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【CONTENTS】

Original articles

Detection of *Giardia* cysts in sewage and estimations of giardiasis prevalence among inhabitants in Hyogo Prefecture, Japan

Oda, T., Kawabata, M. and Uga, S. 1

Epidemiological study on *Ceratomyxa armatus* metacercariae in the Chikusa River, Hyogo Prefecture, Japan

Kimura, D. and Uga, S. 7

Reviews

Review and future perspectives of “development study” in the health sectors

Kobayakawa, T., Nakano, H., Hozumi D., Akashi, H.,
Minamoto, Y., Miyazaki, K. and Ashida, T. 13

Proceedings of the 45th Annual Meeting of Japanese Society of Tropical Medicine 19

Detection of *Giardia* cysts in sewage and estimations of giardiasis prevalence among inhabitants in Hyogo Prefecture, Japan

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Abstract: We recovered *Giardia* cysts from sewage and estimated the prevalence of giardiasis among inhabitants in Hyogo Prefecture, western Japan. Two 200-ml sewage samples were collected once a month from two different sewage plants (A and B) during a one-year period. After pretreating the samples by filtration and centrifugation in the presence of alcohol and diethyl ether, an immunomagnetic separation method was applied to isolate *Giardia* cysts. The mean number of cysts recovered from plants A and B were 880/L and 2,000/L, respectively. Circadian rhythms in the number of cysts detected on each of two examination days were observed with a peak at 10:00 a.m., and seasonal variations in cyst numbers were also observed with two peaks in May and December. By using the data obtained from this survey, we estimated that the prevalence of *Giardia* infection in inhabitants in the areas serviced by plants A and B were 0.1-1.1 and 0.1-1.3%, respectively.

Key Words: *Giardia intestinalis*, sewage, detection method, prevalence

INTRODUCTION

The flagellate protozoan parasite *Giardia intestinalis* is widely distributed throughout the world with a high prevalence in developing countries in the tropics and subtropics. *G. intestinalis* infection is acquired by digesting cysts excreted from infected humans and animals [1]. Approximately 20 to 30% of inhabitants in developing countries in Asia and Africa are infected with this parasite. In developed countries with improved hygiene conditions, meanwhile, *Giardia* infection also constitutes a public health concern [2]. In developed countries, the parasite is brought back from abroad by infected travelers [3]. Many outbreaks of giardiasis due to ingestion of inadequately treated water have also occurred in developed countries; five of these occurred in the United States in 1993 and 1994 [4]. Although a direct smear method is frequently used to detect *Giardia* cysts in stool samples, it does not consistently reflect the accurate prevalence of giardiasis in a given survey area. For instance, 15 to 20% of patients are reportedly diagnosed as false negative [5], despite the use of appropriate fecal examinations. The need for examination of three consecutive stool samples has therefore been indicated for accurate diagnosis [6,7].

The reported prevalence of giardiasis in the metropolis and Tokai regions of Japan ranges from 0.2-0.9% [8,9].

However, the study populations consisted of outpatients and/or inpatients who might not have represented the total population in the area. Furthermore, it is time consuming and expensive to examine thousands of individuals, and it is difficult to obtain consent for stool surveys.

Sykora *et al.* examined 11 sewage plants at different cities in the USA and detected 642 to 3,375 *Giardia* cysts per liter of samples [10], and Jakubowski *et al.* used these data to clarify the relationship between cyst number and prevalence in the respective cities [11]. Although these studies are interesting from the viewpoint of simplicity and/or economy, they do not discuss the recovery efficiency of their method or the circadian rhythm of cyst numbers. In this study, we recovered *Giardia* cysts from sewage samples using the immunomagnetic separation method at a fixed time each month and used the results to estimate the prevalence of the intake of *Giardia* infection among inhabitants in Hyogo Prefecture, Japan.

MATERIALS AND METHODS

Sewage Plants

The present survey was conducted at two different sewage treatment plants (A and B) in Hyogo Prefecture, Japan, during a one-year period (July 2002 to September 2003). Plant A is located on an $44 \times 10^5\text{-m}^2$ artificial island

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constructed in 1981 in the southern part of the prefecture. Approximately 15×10^3 people live on this island, and plant A treats only the sewage from this population. There are dogs and cats but no large animals on the island. On the other hand, plant B is located 22 km west of plant A and treats the sewage of approximately 205×10^3 people. Many livestock animals such as cows, chickens and pigs are bred in this area. The mean daily flow of plants A and B during the study period was 8×10^6 and 66×10^6 L, respectively.

Sample Collection and Concentration

Two hundred ml of sewage was collected from each plant at 10:00 a.m. once a month for one year. Samples were placed in 250-ml conical tubes into which 2 ml of 10% gelatin solution was added to effectively disperse the substances. The tubes were then capped tightly and their contents mixed thoroughly. In an additional experiment, samples were collected from plant A every two hours for 24 hours to clarify the hourly changes in cyst number. We repeated this experiment twice, on May 28 and August 12, 2003. In addition, sampling was performed for 5 continuous days in plant A to compare cyst number before (April 14-18, 2003) and after the holiday week (May 12-16, 2003).

The sewage sample was filtered through a stainless steel sieve with $32\mu\text{m}$ pores, then centrifuged at $1,000 \times g$ for 10 min. The supernatant was aspirated leaving 4 ml of concentrate in the tube into which 4 ml each of ethyl alcohol and diethyl ether were added (total 12 ml). The samples were then mixed and incubated at 60°C for 5 min. After fur-

ther mixing, the contents were centrifuged at $1,000 \times g$ for 10 min, then the pellet was re-suspended in 15 ml of 0.1% gelatin solution and centrifuged again under the same conditions. Finally, the pellet was suspended in 10 ml of distilled water (Fig. 1).

Separation Procedure and Counting of Cysts

Giardia cysts suspended in the 10-ml water sample were purified using an immunomagnetic separation (IMS) method. In brief, samples were incubated with paramagnetic beads (Dynabeads, Dynal, Oslo, Norway) which were coated with antibodies raised against *Giardia*. The resulting complex of cysts and beads was then magnetically isolated and washed. The cysts were then dissociated from the beads, and the beads magnetically removed from the purified sample. The cysts were placed in a well-slide and stained using a fluorescein-labeled monoclonal antibody (EasyStain, Biotechfrontiers, North Ryde, Australia) then observed under a fluorescent microscope (BX50, Olympus, Tokyo, Japan). Round or oval fluorescein isothiocyanate (FITC)-labeled objects measuring $7\text{-}10 \times 12\text{-}15 \mu\text{m}$ with two or more internal characteristic features (2-4 nuclei, median bodies, axonemes) were counted as *Giardia* cysts.

RESULTS

The recovery efficiency of this method was calculated by spiking one hundred *Giardia* cysts labeled with red fluorescent dye (Colorseed, Biotechfrontiers, North Ryde, Aus-

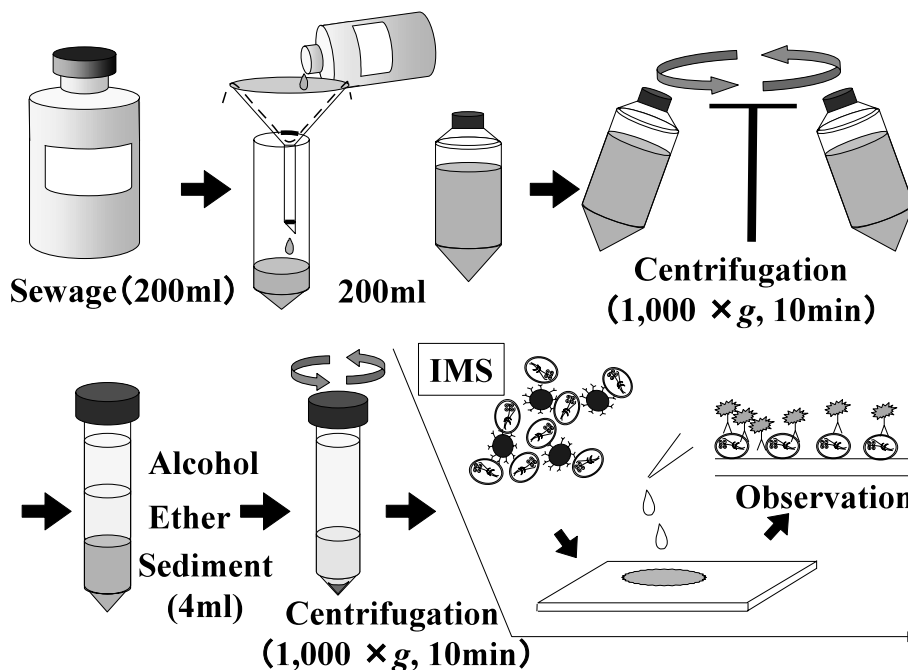


Fig. 1 Method for recovery of *Giardia* cysts from sewage.

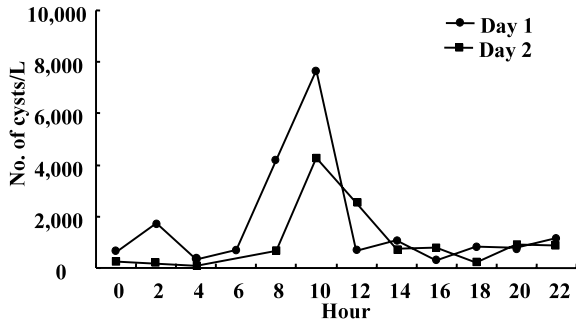


Fig. 2 Hourly change in number of *Giardia* cysts in relation to sampling time. Sewage samples were examined on May 28 (Day 1) and August 12 (Day 2).

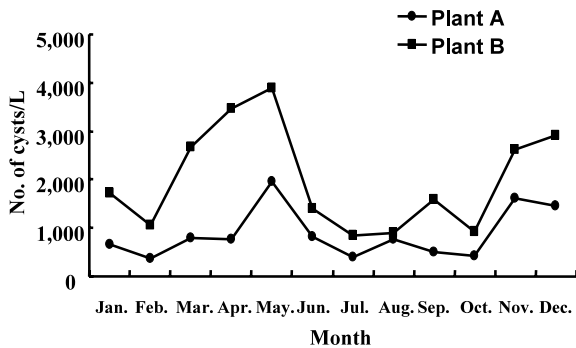


Fig. 3 Monthly change in number of *Giardia* cysts recovered from each plant. Plant A () is located on an artificial island and Plant B () is located in the suburbs.

tralia) into 200-ml sewage samples from plant A; this spiking procedure was repeated three times. The average recovery efficiency was calculated as 40% (37, 42, 40, n=3) and the number of cysts per liter of sewage sample was corrected according to this recovery efficiency.

Circadian rhythms in cyst numbers in plants A and B were observed, with a peak at 10:00 a.m. (4,300/L and 7,700/L, respectively) (Fig. 2). The average number of cysts was 1,700/L and 1,000/L, respectively). The ratio of cyst number at 10:00 a.m. to the daily average cyst number was 0.22 and 0.24 respectively, and the mean was 0.23.

Monthly investigation samplings were performed at 10:00 a.m.; therefore, cyst numbers were adjusted to the

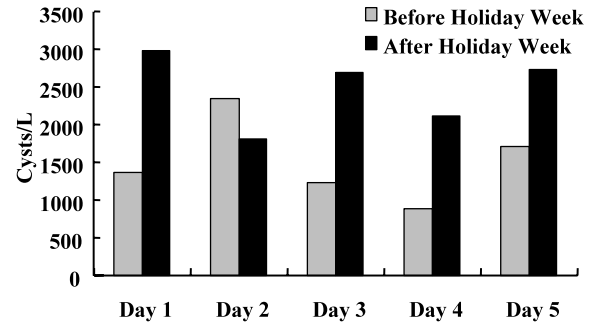


Fig. 4 Comparison between number of *Giardia* cysts before and after a holiday week. Sewage samples were collected for 2 series of 5 continuous days from April 14 to April 18 (before holiday week:) and from May 12 to May 16 (after holiday week:).

daily average number. The daily average number of cysts in each examination was equal to the cyst number detected \times correction factor (0.23; the correction factor is the coefficient used to correct the number of cysts obtained from the 10:00 a.m. sampling period to the daily average).

The seasonal variation in cyst numbers is shown in Fig. 3. The present study revealed that both of the sewage plants studied were positive for *Giardia* cysts, with a mean number of 880/L (range: 370- 2,000/L) and 2,000/L (range: 850 - 3,900/L) in plants A and B, respectively. Cysts were detected throughout the year in both plants, with the number of cysts detected in plant B being higher than that in plant A. Monthly changes in the number of cysts recovered from plants A and B showed two peaks, in one May and the other in December. The number of cysts detected after a holiday week was higher than that before: the average number detected before and after was 1,500/L and 2,500/L, respectively, and the difference was significant ($p < 0.05$ student's t test) (Fig. 4).

The estimated number of infected individuals on each of the examination days was the daily sewage flow \times cyst number of each monthly examination / number of cysts excreted by a patient in one day (1 to 2×10^8 ; according to Tsuchiya, 1931); the estimated prevalence was the estimated number of infected individuals / population in each

Table 1. Estimation of prevalence of giardiasis on examination days in areas covered by plant A and B

Plant	Quantity of sewage treated /day (L)*	Number of cysts found (L)**	Population	Estimated	
				number of patients	prevalence (%)
A	8×10^6	370 (min.)	15×10^3	15- 30	0.1-0.2
		880 (ave.)		35- 70	0.2-0.5
		2000 (max.)		80- 160	0.5-1.1
B	66×10^6	850 (min.)	205×10^3	280- 560	0.1-0.3
		2000 (ave.)		650-1300	0.3-0.6
		3900 (max.)		1300-2600	0.6-1.3

Estimated number of patients= $*$ \times $**$ /number of cysts excreted per patient per day (1 to 2×10^8 , according to Tsuchiya, 1931)

Estimated prevalence=estimated number of patients / population in each plant area.

plant area.

The prevalence of giardiasis in the areas serviced by plants A and B was estimated using the above results (Table 1). Consequently, the average monthly prevalence was 0.1-1.1 and 0.1-1.3%, respectively.

DISCUSSION

Giardia is widely distributed throughout the world with a varying rate of prevalence [2], and it is one of the most common enteric parasites recovered from humans, especially in tropical areas. However, it has become difficult even in these areas, to obtain consent to sample fecal materials. In the U.S.A., attempts have been made to detect *Giardia* cysts in sewage and to estimate giardiasis prevalence [11], but the sucrose floatation and Lugol's iodine techniques used are relatively insensitive. For instance, using the sucrose floatation technique, Sykora *et al.* found highly variable results (0.4 to 77.8%) with a mean of 11.0% [10]. With this in mind, we used a modified immunomagnetic bead method [12] to detect *Giardia* in sewage samples. As a result, the average recovery efficiency from 200-ml of spiked sewage samples was 40% and estimations of cyst concentrations were more precise.

We observed marked seasonal variations in *Giardia* cyst recovery, with two peaks in the cold (December) and wetter seasons (May), in this study. These findings were consistent with previous results. For example, among Guatemalan children, the incidence of giardiasis was highest during the cooler and wetter months [13]. Furthermore, in Saudi Arabia, the highest incidence occurred during September, which is the wettest month of the year [14]. Therefore, cyst numbers in sewage might be related to climatic conditions. Jarroll *et al.* also suggested that *Giardia* cysts find cooler and wetter conditions more favorable for survival [15].

In the present study, the number of cysts detected after a holiday week exceeded those detected before that week. This finding observed might be related to the Japanese life style, that is, many Japanese take vacations from the end of April to the middle of May and travel to foreign countries, including developing countries, and this custom is also seen at the end of the year. It is possible therefore those individuals are infected during these overseas trips, especially during trips to endemic areas. The present findings correlated well with the holiday period in 2003 (April 29 to May 9). According to the Immigration Bureau, 570,000 Japanese traveled overseas during May 2003, and of these, approximately 230,000 traveled to developing countries. This suggests that 290 of the inhabitants in the area around plant A traveled to developing countries.

Kimura *et al.* examined Japanese travelers who visited Nepal and India for more than 10 days and who experienced diarrhea when they arrived back in Japan [16]. Their survey revealed that 69 out of 692 (9%) travelers were positive for *Giardia* cysts. *Giardia* is the most common enteric protozoan parasite in Nepal and India [17,18,19,20] and has remained top of the list for the past decade [17]. Similar imports of *Giardia* infections into developed countries have been reported elsewhere [3]. The reported *Giardia* cyst shedding rates in calves ranges from 80 [21] to 100% [22] in British Columbia, Canada. However, since the area serviced by plant A is an isolated artificial island where livestock are not bred, we can rule out the possibility of sewage contamination by such animals. The hourly variation results obtained in this study also clearly demonstrated that cyst concentrations are related to human activity, and the findings strongly suggest that the *Giardia* cysts were excreted from inhabitants of this island. This result agrees with the earlier study of Gassmann and Schwartzbrod who suggested that a peak of *Giardia* cysts was observed at 10:00 a.m. in Nancy, France [23].

It is difficult to assess the number of cysts excreted daily from observations of a single infected individual. Therefore, we calculated the number of infected individuals based on the recovery efficiency, monthly survey, and variation in cyst numbers at different sampling times. Tsuchiya calculated that the number of cysts excreted from an individual/day varied from 0.7×10^8 with cyst-excreting and non-excreting days occurring alternatively during the course of infection [24]. Using this data as a base, we estimated the mean number of cysts excreted per day per patient. Accordingly, the mean number of cysts excreted for a period of during six weeks after the onset of symptoms was 1×10^8 , but when calculations were conducted using only the cyst-excreting days the value was 2×10^8 .

The infection rate of *Giardia* in the areas serviced by plants A and B was estimated as 0.1-1.1 and 0.1-1.3%, respectively, and these results were consistent with the results obtained from fecal examinations in Japan (0.2-0.9%) [8,9]. This study revealed that sewage samples can be used to estimate giardiasis in a particular service area (community) using the IMS method. The method developed for this survey was easy, cost-effective and time saving, and it allowed for accurate estimations of the prevalence of giardiasis. We believe it can be used for future surveys of the prevalence of giardiasis in other areas.

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Epidemiological study on *Centrocestus armatus* metacercariae in the Chikusa River, Hyogo Prefecture, Japan

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Abstract: The prevalence of *Centrocestus armatus* metacercariae in fresh water fishes were examined in Hyogo Prefecture, western Japan for a period of one year from April 2003 to March 2004. Three species of cyprinoid fish, namely *Zacco temminckii*, *Zacco platypus*, and *Pseudogobio esocinus*, were found to harbor metacercariae of *C. armatus*. The infection rate of metacercariae in *Zacco temminckii*, *Zacco platypus*, and *Pseudogobio esocinus* was 99% (371/377), 100% (8/8), and 100% (2/2), respectively. The mean number of metacercariae recovered was 232 for *Z. temminckii*, 2,123 for *Z. platypus*, and 20 for *P. esocinus*. However, we focused on *Z. temminckii* in this study because it was found to be the most predominant fish species in the Chikusa River and to have high a metacercariae prevalence as well. In *Z. temminckii*, metacercariae were distributed in the brain (37%), viscera (35%), muscles (29%) and gills (0%). No metacercariae were recovered from the scales and fins. This is the first report of the occurrence of *C. armatus* metacercariae in the brain region of the second intermediate fish. The prevalence of *C. armatus* metacercariae was almost constant throughout the year, and no positive correlation was found between the prevalence and seasonal changes. However, a positive relationship was observed between prevalence of metacercariae and fish length, i.e., the intensity of infection increases with the size of the fish host ($p < 0.05$). People in the survey area sometimes eat fish raw, indicating enlightenment as well as the countermeasures to prevent *C. armatus* infection is needed.

Keywords: *Centrocestus armatus*, metacercariae, epidemiological study, freshwater fish, Chikusa River, Japan

INTRODUCTION

The fluke genus *Centrocestus* is a group of minute intestinal trematodes belonging to the family heterophyidae. *Centrocestus* is widely distributed in Japan, Taiwan, Republic of Korea, and Southeast Asia. Four species of *Centrocestus*, namely *C. armatus* [1], *C. formosanus*[2], *C. nycticoracis* [3] and *C. asadai* [4], have been reported to be distributed in Japan. These species normally infect fish-eating birds. They have also been reported to infect mammals, including man except for *C. nycticoracis* [5]. Moreover, *C. formosanus* var. *kurokawai*, *C. caninus*, and *C. armatus* have been reported to infect humans in Japan, Thailand, and Korea [6, 7, 8].

The first intermediate host of *Centrocestus* is a fresh water snail belonging to genus *Semisulcospira* [9], the second intermediate host fresh water fish, and birds such as *Nycticorax nycticorax* the final host [1]. About 20 species of fresh water fish including *Zacco platypus*, *Pseudorasbora*

parva, and *Carassius carassius* are known to serve as second intermediate hosts of *C. armatus* [1, 10].

Rim *et al.* [11] reported that metacercariae of *C. armatus* were found at a rate of 33-100% in five species of fresh water fish from three rivers in Korea. Furthermore, Sukontason *et al.* [12] reported the presence of *Centrocestus* metacercariae in six species of cyprinoid fish in northern Thailand. The prevalence of metacercariae in different locations, seasons, and fish species had also been reported. However, the number of fish examined by Rim *et al.* was limited to only 1-15 samples. Moreover, the prevalence of fish infection was not described and no precise description was provided regarding the identification of metacercariae.

In the present study, therefore, the state of *Centrocestus* metacercariae infection in freshwater fish of the Chikusa River in Hyogo Prefecture, Japan was investigated from April 2003 to March 2004. The correlation of seasonal changes and fish length to metacercariae prevalence was also determined.

MATERIALS AND METHODS

Survey area

The study was carried out from April 2003 to March 2004 in the Chikusa River in Hyogo Prefecture, Japan. The survey area was a midstream section of the river 30 m in width and 0.1 to 1.5 m in depth. Various fish species were captured using a landing net once a month for a period of one year. The collected fish were kept alive in a cooled-box with aeration and brought to the laboratory. Taxonomic identification of the captured fish species was carried out according to the fish atlas by Miyaji *et al.* [13]. In addition, water temperature was measured at the time of each survey.

Examination and observation of metacercariae

The fish brought to the laboratory were kept alive in an aquarium, with artificial aeration, until the examination of metacercariae. The body length of each fish sample was also measured and recorded. To obtain the metacercariae from each sample, fish was minced and digested using a digestive solution (0.1% each of pepsin and HCl in distilled water) at 37 °C for 1 hour. The digested material was filtered through a 325 µm mesh to remove large particles and rinsed twice with saline solution (0.85% NaCl). The sediment was then centrifuged at 150 x g for 5 minutes. The *C. armatus* metacercariae collected were counted under a stereoscopic microscope.

In other experiments, organs such as the brain, muscles, gills, viscera, scales and fins were also dissected and digested artificially to determine the distribution of the metacercariae in the fish body. The counting of circumoral

spines for identification of metacercariae was carried out using a light microscope and a scanning electron microscope (T-330A, JEOL, TOKYO). The size of different organs observed for metacercariae was also measured using a micrometer caliper (OLYMPUS, TOKYO) under a light microscope. In addition, the recovered metacercariae were excysted with artificial intestinal juice (0.4% sodium hydrogen carbonate, 1.0% trypsin, 0.85% NaCl) fixed with 10% neutral buffered formalin and observed under a scanning electron microscope to elucidate morphological detail.

Statistical analysis

The mean abundance was expressed as mean \pm standard deviation (SD). The difference between length of fish and mean abundance was evaluated by one-way factorial analysis of variance (ANOVA), followed by Fisher's PLSD multiple comparison test to identify significant differences among the multiple samples. Differences in the prevalence of infection were tested using χ^2 test. A p value less than 0.05 was considered statistically significant.

RESULTS

Throughout course of the survey we caught 511 freshwater fish from Chikusa River. These consisted of 15 different species as shown in Table 1. Eight of the fish species belonged to the cyprinoid family. Table 1 shows the infection rate of each fish species examined and the mean number of metacercariae per fish. Metacercariae were obtained from only three cyprinoid fish species, namely *Z. temminckii*, *Z. platypus*, and *P. esocinus* with a high infection rate (99-

Table 1. Prevalence of metacercariae and mean number of metacercariae recovered in fish collected from the Chikusa River, Hyogo Prefecture, Western Japan

Species of fish	No. examined	No. of fish infected (%)		Mean No. of Mc** per fish
<i>Zacco temminckii</i> *	377	371	(99)	229
<i>Rhinogobius</i> sp.	50	0	(0)	0
<i>Plecoglossus altivelis</i>	28	0	(0)	0
<i>Silurus asotus</i>	13	0	(0)	0
<i>Cobitis biwae</i>	12	0	(0)	0
<i>Zacco platypus</i> *	8	8	(100)	2,123
<i>Pelteobargus nudiceps</i>	5	0	(0)	0
<i>Coreopera kawamebari</i>	5	0	(0)	0
<i>Odontbutis obscurus</i>	3	0	(0)	0
<i>Pseudogobio esocinus</i> *	2	2	(100)	20
<i>Carassius carassius</i> *	2	0	(0)	0
<i>Puntungia herzi</i> *	2	0	(0)	0
<i>Squalidus gracilis gracilis</i> *	2	0	(0)	0
<i>Tribolodon hakonensis</i> *	1	0	(0)	0
<i>Cyprinus carpio</i> *	1	0	(0)	0

* : Cyprinoid fish

** : metacercariae

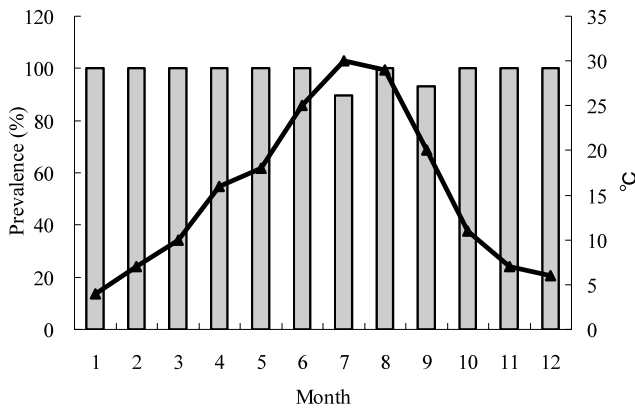


Fig. 1 Monthly changes in prevalence of metacercariae of *Centrocestus armatus* in *Zacco temminckii* () and water temperature ().

100%). *Z. platypus* showed the highest mean abundance of metacercariae. However, *Z. temminckii* was the most predominant fish species collected (74%) throughout the sampling period, and it also showed a high infection rate (99%). Therefore, further examinations were focused on *Z. temminckii*.

The monthly changes in the prevalence of metacercariae in *Z. temminckii* are shown in Fig. 1. The metacercariae infection rate of *Z. temminckii* was nearly 100% throughout the year. No positive relationship was observed between prevalence of metacercariae and seasonal changes and water temperature.

Throughout the survey, 84,921 metacercariae were recovered from infected 371 *Z. temminckii*, with a mean abundance of 229 (Table 1). Of the 11,760 metacercariae found in *Z. temminckii*, 37% (4,306) were observed in the brain; 0% (16) in gills; 35% (4,069) in viscera; and 29% (3,369) in muscles (Table 2). No metacercariae were found in scales and fins. The number of metacercariae was also computed per gram of fish meat. As a result, the brain showed the highest number of metacercariae per gram of meat (2,057/g), followed by viscera (262/g), muscles (20/g), and gills (5/g) (Table 2).

The relationship between fish length and the number of metacercaria per fish is also shown in Fig. 2. The preva-

Table 2. The distribution of *Centrocestus armatus* metacercariae in various parts of fish

Parts of fish	Mean Weight (g)	Mean No. of Mc	Distribution rate (%)	No. of Mc/g
Brain	0.07	144	37	2,057
Gills	0.22	1	0	5
Viscera	0.52	136	35	262
Muscle	5.59	112	29	20
Scale, Fin	0.47	0	0	0

Thirty *Zacco temminckii*, 9-10 cm, were examined.

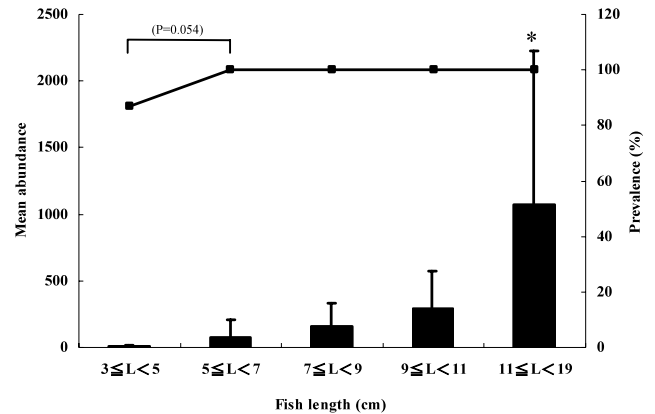


Fig. 2 Relationship of length of *Zacco temminckii*, mean abundance () and the prevalence () of *Centrocestus armatus* metacercariae in the Chikusa River, Western Japan. Data are expressed as mean \pm SD (n: 16-65). *: $p < 0.05$.

lence levels were almost the same irrespective of fish length ($p > 0.05$). Even young fish of four centimeters or less in length showed an infection rate as high as 90%. The results also showed that the number of metacercaria per fish was directly correlated with fish length, i.e., there was an increase in the mean abundance of metacercaria as the fish length increased ($p < 0.05$). The number of metacercaria recovered from fish samples with smaller than 5 cm and more than 11 cm in length was 8 and 1,069 respectively ($p = 0.017 \times 10^{17}$) (Fig. 2).

