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Phylogenetic status of a lung fluke in the Philippines based on mitochondrial genome

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Abstract

Based on a near-complete mitochondrial DNA sequence, the phylogenetic status of a lung fluke collected in the Philippines was evaluated. The lung fluke from Leyte Island, Philippines, resembles *Paragonimus westermani* morphologically and is sometimes regarded as a subspecies, *P. westermani filipinus.* In this study, all mitochondrial genes of the Leyte form were sequenced: 12 subunits of mitochondrial enzymes, 2 ribosomal RNA genes and 21 transfer RNAs. The gene order is the same as that of the previously-published *P. westermani* from Korea. All genes are transcribed in the same direction. The sequence from Leyte was 88.8% identical to the previously-published sequence (accession No. AF219379). This is further evidence that the Philippine form should be regarded as specifically distinct from the form in East Asia (China, Japan and Korea). This conclusion is strengthened by the observation that the molluscan host of the Philippine form is of a different family from that of the East Asian form.

Key words: mt DNA, complete sequence, *Paragonimus westermani*, *P. filipinus,* Philippines

INTRODUCTION

The lung fluke, *Paragonimus westermani* (Kerbert, 1878) is a well known zoonotic agent found in eastern and southern Asia and has been the focus of many taxonomic and biological studies (e.g. Blair *et al.,* 1999). Several previous studies have suggested that some regional populations of *P. westermani* should be regarded as distinct species. For example, Miyazaki (1981) proposed on morphological grounds that the Philippine form should be recognized as a distinct subspecies, *P. westermani filipinus.* Allozyme analysis and partial mitochondrial DNA sequences demonstrated that Philippine isolates are genetically distant from strains found in Taiwan, Japan and North East China (Agatsuma *et al,* 1988; Blair *et al,* 1997). Previous analyses of partial sequences (e.g. Iwagami *et al,* 2000) used only short sequence tracts. Comparisons of complete genome sequences can provide more reliable results and also permit comparisons of features such as gene order and the structure of non-coding regions. In this study, we present and discuss the complete sequence of the coding region of the mitochondrial genome for the Philippine lung fluke. In the light of this, we make recommendations concerning its phylogenetic status.

MATERIALS AND METHODS

Adult specimens of a Philippine species of *Paragonimus* used in this study are those previously reported (Agatsuma *et al.,* 1988). DNA extraction of the specimens is as described previously (Iwagami *et al.,* 2000). Based on the 14,967bp of mitochondrial DNA sequence from *P. westermani* (Korean triploid form - database accession No. AF 219379), several PCR primer sets were designed and applied for the present study. Amplified PCR products were purified by gel-electrophoresis and ethanol-precipitation, ligated into pGEM-T plasmid vector (Promega) and cloned inserts sequenced using a dye terminator cycle-sequencing kit (Applied Biosystems) and an automated sequencer (ABI 310, Applied Biosystems). Multiple sequence alignments were performed using the programs CLUSTAL V and GENETYXMAC.

RESULTS AND DISCUSSION

In total, 14,199bp were amplified and sequenced. As

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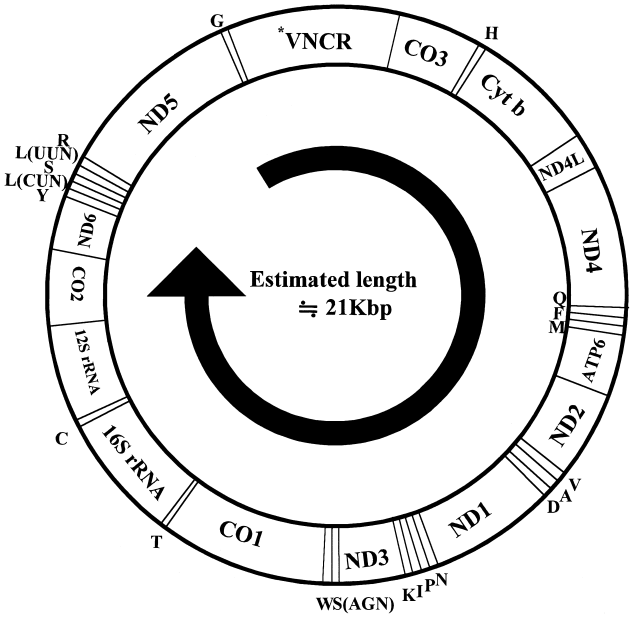

Fig. 1. Circular map of the mitochondrial genome of a lung fluke of *Paragonimus* from Leyte, the Philippines. There are 12 protein-encoding, 2 ribosomal (12S and 16S subunits) and 21 transfer RNA genes. Transfer RNA genes are described with amino acid one letter code. An arrowhead indicates putative direction for transcription. *VNCR: variable non-coding region.

shown in Fig.1, the putative mitochondrial genes included 12 subunits of mitochondrial enzymes (ATP6, CO1～3, Cytb, ND1～6, ND4L), two ribosomal RNA genes (12S rRNA and 16S rRNA) and 21 transfer RNAs (tRNA). As is the case in other trematodes, serine and leucine are each specified by two different tRNAs. No tRNA corresponding to glutamic acid (Glu: E) was found. The gene order and direction of transcription was determined by comparison with the previously reported sequences (Le *et al.,* 2000).

Sequence comparisons between Philippine (2n) and Korean (3n) forms are shown in Table1. The overall difference in DNA sequences of protein-coding genes was 12.40%, in tRNA genes 6.10% and in ribosomal RNAs, 8.03%.

The complete mitochondrial genome of *P. westermani* is estimated to be about 21Kbp (Agatsuma *et al.,* 1994). Of the 14,199 bp we sequenced, 13,196 bp coded for genes and 1,003 bp consisted of non-coding regions. The portion remaining to be sequenced is therefore about 6,800 bp and must constitute the long variable non-coding region (VNCR). Complete sequencing of the VNCR has not been possible to date, probably because of the presence of many repeats. This region corresponds to origin of replication of mitochondrial genomes in many other organisms, attracting interest as to why the length of the site in *Paragonimus* seems to be much longer than in others.

Table 1. Sequence analysis of the mitochondrial genome of a lung fluke from the Philippines, compared to a triploid type of *Paragonimus westermani* from Korea*

Protein-coding region	Number of bases	Number of substitution	Rate of difference (%)
ATP6	513	62	12.09
CO1	1,536	151	9.83
CO2	600	62	10.33
CO3	645	70	10.85
Cytb	1,119	126	11.26
ND1	891	114	12.79
ND2	867	131	15.11
ND3	357	47	13.17
ND4	1,263	180	14.25
ND4L	258	18	6.98
ND5	1,584	225	14.20
ND6	453	65	14.35
Total	10,086	1,251	12.40
ribosomalRNA			
16S rRNA	988	76	7.69
12S rRNA	744	63	8.47
Total	1,732	139	8.03

tRNA	Number of bases	Number of substitution	Rate of difference (%)
Ala	73	0	0
Arg	72	5	4.17
Asn	71	1	0
Asp	67	6	7.46
Cys	64	6	9.23
Gln	62	1	1.61
Gly	71	4	5.71
His	62	11	12.70
Ile	62	1	1.61
Leu (CUN)	62	0	1.61
Leu (UUN)	65	3	4.62
Lys	65	3	7.58
Met	66	0	0
Phe	70	6	8.57
Pro	66	10	13.43
Ser (AGN)	60	5	8.33
Ser (UCN)	65	2	3.08
Thr	66	3	4.55
Trp	64	4	6.25
Tyr	62	3	4.84
Val	63	10	15.38
Glu	Not found	Not found	Not found
Total	1,378	84	6.10

*Accession No. AF219379

As mentioned above, Miyazaki (1981) regarded the Philippine lung fluke as a subspecies of *Paragonimus westermani*, namely *P. westermani filipinus.* The justification was that one lobe of one or both testes is detached from the remainder and that the molluscan host belongs to a different family from that of *P. westermani.* Blair (2000) reviewed phylogenetic relationships among species and strains of the genus *Paragonimus* and showed *P. westermani filipinus* to be distinct from populations from Japan, Taiwan and Malaysia. His study used only 393 bp of the mitochondrial CO1 gene. Partial CO1 sequences previously deposited in the public databases showed 88.7% identity between *Paragonimus* isolates from the Philippines and Korea, suggesting that *Paragonimus* in the Philippines can be regarded as a species distinct from *P. westermani* from East Asia (China, Japan and Korea). However this suggestion was thought to be weak because such short sequence tracts were used. Our results enabled full comparison of all genes of the mitochondrial genome for the first time and found an overall identity of 88.8% between Philippines and Korean worms (Table 1).This result resembles that of the partial sequence analysis discussed above. Despite the lack of clear molecular criteria to determine species-boundaries within a genus such as *Paragonimus*, we consider that our results, and the differences in molluscan host specificity, are good evidence that the Philippine form is specifically distinct. The name *P. filipinus* Miyazaki, 1981 should be applied to it.

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Phylogenetic relationships of snails of the genera *Oncomelania* and *Tricula* inferred from the mitochondrial 12S rRNA gene

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Abstract

The *Schistosoma japonicum* group and *S. sinensium* utilize intermediate snail hosts belonging to the genera *Oncomelania* and *Tricula* (Gastropoda: Pomatiopsidae). In the present study, partial sequences of the mitochondrial 12S rRNA gene from 7 subspecies of *O. hupensis*, two species of *Tricula* (*T. bollingi* and *T. humida*) and *O. minima* were examined to infer a phylogeny for these. Nucleotide differences among subspecies of *O. hupensis* were less than 6.5% and among species from different genera, 10-12%. The phylogenetic tree obtained in this study indicates that *O. hupensis* subspecies fell into four distinct clades ; that is, *O. h. quadrasi* from the Philippines, *O. h. lindoensis* from Indonesia, *O. h. hupensis* from Yunnan, China and the remaining 5 subspecies (*O. h. hupensis* from other parts of China, *O. h. robertsoni* from China, *O. h. formosana* from Taiwan, *O. h. chiui* from Taiwan and *O. h. nosophora* from Japan). The phylogenetic tree also showed that *O. minima* was placed as sister to all of the subspecies of *O. hupensis.* Possible evolutionary relationships among the snail hosts were discussed.

Key Words: *Oncomelania*, *Tricula*, mitochondrial DNA, 12S rRNA gene, phylogenetic tree

INTRODUCTION

Species of *Schistosoma* have been placed in a number of groups based on, amongst other things, egg morphology and/or geographical distribution (Rollinson and Southgate, 1987). For example, African schistosomes of the *S. mansoni* and *S. haematobium* groups develop in pulmonate snails of the family Planorbidae, while the oriental species, namely the *S. japonicum* group (*S. japonicum*, *S. mekongi*, *S. malayensis*) and *S. sinensium*, utilize snails belonging to the family Pomatiopsidae. Pulmonate snails belong to the subclass Pulmonata, while pomatiopsids belong to the subclass Caenogastropoda. These two subclasses diverged a long time ago (Davis, 1980). Generally, specificity for an intermediate host in the genus *Schistosoma* is high (Rollinson and Southgate, 1987). So the phylogeny of these intermediate host snails seems to be very important when the evolution of the schistosome is traced.

Schistosoma japonicum is widely distributed throughout East Asia, China and Japan, and the intermediate host is *Oncomelania hupensis*. Since *O. hupensis* shows geographical variation in morphology, many subspecies have

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been described (Davis *et al.,* 1995). Geographical variation is so great that some researchers prefer to regard the subspecies as independent species (Woodruff *et al.,* 1988; Nihei *et al.,* 1998). In the present study, phylogenetic relationships of the snail hosts of *S. japonicum*, *O. hupensis*, and other related species of *Oncomelania* and *Tricula* were studied using the mitochondrial 12S rRNA gene.

MATERIALS AND METHODS

Snail samples

Sixteen isolates of *O. hupensis*, belonging to 7 subspecies, were examined. We also examined *O. minima*, *T. bollingi* and *T. humida.* The geographical origins and locations of species or subspecies used in this study are shown in Table 1 and Fig. 1.

Preparation of DNA

Genomic DNA from each snail sample was extracted using Easy-DNA Kit (Invitrogen, USA). DNA extracted by this kit contained an inhibitor for PCR, and this inhibitor could not be removed by phenol/chloroform extraction or commercially available spin column etc. So DNA was purified by 0.5% agarose gel electrophoresis with 0.5 X Tris-boric EDTA buffer. After electrophoresis, high molecular weight DNA was cut off with agarose gel and extracted by QIAEX II Gel Extraction Kit (Qiagen, Germany).

Amplification and sequencing of DNA

Purified genomic DNA was used as a template for amplification of DNA fragments by the polymerase chain reaction (PCR). Amplification of a part of the mitochondrial 12 S rRNA gene was carried out using universal primers (Kocher *et al.,* 1989). The sequences of the primers were as follows: L1091; 5'-AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT-3' and H1478; 5'-TGA CTG CAG AGG GTG ACG GGC GGT GTG T-3'. PCR was performed using Ampli Taq DNA Polymerase (Perkin Elmer, USA) according to the manufacturer's instructions. After an initial denaturation step (94 ℃ for 3 minutes) there were 30 cycles of denaturation at 94 ℃ for 30 sec, annealing at 50 ℃ for 30 sec and extension at 72 ℃ for 1min. PCR products were purified with QIAquick-spin PCR purification Kit (Qiagen). Purified double-stranded PCR products were directly sequenced with the same primers as those of PCR from both ends using Dye Terminator Cycle Sequencing FS Ready Reaction Kit and a Model 377A DNA sequencer (Perkin Elmer).

