cytoplasmic elements were conspicuous near the luminal surface of the epithelial cells. The effects of BT: *In vitro,* the cells showed a variety of damage. The tall, columnnar cells in the secretory phase were swollen and vacuolated. Short, dense, pyramidal cells in the absorptive phase appeared to be normal. Active phagocytosis was observed at the luminal surface of the epithelial cells after 3h of incubation. Autophagic vacuoles and membranous bodies were seen throughout the cells. Cellular breakdown was pronounced after 3h of incubation. *In vivo,* autophagic vacuoles were present near the apex of the cells. Cellular breakdown was conspicuous on day 8 post-treatment.

These results suggest that autolytic breakdown of gut epithelial cells induced by both drugs is probably an important contributory factor to the eventual death of the parasite.

B-31

SCREENING METHOD FOR ANTI-*TRYPANOSOMA* AGENTS USING ELISA

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The new method for anti-*Trypanosoma cruzi* (T.c) agents was developed. In this method, the procedure was easy and many test samples were examined at once. Moreover, the results were reproducible. Numbers of T. c were measured by ELISA method using the anti-T.c antibody after T.c was infected to LLCMK2 cells with or without test sample and then infected cells were cultured for 5 days. The optimum conditions for assay were as follows: 0.08% for concentration of SDS, 3200 times for dilution of anti-T.c antibody and 12.5μg/ml

for antigen proteins. T.c proliferated about 30-100 times in the control well. Ragonile, as a standard agent, inhibited the T.c proliferation in a concentration dependent manner. The known antibiotics were examined by this method. Clindamycin, kitasamycin, colistin and amphotericin B inhibited the proliferation of T.c without toxicity to cells, but other antibiotics containing 12 aminoglycoside, 4 tetracycline, 3 macrolide, 3 β-lactam and 5 penicillin antibiotics were not effective in inhibiting the proliferation of T.c.

B-32

EFFECTS OF BERENIL AND OTHER DYSKINETOPLASTIC FORM INDUCING SUBSTANCES ON *TRYPANOSOMA GAMBIENSE* IN MICE

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Berenil(Hoechest) shows a preferential binding to AT-rich DNA and inhibits DNA synthesis. Berenil is known to induce dyskinetoplastic trypanosomes. But, analysis of formation of dyskinetoplastic forms has not been attempted. In the present study, therefore, we examined in detail the mode of formation of dyskinetoplastic form appeared after injection of Berenil into *T. gambiense* p-rosaniline sensitive and resistant clones infected mice. The numbers of appearance of the various forms of trypanosomes were examined at intervals after injection of 10 μg/g of Berenil. The result showed that dyskinetoplastic forms are induced as a result of not only inhibition of kinetoplast duplication, but also disorganization and disappearance of one kinetoplast due to an unequal division of kinetoplast by the drug without any affect on nuclear and cytoplasmic duplication. Some trypanosomes with one nucleus and both a stainable and an unstainable or insignificantly stainable kinetoplasts appeared in p-rosaniline sensitive clone (WS) after the treatment with Berenil. A stainable kinetoplast in which no flagellum is seen near to it, is observed close to the nucleus or anteriorly far from it. Such trypanosomes appeared in WS after the treatment with Berenil, but not in p-rosaniline resistant clone (WR). We have never seen such migration of kinetoplast and undevelopment of flagellum in trypanosomes treated with various chemicals, suggesting that the action of Berenil differs from those of other chemicals. The rate of appearance of dyskinetoplastic forms is 18 % in WS 4 h after the treatment with Berenil, while in WR, the rate is as low as 8 %. The present study indicates that WS is more sensitive than WR to the effect of Berenil in inhibiting kinetoplast division and in inducing disorganization and abnormality of kinetoplasts. Therefore, the following study carried out to examine interrelationship of effects of various dyskinetoplastic form inducing substances on trypanosomes. The clone(WBR) which could be obtained from WS repeatedly treated with Berenil, is used. WBR can still grow after injection of as much as 50 mg Berenil. The rate of appearance of dyskinetoplastic forms 4 h after injection of p-rosaniline, ethidium bromide and acriflavine and of Berenil into WS infected mice was about 25 and 18 %, respectively. In WBR, however, the rate was low as being 2-4 and 8 % after injection of these chemicals. This finding suggests the existence of some relationship between the actions of dyskinetoplastic form inducing substances on the kinetoplast of trypanosomes.

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A LITERATURE SEARCH FOR ETHNOBOTANICAL INFORMATION ON PLANTS TRADITIONALLY USED FOR THE TREATMENT OF PARASITIC DISEASES IN GUATEMALA AND OTHER CENTRAL AMERICAN COUNTRIES (A MINIREVIEW)

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It is by no means easy to reduce the number of patients suffering from parasitic diseases by administering imported drugs in the endemic areas of tropical and subtropical zones where the drugs are inevitably accompanied with difficulties in transportation and expensive for the inhabitants standard. Successful utilization of medicinal plants of local origin is presumed to be appropriate to such countries(Ref. 7). This would be exemplified in Guatemala, one of the Central American countries. Located in the tropical region, Guatemala is a mountainous country the inhabitants of which are mostly infected with a wide variety of parasitic diseases including obstinate ones. In this country medicinal plants have been used traditionally for maladies since the Maya times. Drugs from indigenous trees and grasses play an important role still nowadays in the treatment of parasitic infections among Guatemalan people. However, there are some problems about the utilization of medicinal plants. One of them is that the traditional drugs are administered to patients based on empiricism or popular beliefs. The efficacy of the drugs and the dose-effect relationship should be examined scientifically for the successful treatment of patients with the drugs.

Despite the importance of the scientific validation of plant-origin drugs in antiparasitic effectiveness, the information on medicinal plants believed to be useful in the treatment of parasitic diseases in Guatemala and neighbouring countries is scattered(Refs. 1-5 and 9). Even if the information on medicinal plants utilized for the treatment of parasitic infections is available, it is devoid of botanical identification. With the background of these drawbacks to the information, the compilation of the review articles with scientific names of the plants is now in progress. One of them has appeared recently (Ref. 6). The possibility cannot be denied that there are still a number of the useful plants which have never been recorded. However, so far as the information hitherto collected is concerned, about 300 species of Mesoamerican plants are traditionally believed to be effective in the treatment of parasitic infections. The spectrum of attributable activity of the medicinal plants is such as ameba, leishmania, malaria, trichomonas, plathelminthes, and nemathelminths. Another difficulty is to describe the species names of expelled worms because most of them are recognized usually by inhabitants not parasitologists. The scientific names of the parasites remain to be clarified following experimental and clinical studies and eventually be included into a newer review.

This communication briefly reviewed pivotal articles relating to the importance of medicinal plants for the treatment of parasitic diseases in endemic areas and the information so far obtained on the plants believed to be useful for the treatment of parasitic infections in Central American countries including Guatemala. Now there seem to be three things which should be carried out. First, possible further information should be collected on any other plants customarily used for the treatment of parasitic infections from inhabitants, especially old people familiar with medicinal herbs. Second, the traditionally believed efficacy of plant-origin drugs against parasites should be validated scientifically as mentioned above. And third, those medicinal plants should be preserved and increased that might to extinct being influenced with the decay of tropical forests as a consequence of population growth, worldwide economical crisis, environmental pollution and other factors (Ref. 8).

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ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

VOL. 22 NO. 1 MARCH 1994

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Jpn. J. Trop. Med. Hyg.

JAPANESE SOCIETY OF TROPICAL MEDICINE