In addition, the recovered metacercariae were measured under a microscope. The average length was about 200 μm and the average width about 100 μm . The metacercariae were mounted on glass slides for detailed observation. The oral sucker, ventral sucker, and excretory bladder of mature metacercaria were observed. Light and scanning electron microscopic observations revealed that the oral sucker was located at the anterior end, with 44 circumoral spines arranged in two rows around it (Fig. 3a, 3b). The ventral sucker was located in the midventral part and the excretory bladder was shown as an "X"-shaped organ located posterior to the ventral sucker (Fig. 3a). However, these organs were not observed in immature metacercariae except for the eyespot that was a remnant of the cercarial stage (Fig. 3c).

DISCUSSION

Seven out of 20 known freshwater fish species were observed in this study as second intermediate hosts of *Centrocestus armatus* [1, 10]. In the present study, metacercariae of *C. armatus* were detected in only 3 out of the 15 freshwater fish species obtained from Chikusa River,

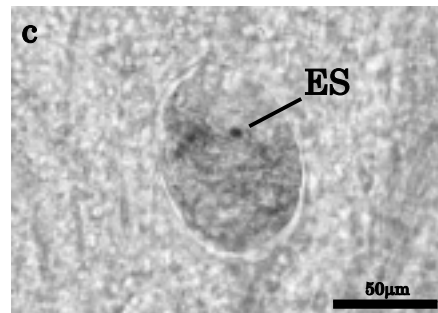
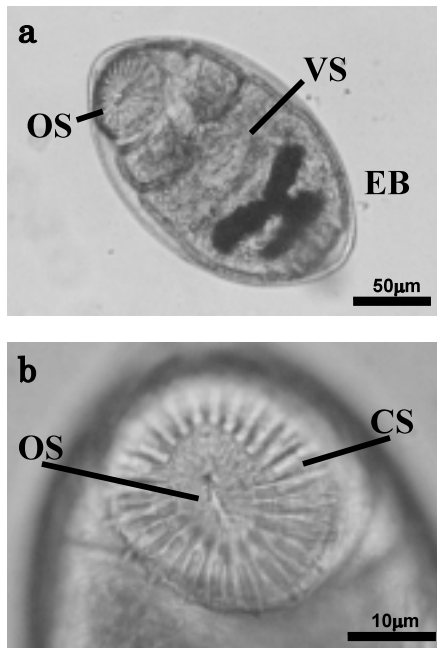


Fig. 3 Microscopic photographs of *Centrocestus armatus* metacercariae.

- A. Metacercariae showing the oral sucker (OS), ventral sucker (VS), and excretory bladder (EB);
- B. Oral sucker showing the circumoral spines (CS);
- C. Immature metacercariae showing the eyespot (ES).

namely *Zacco temminckii*, *Zacco platypus*, and *Pseudogobio esocinus*. The prevalence of metacercaria in these three species of freshwater fish was almost 100%. Among the 3 species, *Z. platypus* showed the highest mean abundance of metacercaria. However, since *Zacco temminckii* was found to be the most predominant fish species collected from the river, it can be inferred that this fish played an important role as the second intermediate host of *Centrocestus armatus* in the Chikusa River.

The prevalence and intensity of trematodes infection to second intermediate hosts such as fish were said to be associated with seasonal changes [14]. Sukontason *et al.* [12] mentioned that trematode metacercariae levels were highest in winter and lowest in the rainy season. Several other studies showed that the emergence of cercariae from the first intermediate host increased as water temperature increased [15, 16]. A study was also conducted on prevalence of cercariae of *C. armatus* in the Chikusa River by Kimura and Uga [17]. They showed that the mean prevalence of cercariae in the snail *Semisulcospira libertina* was low at 6% (1-16%) during winter and spring seasons (October-May) when water temperature was low. In contrast, the period from June to September when water temperature was higher showed a very high prevalence. The mean prevalence value was 39% [17]. The authors conclude that the infection of cercariae to fish as the second intermediate host had occurred during summer when water temperature was high. However, the results of the present survey did not show any seasonal changes in prevalence of metacercariae. Chubb [14] also mentions that changes in the prevalence of metacercariae were not very evident at different climatic

seasons of the year. He states that metacercariae could thrive for a long period of time inside the fish host and therefore that no drastic changes in metacercariae prevalence were observed at different seasons of the year.

Rim *et al.* [11] examined the distribution of metacercariae in the different organs of fish using 22 samples of *Z. temminckii* and *Z. platypus*. They reported that the metacercariae were distributed in the gills, muscles, intestinal organs, and scales and fins, and that the intestinal organs showed the highest mean abundance of metacercariae. In the present study, the brain, muscles, viscera, gills, and scales and fins of 30 *Z. temminckii* were examined for presence of metacercariae. The brain region showed the highest mean abundance (144), followed by the viscera and muscles with abundance of 136 and 112, respectively. However, the gills showed only a mean of 1, and 0 for scales and fins. These results were similar to the observation of Rim *et al.* [11] except for the presence of metacercariae in the brain region. In general, metacercariae of any fluke species can be found in the muscles and viscera of the fish body. In the present study, however, it is interesting that the infection of metacercariae of *C. armatus* was observed in the brain region of the second intermediate host. No related studies have reported the occurrence of metacercariae of *C. armatus* in the brain.

Poulin [18] reported the positive correlation of fish length and mean abundance of metacercariae. Dogiel *et al.* [19] also stated that, in fish populations, the intensity of infection by parasites increased with the age and size of the fish hosts. The same result was observed in the present study, i.e., the longer the fish length the higher the metacer-

cercariae infection. At a fish length of 3-5 cm, the mean abundance was 8, but this increased gradually to 1,069 at a fish length of 11-19 cm ($p=0.017 \times 10^{-17}$). Since a fish with a larger length has a higher contact area, it is likely that cercariae have more chances for attachment to the fish body surface. These cercariae, in turn, could develop into metacercariae inside the fish body and accumulation could occur over time. That is why a higher number of metacercariae was observed in older fish with a greater fish length.

It was reported that metacercaria of the four species of *Centrocestus* distributed in Japan have a different number of circumoral spines: *C. armatus*, *C. formosanus*, *C. nycticoracis*, and *C. asadai* have circumoral spines of 44, 32, 42, and 38, respectively [10]. Throughout the present survey, the recovered metacercariae were found to have 44 circumoral spines and were identified as *C. armatus*.

In conclusion, the prevalence of *C. armatus* in *Z. temminckii* was high in the Chikusa River and the fish *Z. temminckii* is considered to be the most common second host. The questionnaire survey done in our study area [20] revealed that 52% of the inhabitants have eaten fresh water fish including *Z. temminckii*. Moreover, about 10% of inhabitants eat these fish raw, indicating possibility that the people living in this area have been infected by *C. armatus*. Further enlightenment as well as the countermeasures to prevent the infection is needed.

ACKNOWLEDGEMENTS

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Review and future perspectives of “development study” in the health sectors

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Abstract: The research project titled “Developing Technical Approaches for the Master Plan of the Health Sector International Cooperation” has been conducted for the duration of three years from 2002 by the support of grants-in-aid for research on international medical cooperation from the Japanese Ministry of Health, Labor and Welfare. Based upon comprehensive and detailed review on the development studies (DS) in the health sectors so far completed, and throughout extensive and detailed examinations on the various DS related issues at the organized workshops with the parties concerned, the following propositions were made for the orientation and possible improvement of future DS in the health sectors; 1) a master plan in the individual DS to be defined in association with the level of strategy with which the study deals, 2) the instruction of surveys and methods to be more crystallized, 3) appropriate survey methods with reproducibility to be employed, 4) qualitative researches to be complementarily exercised with quantitative researches 5) the ownership of DS to be cultivated by adoption of participatory methods.

INTRODUCTION

This article deals with the study results of the research project titled “Developing Technical Approaches for the Master Plan of the Health Sector International Cooperation”. The government project was supported by the Japanese Ministry of Health, Labor and Welfare which was to be implemented for the duration of three years from 2002. The purpose of this research project was to determine the orientation of future DS in the health sectors by reviewing the studies experienced so far and by introducing various study methods and analytical methods for developing health policies and health planning techniques.

A DS is a form of technical cooperation provided by the Japanese Official Development Assistance (ODA) to assist in the planning of urgent and high priority development projects in developing countries through the preparation of relevant reports [1]. Traditionally, a DS has been conducting feasibility studies prior to assigned projects, mainly in the fields of social infrastructure including electricity power supply and road constructions. In recent years, apart from

these hardware type projects, a DS is also involved in software type projects such as making propositions for the establishment of policies, with which it reflects the diversified and increasing needs of the developing countries. Since the start of the DS projects in health sectors, eight studies which include that of Honduras, have so far been completed (Table 1). DS oriented development assistance aimed at improving the health policy/administrative system is expected to increase. A DS survey is initiated by the implementation of fundamental surveys. Based on the results of those surveys, a master plan is formed. There are two features with which the DS draws the line from other technical cooperation sectors. A DS may propose a policy during the course of a study but any field surveys, analysis and other related studies in the report making are actually conducted by a Japanese consultant. However, the know-how in the investigation, analysis, planning and management of a study by the implementing agency such as the Japan International Cooperation Company (JICA) or the consultants currently appear to be not fully competent. Therefore, the primary objective of this research project is to improve future Japanese

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Table 1. Development study projects with implementation periods in the health sectors

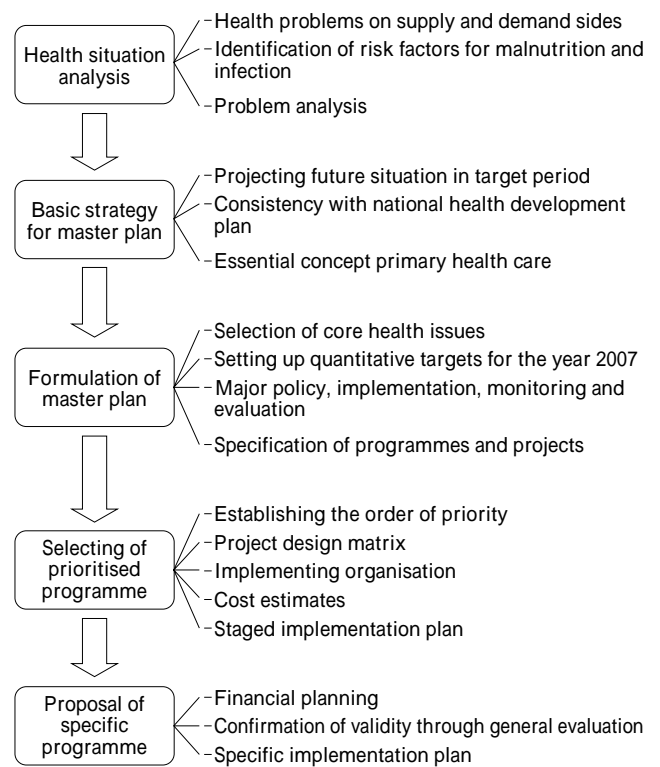
The study on the strategies and plans for the upgrading of health status in the Republic of Honduras [January 1995 ~ October 1996]
The study on strengthening the district health system in the western part of Kenya [August 1997 ~ November 1998]
Master plan study on strengthening primary health care services in the Republic of Malawi [June 1998 ~ January 2000]
The development study on reproductive health in the state of Madhya Pradesh, India [November 2000 ~ March 2002]
The study on the improvement of health and medical services in the Lao People's Democratic Republic [March 2001 ~ November 2002]
The study on enhancement of district health system for Beni Province Prefecture in the Republic of Bolivia [June 2001 ~ February 2003]
The study on the restructuring of health and medical system in the Republic of Uzbekistan [October 2002 ~ December 2003]
Master plan study for strengthening health system in the Democratic Socialist Republic of Sri Lanka [March 2002 ~ December 2003]

ODA projects by means of viewing and subsequently suggesting a future course for the DS in the health sectors. Secondly, the research examines the technical development as well as the analytical and planning methods related to the health sector master plan study.

STUDY METHODS

A comprehensive and detailed review is conducted on the seven DS projects already completed in Honduras, Kenya, Malawi, Laos, India, Sri Lanka and Uzbekistan [2,3,4,5,6,8,9]. Extensive analysis and detailed examinations were carried out on the various issues in the health sectors related to the DS through the workshops organized by the research group which are limited to the consultants and the JICA staff. A continuous review was performed on the operation and the administration in order to assure a smooth and effective implementation of the study. In addition, the review also examined the analytical and planning methods currently employed for the study in the institutions both at home and abroad. Besides, as a separate activity, the research group performed a follow-up of the DS study in Laos. It is now also in the process of re-analyzing and renewing the data and information obtained from that of Malawi in order to search for an appropriate health policy

Figure 1. Process of formulating a master plan in Malawi development study



upon the completion of the study.

For the readers, the implementation procedure of the DS will hereinafter be described as those shown in Figure 1. The main components of the DS is composed of field surveys, analysis of the survey results and other relevant data, the formulation of master plans including the selection of priority programs/projects, and providing technical cooperation. A detailed procedure on the formulation of a master plan will be explained by using the example of the DS in Malawi as is shown in Fig 1.

In developing a master plan, the basic strategies for reaching specific goals need to be drawn up. The strategy framework includes an outline of policies and programs that address the roots of the salient health issues. The first cycle of the study demonstrated that a considerable number of Malawians have relatively favorable access to health facilities, but their health indicators tend to be among the poorest in the world. Accordingly, the basic strategy for developing a master plan was directed towards bridging this gap while at the same time, the future situation of major issues such as population, economy, and disease conditions were projected for the respective target years. More importantly, the basic strategy for the formulation of a master plan was consistent with the national health development plan and was in accordance with the basic concepts of the local primary health

care.

In the first cycle of the study, two major issues were identified: child malnutrition and maternal health. Then, the study came up with a list of specific health problems related to these two core issues and then it determined the cause and effect relationships of these problems. The problem tree exercise aided in proposing interventions to the health problems (a long list), which were viewed as effective solutions. Subsequently, each proposed intervention was considered in detail and was integrated into comprehensive projects (a short list). The process of the integration and the prioritization of the projects were considered under the basic strategy or concept as described. Finally, concrete prioritized projects were proposed by taking priority, feasibility, cost-effectiveness and efficiency into account.

DEFINITION OF A MASTER PLAN

In this research project, the definition of a master plan in the DS was what we described in the beginning of a project. It is a comprehensive and fundamental plan for various public sectors including the health sectors. The definition of a master plan may be ambiguous between that of the JICA and the consultants. Therefore, in reality, the DS achievements may vary in their characteristics, depending on the individual definitions of the studies. The plural studies appear to define a master plan as the process from a strategy proposition for the formulation of a plan to the selection of priority programs/projects [3,4,6]. Specifically, it generically represents a conceptual master plan based upon a basic study with the existent data, field surveys, priority programs/projects, and action plans. Under such circumstances, the current research project group carried out a discussion and an analysis on the definition of a master plan prior to the full research activity.

Goals, strategies, scenarios, resources, programs/projects, and action plans are common components of a master plan. On the other hand, there could be presumably various levels of strategies with which a DS deals: the level of national health policy, the level of a priority health program defined in the national health policy (ex. program for the improvement of childhood malnutrition) and the level of a specific implementation activity in certain priority programs (childhood vaccination program). The mode and the substance of a study may of course vary according to each of the level concerned. In the present state of affairs, an overall framework of the master plan should be clearly revealed. Particularly, it is vital to define the relationship between a proposed master plan with the priority programs and the corresponding national health policy. Furthermore, relevance of the master plan ideas with SWAPs (Sector Wide

Approaches) as well as PRSP (Poverty Reduction Strategy Paper) should also be considered. Further studies will be required to define master plans of other sectors.

REVIEW OF A "DEVELOPMENT STUDY" IN GENERAL

A comprehensive review on the DS was performed. First, the principal issues with respect to the management and the technical subjects of the DS are described. In terms of management, some recipient countries tend to expect a materialization of the project following a completion of the master plan, on the assumption that the DS was a sort of feasibility study prior to a launching of a project. There was also indication that the instructions of surveys and methods used by JICA were extremely comprehensive and detailed without any given specificities while the process of plan formulations were complicated. Since the descriptions of achievements in the context of study were ambiguous and unspecific, a counter-proposal party was necessary in order to describe the difference of terms to JICA. These processes should be related to the preparatory study. However, since the purpose of the DS was not clearly defined at the time of the preparatory study, the actual development of the study was commenced without a conclusive result of the preparatory study. Therefore, it is suggested that specification of principles and fields of the study should be clarified at the time of a preparatory study.

Next, the technical subject on the implementation of the study was viewed. The field survey implemented in the DS is the bottom line for the formulation of a master plan. A field survey is an important opportunity to collect information and data relevant to health sectors in order to understand the health situation of a recipient country. The result of a field study will be extensively utilized and is itself a wider framework than that of a DS. The methods employed by the DS in quantitative researches in the health sectors include household survey, exit interview at health facilities, growth monitoring, drug and medical supply inventory survey, KAP (knowledge, attitude and practice) survey and health manpower assessment [10]. The first issue that one could point out regarding the quantitative research performed in the DS was the insufficient description of the survey and the analytical methods, which might account for the difficulty in verifying the relevance and the accuracy of a study. Similarly, inadequate reference to the planning methods and processes for the formulation of a master plan was found. Furthermore, problematic sampling and inappropriate study methods were employed in household surveys.

Recently, qualitative research techniques such as the Focus Group Discussion, Key Informant Interview and Ob-

servations have been introduced together with those of the quantitative research into the DS in health sectors. The purpose of a qualitative research is to interpret the phenomenon of a situation in question and why it happened, based on the recognition and interpretation of related parties. More specifically, it is an approach to comprehend the sense of value and the concept within the social, political and economical factors through the behavioral patterns of a party of interest such as the inhabitants. In the scene of an international cooperation, which is an external intervention, an effective usage of a qualitative research that reflects local needs without being assertive should be a subject of discussion in the future. When one considers an optimal effect on the practicality of international cooperation in the health sectors, qualitative researches could be complementarily exercised with quantitative researches. For example, the typing of phenomena by a qualitative research could precede a quantitative research or a qualitative research could also be utilized to reassess the appropriateness of the data collected by a quantitative research [10,11,12,13,14].

TECHNOLOGY TRANSFER

Technology transfer, ownership and follow-up in the DS are components closely related to each other and will be described together. In a DS, technology transfer works in different manners according to the level of development and maturity of the counterpart countries. In the countries with a high level of problem consciousness as well as ownership, one could expect their independent operation of the study even at the level of planning formulation. To the contrary, as is seen in many African countries with considerably low level of development, there often can be confusions between a DS and a project finding mission because of the lack of understanding as to what a “development study” is.

For all the DSs conducted in the health sectors so far, with regrets, the ownerships of the studies have not been well cultivated in Honduras, Kenya and Malawi. However, the participating consultants aptly compensated the ownerships with their expertise in formulating the planning. It was perhaps from the DS of Laos that the current ownership was transformed. The Laos study team worked on their task by implementing strong incentives to the counterparts and adopting participatory methods. Throughout the course of the study, the Japanese study team kept taking the position to accord technical supports for the master plan which would be exercised by the Laos side and consequently the counterpart actively participated in the implementation of the study. In the mean time, health forums under the auspices of the Laos Ministry of Public Health were held five times. This was a stakeholder meeting which took place

across the country with approximately 200 participants that included the minister, executive officers of the ministry, local health officers, donors and NGOs. Earnest discussions on programs or strategies were conducted during the course of the master plan formulation. The final report of the study was translated into Laos language immediately after the completion of the study. This translated version was distributed to the divisions concerned for enlightenment as well as brainstorming, especially in the regional areas and this undertaking is still underway. The autonomic movement was enhanced by the efforts of JICA advisor in the ministry. Thus, the implementation of the DS in Laos permitted any disputed points on health policy and programs to be on the grounds for discussion.

In the case of Sri Lanka, the Japanese study team put emphasis on the participation of stakeholders. Specifically, the counterpart independently organized weekly meetings and working groups within the structure of the Ministry of Health in their own realms. Most of the staffs at the position of directors or higher were involved in elaborating the work of the master plan. In addition, a review panel consisting of university members, research institute staff and OBs of the ministry commented on the draft of the master plan. Regional stakeholder meetings as well as seminars were frequently held to discuss the master plan in progress. Those undertakings also gave an opportunity to the follow up of the study.

During the DS of Uzbekistan, the analysis of survey results and the discussion for the formulation of master plan were implemented in workshops with specific minor groups composing of members who were from not only the central government but also the local governments, international organizations and NGOs et al. The introduction of such workshops with diverse members could be a major characteristic of a DS study.

The principle of a Japanese DS implies versatile utilization of a master plan and does not particular assume the executing body in the materialization of a proposed program/project. However, a partner country is generally more interested in whether or not the consequences of DS could lead to the implementation of the proposed programs/projects. Therefore, the Japanese side should unveil the definition of the so-called “batting average”. In other words, they should clarify the ultimate goal of the Japanese DS; by indicating whether the proposed programs/projects by the Japanese parties or other donors including international organizations would be implemented or whether the DS is only restricted to the master plan study itself. Second thought has to be given during the processing of a DS and during coordination with the donors in order to find an optimal way.

At the moment, the accomplishment of a DS cannot be

easily evaluated due to a lack of an established evaluation index. Therefore, it is necessary to crystallize an index with two possible viewpoints: an evaluation of the contract on the actual implementation of a study and an evaluation of the extent a master plan can be realized several years after a DS. The latter should include how a master plan is exploited and materialized. These evaluations should be conducted by not only an internal body, JICA, but also by one or several other third parties.

Throughout the current research project, improved quality of DS in the health sectors is definitely recognized, especially in terms of technology transfer and the development of national ownership. Meanwhile, JICA performed structural reform and reorganized the former principal assistant team system to five subject departments, namely, human development department, global environment department, rural development department and economic development department. This reorganization allowed a single department to take charge of a particular subject regardless of the different modalities for implementation. In addition, JICA defined “program approach” as an aggregate of activities and policies leading to the comprehensive and coordinated planning and implementation of activities under the ownership of the partner government. In the program approach, a variety of modalities, each having different advantages and disadvantages, were made available to meet the different development needs in different countries and sectors. It is expected that projects integrated in the overall coordination framework would contribute more effectively to the objectives of the overall program. At present, DS is being actively utilized under such programs and some DS with clear and designated objectives have already been carried out. It is expected that such trend will be happening in the health sectors soon. With that, it is necessary to prioritize research projects in terms of developing methods of analysis for health policies and health planning as well as developing various study methods based on the program approach.

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PROCEEDINGS OF THE 45TH ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE

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President

Keizo Yamaguchi, M. D, DMSc
Professor & Chairman,
Dept. of Microbiology & Infectious Disease
TOHO University School of Medicine

CONTENTS

Special lecture

K1 International contribution in the control of tropical diseases through new drug development Omura, S.

Educational lectures

E-1 Role of phosphoinositide 3-kinase in parasite infection Koyasu, S.
E-2 Why Influenza Kills and will Kill Again Kawaoka, Y.
E-3 Current status of dengue vaccine development. Kurane, I.
E-4 Bacterial intestinal infection and quorum sensing Kamiya, S.

Prize Winner's lectures

The JSTM Award of Excellence

K2 Quo Vadis: From Bench To Field Kojima, S.

JSTM Young Investigator Award

K3 Drug Susceptibilities and Clinical Manifestations of *Mycobacterium tuberculosis* in Zambia Mitarai, S.

Symposium 1

S1-0 Symposium 1: Learning Clinical Tropical Medicine in the Topics Oishi, K. and Nakamura, T.
S1-1 The short term training course in clinical tropical medicine in Philippines Hasegawa, M.
S1-2 Activities of the student rotation program in San Lazaro Hospital, 2003
Yamashiro, T., Nishizono, A., Kadota, J. and Mifune, K.
S1-3 Clinical clerkship in tropical medicine in Peru. Iwata, K.
S1-4 The clinical course in tropical medicine at Mahidol University in Thailand Matsumura, T. et al.
S1-5 Association of increased platelet-associated immunoglobulins and the severity
of disease in secondary dengue virus infections Saito, M., Oishi, K.
S1-6 Development of Malaria Vaccine based on Recombinant SERA Horii, T.

Symposium 2

S2-1 Role of infectious disease surveillance Taniguchi, K.
S2-2 Risk Management of Infectious Disease at the border.
Role of Quarantine Station in "Borderless" World Iwasaki, E.
S2-3 Crisis Management for Infectious Diseases in Borderless Era
-Role of Central Government- Ushio, M.
S2-4 Infectious disease outbreak and Press reports Minami, N.
S2-5 An outbreak of infectious disease hits civil society hard:
A case study of Japanese expatriate employees and their spouses in Hong Kong Nakamura, Y. and Yokota, S.

Workshop 1

W1-0 Role of Nursing Profession and NGO in Tropical Medicine Ishikawa, N. and Moriguchi, I.

W1-1	Traditional Medicine in Sri Lanka	Higuchi, M.
W1-2	Community Health Nursing in Indonesia	Moriguchi, I.
W1-3	NGO and International cooperation - the African experience	Tokunaga, M.
W1-4	The Role of Social and Behavioral Research on Tropical Diseases Control : A Case Study of Tuberculosis.	Jintana Ngamvithayapong-Yanai,.

Workshop 2

W2-1	Overview	Kamei ¹ , K.
W2-2-1	Coccidioidomycosis	Kawabata, M.
W2-2-2	A case of chronic disseminated histoplasmosis emerged during steroid maintenance therapy for rheumatoid arthritis with secondary amyloidosis and nephrotic syndrome	Takakura, S.
W2-2-3	Paracoccidioidomycosis	Igarashi, T.
W2-3	Imported mycoses related to zoonoses	Sano, A.
W2-4	Causative agents of imported mycoses	Nishimura, K.

Workshop 3

W3-1	Purposes of establishing preventive guidelines against malaria	Kano, S.
W3-2	Mosquito Control for Malaria Personal Protection	Haruki, K.
W3-3	Chemoprophylaxis	Okuzawa, E.
W3-4	Stand-by treatment	Kimura, M.
W3-5	Travel advice based on the guidelines for malaria prevention	Hamada, A.