Table 1. Geographical origins of subspecies or species of *Oncomelania* and *Tricula* examined.

subspecies or species	Location	Country
Oncomelania hupensis hupensis	Anhui	China
O. h. hupensis	Hunan	China
O. h. hupensis	Hubei	China
O. h. hupensis	Yunnan	China
O. h. robertsoni	Sichuan	China
O. h. formosana	Kaohsiung	Taiwan
O. h. formosana	Shuili	Taiwan
O. h. formosana	Yilan	Taiwan
O. h. chiui	Shimen	Taiwan
O. h. nosophora	Kofu	Japan
O. h. nosophora	Kurume	Japan
O. h. lindoensis	Sulawesi	Indonesia
O. h. quadrasi	Mindoro	Philippines
O. h. quadrasi	Bohor	Philippines
O. h. quadrasi	Asuncion	Philippines
O. h. quadrasi	Digos	Philippines
O. minima	Sado	Japan
Tricula bollingi	Fang	Thailand
T. humida	Sichuan	China

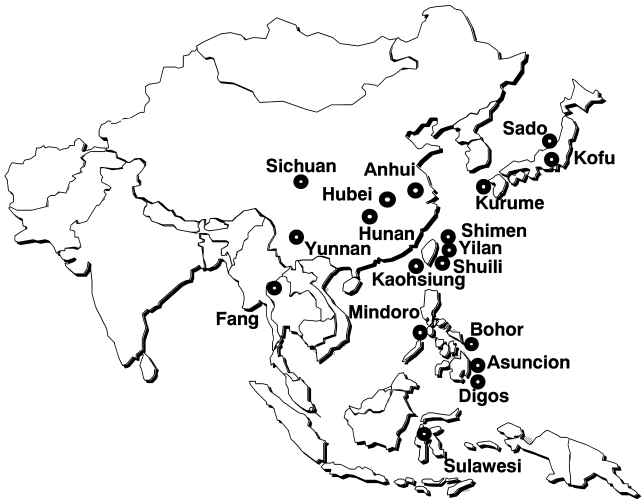

Fig. 1. Geographical locations of species or subspecies of *Oncomelania* and *Tricula* used in this study.

Phylogenetic analyses

DNA sequence data were aligned using the CLUSTAL W computer program. The evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980), and the phylogenetic tree was constructed by the neighbor-joining method using the neighbor-joining computer program in the PHYLIP 3.5 phylogeny package (Felsenstein, 1993). The tree was evaluated using the bootstrap test based on 1,000 resampling. A sequence from *Littorina littorea* was used for the outgroup (Rumbak *et al.,* 1994).

RESULTS AND DISCUSSION

Using this primer pair, fragments of 365 to 368 bp were amplified in this study. Sequences and partial alignments of 12S rRNA gene are shown in Fig. 2. The maxi-

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OhcShimen          1:TCTTGAAGATAAATAAATTTATACCGGGGCACTACGAATAATCT-TTAGATTTAAAACCCAAAGAGCTTGGCGGTGTTTT
OhfKaohsiung       1:.........................A..................-.....................................
OhfShuili          1:.............................................-.....................................
OhfYilan           1:.............................................-.....................................
OhhAnhui           1:.............................................-.....................................
OhhHubei           1:...........G.................................-.....................................
OhhHunan           1:.............................................-.....................................
OhhYunnan          1:.....................................G..T.-.....................................
OhlSulawesi        1:.........................A...T.............T.-...A.....................................
OhnKoufu           1:.....................................G....-.....................................
OhnKurume          1:.....................................G....-.....................................C
OhqAssuncion       1:..............G..........A.................T.-.C.....................................
OhqBohor           1:..............G..G.......A.................T.-.C.....................................
OhqDigos           1:.........................A.................T.-.C.....................................
OhqMindro          1:..............G..........A.................T.-.C.....................................
OhqSorsogon        1:..............G..........A.................T.-.C.....................................
OhrSichuan         1:.............................................-.....................................
OminimaSado        1:........AG........A.......A................T.A...A.....................................
TbollingiFang      1:.........T.....G-.A.......A...T...........--T.A...A.....................................
ThumidaSichuan     1:........AG..........CA.....A..........A.........T...A.....................................
Llittorea          1:....AG.TG............TA.TTTACCAGAGTACT.CG.A.CA-..A.........T..................CC..

OhcShimen         81:AGACTATTTAGGGGAACTTGTTTCATAATCGATAATCCACGAGATACCTAACCTTCTTTTGTAATCAGTATGTATACCGT
OhfKaohsiung      81:.....................................................................................
OhfShuili         81:.....................C.....................A.....................................
OhfYilan          81:.....................................................T.....................
OhhAnhui          81:.....................................................................G.....
OhhHubei          81:.....................................................................................
OhhHunan          81:.....................................................................................
OhhYunnan         81:.....................................T.G..........T........C.....................
OhlSulawesi       81:.....................................................................................
OhnKoufu          81:.....................................................................................
OhnKurume         81:.....................................................................................
OhqAssuncion      81:.........................G.................A.............C.....................
OhqBohor          81:.........................G.................A.............C.....................
OhqDigos          81:.........................G.................A.............C.....................
OhqMindro         81:.........................G.................A.............C.....................
OhqSorsogon       81:.........................G.................A.............C.....................
OhrSichuan        81:.....................................................G.....
OminimaSado       81:.....G..........C.....................................................G.....
TbollingiFang     81:......C.....................................T.A.....................................
ThumidaSichuan    81:...................C......G.................T.....................................
Llittorea         81:.....T..C........C...C..........C.........ACA....C...C.T.....C..A.....

OhcShimen        161:TGTCGTCAGGTAACTTTTTAAAATAAAAAAGTTA-GCGAAAAAGCCATAAGCTTACACGTCAAATCAAGGTACAGCCTAT
OhfKaohsiung     161:.....................................-..A.................T.....................
OhfShuili        161:.....................................-.....................T.....................
OhfYilan         161:.....................................-.........T..........T.....................T...
OhhAnhui         161:.....................................-.....................T.....................
OhhHubei         161:.....................................-.....................T.....................
OhhHunan         161:.....................................-.....................T.....................
OhhYunnan        161:.....................................-...................C.T.....................
OhlSulawesi      161:.....C...............................-..A.....................................
OhnKoufu         161:.....................................-..........T.........T...................A.....
OhnKurume        161:.....................................-..........T.........T...................A.....
OhqAssuncion     161:.............................G.....-.....................................
OhqBohor         161:.............................G.....-.............G.....................................
OhqDigos         161:.............................G.....-.............G.....................................
OhqMindro        161:.............................G.....-.............G.....................................
OhqSorsogon      161:.............................G.....-.............G.....................................
OhrSichuan       161:.....................................-.....................T.....................
OminimaSado      161:.............................G.......-..A.GG..A.T....AT.C.....................
TbollingiFang    161:..............................T.....AC.A.....ATTG...AT...T.....................
ThumidaSichuan   161:.......................C.T.G......GC.A.....ATTT.T.TT.....................C.C
Llittorea        161:C....C............A......T..CT....GCTA.G...CTAT.T..T..T.......G........G.......
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OhcShimen       241:AAGAAAGAGAGAATGAGTTACAATTAAAATTTATAATAACGGAATAGAAAAAGAAAATTTCTATGAAGGCGGACTTAAAA
OhfKaohsiung    241:.....G......G.....................................................................
OhfShuili       241:.....G.....................................................................
OhfYilan        241:.....G.....................................................................
OhhAnhui        241:.....G.....................................................................
OhhHubei        241:.....G.....................................G.....................................
OhhHunan        241:.....G.....................................................................
OhhYunnan       241:.....G....A.....................................................T.A.........
OhlSulawesi     241:..AG.G.....................................................................
OhnKoufu        241:.....G.....................C.....................................................
OhnKurume       241:.....G.....................C.....................................................
OhqAssuncion    241:GG.GGG.....................C.....................................T...............
OhqBohor        241:GG.GGG.....................C.....................................T...............
OhqDigos        241:...GGG.....................C.....................................T...............
OhqMindro       241:GG.GGG.....................C.....................................T...............
OhqSorsogon     241:GG.GGG.....................C.....................................T...............
OhrSichuan      241:.....G.....................................................................
OminimaSado     241:.T.G.G.........G...........C.......G........A............T...................
TbollingiFang   241:.T...G....A...TG...........C............G..A.........AA.T........A..........
ThumidaSichuan  241:.....G.........G...........C..............A..GT......C..T.T......A..........
Llittorea       241:..A.GG....AG...G.....................T.....TAGTGGTC.A....GCCACT......A......G...

OhcShimen       321:GTAAAAAAATTACTATAGAGACTTTTTGAATCAAGCTCTGAAACGTGC
OhfKaohsiung    321:................A.............................
OhfShuili       321:.....................................................
OhfYilan        321:.....................................................
OhhAnhui        321:.....................................................
OhhHubei        321:.....................................................
OhhHunan        321:....................T.....................................
OhhYunnan       321:............T......A.T.........TG.....................
OhlSulawesi     321:..............CA.............................
OhnKoufu        321:.....................................................
OhnKurume       321:.....................................................
OhqAssuncion    321:............T........T.........C.....................
OhqBohor        321:............T........T.........C.....................
OhqDigos        321:............T........T.........C.....................
OhqMindro       321:............T........T.........C.....................
OhqSorsogon     321:............T........T.........C.....................
OhrSichuan      321:.....................................................
OminimaSado     321:.........CA.T....A.A.T.........TG.....................
TbollingiFang   321:..........A.T....T.A.T.........T.....................
ThumidaSichuan  321:..........A......A.A.T.........T............T..-
Llittorea       321:..--....G...G..ATC.A.TGC.......T.........GG....-
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Fig. 2. Nucleotide sequence alignment of the 12S ribosomal RNA gene in the mitochondrial DNA in the three genera, *Oncomelania, Tricula* and *Littorina.*

Table 2. Pairwise differences in nucleotide sequences of the 12S rRNA gene among subspecies/species of the genus *Oncomelania* and *Tricula.*

species/subspecies	O.h.hup.	O.h.h.Yun	O.h.rob.	O.h.for.	O.h.chi.	O.h.nos.	O.h.lin.	O.h.qua.	*O.minima*	*T.bollingi*
O.h.hupensis (excluding Yunnann)	0.8*									
O.h.hupensis (only Yunnan)**	4.2									
O.h.robertsoni	0.7	4.4	-							
O.h.formosana	1.1	3.7	1.0	1.3*						
O.h.chiui	0.0	4.6	0.8	1.3	-					
O.h.nosophora	1.7	4.8	1.6	2.0	1.8	0.7*				
O.h.lindoensis	3.6	6.4	3.4	3.4	3.4	4.2	0.8*			
O.h.quadrasi	5.1	6.4	5.3	5.0	5.0	6.1	6.2	0.7*		
O.minima	9.4	9.3	9.6	9.3	9.3	9.1	8.3	10.5	-	
T.bollingi	10.0	10.2	10.2	10.4	10.4	9.9	9.8	11.7	8.8	-
T.humida	10.7	12.1	11.0	10.7	10.7	10.5	10.8	12.5	9.0	9.9

* : values for intra-subspecies

**: The Yunnan strain of O.h.h. was listed separately from the other strains, because it differed in a high degree from any other subspecies of O. hupensis.

mum levels of nucleotide variations detected between pairs of species or subspecies for the 12S rRNA gene are shown in Table 2. The values are expressed as pairwise differences in percentage. Nucleotide differences within the subspecies of *O. hupensis* were in general very low. However, in the case of *O. h. hupensis,* the intra-nucleotide difference was very large, because a Yunnan isolate of *O. h. hupensis* differed from all of the other isolates. On the other hand, differences among five of the subspecies, *O. h. hupensis* from China except for Yunnan, *O. h. robertsoni, O. h. formosana, O. h. chiui* and *O. h. nosophora,* were less than 2.0%. Nucleotide differences between these 5 subspecies and *O. h. lindoensis,* or *O. h. quadrasi* or the Yunnan isolate of *O. h. hupensis* were larger, being about 3 to 6%. Very large values of nucleotide differences (about 9 to 12%) were obtained between different species.

As shown in Fig. 3, a phylogenetic tree of the snail hosts was constructed using the neighbor-joining method. *Oncomelania hupensis* subspecies are distributed among 4 groups. That is, four specimens of *O. h. quadrasi* from Philippines form a monophyletic clade. *Oncomelania h. nosophora, O. h. hupensis, O. h. robertsoni, O. h. chiui* and *O. h. formosana* form one group. *Oncomelania h. lindoensis* and *O. h. hupensis* from Yunnan make independent clades. In the meantime, *O. minima, T. bollingi* and *T. humida* also make independent clades, and they are genetically distant from each other as well as from all the subspecies of *O. h. hupensis.*

The sequence of mitochondrial 12S rRNA gene has been utilized to infer the phylogeny of various animals (Kocher *et al.,* 1989). Rumbak *et al.* (1994) examined the phylogenetic relationships among 11 species in the genus *Littorina,* which is a widely distributed marine gastropod, belonging to the same suborder Archaeotaenioglossa as the genus *Oncomelania.* In the case of the genus *Littorina,* nucleotide differences between European species and American species were about 3%. And differences between subgenera in the genus *Littorina* were about 5% or more. In the present study, *O. hupensis* were distributed among 4 groups, and nucleotide differences among these 4 groups were 3 to 6%. Although there is no reason to assume that rates of molecular evolution have been the same in *Littorina* as in the pomatiopsids, comparisons of the percentage differences between the two studies suggest that the subspecies in *O. hupensis* may require re-evaluation as suggested by Woodruff *et al.* (1988). Despite the wide geographic area involved, the genetic differences between the subspecies in Japan, Taiwan and China were very small. All of the examined snails in China were collected from the Yangtze basin except for that of Yunnan Province. In the glacial maxima, only approximately twenty thousand years ago (Wang and Sun, 1994), sea levels were much lower than now, the mouth of the Yangtze was considerably closer to Japan and Taiwan, and the Taiwan channel was dry land. This could explain the high levels of similarity among Japanese, Taiwanese and Yangtse basin samples.

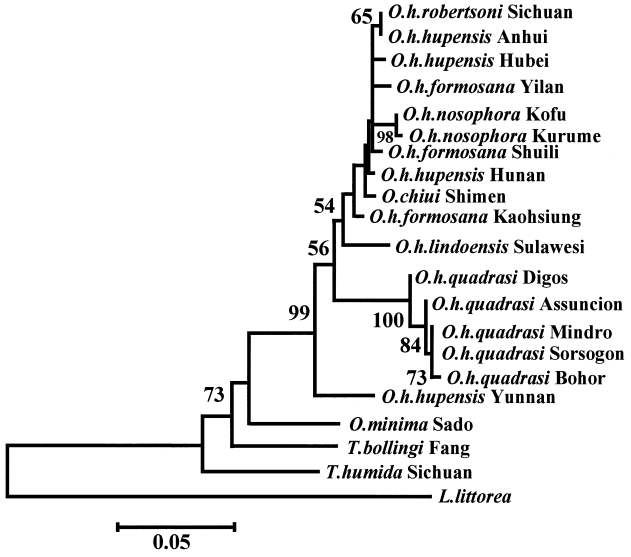

Fig. 3. A phylogenetic tree of the genus *Oncomelania* and *Tricula,* including the snail intermediate hosts of *Schistosoma japonicum,* inferred from 12S rRNA gene in the mitochondrial DNA using the NJ method.

It has been reported that genetic variation among *S. japonicum* populations in Asia, including China, the Philippines, Japan and Indonesia, is very slight (Bowles *et al,* 1993). However, we have shown that genetic variation among their intermediate host snails is quite considerable. Thus, our study supports the idea that *S. japonicum* has been recently introduced to many areas where it now occurs, and has been able to adapt to local strains of *Oncomelania,* as suggested in previous papers (Woodruff, 1988; Davis, 1992; Attwood *et al.,* 2002).

Davis (1980) suggested that *S. sinensium* may constitute a species complex, because of geographical differences in snail host specificity. Our previous studies showed a large difference in egg sizes as well as in nucleotide sequence of CO1 between the two isolates of *S. sinensium* from China and Thailand, supporting Davis's hypothesis (Kawanaka *et al.,* 1998; Agatsuma *et al.,* 2000). The present result of a large distance value between their intermediate hosts may suggest coevolution between the hosts and parasites.

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PROCEEDINGS OF THE 43RD ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE

21-22 November 2002, Kochi

President

Yoshihisa Hashiguchi
Professor, Department of Parasitology
Kochi Medical School

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Prize winner's lecture

THE MALARIA VECTOR IN THE AREA SURROUNDED BY RICE FIELD IN KHAMMAOUNE PROVINCE. LAO PDR WITH THE DISCUSSION OF THE FUTURE ITM STRATEGY IN LAOS AND ALSO MEKONG AREA

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In the 1996, the malaria vector in Laos was not clear. It was suspected that the same vector in the North Thailand; *Anopheles minimus*, *An. maculatus* and *An. dirus*, were existed. In Nhommarath District in Khammouane Province, Lao PDR, a lot of malaria patients were reported in the district hospital located in the center of basin. It was supposed that patient came from the communities around hospital under the consideration of accessibility to hospital, thus the one community around hospital was selected for malaria survey. The prevalence of malaria infection in the inhabitant is high as more than 20%, however, only few number of *An. minimus* was collected, and *An. maculates* and *An. dirus* was not collected. On the other hand, *An. nivipes* is the majority in *Anoheles*, which was collected by human bait collection, and the larva of this mosquito was collected from the rice field. Thus, *An. nivipes* is suspected malaria vector in this basin surrounded by rice field. At present time, the distribution of the malaria vector in Lao PDR is estimated according to the results of a lot of surveys. It is supposed that the existence of *An. dirus* influence to the high endemic of malaria in the southern provinces. Moreover the biting habit of *An. dirus* in this area is one of the causes that an impregnated bed net was affected to control malaria.

The vector control using by impregnated bed net (IBN) is one component of the recent global malaria control strategy. It is well known that the health education is important not only to diffuse IBN but also to sustain the project. In the beginning of 2002, the review of Impregnated Treatment Materials (ITM) strategy was carried out in Roll Back Malaria Initiative in Africa. The new strategy consists of both sides between public and private sector in addition to social marketing. In the Mekong region, the ITM programs were carried out using by mainly public sector only, because the peripheral health administration system was well established in comparison with African countries. The other reason, that social marketing was not conducted in Mekong countries, was supposed that the rural areas without suitable media and market were the serious endemic of malaria. When the project is being carried out in the wide area from now on, however, the only public sector is supposed not to be able to sustain the project. In fact, ITM project was expanded to whole country in Lao PDR, the re-dipping of IBN was not carried out in several communities. It is recommended that the joining of private sectors becomes important to sustain the project from now on in Mekong countries.

President's lecture

TROPICAL DISEASES RESEARCH AND THE ENDEMIC AREAS -A BRIEF REVIEW, BASED ON THE RESEARCH/CONTROL OF LEISHMANIASIS IN SOUTH AND CENTRAL AMERICAS-

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During about 20 years from 1982 to date, we made an intensive investigation on leishmaniasis in Sounth and Central Americas, especially in Ecuador. The research project was supported at the early phase by JICA, and then supported by the Ministry of Education, Science, Culture and Sports, Japan. Such a long term financial support has been playing the role of driving force to disclose the bio-medical features of New World leishmaniasis. In the project, an intensive study has been done, in order to get information on the disease and its transmission, from multidisciplinary points of view. The aim of project at the early phase was to consider a suitable control measure, by disclosing transmission mechanisms of the disease in each endemic area of Ecuador. By performing country-wide epidemiological surveys, factors relating to the transmission of leishmaniasis, such as the causative agents (6 *Leishmania* spp.); *L.(L.) mexicana, L.(L.) amazonensis, L.(L.) major*-like, *L.(V.) braziliensis, L.(V.) panamensis, L.(V.) guyanensis*, the vector sandflies (4 *Lutzomyia* spp.); *Lu. hartmanni*, *Lu. trapidoi*, *Lu. gomezi* and *Lu. ayacuchensis*, and the reservoir hosts (8 spp. of mammals); anteaters, sloths (2 spp.), squirrels (2 spp.), kinkajous, rats and dogs, have been known at different endemic areas of Ecuador. *L.(V.) equatorensis* from arborial mammals (sloth and squirrel) was described as a new species, with no human cases. Andean type of leishmaniasis was reported for the first time in Ecuador; the disease form is very similar to Peruvian uta, but the causative agents and vector sandflies are completely different. We tried to search for suitable drugs and treatments of the disease, which would be effective and easily applicable at field conditions; several ointments, lotions and oral drugs such as antimalarials were found to be effective and useful. Furthermore, comparative study was made to have a suitable sampling method applicable for molecular techniques, such as PCR diagnosis. Besides, in the talk several experiences were mentioned, demonstrating cases we face to the problems at field surveys, taking blood samples, skin-testing and etc.

Special lecture

I THE MUCOSAL IMMUNE SYSTEM FOR THE DEVELOPMENT OF NEW GENERATION VACCINE

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The host immune system is equipped with two arms of immmunity at mucosal and systemic compartments. The basic foundation of the immune system was establised according to the extensive molecular and cellular characterization of lymphocytes and their associated regulatory molecules in the systemic compartment. To this end, thymus, bone marrow, spleen and peripheral lymph nodes are thought to be the immunological nest of the systemic immune system. In addition to the systemic immunity, the host is equipped with the mucosal immune system providing a first line of defense against numerous foreign antigens which invade the respiratory, intestinal and reproductive tracts. The mucosal immune system has a difficult task: it has to protect approximately 400 m^2 of mucosal surface area and it must differentiate between beneficial and unwanted antigens at the site of entry.

For providing a first line of defence at a such large mucosal surface, serectory IgA (S-IgA) is a major antibody. For the induction of antigen-specific S-IgA immune response at mucosal surfaces, a unique concept of the common mucosal immune system (CMIS) need to be considered. The CMIS consists of inductive sites (e.g., nasopharyngeal associated lymphoreticular tissue [NALT] and gut associated lymphoreticular tissue [GALT]) and effector sites (e.g., lamina propria and epithelium of the respiratory, intestinal and reproductive tissues). To this end, nasal and oral immunizations have been shown to be effective for the induction of antigen-specific S-IgA response in the distant mucosal effector compartment. Further, mucosal immunization can induce antigen-specific immune responses in the systemic compartment. Thus, the estabrishment of two layers of the protective immunity can be accomplished by oral or nasal vaccine.

NALT is considered to be an important inductive site for the induction of antigen-specific mucosal and systemic immunity against nasally-administered vaccine antigen. In contrast to Peyer's patch (PP), an example of GALT, cytokine and corresponding receptor regulated molecular machinery for the tissue organogenesis of NALT remains to be elucidated. Based on the result obtained by the histological and growth kinetic study of normal and specific gene manipulated mice (e.g., IL-7R$^{-/-}$ and LT$\alpha^{-/-}$), it was shown that NALT organogenesis is independently operated from a group of known cytokine signaling pathway via IL-7R and LTβR for the generation of secondary lymphoid tissue (e.g., PP).

Our recent investigations have provided new observations that mucosal IgA antibody can be developed in a CMIS-independent manner. IgA committed B-1 cells expressing IL-5R and IL-15R are responsible for the generation of CMIS-independent IgA, while B-2 cells responding to IL-5 and IL-6 are the major source for CMIS-dependent IgA. Taken together, these findings suggested an interesting and new possibility that the mucosal surface in protected by S-IgA generated from the two distinct sources of mucosal B cells.

According to these unique features of the mucosal immune system, our current effort is also focused on the development of novel antigen delivery veihicle for the induction of antigen-specific IgA response. Our recent findings demonstrated that Fusogenic-liposome can effectively deliver antigen to NALT via M cells and epithelial cells. Nasal immunization with Fusogenic-liposome containing vaccie antigen can effectively induce antigen-specific mucosal and systemic immunity. In addition, nasal immunization with rBCG containg vaccine antigen resulted in the induction of prolonged memory type antibody responses. These new findings will extend the understanding of unique feature of the mucosal immune system for the development of mucosal vaccine for the prevention and control of infectious diseases.

II MOLECULAR STRATEGIES FOR DISEASE CONTROL ---- RESEARCH TRIALS IN VECTOR BIOLOGY ----

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Vector borne diseases (VBD) are very important in tropical diseases. The pathological agents of VBD include wide range of organisms such as virus, rickettsia, bacteria, spirochete, protozoa and nematode. Vectors are blood sucking insects such as mosquito, black fly, tsetse fly, sand fly kissing bug and so on, and also ticks and mites. It is difficult to control or to eliminate VBD because the disease are depending on very complicated backgrounds and related deeply to the environments. Vector species and number are numerous and area specific, and easy to develop insecticide resistant.

In the last 30 years of 20th century, molecular biology, molecular genetics and molecular technology have developed very much. Based on these development, some trials to establish strategies for VBD control by molecular technique have been performed. One of the trials is to produce a vector which cannot transmit pathogen by genetic transformation. Technology for producing a transgenic mosquito was established first by transforming EGFP gene by using minos as transposon (Crisanti et al 2000). After some trials, a transgenic mosquito, *Anopheles stephensi*, which cannot transmit malarial parasite *Plasmodium berghei* was produced by transforming a synthetic gene of SM-1 (salivary gland / midgut adhesive molecule) (Jacobs-Lorena et al., 2002).

The genome of a malarial vector mosquito *Anopheles gambiae* was published very recently (in Nature, October 2002). So we can find some more candidate genes to transform a vector mosquito, for instance, malarial resistant genes against malarial parasite, and can produce more mosquitoes not to transmit malaria. However, before releasing transgenic mosquitoes we have to develop our technology to replace the natural population to the transgenic populations of mosquitoes and assess the effects to the natural ecosystem.

In the fruit fly Drosophila, female-specific lethal transgenic insects were developed using a sex specific promoter to drive the expression of a repressible transcription factor, which in turn controls the expression of toxic gene product. This principle is applicable to medically important dipteran insect mosquitoes. Using this strategies insect population is more easily controlled than the sterile insect technique.

Some more example trials for developing strategies to control vector borne tropical diseases by tstudies of molecular and genetic biology of vectors were presented in the lecture.