General presentation

1	Role of costimulatory molecules in host resistance to re-infection with <i>Hymenolepis nana oncosphere</i>	Onishi, K. <i>et al.</i>
2	Taeniasis in Bali, Indonesia	Yamasaki, H. <i>et al.</i>
3	The Study Of <i>Ascaris</i> And Ulcerative Colitis Part 2: Localization Of Mucin 12 On <i>Ascaris Lumbricoides Hominis</i> -	Hayashi, I. <i>et al.</i>
4	Characterization of the 150-kDa cysteine-rich surface proteins of <i>Entamoeba dispar</i>	Tachibana, H. <i>et al.</i>
5	A survey on preparation against possible resurgence of SARS	Ohara, H.
6	Ecological roles of mongoose in transmission cycle of Japanese encephalitis virus in Okinawa island	Saito, M. <i>et al.</i>
7	Shift of Japanese encephalitis virus propagating in Japan, 1935-2002.	Ma, S. <i>et al.</i>
8	Japanese encephalitis in the Philippines	Inoue, S. <i>et al.</i>
9	Immunogenetic analysis of The patients with Dengue Hemorrhagic Fever in Vietnam.	Kikuchi, M. <i>et al.</i>
10	Status of Currently Circulating Dengue Virus Responsible for the Epidemic in Bangladesh in the Year 2002	Islam, M.A. <i>et al.</i>
11	Genetic variation in the 3' non-coding region of dengue virus type 1	Nukui, Y. <i>et al.</i>
12	Real-Time Reverse Transcription Loop Mediated Isothermal Amplification (Rt-Lamp) Assay as a Rapid Diagnostic Tool for Emerging Viruses	Parida, M. <i>et al.</i>
13	Characteristic prevalence of HPV genotypes in penile cancer in northern Thailand	Toriyama, K. <i>et al.</i>
14	A new screening method for <i>Salmonella</i> using lemon slices	Midorikawa, Y. <i>et al.</i>
15	Molecular analysis of <i>Haemophilus influenzae</i> isolated from nasopharyngeal aspirate and cerebrospinal fluid among children in Vietnam	Kaji, C. <i>et al.</i>
16	Buruli and the Ulcers Under the Tropics	Mwanatambwe, M. <i>et al.</i>
17	Epidemiological Study of Leprosy in a local village in Myanmar	Namisato, M. <i>et al.</i>
18	Diagnostic trial for cutaneous mycobacterial diseases by using PCR techniques.	Khan, M.A.K. <i>et al.</i>
19	The influence of circumstances to leprosy skin lesions.	Hosokawa, A. <i>et al.</i>
20	Effect of aphidicolin on <i>Entamoeba</i> excystation and metacystic development	Makioka, A. <i>et al.</i>
21	Molecular cloning and characterization of a protein geranylgeranyltransferase type I from <i>Entamoeba histolytica</i>	Kumagai, M. <i>et al.</i>

- 22 The Curative Efficacy of Metronidazole on Intestinal Amebiasis Kobayashi, S. *et al.*
- 23 The attachment and entry of Leishmania (Lesimania) major to macrophages:
24 Observation by scanning electron microscope Uezato, H. *et al.*
- 24 Development of a sensitive and simple diagnostic method for visceral leishmaniasis using urine samples
Itoh, M. *et al.*
- 25 Establishment of a detection and identification method of Leishmania species
26 within naturally infected individual sandflies Kato, H. *et al.*
- 26 Production of recombinant kinesin-related protein of Leishmania donovani
27 and its application in the urine-based ELISA for the diagnosis of visceral leishmaniasis Islam, M.Z. *et al.*
- 27 Development and evaluation of a new antibody detection kit (Instant (TM) CHEK-Amoeba) for amoebiasis
Ayi, I. *et al.*
- 28 The study of antigen for the new tuberculosis diagnosis and application to the serodiagnosis Yoshidome, A. *et al.*
- 29 N-terminus truncated recombinant SARS coronavirus nucleocapsid protein-based ELISA
is more specific for serodiagnosis of SARS Yu, F. *et al.*
- 30 Paragonimus ohirai M. in the basin of Maruyama River, Hyogo Prefecture Yamanishi, H.
- 31 A simulation of egg output school children infected with
32 *Schistosoma haematobium* by an immigration-death model Hamada, Y. *et al.*
- 32 Current epidemic state of Schistosomiasis mekongi in Lao PDR. Nakamura, S. *et al.*
- 33 Water contact patterns and knowledge of schistosomiasis in north Zanzibar Ataka, Y. *et al.*
- 34 Daily variation of *Schistosoma haematobium* egg excretion Muriu, D.W. *et al.*
- 35 Experimental infections of black flies (Diptera: Simuliidae) with microfilariae
36 of *Onchocerca dewittei japonica*, the causative agent of zoonotic onchocerciasis in Japan Fukuda, M. *et al.*
- 36 Application of urine-based ELISA to monitor the effect of mass-drug administration
37 for lymphatic filariasis control in a low endemic area Kimura, E. *et al.*
- 37 A case study of tuberculosis genetic epidemiological study based
38 on the long-term International Collaborative Field Research in Thailand Yanai, H. *et al.*
- 38 Study on transmission and infection of soil-transmitted nematode in the suburb of Hanoi,
Vietnam: contamination of vegetables and fingers and nail dirts with parasite eggs. Fujimaki, Y. *et al.*
- 39 Problems in Dracunculiasis Eradication Activities in Ogoou District, Togo Afetse, Y.D. *et al.*
- 40 A Serosurvey of Human T-cell Lymphotropic Virus type 1 (HTLV-1) in the Dominican Republic Ono, T. *et al.*
- 41 Descriptive epidemiology of imported infectious disease cases from the national surveillance in Japan
Taniguchi, K. *et al.*
- 42 Case series of imported malaria in Okinawa, Japan Haranaga, S. *et al.*
- 43 Characteristics of Imported Malaria in Japan Miura, T. *et al.*
- 44 Malaria epidemiology in Lombok and Sumbawa islands, Indonesia Kanbara, H. *et al.*
- 45 Malaria control in Lombok and Sumbawa islands, Indonesia Kanbara, H. *et al.*
- 46 Magnitude of Malaria Problem in Cambodia Lek, D. *et al.*
- 47 Accumulated infection risks of malaria during five weeks in a living area of an ethnic minority in Vietnam.
Nakazawa, S. *et al.*
- 48 A real-time PCR assay for quantitative detection of *pfert* in *Plasmodium falciparum*
49 and its application to epidemiology Katakai, Y. *et al.*
- 49 An investigation of the relationship between release from chloroquine resistance and blood GSH levels
Miyata, Y. *et al.*
- 50 Protection effect on lysis of Malaria infected erythrocytes membrane by bisbenzylisoquinoline alkaloids
Kosuke, H. *et al.*
- 51 Reactivity of blood samples spotted onto filter papers in the WST-8 method for screening of G6PD deficiency
Arai, M. *et al.*
- 52 Structural-effect relationships of mother moieties in drug-resistance releasing compounds
53 on the chloroquine resistant mouse malaria Osa, Y. *et al.*
- 53 Interleukin 4 polymorphism and malaria endemicity in Melanesia. Dzodzomenyo, M. *et al.*

- 54 Malaria pressure influenced the frequency of a susceptible allele of TNF-alpha promoter region in Vanuatu
Ubalee, R. *et al.*
- 55 Research of the antimalarial drugs from the natural products in JPMW project
Ishiyama, A. *et al.*
- 56 Antimalarial drug development research against drug-resistant *P. falciparum*
Kim, H. *et al.*
- 57 The problems medical facilities abroad for Japanese expatriates.
Fukushima, S.
- 58 "Research Group on Chemotherapy of Tropical Diseases (abbr.)" during the past and the coming three years.
Kimura, M. *et al.*
- 59 Certification in Tropical Medicine in Germany
Hitani, A. *et al.*
- 60 A preliminary study on the image of tropical medicine and the inclination for medicine
and medical science by the sixth year students of School of Medicine, Chiba University
Naoi, K. *et al.*
- 61 Increasing number of HIV-positive mothers only partly explained the recent decline
of birth weight in Republic of Central Africa
Tokunaga, M. *et al.*
- 62 Physicians' knowledge, attitude and practice toward sexually transmitted infections
and human immunodeficiency virus infection in Fuzhou city, Fujian, China
Cai, G. *et al.*
- 63 The study of health status for the inhabitants of rural areas on Vientiane province in Lao P.D.R.
Nishiyama, T. *et al.*

Special lecture

**K 1) INTERNATIONAL CONTRIBUTION IN THE CONTROL OF
TROPICAL DISEASES THROUGH NEW DRUG DEVELOPMENT**

SATOSHI OMURA

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In 1987, the Research Center for Liver Diseases of The Kitasato Institute was designated as a WHO Collaboration Center for Reference and Research on Viral Hepatitis. The Kitasato team headed by T. Takahashi has greatly contributed to the control of viral hepatitis in South East Asia through the large-scale transfer of plasma-derived hepatitis B virus vaccines, as well as through advances in technical methods for vaccine production.

We organized a joint program in 1999 for the screening of antimalarial compounds with the participation of Japanese Pharmaceutical Companies, The Kitasato Institute, the Japanese Ministry of Health Labour and Welfare, and WHO TDR (JPMW). To this joint program, 14 Japanese companies and our institute have sent a total of more than 26,000 samples for evaluation of antimalarial activity. Several of the screened candidate compounds have been targeted for further development.

Avermectin was discovered in 1979 and developed

through joint research between The Kitasato Institute and Merck Sharp & Dohme Research Laboratories. Ivermectin, a dihydro derivative of avermectin has shown efficacy for the treatment of onchocerciasis, which is one of the serious tropical disease prevalent in tropical Africa and a part of Latin America.

Administration of mectizan, a formulation of ivermectin for human use, at annual or semi-annual doses of 150 mg/kg is now being used to protect against onchocerciasis. In 1999, the Mectizan Expert Committee, a joint project with Merck & Co., Inc. and WHO, decided to accelerate the mectizan treatment program, and in the year 2003, more than 56 million people in countries with endemic onchocerciasis received the drug. All of the mectizan tablets were donated by Merck & Co., Inc., and The Kitasato Institute, as a partner in the invention of ivermectin, officially agreed to Merck's contribution to the onchocerciasis control program.

Educational lectures

E-1) ROLE OF PHOSPHOINOSITIDE 3-KINASE IN PARASITE INFECTIONSHIGEO KOYASU¹¹Department of Microbiology and Immunology, Keio University School of Medicine ²Japan Science and Technology Agency

We have studied the role of class IA phosphoinositide 3-kinase (PI3K) in immune responses using mice lacking the p85 α regulatory subunit of class IA PI3K (PI3K deficient mice). We have previously reported that PI3K is important for the development and functions of B lymphocytes. We have also found that PI3K is involved in the development of mast cells as PI3K deficient mice lack gastrointestinal mast cells but not dermal mast cells. In addition, PI3K deficient mice have some defects in the induction of Th2 response and exhibit enhanced Th1 response. Accordingly, PI3K deficient mice on a BALB/c background were susceptible to in-

fection by the intestinal nematode, *Strongyloides venezuelensis*, but were resistant to *Leishmania major* by eliciting an enhanced Th1 response. *In vitro* studies showed that numerous stimuli inducing IL-12 production concomitantly elicited PI3K activation in dendritic cells (DCs), but both PI3K deficient DCs and wild type DCs treated with PI3K inhibitor demonstrated increased IL-12 production. The presence of negative feedback mechanisms for regulation of IL-12 production during DC activation would contribute to prevention of excessive Th1 polarization causing undesirable immune responses.

E-2) WHY INFLUENZA KILLS AND WILL KILL AGAIN

YOSHIHIRO KAWAOKA

Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science University of Tokyo, Japan

In the past century, there were three influenza pandemics (worldwide outbreaks): the Spanish flu in 1918, the Asian flu in 1957, and the Hong Kong flu in 1968. Among these pandemics, the Spanish flu was the most devastating, killing more than 20 million people worldwide. In addition to these pandemics, yearly influenza epidemics occur, causing increased morbidity and mortality, particularly in vul-

nerable populations like the elderly. Moreover, the economic impact of influenza cannot be disregarded; huge losses occur as a result of lost work and increased health care expenditures during epidemics. In my talk, I will discuss our recent findings that may lead to the development of antiviral measures to quell the impact of this virus.

E-3) CURRENT STATUS OF DENGUE VACCINE DEVELOPMENT.

ICHIRO KURANE

Department of Virology 1, National Institute of Infectious Diseases

There are 4 dengue viruses: dengue virus types 1, 2, 3, and 4. These 4 dengue viruses are antigenically related, but different species. Dengue viruses cause dengue fever (DF) and dengue hemorrhagic fever (DHF). DF is a self-limited febrile illness, while DHF is a fatal illness characterized by hemorrhagic manifestation and plasma leakage. Development of dengue vaccine has been attempted for over 30

years. Several kinds of dengue vaccine candidates are at various development stages. The strategy is that 4 monovalent vaccines are developed against each of 4 dengue viruses and a tetravalent vaccine is prepared by mixing the 4 monovalent vaccines.

Two major groups are working on development tetravalent live attenuated dengue vaccine. Vaccines against each

of 4 dengue viruses induced expected levels of neutralizing antibody and very few adverse events were reported. Tetravalent dengue vaccine candidates are at phase II studies. Yellow fever (YF)-dengue chimera vaccine has also been developed. Yellow fever vaccine 17D strain was used. The preM and E genes of YF 17D strain were replaced by those of each of 4 dengue viruses. Four YF-dengue chimera viruses were mixed and a tetravalent dengue vaccine candidate was prepared. The tetravalent YF-dengue chimera vaccine candidate induced high levels of neutralizing antibodies to 4 dengue viruses. Presence of immunity against yellow fever virus did not inhibit the chimera vaccine from in-

ducing dengue virus-specific antibodies. This vaccine is currently at phase II study. Dengue-dengue chimera vaccine using attenuated dengue 2 and attenuated dengue 3 viruses are also under development and pre-clinical studies have been completed.

It is expected that phase study will be done with some of these candidate dengue vaccines in the near future. Selection of the countries and areas has been started for phase study. It is, however, still not determined whether efficacy should be evaluated for prevention of DF or DHF, or how long vaccinees should be followed to confirm the absence of increase in DHF.

E-4) BACTERIAL INTESTINAL INFECTION AND QUORUM SENSING

SHIGERU KAMIYA

Department of Infectious Diseases, Kyorin University School of Medicine, Tokyo, Japan

Expression of bacterial genes is regulated depending on cell density among wide range of bacteria. This regulation system is called as quorum sensing (QS). QS is controlled by autoinducer (AI) produced by bacteria depending on cell density. As gene expression of bacteria is regulated by AI, it is possible that QS communicated by AI is considered to be the language of bacteria. Some Gram-positive bacteria produce different types of oligopeptide AI. The oligopeptide AIs are secreted via ATP-binding cassette (ABC) transporter exposed on the surface of bacteria. It has been reported that more than 25 species of Gram-negative bacteria have QS. AI related with QS in Gram-negative bacteria is called as AI-1, which is acylated homoserine lactone (HSL). AI-1 is synthesized by LuxI protein. AI-1 freely diffuses through the cell membrane and accumulates at high cell density. At high AI-1 concentration, the Lux R binds to their cognate AI-1. AI-1/LuxR complex binds to promoter of the target gene and stimulate its transcription. AI-2 is produced by both Gram-positive and -negative bacteria. As the function of AI-2 is not limited to the bacteria producing its own AI-2 and AI-2 communicates QS beyond bacterial

species, AI-2 is called as universal language of bacteria. AI-2 has been recently shown to be furanosyl borate diester, and is synthesized by LuxS. More than 30 species of bacteria such as *Vibrio harveyi*, *Vibrio fischeri*, *Escherichia coli*, *Salmonella*, *Shigella*, *Helicobacter pylori*, *Bacillus subtilis* and *Campylobacter jejuni* have been reported to have QS communicated by AI-2. There have been several reports that QS communicated by AI-2 is associated with bacterial pathogenesis. It was reported that decrease in motility and production of Vero toxin (Stx2) was detected in luxS-inactivated isogenic mutant of enterohaemorrhagic *E. coli* (EHEC). Recently, it has been reported that EHEC produces a novel AI-3, and very interestingly, AI-3 might be also produced by intestinal floral bacteria. It is well known that EHEC is able to induce apparent infection including hemolytic uremic syndrome (HUS). It is possible that AI-3 produced by intestinal flora assist QS in the infection with small number of EHEC. Progress in the research on correlation between QS and pathogenesis in bacterial infection is expected.

Prize winner's lectures

The JSTM Award of Excellence

K 2) QUO VADIS: FROM BENCH TO FIELD

SOMEI KOJIMA

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Th2 responses are considered characteristic to helminth infections. Therefore, it is important to clarify the cellular and molecular mechanisms involved in Th2 responses and also the role of the responses in terms of pathogenesis and protection in parasitic infections. We demonstrated IgE-dependent eosinophil cytotoxicity to *S. japonicum* larvae, and established a monoclonal IgE antibody specific to paramyosin. We detected paramyosin in postacetabular glands as well as in the muscle layers and tegument of young schistosomula, suggesting paramyosin as a good candidate for schistosomiasis vaccine. To better understand the role of Th1/Th2 responses at the molecular level in protection against *N. brasiliensis* and *P. berghei* infection, we examined IRF-1^{-/-} mice. Unexpectedly, induction of IFN- γ mRNA occurred in the mutant mice infected with *P. berghei*, and the existence of the IL-12-independent novel pathway distinct from the classical Th1/Th2 differentiation pathway was suggested. Results of a study on a potential role of IL-18 in patients with severe *falciparum* malaria suggested that IL-18 plays a key role in the disease severity through a pathway of elevating IFN- γ rather than its IgE inducing activity. In a comparative study in a cerebral malaria (CM)-susceptible or-resistant strain of mice with *P.*

berghei ANKA infection, we observed that in the brain, both strains expressed IP-10 and MCP-1 genes as early as 24 hr postinfection, and that the expression of these chemokine genes in KT-5 was induced in vitro upon stimulation with malarial antigen, suggesting that the direct involvement of brain parenchymal cells occurs in response to plasmodial infection, and providing a new aspect to analyse possible mechanisms of CM. Apart from bench work, guided by an innermost voice saying "Quo vadis?", the author joined to the JICA-ACIPAC project established under the Global Parasite Control Initiative (Hashimoto Initiative). Many issues came out during the process of the project concerning mainly its management. Fortunately, most of the issues have been solved by support from the advisory board of the project in Japan, in which members of the Japanese Societies of Tropical Medicine and Parasitology are cordially involved. Thus, just like Pandora's box, many parasites and unsolved issues came out even from the author's small experience, but hope still remains there in that we could overcome disaster caused by infectious diseases eventually, if young scientists would consider unsolved issues to be challenges for themselves.

JSTM Young Investigator Award

K 3) DRUG SUSCEPTIBILITIES AND CLINICAL MANIFESTATIONS OF MYCOBACTERIUM TUBERCULOSIS IN ZAMBIA

SATOSHI MITARAI

Bacteriology Division, the Mycobacterium Reference Centre,
the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association

The prevalence of tuberculosis in Sub-Saharan African countries has increased in these 10 years. In Zambia, it is approximately 500 per 100,000 people and 40,417 new patients were recorded in 1996. In Zambia, 73% of the adult

tuberculosis patients are HIV-1 seropositive and 13% of all adult deaths are due to tuberculosis. With HIV epidemic, tuberculosis is a major cause of death. The shortages of both medical resources and knowledge about the disease are im-

pediments to adequate treatment of patients, resulting in an increase in drug resistant *M. tuberculosis*. This study was conducted to obtain data related to the spectrum of drug resistant *Mycobacterium tuberculosis*. A total of 499 strains of *M. tuberculosis* were tested for susceptibilities of isoniazid (INH), rifampicin (RFP), ethambutol (EMB) and streptomycin (SM). Totally 225 patients were also evaluated for their clinical manifestations. The primary and acquired resistances were observed in 13.9% and 16.1% to INH, 11.1% and 12.9% to RFP, 6.7% and 9.7% to EMB, and 3.8% and 3.2% to SM respectively. There were no significant differences between primary and acquired resistances. Multi-drug resistance was observed in 22 strains (4.4%). Clinical manifestation, as measured by chest roentgenograms, was similar in patients with either drug-

susceptible or resistant strains. The clinical and radiological information was not useful for the prediction of susceptible and resistant strains. The HIV serostatus was not associated with drug susceptibility profiles either. Our study has documented a high incidence of drug resistant *M. tuberculosis*, with no obvious clinical correlates, which must be considered when implementing a strategy for chemotherapy. As for clinical practices, many defaults during chemotherapy reduced the treatment successes. It is important to assess the efficient implementation of the directly observed treatment with short course chemotherapy (DOTS) and interrupt the further dissemination of resistant *M. tuberculosis* in the community. The drug resistances must be surveyed continuously to obtain useful clinical perspective and evaluate the effectiveness of tuberculosis control program.

Symposium 1

S 1-0) SYMPOSIUM1: LEARNING CLINICAL TROPICAL MEDICINE IN THE TOPICSKAZUNORI OISHI¹, TETSUYA NAKAMURA²¹Department of Medicine, Institute of Tropical Medicine, ²The Institute of Medical Science, The University of Tokyo

Since tropical diseases such as malaria and dengue are observed only as imported infectious diseases in Japan, Japanese clinicians have relatively little experience of these diseases. In this symposium, six speakers made presentations on tropical infectious diseases in the tropics. Four introduced their experiences in a clinical training course, and two discussed their clinical research.

Dr. Yamashiro, Oita University, described a short-term training course for medical students at San Lazaro Hospital, Metro Manila, Philippines. Dr. Hasegawa also introduced a three-week training course for young medical doctors at the same hospital. This course was supported by the COE program of Nagasaki University. Dr. Iwata presented in a one-month clinical training course at Instituto de Medicina Tropical Alexander Von Humboldt, Lima, Peru. Dr. Matsu-mura described a six-month diploma course in tropical medicine and hygiene at Mahidol University in Thailand.

Mariko Saito, a doctoral student of pharmaceutical science, presented data from the Philippines on the mechanism of thrombocytopenia in secondary dengue virus infection. She also experienced dengue illness as a researcher. Dr. Horii presented exciting data on the successful development of a malaria vaccine called recombinant SERA. He developed this vaccine project and is now evaluating the effects of SERA vaccine in Apac, Uganda. Scientific achievements will be of interest to young clinicians who are studying tropical infectious diseases.

Previous clinical training courses in the tropics were hampered by a lacking of funds and publicity. The information on training courses presented in this symposium should be distributed to Japanese clinicians who are interested in tropical infectious diseases. Awareness of these courses may inspire them to enter the field of clinical research in tropical medicine.

S 1-1) THE SHORT TERM TRAINING COURSE IN CLINICAL TROPICAL MEDICINE IN PHILIPPINES

MAIKO HASEGAWA

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As the increase in the circulation of goods and people is making the connection between different regions of the world tighter, global-scale countermeasures against infectious diseases and enhancement of the disease monitoring systems in every aspect are required. Physicians in Japan are responsible for the early detection and treatment of diseases, the exchange of information, adequate responses to the incidence of the diseases, and the improvement of their own abilities.

The purpose of the short-term training course of tropical medicine in the Philippines is to improve the ability of clinical physicians involved in tropical infectious diseases and emerging/reemerging infectious diseases. A one-week preliminary course was conducted in February, and a three-week training course will be held in September 2004, in which 6 physicians, selected from applicants from all over Japan, will participate. The course consists of lectures and

clinical training by specialists at San Lazaro Hospital, which specializes in infectious disease, and at St Luke's Medical Center, which is equipped with state-of-the-art medical facilities, both of which are in Manila. A visit to the World Health Organization West Pacific Regional Office (WPRO) is also planned.

Fortunately, I had the opportunity of attending the preliminary course and this year's course. I consider that this training course has three advantageous points. Firstly, trainees can observe the course of tropical diseases of interest. The experience of observing the course of diseases that are rare in Japan such as dengue, tetanus, typhoid, and leptospirosis, would certainly strengthen the practical ability of the trainees. Secondly, problems of the health care systems in the Philippines makes us realize the importance of global thinking about the problems of infectious diseases rather than as domestic problems. For example, the appearance of

drug-resistant tubercle bacilli in tuberculosis wards is being recognized as a problem in the near future. Thirdly, it would promote exchange of information about the status of, and problems with, countermeasures against infectious diseases between staff in WPRO and Japanese institutions. We learned about the status of avian influenza and the strategy of the WHO in February.

In the present medical system in Japan, it is impossible to take a leave for 3 weeks to attend the course without the understanding of colleagues. It is hoped that the necessity in such training in tropical infectious diseases will be appreciated by everybody.

In the symposium, I will present the plans of this course held in this September.

S 1-2) ACTIVITIES OF THE STUDENT ROTATION PROGRAM IN SAN LAZARO HOSPITAL, 2003

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Oita University (OU) has a unique educational program in the field of medicine aiming at providing an opportunity for students to learn about infectious diseases not usually found in Japan. The program has started since year 2003 in collaboration with San Lazaro Hospital (SLH), Manila. Implementation of the program is justified by the Academic Exchange Agreement contracted between OU and SLH in year 2001. The program has unique characteristics of: 1) OU gives academic credit for participated students, 2) OU provides an intensive preparatory program, 3) OU offers a financial support for participants, and 4) SLH staff prepare program curriculum and do tutoring. Nine medical students (4th year) participated in the program implemented in the period of Nov 9 to Nov. 22 year 2003, and 4 OU staffs (3 lecturers and 1 administrative personnel) accompanied. The students attended Bed-side training in the morning, and lectures in the afternoon. All tutoring and lectures were made by SLH staffs. In order to facilitate the students participation in the program, we have provided intensive preparatory lectures of infectious diseases and of diagnostics before starting of the practice. We have helped the students for preparing a lecture towards SLH staffs regarding

history and a current status of pulmonary tuberculosis in Japan. Eight departments of the Faculty of Medicine OU have been involved to support the program. All participants were covered by travelers insurance and vaccinated for HAV, Diphtheria, Tetanus, HBV, JEV, Measles, Rubella, Rabies, and Influenza. The evaluation of activities of the program was made in both directions. SLH staff gave high rating to the students in terms of comprehension of lectures and motivation for participating in the program. The students appreciated SLH staff's teaching and lectures. The students studied 31 distinctive diseases or syndrome just for 10 days. Each student prepared 2 case reports for patients with typical symptoms. Adding this, they compiled CD or DVD recording program activities, lecture files, and their own lecture files. The student rotation program is highly effective in terms of providing an opportunity for studying infectious diseases in a short period. In order to improve the program, a sophisticated organization is needed for 1) showing practical and attractive educational contents to the students: and 2) offering proper credit for participating in the project to the staffs of both parties.

S 1-3) CLINICAL CLERKSHIP IN TROPICAL MEDICINE IN PERU.

KENTARO IWATA

Division of Infectious Diseases, Kameda Medical Center

Many facilities provide tropical medicine course. Some provide microbiological expertise, and the others do epidemiological skills. Not a lot of institutes, however, have

tropical medicine course with actual clinical practice, which enable us to see real patients and manage them with diagnostic work up and treatment.

The Gorgas course is one of few these programs, which is affiliated with The University of Alabama at Birmingham. The course is held at Instituto de Medicina Tropical “Alexander Von Humbolt” (IMT) and Universidad Peruana Cayetano Heredia (UPCH), located in Lima, Peru. The author was given privilege to spend a month at IMT and UPCH, learning tropical medicine at bedside. Peru is a country in South America, shaping a long needle running from north to south. It has variety of geographical charac-

teristics, including coastal area where you can see cholera patients, or jungle area where you can experience cases such as malaria or yellow fever. Chagas disease, rabies, and cutaneous leishmaniasis are another examples you can see in Peru. And of course, many many HIV patients. Clinical clerkship at outpatient and inpatient settings is invaluable as a clinician. It is highly recommendable course for many tropical medicine and infectious disease specialists.

S 1-4) THE CLINICAL COURSE IN TROPICAL MEDICINE AT MAHIDOL UNIVERSITY IN THAILAND

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Division of Infectious Diseases and Immunology, Institution of Medical Science, Tokyo University

Mahidol University runs a number of international courses. One of them is “the Graduate Diploma in Tropical Medicine and Hygiene”. An objective of this course is to produce graduates with knowledge of tropical health problems and diseases. Another is to produce graduates who are competent in managing and providing advice about common health problems and diseases of the tropics. The course runs annually from April to September. The course is taught in English and is open to participants from all nations. All the students are required to hold a Medical License and have clinical experience of more than 1 year. This year’s intake comprises of 32 students from 18 different countries, including six doctors from Thailand who are working at the University’s Rajivithi Hospital of Tropical Medicine. Most of the students are from Asia, especially the neighboring Mekong countries.

The DTM&H course consists of 9 compulsory modules and an elective module. The former includes the broad range of tropical diseases with some emphasis on those common in Thailand, for example malaria, dengue haemorrhagic fever, opisthorchis and gnathostomiasis. Multi-media lectures are supported by laboratory sessions, involving examination of clinical specimens and demonstrations. There are also discussion sessions and group work about clinical

cases, literature reviews, and how to address problems in developing countries.

There are many opportunities for clinical experience. In June and July there are two field trips to rural areas. During these trips the students examine stool and blood samples to assess disease burden and run a small clinic for the local people. We also collect mosquitoes, rodents, and ticks to study the different vectors of tropical diseases and assess the control programmes. The Rajivithi hospital has 100 beds and is next door to the faculty. All malaria and other tropical disease cases in the area are referred for treatment. There is formal bedside teaching from our lecturers; outpatients clinics and the junior doctor introduce us to any patients of interest. We have seen numerous adult cases of severe malaria with cerebral, renal and pulmonary complications.

In the last 2 months of the course students study elective subjects. The elective options include areas such as Medical Problems in the Tropics, Paediatric Problems in the Tropics, Nutrition, Vector Control, Immunology, and so on.

The unique features of this course are the wide culture mix of doctors attending; the access to clinical cases and specimens; the field trips and close links to brilliant research units.

S 1-5) ASSOCIATION OF INCREASED PLATELET-ASSOCIATED IMMUNOGLOBULINS AND THE SEVERITY OF DISEASE IN SECONDARY DENGUE VIRUS INFECTIONS

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Dengue virus is a mosquito-borne human viral pathogen that belongs to the genus *Flavivirus* of the family *Flaviviridae*. It has recently become a major public health concern particularly in tropical and sub tropical countries and predominantly in their urban and peri-urban areas. The Philippines is a tropical country and thousands of cases are reported here every year. Dengue infections remain the major cause of morbidity and mortality in this country. Severe thrombocytopenia and increased vascular permeability are two major characteristics of dengue hemorrhagic fever (DHF). The present investigation attempted to elucidate the mechanism of their action by observing the relationship between a) platelet count and platelet-associated IgG (PAIgG) or IgM (PAIgM) and b) disease severity and PAIgG or PAIgM in 78 patients enrolled in a prospective hospital-based study. These patients with secondary infection were admitted in a hospital in Metro Manila. Forty patients were diagnosed to have dengue fever and thirty-eight had dengue hemorrhagic fever based on WHO criteria (1997). A decrease in platelet count was observed during the acute phase of infection, while there was a significant recovery in count during the convalescent phase. With respect to PAIgG or

PAIgM, a significantly high level of these antibodies was found from patients in the acute phase in comparison to that of the healthy volunteers. There was a significant decrease in either of these antibodies during the convalescent phase. An inverse correlation between platelet and PAIgG or PAIgM levels was found in the acute phase. Anti-dengue virus IgG or IgM was eluted from platelets of 10 patients in the acute phase of secondary infection. Increased levels of PAIgG or PAIgM were significantly higher in DHF patients than those of DF. An increased level of PAIgM was associated independently with the development of DHF, representing a possible predictor of DHF with high specificity. Our present data suggest that platelet-associated immunoglobulins involving anti-dengue virus activity play a pivotal role in the induction of thrombocytopenia and the severity of the disease in secondary dengue virus infections.