Educational lecture

THE GREAT NAMES OF HISTORY IN TROPICAL MEDICINE

TERUYUKI KOBAYASHI

Non-fiction writer

In the present talk, many great names of history in Japanese Tropical Medicine were mentioned. They were Drs. Yoshio Sawai, Manabu Sasa, Kanzen Teruya, Hachiro Sato, Yoshito Otsuji and others who worked with tropical medicine-related health problems/diseases such as, snake (*Habu*) bite, Tsutsugamushi disease, malaria, filariasis, schistosomiasis, paragonimiasis and other infectious or parasitic diseases. About 40 years ago, all these diseases were highly prevalent at country-wide in Japan and they were great public health problems, because of poor sanitary conditions and insufficient political atention. For example, snake (*Habu*) bite was one of the most serious health problem in subtropical regions of Japan, especially in Okinawa and Amami-Oshima islands. During 13 years from 1959 to 1971, 3,510 snake bite cases were reported from Amami-Oshima island; the average fatality rate was 0.8% in the area. Dr. Yoshio Sawai, a microbiologist at the Institute of Medical Science at the University of Tokyo was the person who hardly worked with the *Habu* bite. In 1952 he was appointed as Associate Professor and chief of the newly-established laboratory of biological products at the Institute. Antivenin productions including these for *Habu* venom was one of the traditional work in the institute. Naturally Dr. Sawai tried to develop more effective and useful *Habu* toxoid. He frequently visited Naze Health Center, Amami-Oshima island and observed there many patients suffered from serious and aggressive *Habu* bites with severe necrosis at the site of snake bite. He started laboratory investigations to improve the treatment of *Habu* bite, together with his co-workers at the Institute. Their purified and lyophilized antivenin was favorable to decrease numbers of the severe cases of *Habu* bites when applied clinically at Amami-Oshima island. However, yearly occurrence of severe clinical cases were still found in the area, in spite of such a Dr. Sawai's untiring efforts. After analyzing many clinical cases of *Habu* bites, Dr. Sawai reached to the conclusion that there might be a limitation in their serum treatment against the snake bite, because the development of local lesions by the large amount of *Habu* venom was too rapid for the antivenin treatment to prevent. Then, in 1963, Dr. Sawai reported a dihydrothioctic acid mixed with different concentrations of the *Habu* venom; the preparation inhibited lethal effect of venom when tested n experimental animals such as mice, rabbits and guinea pigs. In April, 1965, the first large scale trial of active immunization using this product was undertaken in persons living at remote areas with high-risk of *Habu* bites. In Amami-Oshima and Okinawa islands, 43,446 persons received the *Habu* venom toxoid during 3 years of active immunization from 1965 to 1967. Statistic analysis of these trials showed that the venom towoid used was effective, enough to reduce the rate of necrotic cases by 50%. The improvement of the *Habu* toxoid developed by Dr. Sawai's group has been promoted by the Committee sponsored by the Ministry of Health and Welfare in Japan. Later, Dr. Sawai expanded his research to Southeast Asia and India, also contributing greatly to this world-wide life threatening snake bites; he is 90 years old, living in Ohta city, Gunma, Japan. Other works by the great names mentioned above were also discussed briefly in the session, showing many slides.

Symposium

S－I－1) CURRENT STATUS OF EMERGING PROTOZOAL INFECTIOUS DISEASES: CRYPTOSPORIDIOSIS, CYCLOSPORIASIS, AND MICROSPORIDIOSIS

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Cryptosporidiosis, caused by *Cryptosporidium parvum* infection, is recognized as acute, self-limiting, gastroenteritis in immunocompetent humans and persistent and potentially fatal in immunocompromised subjects, particularly those with AIDS. The number of the infection is estimated to be 250 millions to 500 millions every year, in both developed and developing countreis. Recently, reports of imported cases among travelers to less developed countries, domestic infections among both immunocompetent or immunocompromised hosts, and outbreaks associated with public water supply are increasing in Japan. Diagnostic methods for demonstrating the oocyst or antigens in clinical specimens are well developed. While, we still have noconsistently effective chemotherapeutic agent for use. Development of effective drugs for treament and of control measures for waterborne outbreaks is urgently needed.

Cyclosporiasis is caused by *Cyclospora cayetanensis* infection. The organism is a newly recognized coccidian parasite inhabiting small intestinal epithelial cells and causes prolonged watery diarrhea, fatigue, and anorexia in humans was reported at least as early as 1979, it was initially called a coccidian-like body or a cyanobacterium-like body (CLB). In 1993, the organism was identified as a protozoan of the genus *Cyclospora*, and the species name of *C. cayetanensis* was proposed in 1994. The desease is widely distributed in tropical and subtropical countries. Since 1996, more than 10 cases among travelers to developing countries and a case suspected to be infected by ingesting imported fresh vegetables have been reported in Japan. Symptomatic infections can be treated with trimethoprim-sulfamethoxazole.

Microsporidiosis, caused by infection with several species of the phylum Microspora, is an important opportunistic infectious desease among AIDS patients and appears to have worldwide distribution. At present, 13 species of microsporidia have been identified as potential pathogens in humans. The first report of diarrheal syndromes associated with microsporidiosis and HIV infection was published in 1985, and the number of case reports increased rapidly after 1990. Chronic diarrheas caused by *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* infection are frequently observed among AIDS patients. While, *Enc. hellem* and *Enc. cuniculi* cause keratoconjanctivitis and disseminated infection, *Pleistophora* spp. and *Nosema* sp. cause myositis. Recently, cases of microsporidiosis have been reported in Japan. Microscopic detection of the parasite requires special staining methods and adequate microscopic techniques. Although two agents, fumagillin and albendazole, have been reported to have activity against microsporidia both in vitro and in vivo, treatment efficacy is poor except in the case of infections caused by Encephalitozoonidae.

S－I－2) CRYPTOSPORIDIOSIS AND CYCLOSPORIASIS: THE CLINICAL FEATURES AND OCCURRECE IN JAPAN

GOHTA MASUDA

President, Tokyo Metropolitan Kiyose Children's Hospital

◆Cryptosporidiosis

Cryptosporidium sp. (mainly *C. parvum*) causes transient non-bloody diarrhea in normal hosts, while this pathogen produces severe, intractable, and recurrent non-bloody diarrhea, and in some cases biliary tract and/or respiratory tract infections in immunodeficient hosts. Gut infections with this pathogen tend to demostrate various degrees of diarrhea, abdominal cramps, flatulence, general fatigue, nausea and vomiting. Many sporadic diarrheal cases due to this protozoa are thought to exist in Japan, however, when such patients seek medical care, they are often misdiagnosed to have culture-negative and antimicobial-refractory diarrhea.

Large waterborne outbreaks of diarrheal illness due to this pathogen have been reported in Japan involving 461 patients (in Kanagawa-ken, 1994), 8,812 patients (in Saitama-ken, 1996), 129 and 170 patients (in Niseko and Toyako lake side in Hokkaido, respectively, in 2000).

From a prospective study performed at Tokyo Metropolitan Komagome Hospital from 1990 to 2000, *Cryptosporidium* occysts were positively detected in 18 out of 508 specimens (3.5%) from diarrheal patients who had recently traveled overseas, in none of 605 specimens (0%) with no history of overseas travel, and in 4 out of 140 specimens (2.9%) from HIV infected patients.

◆Cyclosporiasis

Cyclospora cayetanensis causes non-bloody watery diarrhea which clinically resembles cryptosporidial diarrhea except for the fact that diarrhea with *C. cayetanensis* tends to be recurring. The administration of sulfamethoxazole/trimethoprim is usually effective for this kind of diarrhea. Cyclosporiasis in immunodeficient patients tends to lead to diarrhea with longer duration than that observed in normal hosts, and this infection is usually non-fatal even in these patients.

A few sporadic cases of *Cyclospora* enteritis have been reported in Japan. A study based on 1,071 stool specimens observed from patients at Tokyo Metropolitan Komagome Hospital from 1996 to 2001 indicated the positive rate of this protozoa among diarrheal illness to be 0.7% (3 out of 410 stool specimens were positive) in patients with a recent history of overseas travel, none (0/513 specimens) in indigenous diarrheal patients, and 0.7% (1/148 specimens) in HIV-infected patients.

S－I－3) EMERGING AND RE-EMERGING ARBOVIRAL INFECTIOUS DISESES

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Arboviruses are one of the major pathogens of emefging and re-emerging infectious diseases. West Nile fever (WNV) epidemics since 1999 in the United States and dengue outbreak in Hawaii during 2001-2002 are valuable lessons. Global warming is a favorable condition for insects such as mosquitoes and ticks. We need to pay more attention to emerging and re-emerging arboviral infectious diseases in Japan as well as in other areas of the world.

Key words: West Nile virus, dengue virus, imported case

S－I－4) CURRENT STATUS OF EMERGING AND RE-EMERGING BACTERIAL INFECTIOUS DISEASES

TSUYOSHI NAGATAKE

S－II－1) DETECTION OF NEW ENDEMIC AREAS OF CUTANEOUS LEISHMANIASIS IN PAKISTAN: A SIX-YEAR STUDY

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Cutaneous Leishmaniasis (CL) is endemic in Pakistan and is widely spreading. We report some new endemic areas of CL in the country. A total of 1210 cases of CL visited our department from 1996 to 2001. Among them, 760 were the residents of Jacobabad, Larkana and Dadu districts of Sindh province and had never been traveled to the endemic

areas before. These districts have never been reported/recognized as endemic for CL. Others were the residents of endemic areas of Balochistan province. All the patients were between 2 and half months and 65 years of age. Three hundred and ninety two patients were females and 368 were males. Duration of the disease ranged from 2 months to one and half year. Most of the patients had single lesion on the face and/or extremities. Clinically the disease was classified as dry popular type, 407 cases: dry ulcerative type, 335 cases; and wet ulcerative type, 18 cases. No any case of muco-cutaneous or visceral leishmaniasis was found during this period. Diagnosis was made on clinical presentation; Giemsa stained smear test; histopathological; and polymerase chain reaction (PCR) results. Smear test was positive in 845 cases, while 365 cases were histopathologically positive. Ultrastructural study was performed using the specimens of few cases. *Leishmania* parasites were detected in the dermal tissues as well as macrophages. About 200 cases were studied by PCR, among them 92% were positive for *Leishmania* parasite. All the cases were treated with the meglumine antimonate 600 mg/day in adults and 15 mg/kg/day in children as intramuscularly for 20 consecutive days.

On the basis of our findings we propose that Jacobabad, Larkana and Dadu districts could be considered endemic for CL. Wet and dry type lesions indicates the presence of both the *L. (Leishmania) tropica* and *L. (L.) major* in this tropical region. This will be confirmed by sequencing technique in future.

S－II－2) CURRENT STATUS OF LEISHMANIASIS IN ECUADOR

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The leishmaniasis is endemic throughout Ecuador and the incidence is increasing annually. Cases had been reported from all geographical regions mainly in populations living in rural areas. Recent studies found that the disease is wide spread in most provinces and is considered a health problem in the country. National data showed that the rate of cases increased from 4.36 in 1998 to 20.24 in year 2001 per 100000 inhabitants.

The localised cutaneous form (LCL) represents more than 95.0% of the total cases. The common LCL form is ulcer, but non-ulcerated forms such as papulas, plaques and nodules are also seen. Diffuse cutaneous form (DCL) is very rare. Mucocutaneos form (MCL) had only been reported from the Amazonian lowlands. In an active survey we found 13 cases of MCL in the region. A confirmed case of DCL was reported to be produced by *L. (L.) mexicana*.

Using molecular techniques, seven species of the genus *Leishmania* has been identified from the Pacific side: *L. (V.) braziliensis, panamensis, guyanensis, equatorensis, L. (L.) amazonensis, mexicana*, and *L. (L.) major*-like; two from the Andes: *L. (L.) mexicana* and *L. (L.) major*-like and two from the Amazon: *L. (V.) braziliensis* and *L. (L.) lainsoni*.

Confirmed vectors of *Leishmania* in the Pacific side are *Lutzomyia trapidoi, hartmanni* and *gomezi*. More recently Dujardin demonstrate cryptic species in *Lu. trapidoi*. In the Andean foci *Lu. ayacuchensis* has been incriminated as a vector. In the Amazonian no vector has been found infected, though there are several man-biting species *Lu. serrana, Lu. nevesi*, etc. Non-human hosts of *Leishmania* detected to date are sloths, squirrels, anteaters, rats, kinkajous and canines. Dogs are recorded as hosts of Andean *L. (L.) mexicana*.

In most endemic areas, leishmaniasis is well known and patients use several “folk methods” for treating it. The drug used is Glucantime provided by the government free of charge. Other drugs such as mefloquine, artesunate, allopurinol, paromomycin and fosfomycin had been tested in small trials with variable efficacy.

Current control measures rely on chemotherapy to alleviate the disease. No vector control has been implemented. Extensive vaccination trial has demonstrated that a cocktail of five killed *Leishmania* induces significant protection. A new phase III study (Biobras) with killed promastigotes+ BCG, is in final step and under revision by TDR/WHO.

S－II－3) EVOLUTION OF THE CYTOCHROME *B* GENE OF *LEISHMANIA* AND *ENDOTRYPANUM*

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Leishmaniasis is caused by parasites belonging to genus *Leishmania*. Human infections display a spectrum of manifestations ranging from cutaneous, mucocutaneous to generalized systemic visceral disease. The genus *Leishmania* is taxonomically classified into subgenus *Leishmania* and subgenus *Viannia*. The parasites reside in different regions of the digestive organ of sandfly, *Phlebotomus* (in the Old World) and *Luztomiya* (in the New World), and their classification is yet to be completed. The cytochrome *b* gene (Cyt *b*) is contained in the mitochondrial genome and encodes an enzyme in the respiratory chain of mitochondria. The Cyt *b* gene has been used for phylogenic studies for animals, plants and fungi. Here, we report determination of complete nucleotide sequences of the Cyt *b* gene in parasites belonging to the genus *Leishmania* (14 strains) and those belonging to the genus *Endtrypanum* (4 strains). Phylogenic analysis of these parasites, based on the difference of Cyt *b* gene sesquense, agreed well with the previous classification of above three groups. The difference was consistent with different geographic distribution of parasites and with different clinical manisfestations associated with parasites. In addition, we present recent progress in our gene expression profile analysis of *Leishmania*-infected macrophages. We show that expression of the genes encoding MCP (macrophage chemoattractant protein)-5 and STAT (signal transducer and activator of transcription)-1 are up-regulated during infection as examined by the quantitative real-time RT-PCR analysis.