Collaborator: Institute of Tropical Medicine, Nagasaki University; Shingo Inoue, Kouichi Morita, St. Lukes Medical Center; Matias RR, Natividad FF, San Lazaro Hospital; Alera TP, Dimaano EM

S 1-6) DEVELOPMENT OF MALARIA VACCINE BASED ON RECOMBINANT SERA

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Malaria remains as one of the most devastating human infectious diseases. The appearance of malaria parasites with resistance to anti-malarial drugs and mosquito vectors with resistance to insecticides has made it more difficult to prevent and cure the malaria infection. It is therefore of increasing importance to develop malaria vaccines. For developing an effective malaria vaccine, the antigen molecule should be derived from the target antigen in human protective immunity against malaria, however the human malaria immunity is not well defined to identify the major target antigens. We have worked on malaria vaccine development with a recombinant protein based on N-terminal domain of *P. falciparum* serine repeat antigen (SERA). The recombinant

structure was produced in *E. coli* by the synthetic gene that is maximized for the protein production. The field research with the recombinant SERA molecule revealed that the acquired malaria immunity is largely associated with the sero positive against our recombinant SERA. Our findings strongly suggest that the N-terminal domain of SERA is one of Achilles heels of malaria parasite. In collaboration with The Research Foundation of Microbial Diseases of Osaka University, the recombinant SERA protein, SE36, was produced under GMP conditions. SE36 protein adsorbed to hydroxylaluminum was highly immunogenic in Chimpanzees. In addition, clinical grade SE36 provided significant protection in Squirrel monkeys after *P. falciparum*

rum challenge infection (Collaborative work with the Department of Molecular Immunology, School of Agriculture

and Life Sciences, University of Tokyo).

Symposium 2

S 2-1) ROLE OF INFECTIOUS DISEASE SURVEILLANCE

KIYOSU TANIGUCHI

Infectious Disease Surveillance Center, National Institute of Infectious Diseases.

Infectious disease outbreaks continuously occur in the world, and deforestation and urbanization will increase the risk of emergence of novel pathogens which have lived behind nature for a long time. And current globalization of travel and trade will facilitate the global spread of disease. Epidemic of infectious disease in one country might be an immediate threat to the rest of the world.

Under these circumstances, infectious disease surveillance at global level is quite important for early recognition and rapid containment of outbreaks of potential international concern. To investigate and follow up outbreak reports, WHO established an innovative mechanism, outbreak verification in early 1997.

Outbreak verification is based on a broad range of information sources, including national institutes of public health, WHO offices at regional and national level, the United Nations system, non-governmental organizations, WHO collaborating centers, news medias. After picking up some events from thousands of rumors, these events are assessed as of potential international importance. Definition of potential international importance includes (1) Unknown illness, (2) High mortality or morbidity, (3) international disease spread or interference with international travel or trade, (4) International assistance required for containment, and (5) Suspected accidental or deliberate release of bio-

logic agents. Once the event is determined to be public health emergency of potential international importance, the process of verification by communicating with affected countries is initiated. Through this verification process, WHO could get more information and discuss about how international society can support affected country.

But all above activities are voluntary so far. It means that if an affected country is reluctant to providing information to the global community, nothing to do. In order to provide global surveillance with legal background, revision of International Health Regulation is now on going.

Of course, good national level surveillance is a base of this global surveillance. Each country should detect as early as possible for protecting not only people in the country but also people living in global village. To enhance the surveillance system at national level, many technical approaches have been made including syndromic surveillance, OTC surveillance, outbreak surveillance, and so on. But clinician's awareness of rare infectious diseases or tropical diseases is most important for improving surveillance. Nowadays all physicians in Japan should think about global situation of infectious disease for daily outpatients with proactive measures and preparedness as risk management in the hospital.

S 2-2) RISK MANAGEMENT OF INFECTIOUS DISEASE AT THE BORDER. ROLE OF QUARANTINE STATION IN "BORDERLESS" WORLD

EMIKO IWASAKI

Sendai Quarantine Station

Today, with advancement of transportation, international traffics of people and goods have increased dramatically. This "globalization" has also caused global spreads of various infectious diseases.

Especially, in recent years, there have been many outbreaks of severe and highly infectious zoonoses, and these diseases spread rapidly in this globalized world.

The SARS outbreak in Asia in 2003 showed us the potential

danger of infectious diseases in today's world, and it gave us an opportunity to evaluate our policy on infectious disease control.

In fact, during SARS outbreak, Quarantine Stations at airports in Japan asked international travelers entering to the country to answer a questionnaire and also asked them to take their body temperature. In addition, because it is impossible to identify patients in latency period at the border,

any health problems after entering the country had to be reported to Quarantine Station and traced.

In addition, because these situations that many of infectious diseases are entering to the county are new to Japanese medical institutions, it is very difficult to manage it for them. So it is important for Quarantine Stations to support

these institutions to diagnosis and treat infectious diseases.

Furthermore, in this globalized world, every country recognized that it is also important to prevent any patients with serious infectious disease from leaving the country. So, we may need to quarantine anyone leaving the country also.

S 2-3) CRISIS MANAGEMENT FOR INFECTIOUS DISEASES IN BORDERLESS ERA -ROLE OF CENTRAL GOVERNMENT-

MITSUHIRO USHIO

Director, Infectious Disease Control Division, Health Service Bureau, Ministry of Health, Labour and Welfare

One of the lessons we have learned from the SARS worldwide outbreak we experienced in 2003 is that there is no border for infectious diseases, as in the world we live in international movements of both people and goods are so common and rapid with highly developed transportation systems and prosperous worldwide trade. At the same time it once again reminded us how severe consequences animal borne diseases could cause and the need to commit fully to controlling through epidemic alert and responses other animal borne diseases such as avian influenza and West Nile fever which are now increasing in case numbers. We are also seeing increase of some of the re-emerging diseases such as tuberculosis which was once thought to be under control. One of the tricky things about infectious diseases is incubation period; it easily happens that someone is exposed to a virus and get infected in one place and be half a world away a day later when he becomes symptomatic and spread a disease in the opposite side of the world where he got the pathogen. We certainly come to recognize the need to improve preparedness for responding to, and mitigating the public health, economic, and social consequences of any infectious disease that could possibly pose a serious threat

to global health security.

To cope with such a situation, Japanese central government have been taking necessary measures, including establishing the Law concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infectious Diseases in 1999. It is based on the old Communicable Diseases Prevention Law which was established more than 100 years ago, but a completely new concepts were introduced such as surveillance system, laying out proactive plans to combat infectious diseases outbreak, respect for human rights and regulation concerning animal borne diseases including introduction of quarantine for animal import. Recent outbreak of SARS in 2003 and US anthrax bioterrorism in 2001 urged us to amend this law in 2003 to strengthen central government competence to cope with large scale outbreak and also to strengthen quarantine system and regulation concerning animal borne diseases and to improve preparedness for possible use of a biological agent to cause harm.

Here is a general overview of Japanese national plan for infectious disease control from legislation point of view.

S 2-4) INFECTIOUS DISEASE OUTBREAK AND PRESS REPORTS

NAOKI MINAMI

News Commentators Division, Japan Broadcasting Corporation, Tokyo, Japan.

(1) The control of SARS and the role of press reports

To prevent the outbreak of SARS, the role of medical technology and administrative organization is important. But it is difficult to control it only with them.

When the SARS vaccine and the treatment aren't established, the citizen must prevent SARS with fundamental sanitation knowledge, and it is necessary to cooperate with the public health countermeasures. Part of the press is the

receiver of infectious disease information ,and also the sender.

(2) The outbreak of SARS in China and report

The progress of the Chinese SARS outbreak shows that, if sufficient information hadn't been provided, the outbreak caused the spread of groundless rumor, and a social panic followed, when people were sincerely looking for information .

(3) Japanese SARS reports

About Japanese SARS reports, the significant parts were , the occurrence in the foreign countries, the influence on the economy and society,the pathogen of this emerging infectious disease, the route of infection, the diagnostic procedure,and the progress of the research on the prevention and the treatment, the reports collected strong concern.

Furthermore, the role of the press was to grasp the problems of the Japanese prevention system of an infectious disease ,and institute the improvement.

(4) Preparing for the outbreak recurrence.

SARS is the 1 st infectious disease of the infectious disease law, and the role of the national government to the local government was reinforced in Japan.

If it is compared with a moment in 2003, it can be said that the system of health, medical treatment has been expanded, and you must prepare for it more.

It is important to introduce information to the public while giving careful consideration to the protection of privacy and from harmful rumor to avoid the social amplification which the public is anxious about infectious disease outbreak.

S 2-5) AN OUTBREAK OF INFECTIOUS DISEASE HITS CIVIL SOCIETY HARD: A CASE STUDY OF JAPANESE EXPATRIATE EMPLOYEES AND THEIR SPOUSES IN HONG KONG

YASUhide NAKAMURA, SACHIKO YOKOTA

Research Center for Civil Society, Graduate School of Human Sciences, Osaka University, Osaka, Japan

A study by Yokota into “psychosocial support for Japanese Hong Kong residents during the SARS crisis” aimed to investigate the feelings and thoughts of Japanese expatriate employees and their spouses based in Hong Kong at that time. Field research was conducted from July to August 2003 using semi-structured interviews and a cross-sectional questionnaire survey. 116 Japanese businesspeople (response rate: 74%) at 10 Japanese companies and 70 spouses (response rate: 61%) responded to the questionnaire. The fact that they could not tell what information was correct (in spite of the large volume of available information) due to the unknown nature of the disease, stressed 94% of the expatriate employees and 97% of their spouses. 66% of the expatriate employees and 65% of the spouses responded that they felt abandoned by Japan. The businesspeople complained about the Government of Japan and their companies placing first priority on the situation inside Japan. As for their sources of information; while they got information through Japanese media (television or newspapers), most of them also relied on the Hong Kong Department of Health's web-site and the information pamphlets provided by their own companies. The Department of Health disclosed infor-

mation in detail on their web-site, while protecting personal privacy. Companies selected the information that was particularly pertinent for their employees. Crisis management for infectious diseases should be implemented without making those living in infected areas feel unduly isolated. The government and the media should consider the human rights of such people. Psychosocial support offered to them should be strengthened. For example, warm messages from outside the infected area encouraged the people who were fighting against this newly emerging disease or who were facing the possibility of dying from the disease. People in infected areas would mostly welcome visits from health professionals during an epidemic. The study revealed that many people received sympathy from others within the isolated infected area and that they felt an increased solidarity with them as a result. We would like to recommend that governments and the mass media disclose detailed information, while respecting personal privacy, and that they report the facts that are thought to be most reliable at that time. Remember the lessons learned from the experience of Hong Kong!

Workshop 1

W 1-0) ROLE OF NURSING PROFESSION AND NGO IN TROPICAL MEDICINENOBUKATSU ISHIKAWA¹, IKUKO MORIGUCHI²¹The Research Institute of Tuberculosis Japan Anti-Tuberculosis Association, Tokyo, Japan²College of Nursing Art and Science, University of Hyogo, Hyogo, Japan

In this workshop, field based studies on various topics were reported and discussion was made on the roles of nursing professions and NGOs in tropical medicine, which was the first one in the history of Japanese Society of Tropical Medicine (Society hereafter). The speakers were all nurses in basic education and professionally top-level people with PhD as researchers cum teachers cum field workers.

Their presentations were all impressive and provoking as all from long-term studies showed more human aspects than diseases either of the providers at the frontline or clients in the community, which have not been covered, in the conventional tropical medicine. The expansion of the concept of conventional tropical medicine to wider holistic one,

which should be more action oriented, resulting in the expansion of the topics and members of the Society. Dr. Higuchi reported the use and perspectives of traditional medicine from her study in Sri Lanka. Dr. Moriguchi reported the importance of participatory research in health problems with the frontline workers in Indonesia. Dr. Jintana showed the role of social and behavioral science research in community care through her study on TB/HIV care in northern Thailand. Dr. Tokunaga discussed the effective role of NGO at the frontline from her long-term commitment in Africa. This kind of workshop needs to be held further until a specific program or committee for nursing and health is institutionalized in the Society.

W 1-1) TRADITIONAL MEDICINE IN SRI LANKA

MACHIKO HIGUCHI

Graduate School of Nursing, University of Shizuoka

Introduction: Traditional medical system in Sri Lanka was initiated in 3 BC when Ayurvedic medicine was introduced from North India. Currently, Sri Lankans use traditional medicine for the promotion of health and for the prevention and treatment of illnesses even though the modern health-care system has rapidly increased in Sri Lanka. Sri Lankans practice different methods of treatment for illnesses, i.e., supplicating with ritual and supernatural powers, traditional medicine, and modern medicine. The objectives of this research were to determine the reasons for people choosing between Ayurveda and modern medicine and to identify the use of home remedies. A total of 286 patients were interviewed in both Traditional Medical Institutes (TMIs) and Modern Medical Institutes (MMIs) in three characteristic areas. The data was collected using individual survey forms comprising open-ended questions and in-depth interviews which were translated from Sinhala to English. The results show that patients at TMIs had obtained higher education, were from a higher income group, and were older than those in MMIs. Patients who suffered

from pain in the joints, arthritis, or rheumatism sought traditional medication and those with heart problems, hypertension, and diabetes obtained treatment from MMIs. Sixty-three percent of the patients in TMI had previously opted for treatment from MMIs and 38% of the patients in MMIs had gone to other MMIs. The patients had changed the treatment from one MMI to another either because the symptoms had persisted, the former hospital was distantly located thus limiting easy access, the former personal physician had recommended the present MMI, extra expenses were incurred on transportation, or the cost of medicine was too high and made it unaffordable. Patients changed from a TMI to a MMI due to other reasons. They complained that there was no improvement in the symptoms even after medication, or the symptoms reappeared, indicating incomplete cure. The patients said that they changed from one TMI to another because there was no change in the symptoms or that modern medicine could provoke other illnesses, and being too strong, could cause side-effects. In conclusion, the reason behind patients choosing each medicine de-

pended on each research area's characteristic. Patients in urban areas obtained better accessibility to both TMI and

MMI than those in rural areas.

W 1-2) COMMUNITY HEALTH NURSING IN INDONESIA

IKUKO MORIGUCHI

College of Nursing Art & Science, University of Hyogo

The Indonesian government has promoted healthcare policies based upon primary health care (PHC) since 1978, and has been trying to correct regional differences under its 5th five-year plan since 1989. With a commitment to providing health care services and educating health care workers in remote areas so that limited resources can be utilized efficiently, the government has striven to increase budgets and reinforce facilities and manpower in areas outside Java. Healthcare workers are unevenly distributed throughout Java and in urban areas, and newly graduated physicians have been dispatched to health centers in remote areas. Due to a high maternal mortality rate (450 in 1992), trained community midwives have been dispatched to rural villages where there are no healthcare facilities. I concluded that community midwives needed to provide PHC services in order to correct regional healthcare differences. Beginning in 1992, therefore, I examined the actual conditions community midwives were working under and clarified issues concerning their activities in South Sulawesi (population: 7.5 million) in cooperation with the province health department. Training and activity methods were also developed with local community nursing managers to improve the quality of the care provided by community midwives. One of the methods involved an investigation into the circumstances of maternal death conducted by community mid-

wives using a verbal autopsy.

In Indonesia, the actual circumstances surrounding maternal deaths were not known at that time. Community midwives in South Sulawesi received training in verbal autopsy to assist investigations into maternal deaths. The training was held in cooperation with nursing managers of both the province and prefectural health departments and after the training community midwives investigated maternal deaths in the villages where they worked. Investigation reports and case study reviews followed to clarify direct causes and other factors affecting maternal deaths. The midwives thereby came to recognize the serious problems surrounding maternal deaths and began actively considering detailed measures to prevent them. This kind of investigation was formerly only conducted by universities and researchers, and results were rarely provided to frontline health care workers.

Based upon the experience gained in Indonesia, it became obvious that research outcomes should be utilized for local healthcare in the field of tropical medicine. This includes cooperating with local healthcare workers as well as researchers; feeding research results back to the workers; and proposing investigation methods that local workers may use on an ongoing basis and healthcare activities that suit each region.

W 1-3) NGO AND INTERNATIONAL COOPERATION - THE AFRICAN EXPERIENCE

MIZUKO TOKUNAGA

School of Health Sciences, Nagasaki University, Nagasaki, Japan.

A Japanese NGO "Friend of Africa" has been tackling infection prevention of HIV and AIDS patients' support in the Central Africa Republic since 1992.

The followings are some thoughts through experiences of NGO activities.

1. I believe it the big mission of NGO to perform education

and technical transfer of the local staff. Because NGO has abundant field experiences and activities which involve flexible local residents' autonomy.

2. It is hoped that mutual cooperation with NGO and ODA will be performed more powerfully.

3. "Accountability" of NGO has been much discussed.

NGO should make the standard of crisis as the first priority.

In order to respond to the requests of the developing coun-

tries, the cooperation with ODA and NGO is an important subject.

**W 1-4) THE ROLE OF SOCIAL AND BEHAVIORAL RESEARCH ON TROPICAL DISEASES CONTROL:
A CASE STUDY OF TUBERCULOSIS.**

JINTANA NGAMVITHAYAPONG-YANAI

TB/HIV Research Foundation, Chiang Rai, Thailand.

In the traditional model of epidemiological triad (agent, host and environment), which is a causation model for all infectious diseases, the social and human behaviors are involved a great deal with host and environment components and they are determinants of disease occurrence. Tuberculosis (TB) is one of the tropical diseases which is the global health emergency. TB has been well recognized as a disease which is closely associated with social and behavioral factors. The paper describes the role of social and behavioral research in TB transmission, prevention and care. Some key concepts of sociology and anthropology in health and social

science research methods are discussed. These include attitude and perceptions about TB; gender; stigma; adherence to TB treatment and qualitative research. By giving the examples from the field research in Chiang Rai, Thailand, the conference's audiences will recognize how social science research help the health care workers understand patient's attitude and behavior which are associated with TB transmission and prevention. Furthermore, the social science research, which led to intervention and policy for TB control will be discussed.

Workshop 2

W 2-1) OVERVIEW

KATSUHIKO KAMEI

Dept of Pathogenic Fungi, Res Ctr for Pathogenic Fungi and Microbial Toxicoses, Chiba University

Mycoses that have been imported into Japan, i.e. coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, penicilliosis marneffeii, and blastomycosis, differ from domestic Japanese mycoses in many aspects: they are highly contagious, easily disseminated, and able to cause biohazards both in laboratories and in society at large. However, medical workers in Japan generally have limited knowledge about these diseases. The numbers of patients of coccidioidomycosis and histoplasmosis have been rapidly increasing in the past decade, with the total numbers having reached 41 and 42, respectively. The increase seen in coccidioidomycosis is particularly dramatic. Most of the coccidioidomycosis patients were infected in the United States, especially in Arizona. As for histoplasmosis, in contrast, as approximately 16% of the patients had no evident history of visiting endemic areas, domestic infection was suspected. Mainly due to continuing increases in international travel and contact, further increases in both diseases can be expected. A total of 18 patients with paracoccidioidomycosis have been reported. The number of patients has shown a decreasing trend after a sharp increase in the 90's during the "Bubble Economy". Most of the patients were workers mi-

grated from endemic areas such as Brazil, and were on temporary visits to Japan when the disease developed. The patients abruptly increased and then decreased in concert with the Japanese economy heating up and cooling down. Although only a few patients were reported in the past ten years, another rise may be on the horizon if the economy again heats up and many people visit Japan from endemic areas. No cases of penicilliosis marneffeii had been reported until 1998, when the first case was found in an AIDS patient. Then, the second case, also an AIDS patient, followed in 2002. Although the number of patients is still limited, the mortality rate of this disease is very high, and its likelihood of occurrence should not be neglected. No cases of blastomycosis have been reported to date in our country. However, this infection is one of the biggest endemic mycoses in the United States, and therefore is sure to hit Japan sooner or later. We have to stay on a high alert. It is clear that these imported mycoses are getting more and more common. Systems for close monitoring of patients, epidemiological studies, and education of medical staffs for their diagnosis and management are urgently required.

W 2-2-1) COCCIDIOIDOMYCOSIS

MASATERU KAWABATA

Department of Respiratory Medicine, Respiratory Center

53 years old, male was admitted to our hospital complaining of fever, cough and dyspnea on effort after the travel to Arizona in the United States. He consulted his primary care physician and was diagnosed common cold three days before the admission. Although he was prescribed NSAIDs, antitussive and antibiotics, his symptoms had not improved. The chest X-ray revealed some consolidations in the right middle lung field and granular shadows bilaterally. The laboratory data showed increase of white blood cell counts and rising erythrocyte sedimentation rate. Coccidioidomycosis was suspected for the previous travel history to Arizona and the clinical features. The thorascopic lung

biopsy was performed for the pathological diagnosis. The specimen was revealed granulomas consist of remarkable infiltration of eosinophils and giant cell. Some typical spherules, which were strongly stained by PAS, were found in the eosinophilic granuloma. We certainly diagnosed coccidiomycosis and started itraconazole 400 mg/day. After the treatment of three-month regimen of itraconazole, his symptoms, the laboratory and the X-ray findings were improved. Coccidioidomycosis is caused by *Coccidioides immitis*, a dimorphic fungus that grows as a mold in the soil. *C. immitis* is most commonly found in the deserts of the southwestern United States (southern Arizona, central Cali-

fornia, Southern New Mexico, and west Texas) and Central and South America. Coccidioidomycosis is acquired from inhalation of the spores, arthroconidia. Once in the lungs, the arthroconidia transform into spherical cells called "spherules". An acute respiratory infection occurs 7 to 21 days after exposure and typically resolves rapidly. In approximately 40% of infected cases, symptoms present usually as flu-like illness with fever, cough, headaches, rash, and myalgias. Some patients fail to recover and develop chronic pulmonary infection or widespread disseminated in-

fection affecting meninges, soft tissues, joints, and bone. Severe pulmonary disease may develop in HIV-infected persons. Patients with self-limited disease or relatively localized acute pulmonary infections usually do not require antifungal therapy. Antifungal therapy should be given to patients who have disseminated disease or are under risk of complications due to their underlying immunosuppression and other factors. Amphotericin B and azoles, such as fluconazole, itraconazole, and ketoconazole are used for treatment.

W 2-2-2) A CASE OF CHRONIC DISSEMINATED HISTOPLASMOSIS EMERGED DURING STEROID MAINTENANCE THERAPY FOR RHEUMATOID ARTHRITIS WITH SECONDARY AMYLOIDOSIS AND NEPHROTIC SYNDROME

SHUNJI TAKAKURA

Department of Laboratory Medicine, Kyoto University, Kyoto, Japan.

[Case] A 78-year-old man with chronic disseminated histoplasmosis emerged during steroid maintenance therapy without probable travel to endemic area.

[Clinical course] He had received steroid therapy against rheumatoid arthritis for 10 years. Secondary amyloidosis with nephrotic syndrome was diagnosed 4 years ago. Daily dose of steroid was increased temporarily to 60 mg/day but reduced to maintenance dose of 10 mg/day soon after. One-month stay in New York 10 years ago was his only international travel. He has lived in Kyoto and worked as a cargo worker during World War II and a driver after the war. He found to have low-grade fever with malaise. A chest and abdominal CT scan revealed enlargement of bilateral adrenal glands. Pancytopenia and increased acute phase reactant were found in the blood examination. Intense uptake in the bilateral adrenal glands and numerous spotted uptakes in the liver was detected by FDG-PET scan.

[Diagnosis] Ultrasound-guided percutaneous adrenal aspirate and bone marrow aspirate yielded *Histoplasma capsu-*

latum var *capsulatum*. Both urinary and serum histoplasma antigen were positive. We diagnosed him as chronic disseminated histoplasmosis.

[Therapy and course] We started amphotericin B for 0.8 mg/kg/day. Defeverescence and disappearance of subjective symptoms were gained after 1 week of therapy. After 3 weeks of therapy, blood cell count and acute inflammatory markers returned to normal limit. Follow up imaging study revealed that bilateral adrenal enlargement had continued to decrease in size. Unfortunately, sudden-onset MRSA prosthetic arthritis of his left elbow required urgent surgery and administration of vancomycin, which led to rapid deterioration of renal dysfunction. He expired 3 months after the diagnosis of histoplasmosis.

[Conclusion] Histoplasmosis should be suspected even without obvious international travel history, and be included in the differential diagnoses of bilateral adrenal enlargement and pancytopenia.

W 2-2-3) PARACOCCIDIOIDOMYCOSIS

TAKESHI IGARASHI

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Paracoccidioidomycosis (PCM) is known as endemic disease of Central and South America area infected with an

adult man. Inhalation of the infective spores is the usual method of infection. Most patients with the disease are male

agricultural workers in close daily contact with the soil. Chronic pulmonary paracoccidioidomycosis progresses very slowly and develops a cough, sputum, loss of weight, on exertion breathlessness, fever and lung fibrosis progresses when it progresses and develops the respiratory failure. The radiologic patterns of PCM can be micronodular, infiltrative, or linear. The micronodular are usually less than 3 mm in diameter, sharply defined, and have a uniform density. Cavities are found in approximately one third of the cases and are usually small and multiple, and occur in the middle field of the lung. The radiologic abnormalities clear slowly during therapy. Regression may be observed at 3 months or even before that and is usually stable at 6 months. PCM was reported 17 examples in Japan. We experienced PCM with multiple cavity and fibrosis in lungs last year. We will explain in detail about clinical feature of PCM mainly on this case.

Case: A 43-year-old Japanese Brazilian came to Japan in 2001. Since subjective symptoms such as cough, sputum, dyspnea on exertion became severe, he was referred to our hospital because of suspicion of pulmonary tuberculosis in chest x-ray and CT findings. Through chest x-ray of initial examination showed interstitial shadow in bilateral lungs with nodular, infiltrative or cavitary changes. The mycobacterium tuberculosis was not found. The mycetocyte with watched budding resembling steerage of the ship which was characteristic of *Paracoccidioides* was observed in sputum and transbronchial lung biopsy specimens. We could culture fungus to show dimorphism of temperature dependency and diagnosis was established as chronic lung paracoccidioidomycosis. By dosage of ITCZ 200 mg/day, chest x-ray findings and clinical manifestations were improved.

W 2-3) IMPORTED MYCOSES RELATED TO ZOONOSES

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It is well known that dermatophytes cause zoonotic fungal infections by the contact with infected or carrier animals. Recently companion animals became very intimate with their owners' life. Exotic animals imported without veterinary check are now common in Japan. Although quarantines for rabies and some viral infections are well established, fungal infections brought by imported animals have been ignored. Therefore we have to be on the alert of newly imported zoonotic mycoses. Actually, a dermatophyte, *Trichophyton mentagrophytes* var. *erinacei*, which has never been reported in Japan, was isolated from an imported hedgehog in 2001, although the fungus is not highly pathogenic for humans. Its prevalence in household hedgehogs in Japan was approximately 40%, which suggested that the dermatophyte was already spread whole of the country. As anticipated, a human case was reported in 2003. This is just a tip of iceberg.

From the aspect of prevalence in Japan, imported mycoses (coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, penicilliosis marneffeii, and blastomycosis) are not exceptional. They are probably brought into Japan with imported animals. Coccidioidomycosis caused by *Coccidioides immitis* classified as the highest pathogenic fungus is endemic in desert areas of both American continents.

Mammals and reptiles are sensitive to the pathogen.

Histoplasmosis by *Histoplasma capsulatum* is also reported in humans and many species of animals. Although most of clinical cases were imported, some autochthonous ones were reported in Japan. Some native cases in both humans and dogs had skin lesions. The clinical manifestations closely resemble to those of pseudofarcy in horses, which was endemic in Japan before World War II, and this fact suggested that the humans and dogs were infected as heteroecism. Pseudofarcy is known as a notifiable disease.

Paracoccidioidomycosis by *Paracoccidioides brasiliensis* is endemic in Latin American countries. The most important natural carrier is nine-banded armadillos. Some probable cases of dogs were also reported. Penicilliosis marneffeii by *Penicillium marneffeii* is endemic in Southeast Asian countries. Its reservoir is bamboo rats, which became a fashionable exotic animal a few years ago. Blastomycosis by *Blastomyces dermatitidis* is known to be carried by porcupines on their quills. Sometimes dogs in the endemic areas became infected with the fungus by catching porcupines. Although there is no imported mycosis in animals, except for autochthonous histoplasmosis, we should pay attentions to imported or traveling animals.

W 2-4) CAUSATIVE AGENTS OF IMPORTED MYCOSES

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The causative agents of imported mycoses are required to be carefully handled because they cause diseases even in healthy subjects. Imported mycoses in Japan are listed up as follows; coccidioidomycosis caused by *Coccidioides immitis*, histoplasmosis by *Histoplasma capsulatum*, paracoccidioidomycosis by *Paracoccidioides brasiliensis*, blastomycosis by *Blastomyces dermatitidis*, and penicillosis marneffei by *Penicillium marneffei*, which are recognized as biosafety level 3 pathogens as the same as *Bacillus anthracis*.

Among those, *C. immitis* has the highest pathogenicity with many mortal cases of laboratory infections in U. S. A. The species produces abundant arthroconidia within one week, which easily disperse into opened air. It is said that only 10 arthroconidia could cause the disease in humans. Since the fungus produces a whitish cottony colony with conidia within one week, it is very dangerous to open the cover or plug of the culture. In cases that patients would have a possibility to be diagnosed as coccidioidomycosis based on typical clinical manifestation and on visits to the endemic areas, isolation of the causative agent should be

done under a biosafety level 3 condition and avoid the secondary infection. Common laboratories are permitted only to do serological diagnosis and/or detection of spherule cells by cytological and histopathological observations.

The causative agents for histoplasmosis and paracoccidioidomycosis are also dangerous and difficult to isolate, because they require special culture conditions and a long time at least one month to produce characteristic colonies. The observations of thermally dependent dimorphism need one additional week. *P. marneffei* distributed in the Southwest of China and the North of Thailand and Vietnam should be pointed out to produce very dispersible conidia.

Thus we developed some molecular biological diagnostic methods for clinical materials. The PCR-based detection of species-specific genes from paraffin-embedded tissue samples, biopsy tissue, blood clot, and sputum were attempted to diagnose coccidioidomycosis, histoplasmosis, paracoccidioidomycosis and penicillosis marneffei. Furthermore, loop-mediated isothermal amplification (LAMP) method for detecting *gp43*, species-specific gene, for *P. brasiliensis* could confirm the diagnosis within 4 hours.

Workshop 3

W 3-1) PURPOSES OF ESTABLISHING PREVENTIVE GUIDELINES AGAINST MALARIA

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Malaria has been successfully eradicated since 1959 in Japan and we only encounter imported malaria cases now. The number of Japanese people who were going abroad had increased up to 16,357,572 in 2001, but decreased to 13,296,330 in 2003 on account of the terrorism in New York in 2001 and SARS epidemic in 2003, so did the number of imported malaria cases in Japan. In fact, total number of the patients was around 100-150 annually for the last 10 years, particularly in 2000, the number reached 154, however in 2002, the number decreased to 83.

The significance of the chemoprophylaxis has not been well recognized in Japan. After the approval of mefloquine for use by Ministry of Health, Labour and Welfare, physicians are encouraged to prescribe the drug for international travelers not only for treatment but also for prophylaxis.

Still, not so many Japanese physicians are happy with the tropical or travel medicine, the guidelines on the prevention of malaria using appropriate drugs have to be established as soon as possible. Mefloquine resistant malaria has been reported widely in Asian malarious areas, and indeed there are already some reports of its resistance in Japan. We have to prepare another good chemoprophylactic regimen applying, for instance, atovaquone/proguanil, primaquine, or doxycycline. Of course, proper anti-mosquito bite behavior using repellent or mosquito nets are encouraged, in fact, stand-by emergency treatment should be expected for certain travelers. The need for the medical practices on travel medicine using the guidelines is envisaged at the outset now in Japan.

W 3-2) MOSQUITO CONTROL FOR MALARIA PERSONAL PROTECTION

KOSUKE HARUKI

National Defence Medical College, Saitama, Japan

Avoiding mosquito bites are basic protection method of malaria infection. They are resembles to the diet, exercise therapy of diabetes and low salt diet for hypertension. Malaria is transmitted by specific mosquitoes, which are belonging to *Anopheles* spp. The protection methods of mosquito bites are as follows: 1 House and residence protection: Avoiding to live around water holding place such as marsh, pond, old tires, tins and so on. Living in air-conditioned house. Living above first floor. 2 Clothes: Wearing long sleeves and trousers impregnated with insect repellants

(DEET) or insecticide (should not use with under ware). 3 Behaviors: Sleep inside the impregnated mosquito net and should not move around during mosquito active time (usually evening to morning). Using mosquito coils. If it is not feasible to avoid mosquito bites, although most effective method to protect malaria infection is avoiding mosquito bites, most of these cases may be an indication of taking anti-malarials for prophylaxis and emergency stand by treatment.

W 3-3) CHEMOPROPHYLAXIS

EIICHI OKUZAWA

Dept of Research and Information, Japan Overseas Health Administration Center,
Japan Labour Health and Welfare Organization

Recently the number of malaria cases reported in Japan was about 100, and some of them were fatal. Almost of all victims were defenceless travelers to tropical zone. Mefloquine, the only drug for malaria prevention in Japan, has been available since 2001. However, chemoprophylaxis is not widely spread among high-risk travelers, and sometimes used inappropriately. To improve the present situation, Japanese doctors are desired to comprehend the reasonable use of chemoprophylaxis.

1. Legal request for prescription

Mefloquine is under some regulations as well as other prescription drugs.

- Pharmacists, when they sell it, are requested to check doctor's prescription.
- Doctors, when they prescribe it, are requested to check patient's physical condition.
- Use of mefloquine for prophylactic purpose is admitted by MOLH Japan. Only that, medical fees for it are out of coverage by national health insurance.

2. Medical advice to travelers

All travelers to endemic areas should consider the following measures.

- To be aware of malaria risk
- To avoid mosquito bites

- To seek a doctor immediately, if fever develops
- To take chemoprophylaxis, if malaria risk is high

3. Indication for chemoprophylaxis

Chemoprophylaxis is strongly recommended if the both of the following risk present.

- High risk of infection: e.g. Stay in holo-endemic or hyper-endemic areas.
- Risk of serious illness: e.g. Absence of proper medical service at the time of onset.

In the other cases, further assessments are necessary (relative indication). Logically, it is supposed to be beneficial in case with high risk of infection.

4. What should be checked and documented by prescribers

- Travel schedule to see indication for chemoprophylaxis
- Physical condition of the client to exclude contraindication: e.g. drug allergy
- Scheduled dates and dose for medication

5. Travel clinic directory

Number of travel clinic in Japan is small yet. This has limited spread of appropriate chemoprophylaxis. A small list of travel clinics will be attached to the guidelines. It may be useful for doctors who will not or can not prescribe for preventive use.

W 3-4) STAND-BY TREATMENT

MIKIO KIMURA

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Our future Guidelines for Malaria Prevention may not deal with stand-by treatment (SBET) in detail. However, SBET is mentioned in WHO's recommendations and guidelines of Western countries, and therefore, it will be targeted during this workshop, aiming at provoking discussions about its positioning.

SBET is to take antimalarials as an emergency measure when immediate medical attention is not available in case of suspected malaria, because malaria can become severe or even lead to death within a short period. Practically, prerequisites of SBET include 1) > 7 days after entering a

malarious area, 2) fever of > 37.5 °C suggestive of malaria, and 3) inability to seek medical attention within 24 hours of disease onset. Furthermore, travelers should make every effort to seek medical attention even after SBET, because the disease may require other treatments than antimalarial ones, and even if it is malaria, other antimalarials may have to be used because of drug resistance. SBET is used either solely without chemoprophylaxis or as a back-up measure of chemoprophylaxis; however here, we will focus mainly on the former.

Although in general chemoprophylaxis is recom-

mended for travel to areas with high risk of malaria, especially sub-Saharan Africa, SBET could be recommended in case of low risk areas, because the risk of drug adverse effects (AEs) due to chemoprophylaxis may outweigh the benefit of avoiding disease contraction. Furthermore, SBET could be recommended for travel to high risk areas if chemoprophylaxis is 1) contraindicated, 2) not available, or 3) not preferred by the traveler.

In selecting chemoprophylaxis and/or SBET, one needs to understand advantages and disadvantages of both measures as well as other factors including the character of the traveler. Advantages of chemoprophylaxis include 1) preventing the disease acquisition (even if it fails, treatment follows), and 2) less likely to be misused because of the

simple daily or weekly drug intake, and the disadvantages include 1) the possibility of the risk of AEs outweighing the benefit of avoiding infection, and 2) likely to pose false sense of security. On the other hand, advantages of SBET without chemoprophylaxis include 1) the benefit of avoiding death due to malaria outweighing the risk of AEs, and 2) insisting the necessity of early treatment with self-responsibility, and the disadvantages include 1) inability to suppress the disease acquisition (if it fails, death may follow), and 2) likely to be misused because of the complex drug intake.

At present, it is not recommended to advise travelers to practice SBET based on the results of the self-use malaria rapid tests that are available in foreign countries.

W 3-5) TRAVEL ADVICE BASED ON THE GUIDELINES FOR MALARIA PREVENTION

ATSUO HAMADA

Japan Overseas Health Administration Center

The guidelines for malaria prevention were drawn up in response to the authorization of mefloquine in Japan in 2001 by the Minister of Health, Labor and Welfare, which has made it possible to administer chemoprophylaxis and stand-by treatment here in Japan. The first objective of the guidelines is to clearly cite the conditions under which it is necessary for chemoprophylaxis and stand-by treatment to be implemented, so that travelers are able to avoid contracting malaria or possible death. The second objective is to guard against the overuse of mefloquine and to minimize the incidence of its side effects. These guidelines were created for the average clinician and main antimalarial tablets, which could be acquired in Japan, were included. Regarding the two types of malaria prevention, only chemoprophylaxis is clearly detailed in the guidelines. As stand-by treatment has quite a few unsolved legal problems, only a brief summary of it is given in the guidelines.

To begin with, the guidelines emphasize that all travelers staying in or going to regions with a prevalence of malaria should take the necessary personal protection measures to prevent mosquito bites. The guidelines also mention the two conditions under which mefloquine prophylaxis is ad-

ministered, namely absolute indication and relative indication. The essential standard by which absolute indication can be defined is a situation where travelers face a high possibility of death or contracting serious symptoms due to malaria without taking a chemoprophylaxis regimen. There are two required conditions for absolute indication. First, if there is a high prevalence of malaria in the region where the traveler is staying. Second, if adequate medical facilities are not present in the place where the travelers are staying. When both conditions are in existence, absolute indication occurs and under these circumstances we strongly recommend that mefloquine prophylaxis be taken. Relative indication is defined as the situation where travelers stay in a region where malaria is prevalent but all of the conditions for absolute indication are not present. In this case, travelers should take mefloquine only after they have weighed up the risks of infection with the side effects associated with the antimalarial drug.

In this workshop, I hope to discuss the guidelines in detail with the audience and add any relevant contributions from our discussion.

General presentation

1) ROLE OF COSTIMULATORY MOLECULES IN HOST RESISTANCE TO RE-INFECTION WITH *HYMENOLEPIS NANA ONCOSPHERE*KOTARO ONISHI¹, KAZUHITO ASANO², KENJI ISHIWATA¹, NAOHIRO WATANABE¹¹Dept of Tropical Medicine, Jikei Univ, Tokyo, Japan ²Dept of Physiol(I), Showa Univ, Tokyo, Japan

Host's intestine is important on the host resistance to orally infectious parasites. However, immune system to intestinal parasites is yet unclear. Mice receiving a single oral inoculation of *Hymenolepis nana* (*H. nana*) eggs acquire a strong protective immunity and a challenge infection with eggs will be completely rejected by inhibiting larval growth in the intestinal villi. This strong resistance to re-infection is established on 24 hours after immune infection. This acquired immunity is dependent on CD4 positive T cells. We focused our studies on *in vivo* analysis of the role of costimulatory molecules in host defence mechanisms against *H. nana* re-infection in mice. Primary infection of *H. nana* was carried out by giving 1000 eggs on day 0. The mice were challenged orally with 1000 eggs on day 5 and

killed 4 days later. The protective effect was assessed by counting the cysticercoids which had developed in the intestinal villi by day 9, 4 days after challenge. Monoclonal antibodies were intraperitoneal injected at 0.5 mg on 2 hours before and 48 hours after primary infection. These antibodies are neutralizing monoclonal antibodies against mouse CD80, CD86, ICOS L and CD40 L. Treatment of neither antibodies on protective immunity failed to suppress acquired resistance to *H. nana* re-infection. CD28 knock out mice were carried out as above infection. In this knock out mice, protective immunity also failed to suppress acquired resistance. The present results suggest that protective immunity against *H. nana* oncosphere is established independent to known costimulatory molecules.

2) TAENIASIS IN BALI, INDONESIAHIROSHI YAMASAKI¹, TONI WANDRA², THOMAS SUROSO², MINORU NAKAO¹,
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Indonesia is a multi-ethnic nation which consists of more than 17,500 islands and differs in language, religion, culture, eating-custom and biogeographical characteristic by the island. Bali Island, located between Java and Lombok Islands, is one of them and a globally famous resort. The majority of residents is Hindu (93%). From the eating-habit of Balinese who have eat uncooked pork, has the custom of eating uncooked pork, The island where Balinese who eat uncooked pork live was endemic area for taeniasis/cysticercosis due to *Taenia solium* once. In recent years, taeniasis due to taeniid cestodes other than *T. solium* has been reported. However, whether the causative agents are *Taenia saginata* or *Taenia asiatica* is highly suspicious due to the morphological similarity between *T. saginata* and *T. asiatica*. In the present study, the taeniid proglottids discharged from the tapeworm carriers in Bali have been accurately identified using multiplex PCR targeting cytochrome

c oxidase subunit 1 gene (*cox1*). The survey area was the Ketewel village in Gianyar district located in the southeast part of Bali Island. Of 125 inhabitants examined, 32 persons were diagnosed as taeniasis based on the expulsion of proglottids after treatment with praziquantel. For the molecular identification of the parasites, mitochondrial DNAs from the parasites were prepared using DNeasy Tissue kit and multiplex PCR was performed according to the method established recently¹. Consequently, the *cox1* fragments of 827 bp were amplified from all parasites and were identified that these taeniid cestodes are *T. saginata*. DNA sequencing of the PCR products also supported these results. Furthermore, in order to test whether taeniid DNA is detectable in the stool samples, 10 fecal samples from 32 tapeworm carriers were examined. 827 bp-*cox1* products were detected from all stool samples. As mentioned above, mitochondrial DNA analysis of proglottids and stool samples re-

vealed that human taeniasis in Bali is due to the infection of *T. saginata*. Having considered Balinese eating-habits, uncooked beef called lawar is considered to be a possible source of infection. It seems that change of an eating-habits

of Balinese who are Hindu are reflected. So far, no evidence of *T. asiatica* infection has been obtained from Bali, but the further investigation is required. ¹Yamasaki *et al.* J Clin Microbiol 42: 548-553, 2004.

3) THE STUDY OF ASCARIS AND ULCERATIVE COLITIS PART 2: LOCALIZATION OF MUCIN 12 ON ASCARIS LUMBRICOIDES HOMINIS -

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Some researchers reported that the antibody against *Ascaris* was detected in patient's serum of ulcerative colitis (UC). In the 43rd congress (2002), we reported the results of analyzing the genes related with cross-reactive antigens between *A. lumbricoides* and human colon mucosa by screening method. In the results, we got 3 clones, transmembrane mucin12, similar to actin (gamma 2 of enteric smooth muscle) and beta-casein-like-protein. In these three, mucin localizes in the digestive organs, especially in the colon, and was reported that the antibody against colonic mucin might contribute to the epithelial cell injury of colonic mucosa through antibody-dependent cell-mediated cytotoxicity (ADCC), and it was detected that this autoantibody (anti-mucin antibody) titers are high in some patients for UC. *A. lumbricoides* was likely to harbor in the human intestine, because mucin3 localize richly in human intestine, and it was guessed to be a suitable environment for *A. lumbricoides*. In this study, we were investigated by immunostaining method using the human anti-mucin antibody in order to determine the localization of Mucin 12 on *A. lumbricoides*. In addition, we were investigated that which organs Mucin 12 localized in human internal organs at the RNA

levels using Northern Blot analysis.

As the results of immuno-staining methods using the monoclonal anti-human Gastric mucin for first antibody and FITC anti-mouse IgG for secondary antibody, the specific signal was observed in the lateral lines and cluster-like organs of *A. lumbricoides*. Therefore same localizations were detected by immuno-staining method using the DAB (3,3'-diaminobenzidine). These results were demonstrated that these signals were specific and not endogenous fluorescent substance in *A. lumbricoides*. Furthermore in order to confirm the specificity, immuno-staining was performed using monoclonal anti-human antibody pretreated with purified Mucin12. As the results, it was observed that these signals were declined clearly. This result was suggested that Mucin12 had cross-reactive antigenicity to human Mucin in the lateral line and cluster-like organ of *Ascaris*. As a result of Northern Blot, Mucin12 were localized mainly in colon, rectum, and rarely in jejunum in human internal organs. In this report, we specified the localization of Mucin12 in *Ascaris* and human internal organs. We are going to prepare anti-Mucin12 antibody and perform immuno-staining, similarly.

4) CHARACTERIZATION OF THE 150-KDA CYSTEINE-RICH SURFACE PROTEINS OF ENTAMOEBIA DISPAR

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Entamoeba dispar, morphologically similar to *Entamoeba histolytica*, is neither pathogenic nor invasive. Comparative analyses of surface proteins of the two species are important to determine pathogenic factors and to establish a

specific diagnosis for amebiasis. Recently, we have identified intermediate subunits of galactose- and N-acetyl-D-galactosamine-inhibitable lectin (Igl), as 150-kDa cysteine-rich proteins on the surface of *E. histolytica* (EhIgl1 and

EhIgl2). In the present study, molecular characterization of the corresponding proteins in *E. dispar* has been performed.

The open reading frame of two cloned Igl genes from *E. dispar* encoded 1,110 (EdIgl1) or 1,106 (EdIgl2) amino acids. The calculated molecular masses and theoretical pI were 120,886 Da and 5.5 in EdIgl1 and 120,347 Da and 4.87 in EdIgl2. When the deduced amino acid sequences of these Igl genes were compared, identities were 76.9% between EdIgl1 and EdIgl2, 73.7% between EdIgl1 and EhIgl1, and 73.0% between EdIgl2 and EhIgl2. To measure quantitatively the expression of the Igl genes, real-time reverse transcription PCR was performed and the results analyzed by a comparative C_T method using actin genes as internal standards. The expression level was higher in *E. histolytica* than in *E. dispar*, and also higher in Igl1 than in Igl2. These results suggest that the Igl genes are essential proteins even in the non-

pathogenic amoeba. Recombinant Igl genes were expressed in *Escherichia coli* and their reactivity with anti-EhIgl antibodies and patients' sera was examined. Sera from patients with amoebiasis and a polyclonal antibody to the EhIgl genes reacted with the EdIgl genes suggesting the existence of common epitopes between Igl genes from *E. histolytica* and *E. dispar*. On the other hand, two monoclonal antibodies recognizing either the conformation or the N-terminus of EhIgl1 did not react with EdIgl genes showing the existence of *E. histolytica*-specific epitopes. Although we have recently demonstrated that recombinant EhIgl1 is a useful antigen for the specific and sensitive serodiagnosis of amoebiasis, it is important to consider the antigenic similarity between EhIgl genes and EdIgl genes and their species-specific epitopes for increasing diagnostic specificity.

5) A SURVEY ON PREPARATION AGAINST POSSIBLE RESURGENCE OF SARS

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While Severe Acute Respiratory Syndrome (SARS) was in epidemic, a large number of nosocomial infections broke out in hospitals where patients were accommodated. The epidemic was put under control in July 2003, however possibility of resurgence still remains. This survey was conducted with the purpose of contribution to the appropriate response at hospitals in case of resurgence, by analyzing current situation of preparation against SARS and existing problems.

Questionnaire survey was performed for hospitals in the countries where SARS was experienced (41 hospitals in Vietnam and 95 in China), and those in the country without experience (5 in Nepal) on awareness among staff, current situation of nosocomial infection control, ability to accept SARS cases, etc. These hospitals will possibly accept SARS cases in case of recurrence. Among them only 1 hospital in Vietnam actually treated SARS cases, and 2 hospitals in Vietnam and 3 in China examined suspected cases.

In Vietnam and China awareness on SARS among hospital staff was significantly higher (66.7% and 27% of the hospitals, respectively, answered "high") than in Nepal (100% were "low"). Regarding manual, 66.7% of hospitals

in Vietnam and 72.5% in China prepared, while 25% in Nepal. The rate of having nosocomial infection prevention committee was 85.4% in Vietnam, 95.9% in China and 75% in Nepal. In Vietnam 95.1% and 97.5% of hospitals were regarded as having good storage of surgical masks and gloves, respectively. Similar good results were obtained in China. However, in both countries protection attires such as N95 masks and goggles were lacking. In Vietnam and China, 75% and 97.9% of hospitals conducted staff training for infection control against SARS. In Vietnam and China, Infection Control Team (ICT) has been established in 51.2% and 71%, respectively, while in no hospital in Nepal. All the hospitals in Vietnam were requesting Bach Mai Hospital, the hospital that had an experience of SARS containment, for providing technical guidance on SARS control.

In hospitals in the countries that experienced SARS, growing awareness on SARS, steady preparations against possible resurgence, and request of staff for further training were shown. ICT has been established in many hospitals but had problems in management. On the other hand, in the country where SARS was not experienced, both awareness and preparation status were poor.

6) ECOLOGICAL ROLES OF MONGOOSE IN TRANSMISSION CYCLE OF JAPANESE ENCEPHALITIS VIRUS IN OKINAWA ISLAND

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Background

Japanese encephalitis (JE) had been one of the major public health problems before 1970's in Japan, including Okinawa Island. No case has been reported officially in Okinawa Island since 1974, but transmission of JE virus in nature is demonstrated by the high prevalence of JE virus antibody in swine sera, and by the virus isolation from swine sera and mosquitoes.

Around 20 mongooses (*Herpestes javanicus*) were imported to Okinawa Island in 1910 in order to control toxic snake and mice. The number of the mongoose is increasing and estimated as 30,000 at present and hazardous to the unique wild life.

Objectives

To clarify trole of mongooses in transmission cycle of JE virus in Okinawa island.

Materials and methods

102 sera were collected from captured sera in all areas of Okinawa Island between 2001 and 2004. Sera from 3 baited mongooses in agricultural area were collected regularly from July to October, 2004. Virus isolation from all sera mentioned above was carried out by inoculation each sera onto C6/36 cells and Vero cells and incubated for 8 days. Existance of infective virus in cultural fluids was identified by immunostaining method using polyclonal antibodies against JE virus.

Neutralization (N) test were conducted on sera from captured and baited mongooses by 50% focus reduction method using Beijing-1(human vaccine strain, genotype 3), Nakayama (prototype, genotype 3, Oki 431S (Okinawan isolate from swine in 2002, genotype 1)and Na 54 (Okinawan isolate from swin in 1985, genotype 3).

Results

Virus isolation from captured and baited sera was failed.

Prevalence rates of N antibody against JE virus strains were: 14.7% against Beijing-1, 24.5% against Nakayama, 3% against Oki 431S and 1% against Na 54. N-titers against JE virus strains were relatively low as less than 125 except one sera with high N-titers between 400 to 3,100. Temporal change of N antibody titer in sera of one baited mongoose was observed.

Conclusion

This is the first serological report that mongooses have JE virus antibodies, and clarified that mongooses are infected by JE virus.

Discussion

We found N-antibodies against JE virus strains in mongooses, however relatively low N-titer might suggests possibility to be infected by other flaviviruses than JE virus. Furthermore, the role of mongooses in transmission cycle of JE virus or other flaviviruses must be clarified.

7) SHIFT OF JAPANESE ENCEPHALITIS VIRUS PROPAGATING IN JAPAN, 1935-2002.

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We have reported that the dominant genotype of the Japanese strains of Japanese encephalitis virus (JEV) shifted from genotype 3 to genotype 1 (G1) in the early 1990s. However, the origin of these Japanese G1strains remains unclear. A phylogenetic analysis based on the 196-nucleotide (nt) capsid/premembrane region of 77 JE virus

strains isolated during 1935-2002 in Japan revealed the existence of at least six dominant genetic clusters (cluster A-E and GI). The result of the phylogenetic analysis, which used the neighbor-joining method, indicated that these clusters of JEV might have been introduced into Japan from the west of Japan and not from the south via the Ryukyu Islands and

Taiwan. The strains that isolated from Japan in the 1980s, belonged to cluster E, had a 9- to 10-nt deletion in the 5' ter-

minal of their 3' noncoding region.

8) JAPANESE ENCEPHALITIS IN THE PHILIPPINES

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[Objectives]

Japanese encephalitis virus (JEV) belongs to Genus *Flavivirus*, Family *Flaviviridae* and is the causative agent of Japanese encephalitis, a mosquito-borne disease transmitted by *Culex tritaeniorhynchus*. In recent years, no serological and virological survey on JEV has been done in the Philippines. This study aims to determine the seasonality, age distribution, and geographical distribution of JEV infection in the Philippines.

[Patients and Methods]

One hundred fourteen Serum and 329 cerebrospinal fluid (CSF) samples extracted in 2002 and 2003 from patients clinically diagnosed as CNS diseases such as viral encephalitis, aseptic meningitis and other acute paralysis were examined using IgM capture ELISA for JEV. Six hundred eighty four pig sera were also collected in Tanay, Rizal from May, 2002 to October, 2003 and then examined their IgM and IgG against JEV.

[Results and Discussion]

Out of 329 CSF and 114 serum samples, 38 (11.6%) and 21 (18.4%) were IgM positive for JEV, respectively.

These results were almost similar with the results of 1999 to 2001. The analysis of 52 laboratory diagnosed JE patients indicated that among 31 age recorded cases, 25 (80.6%) were younger than 20 years old. The seasonality of JEV infection was seen from July to November that was coincided with rainy season of this area. The geographical distribution of JEV infection was observed in Tarlac province, 150km north from Metro Manila, had higher incidence.

We also monitored the IgM and IgG levels against JEV among pigs in Rizal province, 100km south from Metro Manila, monthly from May 2002 to October 2003. The result of IgG-positive ratio against JEV indicated that the peak season of JEV infection was from July to September in 2002 and 2003. It was synchronized with the incidence of human JEV infection cases.

Although we have not yet isolated the JEV from human, pig and mosquito samples, the results of our serological surveillance on JEV indicated that JEV infection occurred among young age group during hot and rainy season from July to September.

9) IMMUNOGENETIC ANALYSIS OF THE PATIENTS WITH DENGUE HEMORRHAGIC FEVER IN VIETNAM.

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Dengue virus infections are serious public health problems in many regions of the world, especially in the South East Asian countries. Dengue virus infection may lead to Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), or Dengue Shock syndrome (DSS). Two factors are proposed to be important to produce DHF / DSS. One is viral virulence and the other is host genetic factor. In this study, we made an experimental design to identify the host gene(s) contributing to the development of DHF/DSS in Vietnamese by case control study. The patients were clinically diagnosed by WHO criteria (grade I, II or grade III, IV) and their peripheral blood samples were collected at the Center for Preventive Medicine, Vinh long Province (VL), and the Pediatric Hospital No.2, Ho Chi Minh City (NDII) in 2002

to 2003. The patients age ranged between 10 months and 15 years. 200 age and sex matched control samples were collected in Vinh long. The number of the patients with mild cases (grade I, II) was 80 from VL, 107 from NDII, and that of the patients with shock (grade III, IV) was 205 from VL, 66 from NDII. DNA was extracted from each blood sample, then HLA class I (HLA-A, B) and TNF- α promoter SNPs typing were performed. There was no significant difference in HLA-A, HLA-B alleles. However, TNFP-D allele was significantly increased in severe cases ($p=0.0074$, $P_c < 0.04$ OR=2.2895% CI1.23-4.23). The TNFP-D allele might directly contribute to severity of DHF/DSS or might be a genetic marker that has strong linkage disequilibrium with some responsible gene

10) STATUS OF CURRENTLY CIRCULATING DENGUE VIRUS RESPONSIBLE FOR THE EPIDEMIC IN BANGLADESH IN THE YEAR 2002

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【Introduction】 Dengue fever and dengue hemorrhagic fever (DF/DHF) has appeared in Bangladesh as an outbreak since the year 2000. To analyze clinical features of DF/DHF in Bangladesh and to get detailed information about the currently circulating dengue viruses, we conducted clinical data collection, sero-epidemiological surveillance and virus isolation. This is the first report on molecular biological analyses of dengue viruses isolated in Bangladesh.

【Patients and Methods】A total of 200 serum samples were collected from the dengue clinically diagnosed patients in

Shaheed Suhrawardi Hospital, Shere-e-Bangla Nagar, Dhaka, Bangladesh in 2002. All the serum samples were measured the anti-dengue antibody level by IgM-capture ELISA and indirect IgG ELISA. For dengue virus isolation, serum samples were inoculated into mosquito derived C6/36 cells. Isolated dengue viruses were sero-typed by RT-PCR. Based on the envelope gene sequencing data, phylogenetic analysis was done. To clarify the clinical feature of DF/DHF in Bangladesh, clinical records of 200 dengue patients collected in 2002 were analyzed.