S－II－4) ADVANCE IN DEVELOPMENT OF ANTI-LEISHMANIAL DRUGS AND RESEARCH ON MECHANISMS OF DRUG RESISTANCE IN *LEISHMANIA*

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Pentavalent antimonial drugs have been used for treatment of leishmaniasis since 1940's. Antimonial drugs, however, require to be injected and produce side effects, thereby oral safety anti-leishmanial drugs have long been desired. Sterol biosynthesis pathway in *Leishmania* appears to be very similar to that in yeast. Thus, oral anti-fungal drugs have also been used for chemotherapy of leishmaniasis. We found that SS750, a synthesized new triazol anti-fungal compound, was effective on both cutaneous and visceral leishmaniasis in mice. Skin lesions of mice, which orally received SS750, were smaller than those of untreated control mice infected with *Leishmania amazonensis*. Parasite burden in the liver was low in mice orally treated with SS750 than that in control mice infected with *L. donovani*. These results suggest that SS750 may be applicable for treatment of various types of leishmaniasis, although miltefosine (hexadecylphosphocholine) is now the most hopeful oral drug for treatment of visceral leishmaniasis.

Studies of drug resistance mechanisms in *Leishmania* is necessary for more rational use of drugs and drug combinations to minimize or circumvent resistance, since antimony resistant *Leishmania* species are found in endemic areas, especially in India. Studies on multidrug resistance in mammalian cancer cells lead the discovery of the ATP-binding cassette (ABC) proteins, which form one of the largest protein families and its members are present each

kind of organism from bacteria to human. Among the ABC proteins, MDR1 (multidrug transporter or P-glycoprotein) and MRP1 are most involved in drug resistance in human. MDR1 is localized in the plasma membrane and can extrude a wide range of structurally unrelated hydrophobic toxic compounds from the cell. MRP1 transports both hydrophobic anticancer agents and glutathione conjugates. In *Leishmania*, currently two ABC transporters can mediate drug resistance in experimental conditions. PGPA, a member of MPR subfamily, appears to transport antimony- and arsenite-thiol conjugates into intracellular vesicles. Leishmanial MDR1, a homologue of mammalian MDR1, can confer resistance to a number of anticancer drugs of the mammalian MDR1 spectrum and appears to be localized in the multivesicular tubules of lysosomes. We have cloned and characterized a new member of ABC transporter, LaMDR2 in *L. amazonensis*, which can confer resistance to 5-fluorouracil by probably exocytosis. These results suggest that drug resistance mediated by ABC proteins in *Leishmania* is related to secretory and exocytotic pathway of the parasites.

S－III－0) FOR THE FRUITS OF TROPICAL MEDICINE

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The tropics are inevitaby hotbeds of pathogen transmission because of its various natural and socio-economical conditions, where there are all of the disease transmission structures including pathogens, vectors and diseases, which investigators have been pursuing. The final goal of “Tropical Medicine” is “To prevent, diagnose and treat diseases be there, mainly composed of infectious disease”, and “Tropical Medine” is a scientfic field containing all fields concerning it. For the fruits of “Tropical Medicine”, in other words, in order to contribute to the prevention, the diagnosis and the treatment of diseases there, what should we do in future?

In this session, young scientists will introduce their works in the past and now at first, and then survey a future concerning the study. Furthermore, for the fruits of “Tropical Medicine”, we would like to discuss about

1. What are deficient in?
2. How can we get the deficient one (knowledge, technique, skill, idea, personal connections, language ability, funds, a position, fields etc.) ?
3. What kind of strategies we can map out to survive in each organ while pursuing Tropical Medicine?

, as an individual investigator or as an organization, Japanese Society of Tropical Medicine,.

I hope that this session will encourage young scientists in making a dream come true.

S－III－1) SYNTHESIS OF USEFUL ANTIBODY MOLECULES USING THE COMBINATORIAL LIBRALY AND THE PHAGE DISPLAY TECHNOLOGIES

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DEN infection has been a big concern in a DEN endemic society for decades all over the world. According to the investigations conducted by the WHO, the worldwide incidence of dengue fever (DF) is estimated from twenty-five million to fifty million. An incidence of the Dengue shock syndrome (DSS), an atypical and much more serious response to the viruses characterized by hemorrhage is considered more than five hundred thousand in the whole world.

Recently, we have constructed a phage library with material of DEN hyper-immune higher primate and from which a panel of Fab specific to DEN virions were isolated. Biological analysis of those Fabs were performed and described. Those Fabs obtained through a combination of combinatorial library and phage display technologies

should be valuable for use in passive prophylaxis or therapy for humans against dengue infection.

When one attempts a collaborating study with an institute of distinct culture, attention should be paid on the following points. Choose a theme appropriate to demands of the society where the study would be conducted. Have the clear objective. Prepare the detailed work plan. Share detailed information among the study team, Make an effort to reduce mistake-prone steps. Design and perform a pilot study in order to obtain information needed for refining the study. Distribute the results and interpretation obtained from the study to each person or institute involved. Careful considerration should be paid to the human rights when collecting and handling human samples.

We are aiming at the preparation of human Fabs possessing anti-dengue neutralization activity through the combinatorial science. Is it possible to construct an Fab library using human material obtained from healthy volunteers who had been recovered from DEN infection after obtaining full consent to participation in a study in DEN endemic area? The constructed library should contain higher population of phage clones displaying Fab specifically neutralize DEN and has an obvious advantage in isolation of DEN neutralizing Fabs over other libraries.

Curretly, a large number of epidemiological surveys as well as sample collections for determining pathogens or pathogenesis of microorganisms have been conducted in the field of tropical medicine, however, it also possess a potential dimension for supplying useful molecules for prophylaxis or therapeutic applications against certain infectious diseases.

S－III－2) MOLECULAR MECHANISM OF MALARIAL SPOROZOITES INFECTION INTO THE MAMMALIAN LIVER

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Even today, malaria is one of the most devastating infectious diseases, and it kills more than two million people in the world at a year. The emergence of resistant parasites makes it more difficult to control this disease. Moreover, preventive method, such as vaccine, has never developed yet.

Malarial sporozoites injected by mosquito into mammalian host rapidly enter circulatory system, then infect hepatocytes, where they develop to thousands of merozoites. Therefore sporozoite infection of hepatocytes is the obligatory step for malaria transmission to mammalian host. However its molecular mechanism is unclear yet. Aimed at elucidation the molecular basis of parasites infection, we applied a reverse genetial method, as ‘post genome study’.

As it was reproted that infectivity to the liver was acquired after sporozoite invaded the mosquito salivary gland, we constructed EST database of *P. berghei* sporozoites collected from salivary gland. Then, by gene targeting method, function of some proteins expressed in salivary glands sporozoite were analyzed. As focused on parasite-host interactions, putative membrane associated or secretory proteins were selected preferentially. Here we report that a salivary gland sporozoite specific protein was identified and it was involeved in parasites’ infectivity of the mammalian liver.

By the way, it was known that administration of radiation attenuated sporozoites induces preventive immunity against malaria. Thus it was believed that some sporozoite proteins should be good targets for developing malarial vaccine. Our strategy will faciliate on understanding of the molecular basis of malarial of vertebrate hosts, and may lead to the development of vaccines against malarial transmission.

S－III－3)　INVOLVING TROPICAL MEDICINE PRACTICALLY

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The presentation focused on two areas.

The first outlined the various approaches in participating international health in the tropical regions. Having learnt from personal experiences, from working as a staff member in the health service providing facility such as a hospital, to a project co-cooridnator in the ministry of health, the disease control programme onto the existing health infrastructures seems feasible approach. One of these infrastructures is the school system. Schools have increasingly been recognized as one of the most convenient and logical springboards from where to promote healthy behaviours and to deliver certain health services. In the context of de-worming campaigns, schools offer one of the most efficient and effective channels to one of the most vulnerable groups.Moreover, the ability of non-medical personnel to deliver the drugs means that the number of children and the speed at which they can be reached is massively accelerated.

The second part of the presentation focused on the parasite control activities at Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC) of the Kenya Medical Research Institute. ESACIPAC is one of the three centres of the Global Prasite Control Initiative, also referred to as the Hashimoto Initiative. Its mandate is to coordnate capacity building for parasitic diseases control activities in the regional countries, namely, Kenya, Uganda, Tanzania/Zanzibar, Malawi, Zambia, Botswana and Zimbabwe. Its primary functions include training of personnel and networking among them. ESACIPAC will involve implementation of school based parasite control activities in Kenya. The strategy for coodinating effort of all other technical co-operation project funded by JICA (Japan International Co-operation Agency) is also discussed.

S－III－4)　WHAT IS THE TROPICAL MEDICINE IN JAPAN? - A QUETION FROM A YOUNG SCIENTIST -

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So-called “Tropial medicine” consists of two research areas. First area pursuits to study a basic biological aspect of the tropical diseases and develops tools to prevent and/or treat human beings from diseases by biomedical research. The other area pursuits to study a public health system to develop strategies to apply tools produced. These two areas simultaneously contribute to solve the tropical health problem. Increasing funding in the basic reseach area of the infectious diseases has stimulated the activity of this area and the number of young researchers has increased in the past 10 years. Furthermore, based on the Hashimoto Initiative, projects aiming human resource deveopment and information networking to control parasites in Asia and Africa were launched with the strong supports by JICA. However, despite of these situations, there is a big gap between the basic research area and the disease control area. One of the reasons of this gap can be explained by the limited human resources of this area, especially in the research area conducted in the tropical field. Improved health situation in Japan has reduced not only infectious diseases, but also reseachers in this area. As mentioned at the beginning, successful control of the diseases requires tools and strategies, thus our current need is to promote the authentic epidemiology in order to create better strategies to combat tropical diseases. Here I propose 3 activities, 1) Promoting more field studies in the tropical countries to stimulate the academic side of this area; 2) promoting young researchers in this area and recruit researchers from other areas to increase the population of this area; and 3) encouraging to attend international meetings of tropical medicine to upgrade the quality of this area. These activities would produce fruitful outcomes from Japanese Society of Tropical Medicine in the next 20 years.

S－IV－1)　ADEQUATE TREATMENT AND EARLY DIAGNOSIS OF LEPROSY

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In fact, a large number of patients with leprosy rapidly decreased by multi-drug-therapy(MDT). But newly detected patients with leprosy has been increased in number in the endemic areas, especially in India, Brazil and Southeast Asia etc. Three important measures of leprosy control are to be discussed, namely chemotherapy, early detection in the early stage of leprosyand the prejudice for the disease.

1) Chemotherapy; Four leprosy cases with sequelae caused by neuritis which increased after starting MDT and two cases without sequelae were shown. For a patient with borderline group leprosy, who showed severe neuritis and deformity of the fingers after starting of the medication, the chemotherapy was considered to be inadequate. A patient with an annular erythema on the on left buttock, diagnosed as to be tuberculoid leprosy, received a modi-fied MDT, comprising of 450mg of RFP per monthy and 75mg of DDS for a month, healed completely within a few months. Two contrastive patients with borderline group leprosy showed similar infiltrared erythema on the right face were reported. In the course of chemotherapy, the deformity of face became obvious and flacid palsy of the right orbicularis oculi muscle appeared in a borderline tuberculoid-middle borderline leprosy patient. On the other hand, another patient with borderline tuberculoid leprosy healed without any sequelae. As shown in the above mentioned cases, each of them showed different and various responses to chemotherapy during the course of the treatments. Therefore the simplified chemotherapy for the simplified clasiffication considering sensory loss, neuritis and the site of lesion are need-ed to prevent the sequelae. For example, n1 indicate the type of leprosy with sensory loss and minor neuralgie, n2 indicate the type of leprosy with sensory loss and severe neulargie. h1 indicate the site of lesion at the axillary, inguinal, perineal lesion, buttock and scalp, h2 indicate site of lesion at the hands, feet-leg, face and ear.

2) Early detection; Indeterminate leprosy case healed completely in a few months. Therefore, it is very important for elimination of leprosy to detect leprosy patients in their early stage of the disease. For this purpose, paramedical and non-medical staffs may play a great role.

3) Prejudice; Though the prevalence rate of leprosy in Japan became below 1.0 per 10.000 approximately 30 years ago, prejudice for the disease is still remain especially in old people.

Japanese medical personnels can assist to resolve the above mentioned problems and shortening the period for elimination or eradication of the dis-ease in the endemic areas.

S－IV－2)　AROUND THE FUNDAMENTAL LEPROSY RESEARCH －HOW TO PROCEED THE RESEARCH OF A CLASSICAL INFECTIOUS DISEASE IN GENOME ERA －

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Leprosy is a classical infectious disease caused by *Mycobacterium leprae*, which was discovered by G. A. Hansen in 1873. The disease has been documented since antiquity and still continues to be endemic in some developing countries. *M. leprae* is an exceptional bacterium because of its long generation time and no growth in artificial media. Entire sequencing of the bacterial genome revealed numerous pseudogenes which might be responsible for the very limited metabolic activity of *M. leprae*. The establishment of effective combination chemotherapies in 1970s prove a great boon to the victim of leprosy. Multiple drug therapy (MDT), which consists of several antibiotics linked to simplified diagnostic regimen, has become increasingly popular in the countries where the disease is endemic. A significant drop of the disease prevalence has been achieved worldwide, and the endemicity of leprosy remains in few countries. To achieve the worlcd without leprosy, we have many questions tobe answered. How the bacteria are disseminated?

Why intrafamilial infection is likely? Is there any source of infection other than symptomatic patients? Moreover, no diagnostic marker nor vaccines are available. A compendium for the current leprosy research is provided and the future prospects are also discussed.

S－IV－3) PRESENT SITUATION OF HANSEN'S DISEASE IN THE WORLD

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Lrprosy patients in Japan was reported that about 30,000 in 1900, which decreased to about 16,000 by 1919. From roughly 1955, due to the improvement of public health and appearance of remedies, new patients decreased repidly. Recently, there are less than 10 new patients per year. On the other hand, non-Japanese patients (imported cases) have been on the increases since roughly 1991, counting about 10 patients per year. It is, therefore, anticipated that there will be no leprosy patients among Japanese in near future. Non-Japanese patients, however, appear among a large number of employees aged 20s～30s from Brazil and the Philippines where leprosy is still endemic.

Global situation owing to multidrug therapy (MDT) pursued by WHO; more than 10 million leprosy patients worldwide were cured from 1985 to the end of 2001. The registered cases decreased to 635,404 by the beginning of 2002 and the prevalence was 1.0 per 10,000 population, which is a 88% reduction from 1985. After treatment for 6 months in PB cases and for one year in MB cases, they are regarded as cured and withdrawn from the WHO. The relapse rate is about 0.1% per year. Only few MDT-resistant cases have been found. Physical disability may be seen after peripheral nervous impairment (two to three million cases), and many have difficulty returning to and attaining social lives. In spite of the promotion of MDT by WHO, as many as 760,000 new patients are still registered every year, and that is presumably due to improved diagnosis and enhanced surveillance for many new cases including ones overlooked in the past. The countries with many yearly registered new leprosy patients during the year 2001 are; India (617,993), Brazil (41,070), Indonesia (13,286), Bangladesh (10,740), Myanmar (9,684), and Nepal (13,830).

Full control of leprosy has eluded mainly in Angola, Brazil, India, Madagascar, Mozambique, Myanmar and Nepal. These countries are committed to stepping up leprosy control activities. Access to information, diagnosis and treatment with MDT is essential Information campaigns about leprosy in high risk areas are crucial so that patients and their families, who were historically ostracized from their communities, are encouraged to come forward and receive treatment. Today, diagnosis and treatment of leprosy is easy. Essential work is being carried out to integrate leprosy services into existing, general health services. This is especially important for communities at risk for leprosy, which are often the poorest of the poor and under-served.

S－IV－4) PRESENT SITUATION OF LEPROSY DISEASE IN MYANMAR

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Leprosy Control Programme, Myanmar (Leprosy Research Center, NIID, Japan)

The Union of Myanmar is one of the hyper endemic countries with leprosy disease. Although the leprosy disease burden has significantly reduced to a low level in Myanmar, leprosy elimination activities need to intensify to achieve the elimination goal in time. National Leprosy Elimination Programme has been already committed to reach the elimination at the national level by reducing the prevalence below one case per 10,000 populations by the end of year 2003. To achieve the elimination of leprosy, the programme was fully integrated in Basic Health Services. It was decided in 1969 to try a gradual handling over of responsibilities of the vertical leprosy control services to the basic health services personnel. In this presentation I present "the present situation of leprosy disease in Myanmar"

such as disease burden, integration, detection of new cases, prevalence and detection ratio, routine, activities, community awareness and monitoring and supervision. And I also present “cooperation of Myanmar and Japan (JICA) regarding the leprosy disease”. With the cooperation of other organizations such as WHO, JICA and NGOs, we could accelerate the elimination activities towards the goal and also other aspects regarding with leprosy disease. There is a period very near to elimination of leprosy in Myanmar. We conduct the acceleration and sustainability of leprosy elimination activities. The application of multi drug therapy (MDT) is progressing rapidly towards the goal of elimination of leprosy as a public health problem. Preparatory activities for post-elimination strategies are also considered including prevention of disability, disease surveillance, control and prevention tools and etc.

S－IV－5) ACTIVITES OF JICA PROJECT FOR LEPROSY CONTROL AND BASIC HEALTH SERVICIES IN MYANMAR

EIJI NAGAO

National Leprosarium Osima Seisho-en

JICA project team has carried out many kinds of provisions against National Programme for Leprosy Elimination in Myanmar, from 2000.

We have conducted training courses of midwives, laboratory technicians, doctors, physiotherapists, nurses, prosthesis therapists, shoemakers, and persons affected leprosy (PALs).

We recommend (1) that special actions for new case detection should be continued, until true elimination on townships and villages level is achieved, (2) that various kinds of useful training should be continued, and (3) that various kinds of effective enlightenments should be held in communities, because leprosy is a chronic disease with extermely long incubation term and PALs with disabilities need many kinds of aids.

S－V－1) ROLE OF THE JICA ZAMBIA PROJECT IN AIDS CONTROL IN SOUTHERN AFRICA

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The AIDS epidemics is still one of the biggest problems in global health issues. At the end of 2002, over 60 million people were infected with HIV. Forty million are still living with HIV and 3 million died during the year 2002. More than 90% of these infected people are living in developing countries and got infected with HIV through heterosexual contact. Zambia has a high seroprevalence of HIV-1. One forth of the adult population in Zambia is infected with HIV. For the past 10 years education has been emphasized to control the spread of HIV in Zambia. While education is still very important, it is, however, not enough to control the HIV epidemic because the number of infected people is still increasing. Further more over 1 million Zambians are living with HIV.

From 1989 to 2000 JICA Zambia infectious disease and infectious diseases control programs established the University Teaching Hospital (UTH) Virology Laboratory to examine viral diseases such as polio, measles, influenza and HIV. For HIV, we introduced flow cytometory and PCR technology at the laboratory in Zambia. In 2001 the JICA HIV/AIDS and TB control project was started in Zambia and this project is contributing to the control of the HIV epidemic. Currently the Virology Laboratory is the only laboratory which can evaluate quality assurance of HIV serological testing and monitoring the condition of HIV-infected patients.

To control HIV/AIDS, this program is closely working with other working groups such as the national AIDS secretariat, voluntary counseling and testing and care program (VCT), prevention of mother to child transmission program (PMTCT), and treatment and vaccine program. The Virology laboratory is currently conducting training and quality

assurance of HIV serological testing for technologists at VCT sites, monitoring of drug-resistant HIV-1 and HIV-1 strain surveillance for vaccine development.

I hope through this program many young Zambian and Japanese experts will gain experiences to fight infectious diseases and will develop better strategies to control HIV/AIDS.

S－V－2) TUBERCULOSIS CONTROL IN NEPAL AND JAPANESE MEDICAL COOPERATION

KATSUNORI OSUGA

Research Institute of Tuberculosis Japan Anti-Tuberculosis Association

In the early 1960s, a Japanese doctor arrived in the Kingdom of Nepal. His name was Noboru Iwamaura. As a mission doctor, he worked in the mountainous outskirts of the Himalayas, visiting villages searching for tuberculosis patients. Many locals still remember Dr. Iwamura, one of the nost well known Japanese names in Nepal to date. With widespread poverty, however, tuberculosis remained the biggest threat to public health in Nepal.

Following pioneers from the private sector, the Japanese Government started to provide assistance to control tuberculosis in the 1970s. The National Tuberculosis Center was constructed with the Grant Aid scheme in the 1980s. Today, this center functions not only as the headquarters of the National Tuberculosis Program, but also as the regional TB center for South Asia (SAARC TB Center). Coupled with the construction of the TB center, a technical cooperation project by JICA was also carried out. Short course chemotherapy using Rifampicin, which was uncommon in developing countries at that time, was also tried as a pilot study. Technical expertise was provided from the Research Institute of Tuberculosis (RIT), Tokyo. Apart from Japan, no major donors or international agencies provided substantial assistance for TB control in Nepal during this period.

It was in the mid 1990s, when the international arena began to focus on TB control. WHO Iaunched the new strategy called “DOTS” and the World Bank supported its cost-effectiveness. Since then, many new donor partners, such as Norway, UK, and WHO, joined the effort to combat TB in Nepal. With multiple partners providing assistance to the Government of Nepal in different schemes, misunderstanding and friction sometimes occurred. The importance of coordination among donors and UN agencies was recognized.

Today, nearly 90% of the population in Nepal has access to DOTS, with over 85% treated successfully. Japanese technical coooperation in Nepal now has broader aspects. The current JICA project is focusing on “lung health”, targeting childhood ARI and smoking cessation, as well as TB control in difficult population groups and areas. Every year, medical doctors and paramedics from Nepal are trained at RIT; more than 70 Nepalese have been trained in the past 40 yaers. It is now well recognized that Japanese technical cooperation, both through governmental and non-governmental sectors, has made a significant contribution to human resource development and appropriate technology transfer for TB control in Nepal. Howrver, the approaching HIV epidemic, apreading poverty, and social instability in recent years, all pose a major threat to TB control in Nepal. Long-term collaboration appears necessary to sustain the fight against tuberculosis in Nepal.

S－V－3) PROBLEMS OF IMPLEMENTING THE MALARIA CONTROL PROJECT

HIROJI KANBARA

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We, the Institute of Tropical Medicine, Nagasaki University have began the JICA partnership project "Malaria control at Lombok and Sumbawa islande in Indonesia" since November, 2001. Since activities necessary for the project included various fields, we, first, formed necessary sections and appointed a responsible person in each section as follows. 1) Section for malaria parasite survey and control. The section was devided into two pars ① Regular monitoring survey and ② Case detection and treatment 2) Section of entomological survey for vector mosquitoes 3) Section of vector control 4) Section of social survey including education programs for villagers and concerned staff.

Besides, the regular meetings were held four times a year in which Japanese representatives including me, representatives from Ministry of Health, Jakarta, from Tropical Disease Center, Airlangga University, from Nusa Tenggara Barat (NTB) provincial health office and from two district health offices at West Lombok and Sumbawa participated. These activities evoked the following problems.

1) Malareia epidemiological conditions were different according to geographycal and environmental conditions. In Muninting area of West Lombok district two distinct malaria endemics were found, one in the coastal areas and the other in the forested and hilly areas.
2) Although we must adopt proper control methods based on endemic characters in each area, we have only limited number of control methods, and don't have a fixed control manual for each character.
3) Malaria control activities are feasible only under systematic coopereation of various organizations, especially the comprehensive cooperation from villagers.
4) Intimate relationship between the central government and local governments must be established.

In the present project we are acting as a coordinator and as a financial supporter to establish a model of control strategy fitting for endemic conditions in each area. For this purpose we despatched two Japanese young staff for long stay after training them. This typr project will become important for our country to pactise medical cooperation or support in developing countries. However, the following problems in our country should be considered for further development of medical cooperation.

1) Shortage of specialists and practical personnel for control activities.
2) Low evaluation for persons concerning practical works in the field of preventive medicine including malaria control.
3) Shortage of educational organs to teach public health in developing countries.

S－V－4) HASHIMOTO INITIATIVE AND OKINAWA INFECTIOUS DISEASE INITIATIVE: JAPAN'S CHALLENGE TO GLOBAL PARASITE CONTROL

TSUTOMU TAKEUCHI

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In May 1998, at G8 Summit in Birmingham, Mr. R. Hashimoto, the prime minister of Japan, made a proposal that the G8 should take a leading role in promoting parasite control efforts and contributing to the world health. Mr. Hashimoto's proposal was endorsed and the resolution to this effect was incorporated into the official communique with the statement that "Control of parasitic diseases including malaria should be implemented". Since then, Mr. Hashimoto's proposal has been called "Hashimoto Initiative"(HI).

As the basic strategy, Hashimoto Initiative raised 4 issues, First, it stressed the necessity of effective international cooperation for parasite control. Than it raised the active pursuit of research as the scientific basis for parasite control and active implementation of the control projects. Finally, the initiative requested each of G8 countries to reinforce the capability to deal with parasitic diseases.

Among Japan's own activities by HI, the most important contribution is the establishment of research and training centers for parasite control in Asia and Africa. As the

foothold for this purpose, Mahidol University in Thailand, and the Kenya Medical Research Institute (KEMRI) in Kenya, and the Noguchi Memorial Institute of Medical Research (NMIMR) in Ghane were chosen. These centers will have functions of effective training, technical support, operational research and information/human networking. To promote such activities, collaboration with international organizations will be accelarated. More importantly, the south-to-south cooperation will be strongly promoted. Among them, the center at Mahidol University became functional earlier. It is called the Asain Center of International Parasite Control (ACIPAC). Those at KEMRI and NMIMR are called East and South African Center of International Parasite Control (ESACIPAC) and West African Center of International Parasite Control (WACIPAC), respectively, and have already initiated their own activities.

In addition to HI, Japanese Government Iaunched Okinawa Infectious Disease Initiative at the Kyushu-Okinawa G8 Summit in 2000, where HIV/AIDS, tuberculosis, malaria were listed as the major target of international collaboration. As the chairman country, Japan also proposed parasitic diseases and polio. In this sense, both Hashimoto Initiative and Okinawa Infectious Disease Initiative are expected to be effectively coorodinated in near future, taking a leading role in parasite control at the global level.