【Results and Discussion】 IgM positives and IgG positives against dengue virus were 59.5% and 65.5%, respectively. Eight strains of dengue virus were isolated and all of them were DEN-3. All 8 DEN-3 Bangladeshi strains clustered within genotype II along with some DEN-3 strains from Myanmar, Thailand and Malaysia and they were closely related to Thailand's isolates from the 1990's. It suggests that DEN-3 might have entered Bangladesh from Thailand during the last decade. Besides, 8 isolates presented in a distinct sub-cluster of the genotype, indicating some independent evolu-

tion occurred in Bangladesh. Clinical profiles of 200 dengue patients indicated that most patients showed high frequency of prodromal symptoms such as fever, headache and vomiting. Also relatively high percentage (70%) of patients showed hemorrhagic manifestation. Six patients (3%) also showed plasma leakage (edema and ascities). Peak age distribution of the dengue patients belongs to 21-30 years old age group. Most of the dengue cases were reported in August and September.

11) GENETIC VARIATION IN THE 3' NON-CODING REGION OF DENGUE VIRUS TYPE 1

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We determined the complete genome sequence of dengue-1 virus, DENV1/hu/Seychelles/NIID41/2004 (NIID 03-41), isolated from a traveler to Seychelles. Sequence analysis showed that the 10,718-nucleotide-long RNA genome of NIID03-41 was shorter than that of other dengue -1 (DEN-1) isolates, because there was a 17-nucleotide deletion in the 3' non-coding region (NCR). A region of approximately 60 nucleotides, which located at the down-

stream of the open reading frame stop codon, showed high sequence variability as compared with other DEN-1 isolates. Phylogenetic analyses along with the full-length sequences of 20 DEN-1 virus genomes indicated that the NIID03-41 strain was closely related to those included in the Pacific group, not the African group. This is the first report of DEN -1 isolate with a nucleotide deletion in the 3' NCR.

12) REAL-TIME REVERSE TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) ASSAY AS A RAPID DIAGNOSTIC TOOL FOR EMERGING VIRUSES

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Loop Mediated Isothermal Amplification (LAMP), a novel method of gene amplification is emerging as a valuable rapid diagnostic tool for rapid detection of infectious diseases. We have developed and evaluated one-step single tube real-time accelerated quantitative reverse transcription loop mediated isothermal amplification (RT-LAMP) assays for rapid detection of some of the recently emerged human viral pathogens viz; West Nile, SARS and Dengue viruses. The whole procedure is very simple and rapid wherein the amplification can be obtained in 20-30 min by incubating

all the reagents in a single tube with reverse transcriptase and Bst DNA polymerase at 63 °C. The RT-LAMP assay was found to be 10 to 100 fold more sensitive as compared to conventional RT-PCR, with a detection limit of 0.01 to 10 PFU of virus. The specificity of the RT-LAMP assay systems for respective viruses was established by cross-reaction studies with other closely related viruses. The evaluation of SARS RT-LAMP assay with 49 suspected and 10 healthy throat wash specimens revealed 100% and 87% sensitivity and specificity respectively with regard to RT-

PCR. The evaluation of West Nile RT-LAMP assay with 8 of spiked and 6 negative human serum samples revealed 100% concordance with RT-PCR in terms of sensitivity and specificity. The applicability of RT-LAMP assay for clinical diagnosis of dengue virus infection was validated by examining limited number of DF and DHF patient serum samples of each serotypes. The RT-LAMP assay could correctly identified the serotypes with a 100% concordance with one step serotype specific direct RT-PCR. The field application

of RT-LAMP assay made easy by employing the SYBR Green-I dye mediated naked eye visualisation protocol following incubation in a water bath. Thus the RT-LAMP assay reported here has the advantages of rapid amplification, simple operation and easy detection with potential usefulness for clinical diagnosis and surveillance of viral diseases in developing countries without requiring any sophisticated equipments or skilled personnel.

13) CHARACTERISTIC PREVALENCE OF HPV GENOTYPES IN PENILE CANCER IN NORTHERN THAILAND

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PURPOSE: Penile cancer(PC) is uncommon in the developed countries such as the US, Japan and European countries. The incidence is higher in some of the African, South Asian, Southeast Asian and South American countries. The etiology of PC is not well understood. Lack of neonatal circumcision and phimosis are considered to be the most significant risk factor for PC and also hygienic condition in each country influences the occurrence of PC, however the causal relationship has never been established. **MATERIALS AND METHODS:** To clarify the role of human papillomavirus(HPV) in PC, we studied the prevalence and genotypes of HPV DNA in the paraffin blocks from surgical specimens of PC and control tissues in northern Thailand, using Short-Fragment PCR assay. We analyzed 31 cases of invasive squamous cell carcinoma (SCC), 1 of verrucous carcinoma (VC), 1 of epithelial dysplasia (ED) and 1 of paraffinoma. **RESULTS:** Overall, the detection rates for HPV DNA in our study were 81% (25 of 31 cases) for invasive SCC, 100% (1 of 1 case) for VC, 100% (1 of 1 case) for dysplasia and 100% (1 of 1 case) for paraffinoma. In all

SCC groups, including VC, 42% (11 of 26 cases) for a solitary infection of HPV-18, 23% (6 of 26 cases) for a solitary infection of HPV-6, 15% (4 of 26 cases) for double infection of HPV-6 and 18 and 4% (1 of 26 cases) for mixed infection of HPV-6, 18 and 22. 100% (1 of 1 case) of ED and paraffinoma showed solitary infection of HPV-6 and HPV-18, respectively. **CONCLUSION:** The prevalence rate of HPV DNA in PC in northern Thailand is much higher than previous reports from the US and European, South American and African countries. They described that HPV-16 which belongs to high risk types is the most dominant HPV genotype, HPV-18 which also belongs to high risk types is minor HPV genotype in HPV-positive PCs and HPV-6 which belongs to low risk types are detected mainly in the benign and premalignant lesions in these countries. However, in our study no HPV-16 is found in HPV-positive PCs and a premalignant lesion. HPV-18 and HPV-6 are the most common genotypes in HPV positive PCs in northern Thailand.

14) A NEW SCREENING METHOD FOR SALMONELLA USING LEMON SLICES

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The salmonella was inoculated in DHL agar and the circular slice lemon was put in the top, and it was cultivated at 35C for 18 hours. In 18 hours and circumference of the circular slice lemon, the black band where 4-5 mm of the ferric sulfide was observed. In addition, the part under the

circular slice lemon pinkly changed. In case of *Citrobacter freundii* and *Proteus mirabilis*, there was the dispersion of the result. This research indicated the method for examining the Salmonella only in the DHL culture medium.

15) MOLECULAR ANALYSIS OF HAEMOPHILUS INFLUENZAE ISOLATED FROM NASOPHARYNGEAL ASPIRATE AND CEREBROSPINAL FLUID AMONG CHILDREN IN VIETNAM

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The goal of this study was to investigate the characteristics of *Haemophilus influenzae* among children less than 5 years of age in Hanoi, Vietnam. Thirty-seven *H. influenzae* strains were isolated from the nasopharyngeal swabs (NP) of 37 children with acute lower respiratory tract infections and 44 *H. influenzae* strains were isolated from the cerebrospinal fluid (CSF) of 44 children with meningitis. Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c and one was type a, whereas those of the 44 isolates from CSF were all type b. Twenty-six strains (70.3%) from NP and 23 strains (52.3%) from CSF were b-lactamase-producing, and the remaining strains were b-lactamase-negative. PCR analysis to identify the resistance genes indicated 25 strains from NP and 21 strains from CSF had the TEM-1-type b-lactamase gene, 11 strains from NP and 22 strains from CSF lacked all resistant genes, and each strain from NP and CSF had the

TEM-1-type b-lactamase gene and the *ftsI* gene with the same substitution as the low-b-lactamase-negative ampicillin-resistant (low-BLNAR) strains. No BLNAR strain was found. The MICs against 16 antibiotics between the *H. influenzae* isolates from NP and CSF were similar, except for penicillin G and ampicillin. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns without any predominant pattern and 44 *H. influenzae* strains from CSF had 9 PFGE patterns with predominant patterns a (22 isolates) and b (12 isolates). The PFGE patterns of the 4 *H. influenzae* type b strains from NP were quite different from those of 44 *H. influenzae* type b strains from CSF. Our results indicate that the BLNAR strains are not prevalent and that 2 predominant types of *H. influenzae* type b strains have the potential to spread among children with meningitis in Hanoi, Vietnam.

16) BURULI AND THE ULCERS UNDER THE TROPICS

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Buruli ulcer, caused by *Mycobacterium ulcerans* is considered the third most prevalent mycobacteriosis after Tuberculosis and Leprosy. A largely neglected disease, it represents a serious treat to public health in poor and remote rural areas of endemic countries. Emerging since the eighties as an important cause of human suffering, the disease has been reported or suspected in more than 30 countries in the world. Although Africa remains the most affected region, Asia bears as well some endemic regions like Australia, India and Malaysia whether Buruli ulcer is an emerging or re-emerging remains subject of controversy. But, that Buruli ulcer is a neglected disease fits perfectly in the WHO definition of Neglected diseases. Although Buruli ulcer can prove extensively damaging to the skin, its awareness is very limited in the medical community as well as in

the general public. This results in its under-recognition and thus its under-reporting. Its under-diagnosis or most frightening, its misdiagnosis can lead to life threatening consequences. We report here a series of 96 specimens of skin biopsy, obtained from the Agroyesum Catholic Mission Hospital in the Ashanti district of Ghana. Thirty % of the specimen in the series proved to be misdiagnosed lesions, other than Buruli ulcer. Among these, 1/3 were malignant lesions ranging from fibrosarcoma to malignant melanoma. The remaining were inflammatory tumorous conditions. The aim of the present study is to emphasize that Buruli ulcer can lead to confusion with the wide spectrum of tropical both parasitosis and infectious conditions that can mimic its different clinical presentation.

17) EPIDEMIOLOGICAL STUDY OF LEPROSY IN A LOCAL VILLAGE IN MYANMAR

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In many developing countries where life threatening diseases such as malaria, tuberculosis have been getting big attention of government and international organizations, there are also other diseases which have less prevalence and less severity. They are called neglected diseases and are usually not acutely fatal. However, the QOL of sufferers are often greatly impaired leading to the vicious cycle of poverty. Leprosy typifies a neglected disease.

After introduction of Multidrug therapy in 1982, global prevalence of leprosy has greatly decreased. However, yearly number of new cases has not decreased satisfactory. Although early detection and treatment is in the main-

stream of leprosy control, another intervention is desired based on the recent epidemiological studies.

We have been supporting leprosy control activity in Myanmar where leprosy is highly endemic.

Materials & Methods

1 From 2000, prevalence of *Mycobacterium leprae* (ML)-infection has been studied in K-village (population: 306), except in 2002 and 2003, study was difficult because of the restricted situation. ML-infection was identified by ML specific anti-PGL-I antibody titer, measured by ELISA.

2 Drug resistance-related mutation was examined in leprosy patients visited CSSC. Cardinal drugs for chemotherapy;

DDS, RFP, and OFLX were examined. This time, preliminary study can be shown.

Results

1 From the prevalence of PGL-I-positivity, about 40% of villagers were once infected with *ML* until 20-30 year-old.

During study period, 3 villagers developed leprosy. Case 1 was found in 2001 to have MB (multi-bacillary) leprosy. She presented high level antibody in 2000 and 2001, and then it decreased in 2004. Case 2 was diagnosed in 2002 to have PB (pauci-bacillary) leprosy. She presented high titer in 2000 but low in 2001 and 2004. Case 3 was found in 2003 to have PB leprosy. His antibody was negative during all study-period. (The shift of antibody is shown in the graph.)

2 Drug resistance-related mutations were found in 2 out of 29 cases examined. From the past history, they were considered to be primary resistant cases.

Conclusion

1 High prevalence of *ML*-infection was shown in K-village. We may predict some possible cases of developing MB leprosy by measuring PGL-I antibody. However, PB candidates cannot be predicted with this method. Chemoprophylaxis targeting the villagers having high antibody titer might be one way to prevent new MB cases, but cannot cover the PB cases.

2 It's a big threat that primary drug resistance was detected in endemic situation. More vigorous study is required in this field.

18) DIAGNOSTIC TRIAL FOR CUTANEOUS MYCOBACTERIAL DISEASES BY USING PCR TECHNIQUES.

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For the diagnosis of *Mycobacterium tuberculosis* (TB) infection, identification of bacteria by Ziehl-Neelsen stain and culture methods are very useful. Eventually, sometimes it is very difficult to detect bacteria especially in skin samples. Now-a days, DNA-DNA hybridization and amplification of *Mycobacterium tuberculosis* DNA have been considered as a new method for the diagnosis. This new method requires cultured samples. When culture of bacteria fails, then PCR analysis become the more important useful method, which requires a small quantity of DNA taken from the tissue samples. In this study, we reported a total of five cutaneous mycobacterial patients, in which four cases

were scrofuloderma and another was warty type. All cases were confirmed as positive reactivity to Ziel-Neelsen stain and culture. The confirmation can be made on positive culture only in our cases, but we performed PCR analysis and direct-sequence analysis, by using DNA product of the skin tissues instead of cultured sample for species specificity. PCR analysis is the easy and simple method as compared to other diagnostic tools. We confirmed the all five cases were infected with *mycobacterium tuberculosis*. From these observations, we considered that PCR analysis might be useful for the diagnosis for cutaneous tuberculosis.

19) THE INFLUENCE OF CIRCUMSTANCES TO LEPROSY SKIN LESIONS.

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Today, most of countries endemic for leprosy are situated in the tropical zone. Therefore, in consideration of the histopathology and epidemiology of leprosy, the evaluation of its temperature and UV etc. are considered to be important.

The purpose of our study is to find out the correlation between conditions of circumstances and skin lesions of leprosy patients who were observed at the Ryukyu University Hospital.

Material and method; From 1982 to 2004, 147 new patients and seven relapsed patients with leprosy visited at outpatient clinic of dermatology. We recorded the sites and symptoms of skin lesions and the date of outbreak of the

disease written in their charts.

Result: 26 cases showed the eruption on the face (17.7%). In 32 cases (21.6%), the first site of lesion was face and/or neck, and in 16 cases (10.8%, 16/147) lesions were localized at face and/or neck. Number of patients who noticed the disease was largest in June. In addition, relatively many cases showed the eruption at the knee joint area and elbow where skin temperature was slightly low, and no lesion was observed at the scalp, axillary, inguinal, perineal and anal regions.

Summary; Clinically, we could observed the correlation between the cutaneous changes of leprosy and UVB, temperature and skin temperature.

20) EFFECT OF APHIDICOLIN ON ENTAMOEBA EXCYSTATION AND METACYSTIC DEVELOPMENT

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The effect of aphidicolin, a specific inhibitor of the replicative DNA polymerases, on the excystation and metacystic development of *Entamoeba invadens* was examined. The protein profile of metacystic amoebae and their immunogenicity in the presence and absence of aphidicolin were also examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Excystation, which was assessed by counting the number of metacystic amoebae after the induction of excystation, was inhibited by aphidicolin in a concentration-dependent manner during incubation compared to the controls. Metacystic development, when determined by the number of nuclei in amoeba, was also inhibited by aphidicolin, because the percentage of 4-nucleate amoebae in cultures with aphidicolin during incubation was higher than that in cultures without the drug. The addition of aphidicolin to cultures at day 1 of incubation

reduced the number of metacystic amoebae thereafter compared to cultures without the drug. The inhibitory effect of aphidicolin on excystation and metacystic development was reversed by removal of the drug. Pretreatment of cysts with aphidicolin before transfer to a growth medium containing the drug had no further effect on the excystation and metacystic development. Cellular proteins of metacystic amoebae with 4 nuclei, which were predominant even at day 3 in the cultures with aphidicolin, reacted strongly with rabbit anticyst serum absorbed with trophozoite proteins. In contrast, those of metacystic amoebae with 1 nucleus, which were predominant at day 3 in cultures without aphidicolin, no longer reacted with the absorbed anticyst serum, suggesting change in the expression of proteins during metacystic development.

21) MOLECULAR CLONING AND CHARACTERIZATION OF A PROTEIN GERANYLGERANYLTRANSFERASE TYPE I FROM ENTAMOEBA HISTOLYTICA

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Ras superfamily small G proteins act as a molecular switch of signal transduction involved in cell proliferation and differentiation, cytoskeletal reorganization, and vesicular transport. Protein prenylation, a class of posttranslational lipid modifications, is necessary for these proteins to associate membrane structures and function. Protein prenylation includes protein farnesylation and protein geranylgeranylation, which are catalyzed by protein farnesyltransferase (FT), protein geranylgeranyltransferases type I (GGT-I) and type II. It has been revealed from the study of mammals and yeasts, that these enzymes are consisted of α and β subunits, and that FT and GGT-I have a common α subunit. The inhibitors for protein prenyltransferases inhibit the carcinogenesis caused by mutations of Ras proteins, and also inhibit the proliferation of protozoan parasites such as *Trypanosoma*. We have been studying on the protein prenyltransferases of *Entamoeba histolytica* (*Eh*), as a control molecule of cell proliferation and differentiation, and as a possible target of chemotherapy. We previously cloned and sequenced *EhFT* and characterized the recombinant pro-

teins. Here we reported on *EhGGT-I*.

The β subunit of *EhGGT-I* had 337 amino acids, and showed 22 to 28% identities to those of human, yeasts and *Arabidopsis thaliana*. The recombinant *EhGGT-I*, expressed by *Escherichia coli*, was obtained as a complex of 38 kD and 35 kD proteins. The rabbit antiserum raised against the recombinant *EhGGT-I* reacted with *EhGGT-I*, but did not react with rat GGT-I. In contrast to rat GGT-I, which geranylgeranylates human H-Ras-CVLS, but does not geranylgeranylate the mutant H-Ras-CVLL, *EhGGT-I* geranylgeranylated both substrates. In mammals, Ras proteins are farnesylated by FT, and Rac proteins are geranylgeranylated by GGT-I. *EhGGT-I*, however, geranylgeranylated Ras proteins such as *EhRas 1-CIMF* and *EhRas 2-CELL* as well as Rac proteins including *EhRacA-CLLF* and *EhRacG-CSLF*. *EhGGT-I* was much more resistant to inhibitors for mammalian GGT-I than rat GGT-I.

In conclusion, *EhGGT-I* was significantly different from mammalian GGT-I, and thus would become a possible chemotherapeutic target of amoebiasis.

22) THE CURATIVE EFFICACY OF METRONIDAZOLE ON INTESTINAL AMEBIASIS

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The prevalence of amebiasis of these three years tends to increase in Japan. And over five hundred cases of mainly symptomatic amebiasis have been reported per year. The majority of these reported cases were intestinal amebiasis. Metronidazole (Mz) is usually single used as a medicine for treating amebiasis in Japan. Mz has significant therapeutic effect especially on invasive amebiasis because of its immediate absorption from intestine. And Mz is also used for treatment of noninvasive intestinal amebiasis by means of increasing its dosage and dosing period. However, recently measurable failure cases of Mz have been reported. We have also experience of Mz failure in treatment of mass infection

of *Entamoeba histolytica* (prevalence of cyst positive cases/80 persons; 30%) in a rehabilitation facilities for persons with intellectual disabilities, despite of repeat prescriptions of Mz, maximum 8 times for 2 years. Coprophilia was estimated one of the reason of reinfection of cysts of *E. histolytica* and the reason of failure of Mz treatment. Finally, the mass infection of *E. histolytica* was eliminated by combination chemotherapy of Mz and Diloxanide flooate (Dlx) as lumen drug. By the result of Mz treatment in mouse (C3H/HeJ) intestinal amebiasis model, Mz (12.5mg/kgx7days) administered via intraperitoneal was more effective than via orally administered Mz with same dose. This drug kinetics

may indicate that amebae are colonized and proliferated by adhesion with or by invasion in intestinal mucosa. These results showed some difficulties of monotherapy of Mz. And,

if unlicensed Dlx is unobtainable, some combinations of probiotics for improvement of intestinal bacterial flora and constipation with Mz may be effective.

23) THE ATTACHMENT AND ENTRY OF *LEISHMANIA (LESIMANIA) MAJOR* TO MACROPHAGES: OBSERVATION BY SCANNING ELECTRON MICROSCOPE

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Leishmaniasis starts with the injections of the *Leishmania* protozoan promastigates into the skin at the time of blood suction by a female sandfly. The infection with leishmania is established when the *Leishmania* protozoans start their own intracellular multiplication after having been phagocytized by the host macrophages. In the earliest stage of the infection of leishmaniasis, therefore, the attachment of the promastigates to the macrophages is es-

sential. We incubated in vitro a mixed culture of macrophages (JM774-1A) and *Leishmania (Leishmania) major* for 6 hours and observed the form of the attachment between the two by a scanning electron microscope. We found that the attachment between the two occurred at the site of the parasite body in addition to the previously reported sites such as the flagellar tip, flagellar base, and aflagellar tip (posterior pole).

24) DEVELOPMENT OF A SENSITIVE AND SIMPLE DIAGNOSTIC METHOD FOR VISCERAL LEISHMANIASIS USING URINE SAMPLES

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We previously reported a sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the diagnosis of visceral leishmaniasis (VL) using urine samples that can be easily and safely collected.

Antibodies specific to *Leishmania* antigens were detected by the ELISA in non-concentrated urine samples from VL patients. However, the urine-based ELISA needs a plate reader to measure optical density. On the other hand, a direct agglutination test (DAT), which uses *Leishmanis donovani* promastigote as antigen (DAT antigen), does not require sophisticated equipment and has been used widely. In this study, we report a new DAT that has enough sensitiv-

ity to detect antibodies in urine samples.

A96-well microtiter plate was coated with anti-human IgG. Urine samples were applied to each well and incubated overnight. After washing the plate, DAT antigen was applied to each well. Agglutination was observed after overnight incubation.

With this new DAT, 55 out of 56 VL patients (98%) were diagnosed as positive and all endemic normals (n=59) and Japanese controls (n=24) were negative. Adaptation of this sensitive, specific and simple method to a mass survey of VL is now in progress.

25) ESTABLISHMENT OF A DETECTION AND IDENTIFICATION METHOD OF LEISHMANIA SPECIES WITHIN NATURALLY INFECTED INDIVIDUAL SANDFLIES

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The surveillance of prevalent *Leishmania* and sandfly species in endemic areas is important for prediction of the risk and expansion of leishmaniasis. Usually, the infection of sandflies with *Leishmania* promastigotes has been examined by dissection of each sandfly under a microscope.

However, for this purpose, sandflies should be fresh and highly skilled technique is required for dissection of tiny sandflies. In addition, this process takes a relatively long time and is not suitable for examination of a large number of sandflies. In the present study, we attempted to establish a PCR-based detection and identification method of *Leishmania* species within naturally infected sandflies.

First, we tested the specificity and sensitivity of our PCR method with primers specific for *Leishmania* minicircle kinetoplast DNA using genomic DNA equivalent to 10, 1 or 0.1 parasites from five reference strains of *Leishmania* species distributed in Ecuador as templates. As the result, we amplified minicircle kinetoplast DNA from all the *Leishmania* species examined and detected 0.1-1 parasites in these species. In addition, we successfully detected *Leishmania*

minicircle DNA by PCR from ethanol-fixed sandflies infected with *Leishmania* promastigote under microscopy.

We next applied this method to ethanol-fixed field-captured sandfly samples. After extraction of DNAs from individual sandfly samples, PCR amplification was performed as described above. We detected the minicircle DNA in 6 out of 183 (3.3%) sandflies with this method, while 5 out of 143 (3.5%) were positive for *Leishmania* promastigotes in the same areas under microscopic examination. The species were determined *Leishmania (Leishmania) mexicana* by nucleotide sequencing of the Cytochrome *b* gene. Additionally, all the *Leishmania*-positive sandflies were identified as *Lutzomyia ayacuchensis* by the restriction enzyme digestion of the PCR-amplified 18S rRNA gene fragments. Since this combined method is relatively easy and process a large number of samples, it will be a powerful tool for rapid identification of prevalent sandfly and *Leishmania* species as well as monitoring the infection rate in sandfly populations in endemic areas.

26) PRODUCTION OF RECOMBINANT KINESIN-RELATED PROTEIN OF LEISHMANIA DONOVANI AND ITS APPLICATION IN THE URINE-BASED ELISA FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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For the serodiagnosis of visceral leishmaniasis (VL), a recombinant antigen, rK39 (a part of *L. chagasi* kinesin related protein), has shown very good sensitivity and specificity. Here we report the production of recombinant kinesin-related protein with a molecular weight of 42-kDa (rKRP

42) of *L. donovani*, a homologue to rK39, and the value of this antigen in urine-based ELISA for the diagnosis of VL.

To obtain the gene coding the rK39 homologue, PCR amplification was performed from *L. donovani* genomic DNA. Then the amplified PCR product was subcloned in

pBluescript KS (-) and sequenced. The PCR product was 1071 bp, which was just one repeat (39 amino acid) larger than the rK39 antigen. Amino acid sequence showed 89.3% identity and 98.7% homology to the rK39 antigen. The PCR product was cloned in pTYB12 expression vector. The rKRP42 was purified with Ni-NTA column, then with Chitin column, and used in the urine-based ELISA for the diagnosis of VL.

The ELISA showed 94.3% sensitivity (66 positives

among 70VL samples) and 99.6% specificity (1 positive among 240 non VL samples). The specificity was determined with urine samples of 59 endemic healthy controls, 80 Japanese controls, 58 malaria, 13 tuberculosis, 23 cutaneous leishmaniasis, and 7 other diseases. This assay system showed similar sensitivity and specificity to our previously reported urine-based ELISA with acetone-treated *L. donovani* promastigotes antigen. The use of this rKRP42 will ensure the stable supply of antigen.

27) DEVELOPMENT AND EVALUATION OF A NEW ANTIBODY DETECTION KIT (INSTANT(TM)CHEK-AMOEBA) FOR AMOEBIASIS

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After the enforcement of the Infectious Diseases Control Law in April 1999 (partially amended in November 2003), amoebiasis was included in Category V of the law and all physicians were to take note that it was no longer a quarantinable disease. Since then, the chances of medical examination and treatment of amoebiasis in out-patients clinics have increased, and antibody tests of the disease are in higher demand nowadays. Diagnostic tests based on existing methods like plate ELISA, gel precipitation test and immunoblot assay require a couple of days to obtain laboratory results. In addition, commercially available test kits for amoebiasis often show false negative results. We hereby present a report on a rapid test kit Instant (TM) CHEK-Amoeba for the detection of anti-*Entamoeba histolytica* antibodies from patients' serum. The kit does not need any special apparatus and the result of a test could be decided within one minute.

The test is easy to perform. Twenty microliters of test serum diluted fourfold (1:3) is added to a nitrocellulose membrane unto which *E. histolytica* HM-1 strain antigen has been adsorbed and allowed to be naturally absorbed

(approx. 15 sec). A solution of Protein A conjugated colloidal gold particles is immediately added and allowed to be absorbed (approx. 15 sec). Then, 3 drops of washing buffer A and 3 drops of washing buffer B (included in kit) are added in that order to wash off excess reagent (30 sec). In the case of antibody positive reaction a red/pink spot appears in the center of the device at the point of washing with buffer A. It is that simple to conduct the test.

This new kit was used in testing dot-ELISA-tested serum samples from 44 hepatic and 64 intestinal amoebiasis patients and a 100% agreement rate was obtained. Sera from patients with non-amoebic protozoa infections, ulcerative colitis and normal individuals, consisting of both males and females, were used in investigating the kit and no positive results were observed in all the cases. We believe that so far the Instant (TM) CHEK-Amoeba is the only antibody detection system that has the peculiarity of a matching sensitivity to the already established dot-ELISA and besides it is very handy and the results can be decided in the shortest possible time.

28) THE STUDY OF ANTIGEN FOR THE NEW TUBERCULOSIS DIAGNOSIS AND APPLICATION TO THE SERODIAGNOSIS

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In order to identify the active tuberculosis, it is necessary to search the antigen that has the high specificity and good sensitivity for the diagnosis. In the field of developing countries, to establish the screening method for the active tuberculosis patients is very important. At first, we established the BCG cDNA library. Briefly, RNA was extracted from the *Mycobacterium bovis* (BCG Tokyo strain), and cDNA was synthesized using the random primer and the adapter with restriction enzyme was attached to both end of the fragments, and ligated into the expression vector. As a result of immuno-screening using the tuberculosis patients' sera, 15 positive clones were identified. The sequences of these clones were examined and searched their homologies by GenBank. One of their clones was identified as CONSERVED HYPOTHETICAL PROTEIN of *M. bovis* (ORF of this gene is 288 bp and the molecular weight is about 12 kDa). This region was highly preserved between *M. bovis* and *M. tuberculosis*. This result suggested that this antigen could be applied to the diagnosis of the human tuberculosis

and was named BCG1. We confirmed the expression of recombinant BCG1 as a fusion protein (40 kDa) with GST by SDS-PAGE. Therefore, the reactivity of this antigen against the patient's serum was examined by Western Blotting, and specific signal was detected by patient's serum but not by healthy subjects. These results were suggested that this antigen BCG1 was reacted with the patient of tuberculosis specifically. Furthermore, we examined the specificity of this antigen by Dot-blot analysis. The dilutions of the antigen were prepared by each 4 fold from 156 mg/ml. The reaction of patient's serum was disappeared at 4,096 fold dilution and healthy subject disappeared at 64 fold dilution. The difference of the reactivity was 64 fold between patient and healthy subject. These results were suggested that BCG1 antigen was useful for diagnosis of active tuberculosis patients in view of the peculiar sensitivity with patient's serum. We are going to examine the reactivity of BCG1 antigen against each condition of patients.