S－V－5) THE PROGRESS AND THE DIRECTION OF THE ASIAN CENTRE OF INTERNATIONAL PARASITE CONTROL PROJECT

NOBUHIKO NAGAI, NORIAKI TOMONO, MITSUHIKO IWASHITA and SOMEI KOJIMA

Asian Centre of International Parasite Control (ACIPAC)

At the G8 Summit Meetings in 1997 and 1998, former Prime Minister Ryutaro Hashimoto proposed that the G8 countries should cooperate to support countries affected by parasitic desease. Under the Hashimoto Initiative, Japan International Cooperation Agency (JICA) decided to set up one centre for parasitic disease control in Asia (Asian Centre of International Parasite Control, ACIPAC) and two in Africa. ACIPAC was launched as a five-year project in March 2000. The partner countries include the Greater Mekong Sub-region countries of Cambodia, Lao PDR, Myanmar, Thailand, and Vietnam. The overall goal is to strengthen parasite control programmes in the partner countries through human resource development. JICA's regional technical cooperation programme supports ACIPAC activities.

The slogans "Save Wormy Schoolchildren" and "Save Schoolchildren from Parasites" have been chosen to promote this effort. ACIPAC will advocate parasite control through school health programmes. ACIPAC will utilize South-South Cooperation through information sharing and human resource networking, to help achieve the goal of parasite control.

In the second fiscal year (2001), the first three-month training programme "School-based Malaria and Soil.-transmitted Helminthiases Control for Programme Managers" was conducted from September 17 to December 7. Twenty-eight trainees who are administrators and technical officials from MoH, MoE and provincial Health Offices participated in this training. Five participants were invited from each of ACIPAC's partner countreies. There was one participant each from Japan, the UNICEF Laos office and the Kenya Medical Research Institute (one of the Hashimoto Initiative African Centres).

After the ACIPAC training, ACIPAC started a small-scale pilot project in each country. Planning for these pilot projects was done by the students during the ACIPAC International Training. In February and March 2002, as a first step, baseline surveys of malaria and parasite infection and socio-behavioral studies were conducted in Laos, Cambodia, and Vietnam. Myanmar will start its baseline study early next fiscal year. At the end of March, ACIPAC held an international workshop, and some of the graduates presented the results of baseline study.

In Thailand, ACIPAC has two model projects. They are in Ratchaburi province and Nakorn Si Thammarat province, which are endemic areas for malaria and soil-transmitted helminths, respectively. These model projects were utilized for field practice in the ACIPAC Intermational Training Course.

ACIPAC is also planning an information-sharing network for parasitic disease control. ACIPAC has launched its Web site (http://www.tmacipac.mahidol.ac.th).

S－V－6) ROLES AND CHALLENGES OF JAPAN'S ODA FOR GLOBAL HEALTH

OSAMU KUNII

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[Background] Japanese government placed health sector among priority areas in its Official Development Assistance (ODA), and announced "Global Issues Initiative on HIV/AIDS and Population" in 1994, "International Parasitic Diseases Control (Hashimoto Initiative)" in 1998 Birmingham Summit and "Okinawa Infectious Diseases Initiative" in 2000 Kyushu-Okinawa Summit.

The Okinawa Infectious Diseases Initiative (IDI), reinforcing the Hashimoto Initiative, has six basic strategies: (1) Supporting institutional building of health sector with ownership of the recipient countries, (2) Focusing human resources development, (3) Strengthening partnership with civil society, donor countries and international organizations, (4) Supporting South-South cooperation, (5) Promoting research activities, (6) Promoting public health approaches at the community level.

[Achievements] IDI pledged US$ 3 billion in five years and has already spent US$ 1 billion in 1.5 year. It has provided over 50 bilateral projects and has contributed to international organizations such as WHO, UNICEF, UNAIDS, and to Japanese/International NGOs. In particular Japan established "Policy and Human Resource Development Fund (PHRD Fund)" in World Bank, "Human Security Fund" as the trust fund of UN to support their programs, which have been made use of over 100 countries. Besides IDI, Japan has pledged US$ 200 million to the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), 2nd largest contribution following the U. S..

[Challenges] More collaboration with stakeholders at the international/national/regional level is required to make most of Japan's ODA. Especially it is crucial to make systematic and strategic coordination in (a) human resources from Japan Overseas Cooperation Volunteers (JOCV), NGOs to professionals, (b) organizations from JICA/JBIC to private enterprises, (c) programs from infrastracture buildings to community development. Japan could contribute to global partnerships to promote cooperation in development assistance.

I hope the Japanese Society of Tropical Medicine would play more active role in Japan's ODA and NGOs' assistance, providing technical assistance, researches directly contributing to ODA, and human resource development of the tropics and Japanese young generation.

S－VI－1) DIFFICULTIES ENCOUNTERED IN FIELD RESEARCHES, WHICH WERE USED AS EXCUSES

MASAAKI SHIMADA

Research Center for Tropical Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

For the symposium I was given an assignment. What the chairperson requested me to talk was my personal experience in field works. Therefore the subjects I point out here are mostly on my personal experience.

1. Selection of target population

As is usual in any field work, target population cannot always be selected based on scientific evidences. A community is usually selected according to the administrative boundaries and therefore a political will always influences the selection. Sample size may not be satisfactory. Sampling methods we apply sometimes do not work well because of the complaint of both selected and unselected people.

2. Members of the population

After the selection, several persons or one in the community are recruited as informants or field workers. It is sometimes difficult to choose the best person by the research team. There is always a hierarchy in any community, which may disturb the best selection.

3. Identification of individuals

It is not easy to identify a person at different times and places. Demographic data such as names, sexes, birthdays sometimes do not help because different names and birthdays could be used at different circumstances. Even pictures may not help especially in case of children. Population movement is another factor that makes the identification difficult.

4. Expectation of the community

What we are expecting in the field research and that the community is expecting in the work are sometimes different. Not all of the members of the community are cooperative. The demand of the community, excessive expectations and the existence of uncooperative people may influence the results of the study.

5. Heterogeneity of the population

The target population is not homogeneous like laboratory animals. This heterogeneity is a treasure of field work to obtain interesting results. However it is not easy to collect the data that properly show the heterogeneity of the population. There are usually limitations in collecting data on the characteristics of individuals.

S－VI－2) FROM VOODOO EPIDEMIOLOGY TO RELIABLE EPIDEMIOLOGY

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An epidemiological study is essential and inevitable in the field study of tropical medicine, because an epidemiological study shoud only be the method to obtain necessary information for the people live in the target area. But an epidemiological study is not so appreciated in the society of tropical medicine because of uncertainty of date. In this presentation, four points will be discussed in order to establish reliabe epidemiology.

1. Two types of epidemiological studies should be distinguished. One is descriptive type epidemiological study (to describe epidemiological characteristics of target population in a specific area.) The another is hypothesis testing type epidemiological study (to test hypothesis among human subjects). So called epidemiological study can be referred as one of two types. Study type should be clarified for further discussion.

2. Goal of the study should be clarified. For descriptive type epidemiological study, representativeness, degree of data accuracy should be carefully considered.

On the other hand, for the hypothesis testing type epidemiology, comparability between study populations should be of most importance.

3. Study design should be carefully considered. Study design should be a balance between informativeness and feasibility of the study. Which level of information should we obtain for further action? Feasibility of a study (human subjects availability, cooperability, human resources, money, time, etc.) is fundamental for operation of the study.

4. Obtained results should be criticized for reasonable interpretation in terms of three points of view.

1) Is there any biases (Information bias, Selection bias)?
2) Are confounding factors in the study controlled properly?
3) Are statistical test and power problems properly considered?

For reliable epidemiology rather than Voodoo epidemiology, four points stated above should be considered.

S－VI－3) FILARIASIS CONTROL: HOW WERE BLOOD COLLECTION AND MASS DRUG TREATMENT DONE?

EISAKU KIMURA

Dept. Parasitol., Aichi Medical Univ.

In 1978-81, I worked for the filariasis research and control program in Samoa as a WHO staff member. Blood collection by finger prick and venipuncture was repeated day after day. Under the WHO project, I never thought of ethical issues in relation to blood sampling, and cannnot remember such things were discussed among WHO staff. As an international organization, there should never be any unethical affairs with WHO activities. It was an established

fact. In Samoa, there is a “matai” system, where decision by village matais, or chiefs, will overpower individual opinions of villagers. If matais consent blood collection, no villagers can protest. Although it may not be unethical, our program apparently took advantage of the system to carry out researches more easily than in other countries.

In 1973-75, I worked in Ethiopia for smallpox eradication program under WHO. What we did was to vaccinate as many children as possible. We worked hard in very remote mountainous areas where people often could not expect even the minimum level of medical care. In 1980, WHO proudly declared the eradication of smallpox, and published the final report. One sentence in it was shocking: Vaccination will no longer be justified because it has serious side reactions which may be fatal. We vaccinated many children without discussing any about the danger of the vaccine. Was it ethically acceptable to vaccinate when we know some could suffer from serious reactions and they could never be attended? Can some people be sacrificed if the aim is for the international benefits and betterment of all people? We know that benefits are often more for the rich than the poor.

Ethical standards are moral prerequisite for all researchers, but for some civilized people, they have other aspects of avoiding legal complications, and self-satisfaction. It will be very important for us to participate positively in the formation of morally sound and practically workable standards with which field studies in developing countries can be performed with more confidence of scientific contribution.

S－VI－4) BIOETHICS AND BIOMEDICAL RESEARCH IN DEVELOPING COUNTRIES: WHAT SHOULD RESEARCHERS DO FROM THE VIEWPOINT OF BIOETHICS?

SATOSHI KANEKO

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The fundamental principle of ethics for medical research on human body had been based on protection of human right, and voluntarily participation to research. Along with rapid changes in modern societies, new concepts on human right have appeared on the field of biomendical research; that is, right to be let alone and right to control personal information. Furthermore, a process of informed consent to support voluntarily participation has been weighted in recent biomedical research. These new ethical issues have been considered to conflict with procedures in biomedical research. To balance the friction between them, several ethical guidelines for biomedical research using human subjects have been published based on the Declaration of Helsinki. Such guidelines recommended strengthening the review function of ethical review committees (ERCs) to make an applied biomedical research ethically justifiable. Although the ethical acceptability of biomedical research in developing countries supported or undertaken by developed countries must have followed the guidelines, it is not well discussed until the debates in 1997 on the zidovudine (AZT) clinical trials conducted in developing countries. These trials were implemented to see whethere zidovudine (AZT) treatment for HIV-infected women prevented perinatal taransmission of HIV. Although these debates resulted in the revision of the Declaration of Helsinki and an ethical standard to clinical trials in developing countries were given, they did not expand to ethical issues in general biomedical research or genomic research in developing coutries.

In Japan, the bioethical guideline for epidemiological research has recently proclaimed by governmental ministries. Since the guideline has just come into effect, there has been no standard on ERCs and the abilities to review study protocols are not well evaluated. When it comes to bioethical issues for research in developing countries, the situations becomes more chaotic. Most institutional ERC members do not know the situation in developing countries. Thus researchers must make an effort to inform the particular environments in developing countries to the ERC members. Sometimes, researcheres might have to scientifically or logically assert reasons to ERC members when the protocol could not exactly follow the bioethical guidelines. Through such constructive debates, the study protocol would become more refined, and persuasive. Mutual evolution of ERC members and researchers would not only promote the bioethical standard, but also improve the quality of the biomedical studies in developing countries. It is time for ethics and science to shake hands. The Japanise Society of Tropical Medicine must take a measure to enhance such mutual evoluion.

W－I－1) PREVENTIVE BEHAVIOR AND MORBIDITY OF JAPANESE TOURISTS FOR AFRICA

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TSUTOMU YOKOTA AND YOSHIE TANAKA

Narita Airport Quarantine Station

In many advanced countries, travel medicine is developed and taravelers who visit risk areas can access travel medicine information. But, in Japan, travel medicine is developing and many tourists visit risk areas without or insufficient travel medicine knowledge. To assess the preventive behavior and health risks of Japanese tourists, a questionnaire study was carried out. Japanese tourists for Africa are focused. With a cooperaton of a tour company, three hundred tourists of African tours were asked to complete questionnaire. 135 questionnaires ware collected and were analyzed. Male was 58(37%) and the average age was 40.9±12.4(mean±SD), ranged from 8 to 76. As the infectious disease information sources, 96(62%) tourists get information from guidebooks, 66(42%) from tour agency, 36(23%) from internet web, and 30(19%) from friends. TRAVELER'S DIARRHEA: The attack rate of diarrhea was 24% and food score proposed by Sonneburg was 4.7±2.6. Compared with the Sonneburg's data of Kenyan traevlers that the attack rate was 65.7% and the food score was 5.5±8.6, the attack rate of Japanese tourists was lower. MARARIA. The knowledge of malaria was asked and 130 (80%) knew malaria and 131(84%) intend to prevent for malaria. To prevent malaria, 117(87%) planned to use repellent before travel and 105(78%) actually used, 94(70%) planned to wear long sleeved and 86(63%) were, 93(69%) planned to use mosquito coil and 104(77%) used, 26(19%) planned to use mosquito net and 61(45%) acutually used. 28(20%) planned to take malaria prophylaxis before travel, 13(9.6%) actually took, 12(8.9%) take regularly, 5(3.7%) stopped after return. These indicate that most of the tourists know malaria and try to prevent mosquito bite, but the knowledge of malaria prophylaxis is insufficient. VACCINE: Vaccination rate was as follows: yellow fever 108 (80%), tetanus 12(8.9%), rabies 6(4.4%), hepatitis A 5 (3.7%).