29) N-TERMINUS TRUNCATED RECOMBINANT SARS CORONAVIRUS NUCLEOCAPSID PROTEIN-BASED ELISA IS MORE SPECIFIC FOR SERODIAGNOSIS OF SARS

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Severe acute respiratory syndrome (SARS) is a recently emerged human disease associated with pneumonia. The disease has affected 30 countries in 5 continents, with more than 8400 cases and more than 910 deaths. Since the recognition of the SARS coronavirus (SARS-CoV) as the agent causing SARS in April 2003, enormous international efforts to develop laboratory tests for SARS have occurred. Despite sensitive methods for detecting virus genetic material, detection of virus in the early stages of illness may still be difficult, and the final assessment of whether an individual has been infected may depend on detection of antibody to virus. To develop a safe and reliable diagnosis method for SARS, we cloned the whole range and the N-terminal 121 amino acids truncated SARS-CoV nucleocapsid protein (N

protein and N₋₁₂₁protein) gene into plasmid and expressed them in *E. coli*. Serum samples collected from 175 healthy volunteers in Vietnam before the SARS outbreak occurred were used to for the assessment of our newly developed indirect IgG ELISA using recombinant N protein and N₋₁₂₁ protein as assay antigens. While ELISA using N protein showed relatively high nonspecific reaction which was 38/175 (21.7%) and the titer ranging 1:100-1:3200, however N₋₁₂₁ protein could drastically reduce it up to 11/175 (6.3%) and the titer ranging 1:100-1:200. To assess the sensitivity of IgG ELISA using N₋₁₂₁ protein, serially collected serum samples from 37 SARS probable (including 15 cases confirmed with RT-PCR) cases were examined. Thirty six patients (97.3%) showed IgG seroconversion and the titer was

ranging 1:600-1:204, 800. The timing of IgG seroconversion against N₁₂₁ protein after onset of illness were 22.2% in the first week, 69.4% in the second week and reached to 100% in the third week. Our data indicate that our newly developed SARS-CoV N₁₂₁ protein-based IgG

ELISA is a safe, specific and sensitive test for diagnosing SARS-CoV infection. It could serve as a simple, sensitive, and specific test for clinical diagnosis and large-scale sero-epidemiological studies.

30) *PARAGONIMUS OHIRAI* M. IN THE BASIN OF MARUYAMA RIVER, HYOGO PREFECTURE

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In Maruyama River in the north Hyogo Prefect., the epidemiological study of *Paragonimus ohira* is carried out by Yoshida (1955) and Miyamoto (1961) 50 years ago. The investigation was carried out in the holm of Kikuyasima in Maruyama River. *Seasarma dehaani* which was second intermediate host of *P.ohira* in the Kikuyasima lived in great numbers. In April 2002, the infection rate of metacercaria (MC) of *P.ohira* in the *S.dehaani* was 84.9 per cent, 68.8 per cent in May, 61.5-67.4 per cent in July, 78.0-95.6 per

cent in August, 92.0-93.3 per cent in September and 100 per cent in November. The average infection rate was high with 78.6 per cent. It tended to be same in 2003 and the average infection rate was 87.0 per cent. In this holm, *Assimineia japonica* and *A.parasitologica* were distributed. Cercariae of *P. ohira* could not be detected. In comparison with the reports 50 years ago, the distribution of the *S.dehaani* in Maruyama River had been limited and the infection rate of MC were high 50 years ago similarly in Kikuyasima.

31) A SIMULATION OF EGG OUTPUT SCHOOL CHILDREN INFECTED WITH *SCHISTOSOMA HAEMATOBIMUM* BY AN IMMIGRATION-DEATH MODEL

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Monthly *Schistosoma haematobium* egg counts in 10 ml of urine after treatment fluctuated remarkably within a subject of schoolchildren in an African community. This kind of fluctuation has been thought as a technical limitation of the urine examination. It has been assumed 1) that the number of pairs of adult worms of *S. h* increases relatively constantly after the treatment and it does not fluctuate much, 2) that the egg production per pair of worms is relatively unchanged (although crowding induces reduction of egg production per female worm), and 3) that the observed fluctuation of egg output, therefore, is caused both by uncertainty of egg excretion to urine and by volume change of urine.

Daily fluctuation of egg counts for consecutive days, however, was smaller than monthly fluctuation in this community. This suggests that the large monthly fluctuation is not due to the technical limitation. There is a possibility of larger fluctuation of number of adult worm pairs in a human

body than it has been thought. To simulate and verify this, a mathematical model was applied. We built an immigration-death model that assumed that the observed egg output in urine reflected the real number of egg production. We created two parameters in a stochastic process: Mortality (or life expectancy) of adult worms, and frequencies of infection. The number of cercariae penetrating at one time of infection was adjusted by the frequency to have the same acquisition per unit of time. Egg outputs of four patterns, combination of mortality (high or low), and frequency (constant and less constant), were calculated for 1000 days. The simulation model with high mortality of adult worms and less frequent infection matched most with the observed pattern of egg counts. The mean life expectancy is as short as six months in this model. Because this study is based on a simplified assumption, further studies are needed to verify the plausibility of this model/hypothesis.

32) CURRENT EPIDEMIC STATE OF SCHISTOSOMIASIS MEKONGI IN LAO PDR.

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Abstract

A preliminary investigation on *Schistosoma mekongi* infection in human, animal and snail host was performed at Khong district, Champasack province in Lao PDR in May 2004. The results of stool examination conducted at 7 villages that contained 2 newly surveyed ones, revealed in between 20% to 50% of high endemic prevalence. Children in 10-15 years of the age group were the most prevalent at 60% was confirmed. Adult developed stage patients were

also confirmed in the area. No egg was detected at 19 samples of fresh pig stools collected among the villages. On the snail vector collections, both alpha- and gamma- race *Neotricula aperta* snails were also detected at the shallow rocky streams of the Mekong basin.

Key words: *Schistosoma mekongi*, *Neotricula aperta*, infection, Khong, Champasak, Lao PDR.

33) WATER CONTACT PATTERNS AND KNOWLEDGE OF SCHISTOSOMIASIS IN NORTH ZANZIBAR

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Water contact patterns have been one of the foci in the schistosomiasis epidemiological studies. The villagers living in the schistosomiasis endemic area contact cercarial infested water frequently for various domestic purposes in their daily life. These contacts are, however, difficult to avoid due mainly to the unavailability of alternatives, such as safe water supply. Furthermore, the villagers especially children and young adolescent wittingly contact risky water for bathing/playing purposes. Although villagers' knowledge and perceptions of schistosomiasis is critical information for planning control program, they were ignored in many occasions. As a result, little is known about the relationships between exposure to risky water and knowledge and perceptions of this disease. In this study, we conducted researches to elucidate the interrelationships between water contact behaviors and knowledge and perceptions of schistosomiasis among villagers (N=648) living in Bandamaji,

an urinary schistosomiasis endemic area in the north Zanzibar. For water contact behaviors, direct observation was performed using time-saving spot check method with six visits/day from 6:00 till 18:00 for all water contact sites (N=41), and knowledge and perceptions on schistosomiasis were collected by interview survey with systematic questionnaire. The urine examination was also conducted for egg counts. High mean score (8.6/10.0: SD=1.8) for the knowledge of schistosomiasis etiology among villagers excluding small children (N=537) implied that the villagers understood considerably about the risk of infection in the river/pond water. The questionnaire on risky water contacts also clearly indicated that the villagers contacted risk water frequently in spite of their awareness of infection risk. As the result of logistic regression analyses, "washing utensils/tools/bicycle" ($p<0.01$) and "fishing" ($p<0.001$) were the significant factors related to infection for males and "wash-

ing clothes" ($p < 0.01$) for females, in addition to "age-group" factor. The direct observation clarified the frequency of risky water contacts, 6% (104:male=81, female=23) of 1791 water contacts (male=304, female=1487) during 14 consecutive days, and the risky water contacts were observed more among males (27%: 81/304) than females (2%: 23/1487). These results corresponded with the percentage and intensity of egg positives, and with villagers' answers to

the questions on water contacts. In conclusion, control programs which simply recommend avoidance of contacts with risky water contribute little to reducing infections, and therefore the reasons of risky water contacts should be carefully considered; for instance, a program with supply of soaps which are soluble and will lather easily even in hard water of the wells in this area.

34) DAILY VARIATION OF *SCHISTOSOMA HAEMATOBIIUM* EGG EXCRETION

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Eggs of *Schistosoma haematobium* were collected by two methods for 62 schoolboys for 3 days of a week in a urinary schistosomiasis endemic community of coastal Kenya. Spontaneous urine after 10:30 am was collected and 10 ml of the urine was filtered for collecting eggs. This method is referred as the 10 ml spontaneous urine method (eggs/10 ml). The second urine, one hour after the previous spontaneous urine was collected and all the volume of the urine was filtered for collecting eggs. This method is referred as the one-hour urine method (eggs/hour). Prevalence of egg-positive after three urine examinations was 95.2%

for egg/10 ml and 93.5% for egg/hour. Three-day mean of log converted eggs/10 ml and eggs/hour of each boys correlated very well ($R=0.866$), although the value of egg/hour was twice larger than that of egg/10 ml (mean of log (1+eggs/hour) was 2.33 with standard deviation (SD) of 1.04, and mean of log (1+eggs/10 ml) was 2.01 with SD of 0.92. paired t-test, $p < 0.001$). Mean of coefficients of variance (CV) of log converted egg/hour was 23.5% and smaller than that of egg/10ml was 32.4% ($p < 0.05$). The value of the CVs measured in a week seemed smaller than that of three repeated measures over three months.

35) EXPERIMENTAL INFECTIONS OF BLACK FLIES (DIPTERA: SIMULIIDAE) WITH MICROFILARIAE OF *ONCHOCERCA DEWITTEI JAPONICA*, THE CAUSATIVE AGENT OF ZOONOTIC ONCHOCERCIASIS IN JAPAN

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Zoonotic onchocerciasis is very rare in humans. Only 11 cases including five cases in Oita, Japan, have been reported. The causative agent in Japanese cases was all *Onchocerca dewittei japonica* Uni, Bain et Takaoka, from wild boars. To determine the vector (s) of this parasite, microfilariae of *O. dewittei japonica* were injected intrathoracically into 40 newly-emerged females of five *Simulium* spp. from Oita. The flies were maintained with sucrose solution at 25 °C. After 10 days post-infection, the third-stage larvae were found in 2 of 14 *S. aokii* (Takahasi) dissected, 4 of 6 *S.*

arakawae Matsumura, 2 of 4 *S. bidentatum* (Shiraki), 1 of 1 *S. japonicum* Matsumura and 3 of 4 *S. rufibasis* Brunetti. The infective third-stage larvae were 750-1,000 µm long by 18-20 µm wide. It has been proved for the first time that these five black-fly species are able to support the larval development of *O. dewittei japonica*. This finding may suggest a possibility that black flies play a role in the transmission of zoonotic onchocerciasis due to *O. dewittei japonica* in Japan.

36) APPLICATION OF URINE-BASED ELISA TO MONITOR THE EFFECT OF MASS-DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS CONTROL IN A LOW ENDEMIC AREA

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The 50th World Health Assembly made a resolution to eliminate lymphatic filariasis as a public health problem from the world by 2020. The basic strategy for the elimination is mass treatment of all the people in endemic areas with a drug combination of diethylcarbamazine and albendazole. In Sri Lanka, the mass drug administration (MDA) campaign was launched in 2002. For the evaluation of MDAs, many countries, including Sri Lanka, employ a traditional night blood survey to detect microfilaria (mf) carriers, which is one of the most difficult field activities. An ICT card test that detects filarial antigen with day blood in 10 minutes is very effective but not popular due to high cost. Blood sampling that people (especially young children) dislike is another barrier, which is getting higher as the number and seriousness of filarial infections are going down. Considering these problems and importance of children as a sentinel group, we have developed a new ELISA that uses urine as a sample for serum. The ELISA (urine ELISA) detects filaria-specific urinary IgG4, and its high sensitivity and specificity have been shown in various studies. As a

first trial to study the usefulness of urine ELISA as a monitoring tool of nationwide MDAs, it was applied in Wucheria bancrofti-endemic areas in Deniyaya, a mountainous tea plantation district in southern Sri Lanka. Urine samples were collected in the daytime from 699 schoolchildren aged 6 to 16 years in 2002 and 2003, before and about 1 year after the first MDA. The MDA reduced the IgG4 positive rate from 7.9% to 3.3%. Thirty-eight (69%) of 55 positive children in 2002 became negative after treatment. On the other hand, 6 of 644 negative children turned positive in 2003. The results indicate that IgG4 antibodies among children reduce relatively quickly by treatment, implying that the antibody detection is as good as antigen detection in terms of negative conversion. Urine ELISA is a very sensitive method to detect early infection. Sample collection is non-invasive, and therefore most suitable for pediatric subjects. The ELISA needs well-equipped facilities, however. It is necessary to improve the method to be more simple and less costly so that it can be applicable in filariasis endemic areas.

37) A CASE STUDY OF TUBERCULOSIS GENETIC EPIDEMIOLOGICAL STUDY BASED ON THE LONG-TERM INTERNATIONAL COLLABORATIVE FIELD RESEARCH IN THAILAND

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Background and Objective: Since we do not have an exact animal model for tuberculosis (TB), we need to study the human tuberculosis in the field. Genetic epidemiological research may contribute the breakthrough finding. We have conducted a study of the human genetic susceptibility to

Mycobacterium tuberculosis based on the long-term collaboration with Thai Ministry of Public Health and other agencies (since 1993) in Northern Thailand.

Methods: The project set up an active computerized population-based surveillance on TB and HIV/AIDS since

1987 in collaboration with the Chiang Rai provincial health office. Chiang Rai province is the northernmost remote province in addition to the database; the project started the collection of the culture isolates of *M.tb* since 1996. We had begun the study on the genetic susceptibility on tuberculosis in 2002 after a receiving the approval of the ethic committee of Thai Ministry of Public Health. Since our study is of familial-based in nature, we began collecting complete or incomplete TRIO (TB patient and their parents or substitutes) for TDT (transmission /disequilibrium test), ASP: affected sib-pair for Sib-Pair Analysis, and ARP: affected relative pair for alternative methods to detect the linkage.

Results: We identified 17,216 who were registered in 1987-2001 in the surveillance. Among them, we detected 8,586 pulmonary AFB-smear-positive TB cases and we found 144 families to that were affected by TB and thus met our enrollment criteria (ASP 25, ARP 49, Trio 64, Incomplete Trio

6). We confirmed 811 TB patients who did not meet the criteria, 73 who were in the prison, 260 who had moved to other provinces, and 3,756 who had already died. 1,725 TB cases could not be located. We began a prospective study since 2002 January and we have identified 20 ASPs, 34 ARPs, 97 Trios, and 67 In-complete Trio by the end of 2003. We identified one family with 10 TB patients (among them 5 were sibling). We had made the continuous dialogues with Thai counterparts and made the Material Transfer Agreement in August 2003.

Discussion: We were able to set up the field research site, which is possible to conduct the sensitive researches like human genetics based on the mutual trusts. Thai counterpart emphasized the equal partnership based on the long-term collaboration and in particular requested training and education for young Thai scientists. In addition, issue of the intellectual property need to be clear.

38) STUDY ON TRANSMISSION AND INFECTION OF SOIL-TRANSMITTED NEMATODE IN THE SUBURB OF HANOI, VIETNAM: CONTAMINATION OF VEGETABLES AND FINGERS AND NAIL DIRTS WITH PARASITE EGGS.

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To establish more efficient and effective control strategy against soil-transmitted helminth (STH) infections, mode of transmission of STH infections in endemic area should be elucidated. We have been carrying out the project to know how or to what extent the environment is contaminated with STH eggs in a village at the suburb of Hanoi, Vietnam. We report here parasitic contamination level of vegetables, fingers and nail dirts in the study site. The vegetables (nine leafy type, four root type and two fruit type) were examined. Most of them are eaten raw by villagers. Samples of finger-washing fluid and nails were obtained from school children and inhabitants in 33 households. Parasitic eggs were separated from these samples by soaking in 0.5% Tween 20 and recovered by floatation method using saturated NaNO₃. Examinations of these samples were done once in both rainy and dry seasons. Six and seven species of parasite eggs were recovered from sample of vegetables and fingers/nails, respectively. The recovery rate of the eggs from these samples was not different by season. Out of a to-

tal number of 317 vegetables examined throughout the year, 25.9% was positive for any of the eggs. Leafy type of vegetables was most highly contaminated (30.5%, 73/239). Out of the six species recovered, *Ascaris* and *Trichuris* eggs were detected in 20.5% and 7.9% of the samples, respectively. Mean number of eggs recovered from egg-positive vegetables was 2.7-7.0 for *Ascaris*, and 2.0-2.2 for *Trichuris* in rainy and dry seasons, respectively. For examination of fingers and nail dirts, the total number of 508 people, 506 finger-washing and 326 nail dirts samples was examined throughout the year. Out of them, 8.5% of people, 7.5% of finger washing and 3.1% of nail dirts were positive for any of the eggs. *Enterobius vermicularis* eggs were most highly recovered (3.5%), followed by *Ascaris* (1.0%) and *Trichuris* (0.6%). The number of *E. vermicularis* eggs recovered from egg-positive samples was 1-37. Those of *Ascaris* and *Trichuris* eggs were only 1 in all positive samples. In conclusion, leafy type of vegetables is considered to be one of important sources of STH infections in our study

area. Fingers and nail also are one of possible sources but the possibility might be lower.

39) PROBLEMS IN DRACUNCULIASIS ERADICATION ACTIVITIES IN OGOU DISTRICT, TOGO

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Togo is one of the 14 remaining dracunculiasis-endemic countries of the world. In 2000, 75,223 cases of dracunculiasis were reported from these 14 African countries, and Togo reported 811 indigenous cases from 147 villages, the six largest numbers after Sudan, Nigeria, Ghana, Burkina Faso, and Niger. About a half of the cases (386) were reported from Ogou district. In the present study we obtained monthly reports on dracunculiasis eradication activities in Ogou district for 22 months in 2002 and 2003. During this period, 51867 households were visited, and 380 dracunculiasis cases were found, of which 327 (86%) were contained cases. Contained cases mean 1) the infected per-

son was detected within 24 hours of the emergence of guinea worm through the skin, 2) actions (i.e. occlusive bandages, counselling, and care of the patient until the worm is pulled out) were taken to prevent the person from contaminating sources of drinking water, and 3) these two conditions were confirmed by a supervisor within 7 days of occurrence. The eradication programme distributed more than 30,000 clothes for filtering drinking water. Although the number of dracunculiasis cases is decreasing, integration of this vertical eradication programme with the primary health care system of communities is necessary for successful eradication of the disease.

40) A SEROSURVEY OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) IN THE DOMINICAN REPUBLIC

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HTLV-I is known as a causative virus of ATL, HAM/TSP, HAB, HAAP and Uveritis and distributed in the world wide. The people infected with HTLV-I are also widely distributed in the Caribbean region. However, the investigation concerning the HTLV-I in the Dominican Republic (R.D.), located in the Caribbean region, has not done up to the present time and the situation of the HTLV-I infection remains uncertain. Then, we investigated to grasp the situation of infection by using the serum from a total of 3,075 people, 1,069 from men and 2,006 from women, at the 6 cities and the capital in the R.D. during the period from 2001 to 2002. The measurement of the HTLV-I antibody was carried out

by the indirect fluorescent antibody method using the MT-2 cell an antigen. An HTLV-I antibody titer of 40 or over was considered positive. A total of 55 (1.82%), 41 women (2.09%) and 14 men (1.33%), revealed antibody positive to the HTLV-I. The antibody positive rates among the cities were 0.78%-2.88%. A low tendency was seen in the southern part of cities compared with northern part of cities. Although the antibody positive rate of the women appeared higher than men, but a no significant difference was seen. A big difference (0%-5.97%) was observed among the different age groups.

41) DESCRIPTIVE EPIDEMIOLOGY OF IMPORTED INFECTIOUS DISEASE CASES FROM THE NATIONAL SURVEILLANCE IN JAPAN

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Some diseases listed on the national surveillance in Japan have high reporting rates of imported cases. For example, among six diseases, cholera, bacterial dysentery, typhus, paratyphus, dengue fever, and malaria, 62 to 100 percents of cases were imported from the year 2000 through 2003. Many have no effective vaccine available and personal protection through behaviour change is the only preventive measure. Consequently, the information on health issues in destination as well as disease incidence among Japanese became more important for travel planning.

The data of imported cases was analysed in order to illustrate epidemiological characteristics of the diseases. In total, 2786 cases were reported as acquired abroad. Peaks of cases by diagnosed date were observed during holiday seasons and the period shortly after, March to May and August to September in most of the six diseases. Besides this seasonality, some clusters attributing outbreaks and epidemic in destination country observed.

All other diseases except Malaria, major travel destinations prior to the onset were Thailand, Indonesia, India, Vietnam and China which counted as endemic area of tropical diseases. Malaria, on the other hand, most of the cases reported visits to countries in African continent. Young adult was the most affected age group (20 to 39 years), although

the outbound travellers were evenly distributed by approximately 20% among four age groups of adults. Speculating from the months of visits and age distribution of reports, persons suffered from bacterial dysentery and cholera seemed to be travelled for holiday. However, those suffered from dengue fever could speculate as composed from people travelling for holiday and for business in same way.

This descriptive epidemiology of cases reported through mandatory reporting system well supports the results from other studies. Those from India and Nepal reported about ill-prepared Japanese travellers, unvaccinated, no preparation for repellent, and without knowledge of endemic infectious diseases in advance. Especially among young adults, preference of adventurous and more interactive travel style resulting in increased opportunity of exposure to the pathogenic agents. At the same time, travel as daily life attitude of senior traveller also leading to the chance of infection.

Despite several recent health threats in international society, such as severe acute respiratory syndrome and H5N1 avian influenza, the number of Japanese visiting abroad demonstrates upward trend. Therefore, we have obligation to provide, accurate and ready to use information from surveillance to travellers in need in timely manner.

42) CASE SERIES OF IMPORTED MALARIA IN OKINAWA, JAPAN

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The aim of this study was to clarify the characteristics of cases of imported malaria. We reviewed the charts of all 27 patients (18 males and 9 females) who discharged with a diagnosis of imported malaria from the University Hospital Ryukyu and the related hospitals in Okinawa, between 1989 and 2004. Malaria was diagnosed by identification of malaria parasites using Giemsa staining method of thin and thick blood smears, and also using new method of antigen detection kit and of PCR for these 5 years. The mean age of

patients was 31 years old (range: 5 to 64), and 16 patients of them were Japanese who traveled foreign countries. All patients were given diagnosis of malaria within 24 hours after their access to hospitals. The majority of malaria species was *Plasmodium vivax* (13 patients), followed by *Plasmodium falciparum* (12) and *Plasmodium malariae* (1). Mixed infection, identified using PCR method in addition to Giemsa staining, was found in two patients with *P. falciparum* and *P. ovale*, and with *P. vivax* and *P. malariae*. Appropriate

treatment with antimalarial drugs was started immediately after making accurate diagnosis. There were no patients with adverse effects of the drugs. Recurrence of falciparum malaria occurred in two patients, and relapse of vivax malaria was suspected in one patients. Chemoprophylaxis failure was found in 10 patients because they did not take drugs according to recommended prescription. Twelve patients

experienced malaria infections in several times, but no one prepared for prevention of re-infection in correct way. Microscopy, rapid antigen test, or PCR diagnosis where possible, should be carried out in order to confirm malaria infection because these rapid diagnostic tests for malaria may be useful. Education on correct prevention and chemoprophylaxis for malaria should be much given to travelers.

43) CHARACTERISTICS OF IMPORTED MALARIA IN JAPAN

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Whilst exchange of human resources between Japan and developing countries increases, imported malaria is getting more important for public health in Japan. We analyzed 153 of new malaria cases presented to our hospital from 1992 to 2001 for further understanding of current situation of malaria in Japan. The total number of malaria cases in Japan has been constantly over 100, and our cases has accounted for 14.9% of all cases in Japan. The predominant malaria species has been *Plasmodium falciparum* through the decade (51.9%). The most frequent contraction area was Africa (54.2%), followed by Asia (20.9%) and Oceania (19.6%). For the treatment of falciparum malaria, mefloquine was the most commonly used antimalarial drug (82.5%), and atovaquone/proguanil has been in use since 1999. The analysis of 101 Japanese cases revealed clinical

issues that had to be kept in mind to manage malaria patients in Japan. Firstly, high relapse rate of vivax malaria after completion of primaquine treatment was observed in cases imported from Oceania. Secondly, although the number of chemoprophylaxis failure due to inadequate regimen decreased, substantial number of travelers without chemoprophylaxis still suffered from malaria. Finally, median duration from onset of illness to first visit to clinics was 3.0 days, which was unacceptably long for better prognosis. These results indicate that development of travel medicine to provide current information to health care providers, and education campaign to enterprises, travel companies and individual travelers, are warranted to reduce morbidity and mortality due to imported malaria

44) MALARIA EPIDEMIOLOGY IN LOMBOK AND SUMBAWA ISLANDS, INDONESIA

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Introduction; This work was done as the cooperative project between Indonesia and Japan under the JICA partnership program.

Purpose; Malaria epidemiological conditions are clarified to decide the most suitable control measures at the project areas.

Project areas; The subdistrict Batulayar under Meninting health center in Lombok island and the subdistrict Utan under Utan Rhee health center in Sumbawa island were chosen.

Methods; Spleen examination was applied to school-children in the first and second grade of all the elementary schools. Regular blood examinations and entomological surveys were done at subvillages selected based on the above results. Case detection and treatment was practiced by newly organized teams in health centers.

Results and discussion; In the subdistrict Batulayar two different types of malaria endemics were found, one was at hilly forested areas and another was at coastal areas. The

former was found to be more prevalent and to be maintained by a vector mosquito species, *Anopheles balabacensis* which breeds in stagnant water along rivers through valleys and small water pools formed around streams or springs in the forest. The transmission season was obscure, because seasonal difference in transmission seemed to be varied owing to small environmental differences between subvillages. The latter was maintained by a vector mosquito species, *An. sundaicus* which breeds in brackish water mainly of lagoons formed at exits of rivers to the sea in the dry season and was relatively suppressed by long control activities executed by the local governments. In the subdistrict Utan malaria endemics were found only along the coast. The transmission vector was determined as *An. subpictus* that breeds in brackish water mainly of fish ponds developed in the mangrove forest after cutting trees. No malaria endemic was found on hilly areas. These results indicated that efforts of malaria control should be focused on forest malaria endemic.

45) MALARIA CONTROL IN LOMBOK AND SUMBAWA ISLANDS, INDONESIA

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Introduction; This work was done as the cooperative project between Indonesia and Japan under JICA partnership program.

Purpose; Malaria control activities are carried out based on epidemiological data. Subsequently the most suitable ma-

laria control methods are proposed.

Project areas; The subdistrict Batulayar under Meninting health center in Lombok island and the subdistrict Utan under Utan Rhee health center were chosen.

Methods; 1) Reduction of human sources, The case detec-

tion and treatment teams visit subvillages according to their schedule which are informed to villagers through influential persons in advance, find out malaria patients from clinically suspected patients using ICT malaria diagnostic kits and treat them. 2) Distribution of insecticide treated bed-nets and insecticide indoor residual spray are executed to cut the transmission of malaria parasites between vectors and humans. 3) Trials for larval control are done. 4) Appearance of drug-resistant *Plasmodium falciparum* is examined.

Results and Discussion; 1) The activity of case detection and treatment was shown to be universally effective for malaria control, specially under the meso-endemic condition. 2) Anti-adult-mosquito methods were effective against

coastal malaria but not forest malaria. Besides, these methods were costly and laborious. Bed-nets were more acceptable to villagers than the indoor spray. 3) Larval control methods are limited in practical use. 4) Chloroquine resistant parasites were very prevalent in both project areas. SP-resistant parasites also seemed to be common considering the follow-up study in Sumbawa. From these results we proposed that the most effective and economical control method is the continuous activity of case detection and treatment. The activity is also useful to educate villagers and to obtain trust from villagers. Bed-nets should be used as a supplemental measure based on entomological studies and budget size.

46) MAGNITUDE OF MALARIA PROBLEM IN CAMBODIA

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Malaria continues to be a major public health problem in Cambodia. It is the main obstacles to the country's development. About 1.6 million people out of 12.3 million total population are at risk of malaria, who are living in villages located within or with the distance of 1 km from the forest. These people can therefore be classified into the following 4 categories; (I) people reside in forested villages, (II) people reside in the villages at 200 m distance from forest, (III) people reside in the villages between 200 to 500 m distances from the forest and (IV) people reside in the villages between 500 to 1,000 m distances from the forest. About half a million people live in the high transmission forest areas defined as the category (I) with intense malaria transmission; malaria is most severe in isolated villages surrounded by forests. Prevalence rates range from about 15% to 40% in villages near or in forested areas to 0-3% in the plains and rice field areas. Factors that contribute to malaria risk include predominance of falciparum malaria, poor health infrastructure especially in the high transmission areas, continuing shortages of appropriate diagnostics and drugs, poor communication and transportation systems, re-

sulting in treatment delays, presence of specific high risk groups including military personnel, forest and mining workers, in forest villages, children under the age of five years are the primary risk group, predominance of unqualified private medical practitioners and pharmacists who still do not follow national treatment guidelines, abundance of fake drugs on the market and spreading drug resistance in parasites in different pockets of the country. To reduce malaria disease burdens in the high transmission areas, early diagnosis and treatment approach should be improved in public health sector systems, and also private sector through social marketing strategy, improve coverage of preventive measure using impregnated bed nets and social marketing of hammock net with insecticide K-O TAB, increase awareness and care-taking practices on malaria prevention and proper health seeking behavior, improve the behavior change communication in promoting malaria safer behavior in target population and strengthen the institutional capacity of the national malaria control program at central, provincial, operational district and commune levels.

**47) ACCUMULATED INFECTION RISKS OF MALARIA DURING FIVE WEEKS
IN A LIVING AREA OF AN ETHNIC MINORITY IN VIETNAM.**

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Eighty-three of ethnic minority and twenty-five Kinh school children were diagnosed on malaria by microscopy. The children with slide positive were treated with artesunate and observed for the following five weeks. Thirty children out of 83 were slide positive at the first examination. Fifty-nine children were slide positive at any point during the period. The remaining 24 out of 83 ethnic minority and 25 Kinh children were slide negative throughout the period.

Cumulative positive rate was compared between the two ethnic minority children with slide positive and negative at the first examination. The former was not positive one week after treatment but small number of them began to turn positive again from the second week after treatment.

Finally, 50% of them were positive during the period. The latter became positive constantly from week 1 and the increment curve of cumulative positive rate from week 1 to 4 was parallel to that of the former group from week 2 to 4.

Body temperature was not significantly different between Kinh school children and minority school children and even between minority school children with positive and negative slide.

The results suggested that the minority school children should be asymptomatic carriers and the effect of treatment to such people in an endemic area should not be maintained for a long time.

**48) A REAL-TIME PCR ASSAY FOR QUANTITATIVE DETECTION OF *PFCRT* IN
PLASMODIUM FALCIPARUM AND ITS APPLICATION TO EPIDEMIOLOGY**

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It has been reported that mutations in the transporter gene *pfert* of *Plasmodium falciparum* had strong correlation to its chloroquine (CQ) resistant phenotype. Individual malaria patients often harbor a mixture of genetically distinct parasites, i.e. parasite population with wildtype allele of *pfert* (susceptible to CQ) and that with the mutant allele (resistant to CQ). Treatment of the patients with CQ probably selects parasite population, which has the mutant allele. However, currently used typing methods for *pfert* are unable to quantify the proportions of the wildtype and mutant alleles in one patient. In this study, we adapted a real-time PCR with Taqman (R) probes (TaqMan (R) PCR) for differentiation of the *pfert* alleles in *P. falciparum*-infected blood samples.

The mutation in *pfert*, which has the most significant association with CQ resistance, is substitution of threonine

for lysine at codon 76 (K-76T). The TaqMan (R) PCR was thus used *pfert*-specific primers and the probes each specific to wildtype (K76) and mutant (T76) alleles. Plasmid DNAs with sequences from 76K and 76T alleles were prepared as standards. In vitro cultures of two laboratory strains, FCR-3 (K76 allele) and K-1 (T76 allele), were mixed in various proportions. DNA samples were extracted from them, and subjected to the TaqMan (R) PCR. Proportions of K76 and T76 alleles estimated by the PCR were consistent with those expected.

Thirteen blood samples collected from malaria patients in Palawan island of Philippines were subjected to the TaqMan (R) PCR. K76 alleles were detected in 11 samples, of which 6 had T76 alleles simultaneously. There was no sample, which had T76 allele alone. Two samples contained only small amount of DNA and any alleles were detected.

Ratio of K76 and T76 alleles varied from 8.7:91.3 to 44.5:55.5. This result may reflect moderate prevalence of CQ resistant malaria in the island.

Although decisions for antimalarial drug policy will

never be entirely based on molecular survey alone, such data can offer important information. TaqMan(R) PCR may be useful method for estimating the prevalence of CQ resistant *P. falciparum* in malaria-endemic region.

49) AN INVESTIGATION OF THE RELATIONSHIP BETWEEN RELEASE FROM CHLOROQUINE RESISTANCE AND BLOOD GSH LEVELS

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It is a serious problem for the role-back malaria protocol that chloroquine-resistant malaria spreads to all over the world. And also it is expected to conquer the chloroquine-resistance in malaria therapy. To this aim, there is one important strategy, release from the chloroquine-resistance by drugs, in order to make malaria protozoan sensitive to chloroquine. It is said that the resistance of malaria to chloroquine is related to ABC (ATP binding cassette) transporters. We have revealed that it could release this resistance to inhibit ABC transporter by Dibenzo-suberyl piperazine analogs. Dibenzo-suberyl piperazine analogs have little circula-

tory effect and also little central nervous effect. Dibenzo-suberyl piperazine analogs would have specific inhibition effect on the ABC transporters, which are related to the chloroquine-resistance of malaria. To assign those ABC transporters, we investigated about the influence to GSH levels in blood by Dibenzo-suberyl piperazine analog (D67). It is said that GSH levels in resistant malaria are higher than sensitive ones, and it is suggested that SG-X pump, one of ABC transporters, whose substrates are glutathione-conjugates, would be related with the mechanism of the chloroquine resistance.

50) PROTECTION EFFECT ON LYSIS OF MALARIA INFECTED ERYTHROCYTES MEMBRANE BY BISBENZYLISOQUINOLINE ALKALOIDS

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We are investigating the reversal effect by bisbenzylisoquinoline alkaloids on chloroquine resistant *Plasmodium falciparum* so far. The alkaloids have membrane stabilizing activity and also inhibit P-glycoprotein on multi drug resistant cancer cells. We are considering that the mode of action of reversal by alkaloids is different from P-glycoprotein inhibition. The reasons are as follows: 1 Chemosensitizer such as verapamil, which inhibits P-glycoprotein at the or-

der of micro M, but the alkaloids inhibit at the order of nM. 2 The alkaloid reverse even chloroquine sensitive strain whilst verapamil does not. Here, we investigated the membrane stabilizing effect due to the alkaloids using haemolysis technique with sorbitol (osmotic) and KSCN (non-osmotic). We examined the strain TM6 at late trophozoite stage with 5% sorbitol and KSCN by photometer at 540 nm observing haemoglobin release. Either with sorbitol and

KSCN, haemolysis was inhibited by 200 nM and 50 micro M of the alkaloids. As a consequence of these results are suggesting that membrane stability effect of the alkaloid may

play partial role of reversal effect of chloroquine resistant malaria parasite. Further investigation will be required to elucidate this phenomenon.

51) REACTIVITY OF BLOOD SAMPLES SPOTTED ONTO FILTER PAPERS IN THE WST-8 METHOD FOR SCREENING OF G6PD DEFICIENCY

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Deficiency of glucose-6-phosphate dehydrogenase (G6PD), the most common enzymopathy, causes acute haemolytic anaemia usually triggered by oxidative drugs or foods. It is therefore very important to assay G6PD activity, particularly in malaria-endemic areas where primaquine, a typical oxidative drug, is widely used for treatment. Recently, a simple and rapid screening method for G6PD deficiency which utilises a water-soluble tetrazolium salt, WST-8, was established. The WST-8 method has been demonstrated to be suitable for field conditions, particularly for on-site malaria surveys. Although the original WST-8 method uses whole blood as a sample, it would be worth using blood samples spotted onto filter paper, because processing many samples at the same time in the laboratory will enable to make more precise evaluation of individual G6PD activity than on-site conditions. We tested the reactivity of blood

samples spotted onto four types of filter paper, 31ETCHR (31ET), 3MM Chr (3MM), and P81 filters from Whatman, and Advantec No.2 (AD2) filter. The reactivity with fresh samples was highest in 31ET, followed by AD2 and 3MM at similar levels, and lowest in P81. In order to examine the stability of the blood spotted onto each filter, the blood-spotted filters were kept at 4 degrees centigrade or room temperature (25-28 degrees centigrade) for 1-56 days, and examined for their reactivity by the WST-8 method. Within 14 days after blood-spotting, all four types of spotted-blood samples stored at room temperature showed significant reduction (31-45% of the fresh sample) in reactivity, while samples kept at 4 degrees centigrade retained their reactivity (70-92% of the fresh sample). Our results indicate that Whatman 31ET-CHR filter is the most suitable for the WST-8 method with using spotted-blood samples.

52) STRUCTURAL-EFFECT RELATIONSHIPS OF MOTHER MOIETIES IN DRUG-RESISTANCE RELEASEING COMPOUNDS ON THE CHLOROQUINE RESISTANT MOUSE MALARIA

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The spread of malaria and resistance to antimalarial are becoming a serious problem. Since the study on the drugs reducing the drug-resistance of malaria would be useful to overcoming the problem of the multidrug-resistant (MDR) *Plasmodium (P.) falciparum*, we designed and synthesized new compounds that have both reduction activity of chloroquine (CQ)-resistance and antimalarial activity.

The compounds were composed of piperazine (P), aromatic moiety of dibenzosuberanyl, diphenylacetyl and diphenylmethyl (A) and aliphatic moiety (B). A-P structure reduced CQ resistance, but its action was weaker than the compounds of A-P-B, A-P-A and P-B structure had no reduction effects. In conclusion, the structure having A-P-B is necessary to both reduction effects.

53) INTERLEUKIN 4 POLYMORPHISM AND MALARIA ENDEMICITY IN MELANESIA.

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Previous studies in sub-Saharan Africa have suggested the protective role of the Th-2 cytokine IL-4 in malarial disease and associations between the IL4-590T mutation and the elevated levels of *Plasmodium falciparum*-specific IgE. The frequency of the IL4-590T allele varies among geographical populations; eg. 0.14 in Japanese and 0.71 in the UK. We conducted a population-based study to investigate the distribution of the IL4-590T allele and its relation to malaria infection and disease in East Sepik of Papua New Guinea (hyper-endemic, n=80) and on three islands in the Vanuatu archipelago: Malakula (meso-endemic, n=272), Aneityum (hypo-endemic, n=476) and Futuna (non-endemic, n=138). DNA was extracted from blood spotted

on filter paper collected during mass blood surveys and 200-bp fragment of the 5'-flanking region from positions-1111 to-1310 of the IL4 gene was amplified using PCR. Pyrosequencing using specific designed oligonucleotide sequences was used for genotyping IL4-590 position. IL4-590T allele frequencies were 0.39, 0.40, 0.48 and 0.42 in East Sepik, Malakula, Aneityum and Futuna respectively. The observed allele frequencies of the IL4-590T are rather consistent among the surveyed Melanesian populations but different from those reported in far Eastern Asian and European populations. We could not see any association between IL4-590T allele frequency and malaria endemicity.

54) MALARIA PRESSURE INFLUENCED THE FREQUENCY OF A SUSCEPTIBLE ALLELE OF TNF-ALPHA PROMOTER REGION IN VANUATU

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Tumor necrosis factor- alpha (TNF- alpha) is one of the key cytokines that influence the pathology of microbial infections. The genetic susceptibility to severe form of falciparum malaria is associated with TNF- alpha promoter gene polymorphism (TNFP alleles). In our previous study, we identified a TNFP-allele characterized by a T to C transition at position -857 (TNFP-D allele) as a strong susceptibility marker for cerebral malaria in Myanmar. To investigate whether malaria selection pressure on this susceptibility marker has influenced its prevalence, the frequency of the TNFP alleles was estimated in six islands in Vanuatu, Mel-

nesia within the South Pacific. Within the Vanuatu archipelago there is an overall decreasing North to South cline of malaria endemicity. Of the four alleles of the TNFP gene detected, the susceptible TNFP-D allele frequency decrease corresponded to the malaria endemicity increase; TNFP-D varied from 0.55 in the lowest malarial endemicity population to 0.26 in the highest malarial endemicity population ($r = -0.855$, $p=0.03$). This is the first report indicating that malaria selection pressure has selected an allele related to immunity.

55) RESEARCH OF THE ANTIMALARIAL DRUGS FROM THE NATURAL PRODUCTS IN JPMW PROJECT

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The JPMW project was established in October 1999 through a public/private partnership (PPP) among the Japanese Pharmaceutical Companies, Ministry of Health, Labour and Welfare, Japan (MHLW), and WHO/TDR, in order to discover new type of antimalarial drugs in Japan by the Japanese efforts. Our center (KSC) is carrying out the evaluation of antimalarial activity for the compounds from the chemical libraries of 14 Japanese companies and the natural products of our institute, both *in vitro* and *in vivo*, using the multi-drug resistant strain of *Plasmodium falciparum* and the rodent malaria model, respectively. In this presentation, we report the antimalarial compounds from the natural products in this project. By 15 October 2004, KSC has assayed 16,446 natural products etc as *in vitro* screening. As a result, we selected the candidate materials as 23 natural products that had selective and potent antimalarial activities, which must be examined further isolation and purification of active compounds from those materials. In the course of the research from the microbial metabolites, we found that the five known antibiotics (X-206, K-41, polyketomycin, borrelidin and leucinoastatin A) produced by

actinomycete strains K99-0413, KP-4050, K99-5147, OM-0060 and a fungal strain FKI-0266, respectively, had potent and selective antimalarial activities *in vitro*. Further, we found that takaokamycin (identified with hormaomycin) etc, own compounds in the antibiotic library of our institute, had potent activity *in vitro*. In the course of the research from the plant resources, we found that two known biflavonoids as sikokianins B and C were isolated from the root of *Wikstroemia indica*, had moderate antimalarial activities *in vitro*. Furthermore, the *in vivo* antimalarial activities of X-206, K-41 and borrelidin exhibited more potent activities than those of the existed antimalarial drugs as artemether, artesunate and chloroquine. Especially, K-41 and borrelidin has the possibility of the lead compounds as the new type of the antimalarial drugs, further detail studies of K-41 and borrelidin are in progress. There are the first reports to show antimalarial activities of the above compounds. Now, we are ongoing to measure the antimalarial activities of other natural compounds and synthetic compounds derived from the natural products, and also ongoing to isolate active substances from the other natural products.

56) ANTIMALARIAL DRUG DEVELOPMENT RESEARCH AGAINST DUG-RESISTANT *P. FALCIPARUM*

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Emerging and re-emerging infectious diseases have recently been considered to be worldwide problems, and malarial infection, which is a re-emerging infectious disease, has been attracted attention. Three hundred million or more people are infected with malaria and 1.5-2.7 million people die every year, and malarial infection is a major cause of death in developing countries (WHO report). Malaria para-

site (*Plasmodium falciparum*, which contributed most of the death) has been acquiring resistance to various antimalarial drugs. For malaria control, the development of a novel drug, that overcomes the drug-resistance malaria parasite, is most important. We are screening about 5,500 samples containing natural products, organic compounds, microorganism-derived products, combinatorial compounds

and marine products that are supposed to have antimalarial activity. As the results, several compounds with high selective antimalarial activity were obtained using the in vitro

and in vivo assay against *P. falciparum in vitro* and *P. berghei in vivo*. In this conference, the current status of the development of novel antimalarial drugs is introduced.

57) THE PROBLEMS OF MEDICAL FACILITIES ABROAD FOR JAPANESE EXPATRIATES

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Introduction

Our objective for carrying out this survey was to shed some light on the standard of consultation at the local medical facilities and the problems Japanese expatriates and their families had experienced related to the consultation.

Method and Materials

This survey was carried out between November 2002 and October 2003 and 393 subjects took part. (One family was treated as one person). The subjects were Japanese expatriates and their families, who had received a health check-up at the Japanese Overseas Health Administration Center after returning to Japan from abroad. This included both permanent and temporary returnees. We conducted interviews with the subjects for this study, which dealt with the following issues:

- 1) whether they had received a consultation at a local treatment facility
- 2) if they had experienced any problems during the consultation
 - medical service (language difficulties, communication problems, the consultation system itself, the payment system of medical costs)
 - quality of medical treatment (the standard of the staff at the medical facilities, the hygiene of the medical facilities and equipment)
 - medical costs
 - drug

Results

The average age of the subjects who took part in our survey was 42.9 years of age. Their average length of stay in a foreign country was 35 months. Out of the 393 expatriates, 125 had lived in developed countries and 268 had lived in developing countries. (The developed countries included North America, Western Europe, Australia, New Zealand). In total, 306 subjects (77.9%) had received a medical consultation in the foreign country in which they had lived. Of these, 99 subjects had lived in developed countries and 207 in developing countries. Of the 306 subjects, who had received consultations abroad, 189 of them (61.8%) complained about problems associated with the consultation(s). 67.7% of the complaints came from subjects who had lived in developed countries, 58.9% from those who had lived in developing countries. The majority of complaints in developed countries were related to the medical service and medical costs ranked second. Whereas most of the grievances in developing countries were connected with the quality of the medical treatment and this was followed by the medical service.

Discussion

The number of expatriates who received consultations in local medical facilities was relatively large and based on the results of this survey it appears that many of them experienced problems or difficulties related to the consultation.

58) “RESEARCH GROUP ON CHEMOTHERAPY OF TROPICAL DISEASES (ABBR.)” DURING THE PAST AND THE COMING THREE YEARS.

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The Research Group on Chemotherapy of Tropical Diseases (abbr.) was launched in 1980 as one of research projects of the then Ministry of Health and Welfare, and since then, it has been contributing greatly in the treatment of tropical and parasitic diseases using medicines that are not licensed in our country. During the fiscal years Heisei 13-15, the Research Group was funded by a research grant for “Researches on Health Sciences Focusing on Drug Innovation” from the Japan Health Sciences Foundation, and contributed to licensing mefloquine as a therapeutic and prophylactic antimalarial, and ivermectin as an anti-strongyloidiasis medicine. Since the fiscal year Heisei 16, again funded by the Japan Health Sciences Foundation, we are expected to conduct activities, reconsidering the researchers and the medicine stockpiling institutions.

During the past three years, we created our web site aiming at facilitating access to those medicines. Furthermore, we created a listserv aiming at efficient use of the medicines, and promoting the consultation activities about diagnosis and treatment of tropical and parasitic diseases. We exchanged information with specialized European hospitals and succeeded to introduce three kinds of anti-African trypanosomiasis (sleeping sickness) medicines through contacts with the European surveillance network

for travelers’ diseases, TropNetEurop. We treated patients with scabies, malaria, amebiasis and strongyloidiasis, and used ivermectin, diloxanide furoate, chloroquine, primaquine, in descending order. Injectable metronidazole was used in severe amebiasis cases that could not intake oral medicines, and showed excellent efficacy. We are always trying to analyze our data on the medicine use for feedback to the improvement of therapy. According to our data, atovaquone/proguanil combination showed almost equal efficacy as compared with mefloquine in the treatment of falciparum malaria, and it showed a better safety profile due to the absence of neuropsychiatric adverse effects (AEs) that were occasionally seen in mefloquine. When patients with scabies were treated with ivermectin, the medicine showed excellent efficacy, and the occasionally observed AEs were tolerated.

In the fiscal year Heisei 16, under the renewed Research Group, we introduced nitazoxanide for cryptosporidiosis, and will start a therapeutic study targeting this protozoal disease in immunosuppressed individuals, *e.g.*, HIV infected ones. We will also promote consultation activities on our listserv with the “telemedicine” approaches, especially by attaching images such as microscopic photographs of malaria parasites and various CT/MRI images.

59) CERTIFICATION IN TROPICAL MEDICINE IN GERMANY

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There are two certifications related to tropical medicine in Germany: (1) certification of tropical medicine approved by the German Medical Association; (2) certification of travel medicine approved by DTG (German Society of Tropical Medicine and International Health). Physicians, who have previously obtained a board certification in specialties such as internal medicine, pediatrics, surgery, and gynecology, are eligible to apply for the postgraduate resi-

dency training that are approved by the German Medical Association. The certification requires at least one year of residency in Germany, over one year of clinical experience with patient care in tropical countries, and taking a course to obtain the Diploma of Tropical Medicine (DTM) degree or its equivalent in Europe. During the given period of residency, applicants must diagnose over 100 cases of tropical diseases microscopically by themselves, and experience

over 300 travel health consultations including vaccination and malaria prophylaxis. Tropical diseases designated for the residency program consist of 16 major ones. Applications are examined by the accreditation council of the German Medical Association. Certification of travel medicine requires a qualification of physician. This training program consists of lectures of 32 hours for a 4 day period, and a multiple choice examination. Lectures cover topics such as the basic knowledge of travel medicine, infectious diseases that are essential for travel medicine practice, vaccinology, malaria prophylaxis, aviation medicine, and management issues of diving. After successful completion of the course, certification is awarded by DTG. Recertification is needed every three years by participating in a training session for

one day. At present, approximately 500 physicians have obtained the certification of tropical medicine, and are permitted to advertise their designation. The lists of physicians certified as a travel health practitioner by DTG are distributed widely among travelers, travel agencies, and pharmacies. As the acquisition of those certifications could lead to an increasing number of patients/clients, many physicians are willing to participate in the training programs. In Germany, specialists of tropical medicine and travel medicine work in closer cooperation with travel related industries such as travel agencies and airline companies. This networking has a significant influence on the surveillance, prevention, and treatment of diseases.

**60) A PRELIMINARY STUDY ON THE IMAGE OF TROPICAL MEDICINE AND
THE INCLINATION FOR MEDICINE AND MEDICAL SCIENCE
BY THE SIXTH YEAR STUDENTS OF SCHOOL OF MEDICINE, CHIBA UNIVERSITY**

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In this research, the image on tropical medicine by medical students and their inclination for medicine and medical science were investigated. 108 sixth year students (male: 61, female: 47) of School of Medicine, Chiba University, were encouraged to participate in the study, of which 70 students (male: 41, female: 29) replied to the questionnaire in June 2004. Besides their attributes (age, sex, etc.), their interest in and image on tropical medicine (ex. narrow-wide), and their inclination for medicine and medical science (ex. domestic-international) were evaluated with a scale of 1 to 5. Adding to basic statistical analysis on each factor, the association between interest in tropical medicine and 18 factors (12 for image, 6 for inclination) was analyzed with a multiple regression model.

The average of interest in tropical medicine was 2.88, indicating that medical students are relatively less interested in it. The general image on tropical medicine was relatively wide, warm, relatively soft, relatively noisy, relatively bright, relatively prospective, international, developing nations, scarce budget, relatively clinical, relatively new tech-

nology and relatively specialist. The inclination of students was clinical, relatively domestic, relatively specialist, relatively acute-disease-treatment and relatively developed nations. No statistical difference by sex was observed in almost all factors.

Multiple regression model indicated the inclination for developing nations, the image of generalist, bright and hard on tropical medicine as the factors to raise up the interest with statistical significance. The inclination for generalist and the image of warm, basic science, plentiful budget, calm and new technology was also implied as the factors without statistical significance.

This preliminary study suggests that relatively lower interest on tropical medicine by medical students is backed by their own inclination for developed nations, domestic and clinical medicine as specialist. It is impressive that the image of tropical medicine as basic science of new technology backed by plentiful budget with the mind of generalist would increase the interest of medical students.

61) INCREASING NUMBER OF HIV-POSITIVE MOTHERS ONLY PARTLY EXPLAINED THE RECENT DECLINE OF BIRTH WEIGHT IN REPUBLIC OF CENTRAL AFRICA

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Birth weight of 6,904 singleton babies in Boy-Rabe Maternity Clinic of Bangui of Republic of Central Africa between 1998 and 2003 was analysed for their recent decline. Mean birth weight was 2,867 gram and its standard error (SEM) was 6 gram. Low birth weight (LBW) babies were 17.0%. Mean age of mother was 23.2 years old and mean parity was 3.0. Multiple regression analysis revealed that birth weight was independently related with sex of babies, age of mothers, parity of birth, and year of birth (all, $p < 0.001$). Mean birth weight was the heaviest in 1998 (2934gram with SEM of 15 gram) and the lowest in 2002 (2827gram with SEM of 16 gram). Mean birth weight of the same clinic between 1988 and 1990 was reported 2963 gram with SEM of 6 gram, heavier than the value in 1998 ($p < 0.001$). Decline of birth weight between 1998 and 2003 was observed most obviously among mothers of 30

years old with their third parity or more, and among those of 20 years old with their second parity. Between 2001 and 2003, 3995 pregnant women were tested for HIV in this clinic and 539 of them (13.5%) were HIV-positive. There were 101 babies (56 boys and 45 girls) born to confirmed HIV-positive mothers among the 6904 mothers, and their mean birth weight was 2763 gram with SEM of 52 gram, being significantly lighter than the mean birth weight of the other mothers between 2001 and 2003 (2841 gram with SEM of 9 gram, $n=3352$). The proportion of low birth weight among babies born to confirmed HIV-positive mothers was 23.8%. The increasing number of HIV-positive mothers, however, only partly explained the recent decline of birth weight. Major causes of decline of birth weight must be further studied.

62) PHYSICIANS' KNOWLEDGE, ATTITUDE AND PRACTICE TOWARD SEXUALLY TRANSMITTED INFECTIONS AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN FUZHOU CITY, FUJIAN, CHINA

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A cross-sectional questionnaire survey concerning human immunodeficiency virus infection (HIV) and sexually transmitted infections (STIs) was carried out in Fuzhou city, capital of Fujian province, China, and 461 physicians working in 9 general hospitals, 8 health centres and 86 private clinics participated. The proportion of physicians graduated from medical college or over was 90% in general hospitals, 35% in health centres, and 28% in private clinics. Accordingly, the proportion of respondents who stated HIV-related knowledge items correctly over 60% was 86%, 73% and 52% respectively. Gonorrhoea, condyroma acuminata, syphilis, Chlamydia urethritis and genitalia herpes were the most treated STIs by them. HIV treatment was rare except

large hospitals. About two-thirds of the respondents believed that they had risk of HIV occupational exposure, and 59% of them are not willing to take care of HIV/AIDS patients. Over 90% of respondents requested additional education about HIV, particularly information about the prevention of HIV occupational exposure (75%), despite more than a half of them had HIV re-education in the past 3 years. This study revealed the gap between knowledge and attitudes among health care workers about HIV infection, especially in the bottom of the healthcare system. Such attitudes and knowledge deficiencies have the potential to impact negatively on the quality of care, as well as on patient-practitioner safety. More effective training on HIV knowl-

edge and attitude is necessary for clinicians in China.

63) THE STUDY OF HEALTH STATUS FOR THE INHABITANTS OF RURAL AREAS ON VIENTIANE PROVINCE IN LAO P.D.R.

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The investigation of mobile clinic at rural areas in developing countries has several difficulties, for example to use electric power, to carry heavy medical instruments and so on. In this study we tried to make up an integrated mobile clinic for the rural areas in developing countries. And we found the results of general physiological data, serum biochemistry data by filter paper method, and parasitological data by stool examination (Kato method, Formalin ether method) and blood rapid tests (OptiMAL etc.). The subjects were the 387 inhabitants (uplander; male: 203, female: 184) at Phone Muang village on Hinheup district in Vientiane province, Lao P.D.R. In the results of biochemistry: ALT (22.7 ± 7.1 IU/l, n=221), AST (29.2 ± 10.9 IU/l, n=221), γ -GTP (38.8 ± 32.6 IU/l, n=221), BUN (11.8 ± 4.4 mg/dl, n=221), Creatinin (0.56 ± 0.23 mg/dl, n=221), Total cholesterol (146.1 ± 0.23 mg/dl, n=220), Triglyceride (121.3 ± 30.1 mg/dl, n=220). In the data of blood examination: Hb

(13.1 ± 1.7 g/dl, n=221). In stool examinations, several parasite eggs were found, and results as follows: ascariasis (21.8%), trichuriasis (31.2%), Hook worm diseases (20.5%) etc. All inhabitants treated with mebendazole (adults: 500 mg, single dose; children: 250 mg, single dose). And the results of blood test of parasitic diseases were malaria (5.0%) and amebiasis (3.6%).

Clinical diagnosis in the field were respiratory diseases (21.0%), muscle & bone diseases (13.0%) and digestive diseases (10.5%). In conclusion, this mobile clinic was performed high medical levels as well as urban areas in Lao P.D.R. And the health status of this village inhabitants were relatively better conditions than other rural villages in Lao P.D.R. Because this village are located near the main road (Rute 13) and peoples have a jobs as woodcutter and farmer and income were relatively higher than other rural villages.