These data support that guidebooks and the travel agency made a major role for tourists' prevention. Tourists have tendency to keep general preventive measures. The travel agency recommended general precautions actively, but had difficulty to recommended medical prevention because they could not treat medical problems and travel medicine facility is very limited in Japan.

W－I－2) KNOWLEDGE AND PREPARATION REGARDING THE RISK OF MALARIA AMONG JAPANESE INTERNATIONAL TRAVELERS

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HAMADA MASARU[4], TANIHATA TAKEO[1]

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BACKGROUND: Although Japanese citizens travel increasingly to malaria-endemic areas, little has been described on their knowledge and preparation regarding the risk of malaria. This prompted us to conduct a questionnaire study on these issues targeting this population.

METHODS: During a 2-month period, travelers ≧ 16 years of age vitising Tokyo or Osaka Quarantine Station for vaccination were asked to answer a self-administered questionnaire. Most of them attended to receive yellow fever vaccine and therefore, scheduled to visit areas that are, to a greater or lesser extent, malarious. Questions included knowledge of the transmission route, main symptom and praimary preventive measure of malaria. They were also asked whether and how they were informed of the current malaria situation at their destinations, what they would do in case of common cold symptoms after return, and whether they know a medical facility they should visit in case of suspected malaria.

RESULTS: Among a total of 468 individuals visiting the quarantine stations, 284 (61%) responded the questionnaire, with the response rate in Tokyo (questionnaires not delivered individually) and Osaka (delivered individually) being

40% and 99%, respectively. Of those, 48% cases were subjected to analysis. Those who know the transmission route (mosquito bite), main symptom (fever) and primary preventive measure (avoiding mosquito bite) of malaria accounted for 85%, 83%, and 69%, respectively. Eighteen percent stated that the primary preventive measure is vaccination. Only as few as 40% knew whether their destination is malarious, 19% would visit a hospital specialized for infectious diseases in case of common cold symptoms, and 19% knew a medical facility they should visit in case of suspected malaria.

Very few travelers (11%) got the information from travel agencies on malaria prevalence in their destinations.
CONCLUSION: Japanese international travelers visiting malarious areas have moderate knowledge of malaria; however, their preparation for the disease is unexpectedly insufficient and needs to be impromved by education.

W－I－3) DISEASE TRENDS AMONG JAPANESE PEOPLE LIVING IN SOUTHEAST ASIA

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Japan Overseas Medical Fund

Purpose and Method: The number of Japanese people living abroad is increasing every year. We conducted research at two different hospitals in South East Asia (Ram Hospital in Chiang Mai, Thailand, and the Subang Jaya Medical Center in Kuala Lumpur, Malaysia) and have investigated disease trends among adult Japanese patients. We gathered disease names of adult Japanese (≧16 years old) who visited outpatient clinic in both hospitals from January 2000 to December 2001, and the data was classified according to the International Classifications of Deseases (ICD-10).
Results: At Ram Hospital the research covered a total of 4315 Japanese patients. The most common were respiratory diseases (739 cases, usually including respiratory tract infections), which was followed by digestive tract diseases (652 cases, often including dental caries) and infectious diseases (456 cases, many including traveler‘s diarrhea). At Subang Jaya M. C., the reserch period included 4570 Japanese patients. The most comomon were respiratory diseases (239 cases, mostly respiratory tract infections), which was followed by ocular diseases (237 cases,showing symptoms of sore and/or swollen eyes) and unclassified clinical symptoms (237 cases, often including fever probably due to respiratory tract infections). During times of seasonal change, respiratory diseases were common in both hospitals during dry periods. At Ram Hospital, many patients suffered from infectious diseases during the monsoon season. At Subang Jaya M. C., patients suffered from ocular diseases during the dry seasons.
Conclusion: Most of adult Japanese visited the hospitals because of acute diseases such as respiratory tract infections, traveler‘s diarrhea, dental caries and sore eyes. However, the number of Japanese who visited the hospitals for chronic diseases (i. e. metabolic disorders or circulatory diseases) was low. During the research period, we offered health consultations to adult Japanese living in the cities, and many of them suffered from the above-mentioned chronic diseases. It is necessary to urge them to visit the hospitals for taking medical cares of the diseases.

W－I－4) THE STORAGE AND SUPPLY SYSTEM OF ORPHAN DRUGS FOR TROPICAL AND PARASITIC DISEASES

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Despite a significant number of cases with tropical and parasitic diseases, major drugs for those diseases including several antimalarials are not licensed in our country. The foundation of our research groups that import, store and supply those orphan drugs dates back to the year 1980. The present research group, the Research Group on Chemotherapy of Tropical Diseases, is funded by the Japan Health Sciences Foundation. Recently, the number of institutions we designated to store and supply the orphan drugs has been increased to a total of 23 throughout our country, aiming that every Japanese physician who needs the drugs can have access without significant delay. We also initiated communications with some European hospitals that are specializing in tropical and parasitic diseases in order to update trends of the worldwide drug usage. We also publicized our web page on which the drugs we introduced, institutions storing those drugs and other useful information on travel-related illnesses are described. On our closed listserve our member physicians are discussing various issues, *e.g.* the availability of some drugs from other member physicians and the diagnostic problems of actual cases.

Until October 2001 when mefloquine was marketed, almost all mefloquine that was used for the treatment of falciparum malaria in our country must have been supplied from our research group, as chloroquine and primaquine are still now in the case of vivax malaria. Lives of many patients with severe malaria have been saved by injectable quinine, and recently with artesunate, supplied from our research group. In addition, we recently experienced four cases of severe amebic dysentery, three of which were associated with HIV infection, that showed dramatic improvement after receiving injectable metronidazole supplied from our research group. We also succeeded to introduce three kinds of drugs for African trypanosomiasis from a responsible section of WHO.

Thus, we are intending to grow our reseach group network into a comprehensive one in which we, not only supply the drugs upon request, but also accept and manage consultations regarding various clinical issues of tropical and parasitic diseases including diagnostic and therapeutic matters.

W－II－1) MOLECULAR ANALYSIS OF α-THALASSEMIA IN NEPAL: CORRELATION WITH MALARIA ENDEMICITY (II)

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Thalassemia is a group of hereditary disease caused by impaired synthesis of specific globin chain. The disease has a high frequency in regions endemic for malaria including the Mediterranean Basin, Africa, Middle East, Southeast Asia and South China. It has been suggested that the high frequency of thalassemia might reflect advantage due to reduced susceptibility to malaria. To evaluate this malaria hypothesis, we analyzed thalassemia mutations in four neighboring populations of Nepal, the Tamang, the Newar, the Parbate and the Danuwar. The settlements of the Tamang are lacated in the malaria free upland, while those of the Danuwar are below the limit of the malaria zone. The Newar and the Parbate had been livid in the malaria free upland up to 1960s, when malaria eradication program was

accomplished, and then transferred to the malaria zone, where is convenient to cultivation. We detected the 3.7 kb deletion type of α-thalassemia (-$\alpha^{3.7}$) with a high gene frequency, 0.54 in the Danuwars, while only 0.05 in the Tamangs, 0.14 in the Parbates and 0.28 in the Newars, Haplotype analysis of α-globin gene cluster showed that the α-thalassemia mutation has four different genetic background in the Danuwars. These results suggest that the high frequency of α-thalassemia in the Danuwar is due to the adaptation to malarial environment rather than to events such as a bottleneck.

To examine the relationship between α-thalassemia and the actual malaria infection, we analyzed the malaria infection rate by genotype in the malaria zone mentioned above since 2000. In September 2000, 3 of 12 (25.0%) in αα/αα, 1 of 35 (2.9%) in -$\alpha^{3.7}$/αα and 5 of 37 (13.5%) in -$\alpha^{3.7}$/-$\alpha^{3.7}$ were positive for *plasmodium* by PCR. In March 2001, 20 of 63 (31.7%) in αα/αα, 5 of 38 (13.2%) in -$\alpha^{3.7}$/αα and 5 of 26 (19.2%) in -$\alpha^{3.7}$/-$\alpha^{3.7}$ were positive, and in February 2002, 21 of 82 (25.6%) in αα/αα, 6 of 39 (15.4%) in -$\alpha^{3.7}$/αα and 2 of 29 (6.9%) in -$\alpha^{3.7}$/-$\alpha^{3.7}$ were positive for *plasmodium*. These results showed that the malaria infection rate was significantly lower in the population with α-thalassemia than those without α-thalassemia.

W－II－2) KNOWLEDGE AND PREPARATION REGARDING THE RISK OF MALARIA AMONG JAPANESE INTERNATIONAL TRAVELERS

KAWAKAMI, K.

W－II－3) MOLECULAR ANALYSIS OF CHLOROQUINE RESISTANT RELATED GENE IN MALAWI

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Although chloroquine resistant *Plasmodium falciparum* has been spread in Africa since 1970, chloroquine is still important compound because of its inexpensiveness and safeness. In Malawi, first line drug for treatment of *P. falciparum* was officially replaced from chloroquine to sulfadoxine/pyrimethamine in 1993 because of high rate of treatment failure (80%) and *in vitro* resistance (48%). To date, the use of chloroquine has been strictly enforced. We conducted two drug efficacy surveys of *in vitro* in 1998 and *in vivo* in 2000 at Salima district in Malawi and found significant reduction of chloroquine resistant isolates both *in vitro* (3%) and *in vivo* (9%).

We assayed two genetic mutations implicated in chlroquine resistance (*pfmdr1* and *pfcrt*) from 128 *P. falciparum* isolates collected during the 1998 and 2000 resistance studies *P. falciparum* DNA was extracted from filter paper with finger prick blood Nested PCR followed by RFLP methods were performed to determine *pfcrt* polymorphisms at position 76, 220, 271, 326, 356 and 371, and for *pfmdr1* at position 86, 184, 1034, 1042 and 1246.

In *pfcrt*, mutant type alleles were detected in 2 to 21% of isolates at position 76, 220, 271, and 371. The prevalence of mutant alleles significantly decreased from 1998 to 2000 at position 76 (P=0.007), 220 (P=0.009), 271 (P= 0.007) and 371 (P=0.007). Nearly all of mutant alleles were detected in isolates with mixed infections; i.e. including both wild and mutant. In *pfmdr1*, high prevalences of mutant type alleles were detected at position 86 and 184. No defference was found in these prevalences between 1998 and 2000. In *pfcrt*, Fst value were significantly high and similar level (0.051-0.055) at position 76, 220, 271, and 371 between two populations (P<0.02). Contrary to *pfcrt*, the Fst value at position 86, 184, 1042, and 1246 were not significant in *pfmdr1*. These results suggested that *pfcrt* has four distinct regions (76, 220, 271, and 371) of loci with respect to allele frequency between parasite population in 1998 and 2000.

Our results support one possibility that the elimination of chlroquine pressure has reduced the prevalence of mutant *pfcrt* alleles and resulted in a substantial recovery of chloroquine sensitivity. Furthermore, in addition to the K76T polymorphism chloroquine sensitivity is highly dependent

on other as yet unidentified polymorphisms or loci.

W－II－4) GENERATION OF TRANSGENIC ANOPHELINE MOSQUITOES EXPRESSING A GENE ENCODING ANTI-MALARIA MOLECULE

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The recent generation of genetically transformed mosquitoes has raised hopes for the production of mosquito strains that are unable to transmit various parasites and viruses.

We have previously constructed a gene encoding of a single-chain antibody fragment (scFv) specific for Pbs21 molecule expressed on the ookinete surface. The scFv, designated 13.1 scFv, bound to the surface of *P. berghei* ookinete, and blocked oocyst development in the mosquito midgut by at least 93%, as assessed by oocyst counts in mosquitoes. To examine whether expression of 13.1 scFv in the mosquito midguts could lead to transmission-blockade of malaria in the mosquito stage, we generated 5 lines of transgenic *Anopheles stephensi* mosquitoes that express the 13.1 scFv-Shiva gene under the control of the gut-specific and blood-inducible *An. gambiae* carboxypeptidase (AgCP) promoter using *Minos* germ line transformation. The embryos were microinjected by using glass needles with a mixture of *Minos*-13.1 scFv-Shiva transfer plasmid and *Minos* transposase plasmid. The *Minos* transposase mediates integration into the genome of *An. stephensi* cells. Hatched larvae were analysed on an inverted microscape at a wavelength of 490 nm to detect EGFP expression.

Real-time PCR analysis showed that expression of 13.1 scFv-Shiva mRNA is specifically induced (8-fold) in the guts of transgenic mosquitoes, with peak expression at ～3h after a blood meal. By 48h after a blood meal, mRNA abundance retums to a level close to that present before a blood meal. By 48h after a blood meal, mRNA abundance returns to a level close to that present before a blood meal. To examine inhibition of oocyst formation in the transgenic lines, wild-type and transgenic mosquitoes were allowed to feed on the same *P. berghei*-infected mouse. Oocyst formation in one of transgenic lines were significantly reduced (50%) compared with that in wild-type.

W－III－1) INTRANASAL INOCULATION OF YEAST-PRODUCED MALARIA OOKINETE SURFACE PROTEINS CO-ADMINISTERED WITH CHOLERA TOXIN INDUCES COMPLETE TRANSMISSION-BLOCKING IMMUNITY AGAINST A RODENT AND A HUMAN MALARIA PARASITE

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Transmission-blocking Vaccines against sexual-stage malaria parasites have been considered one strategy for malaria control. Recently, several genes encoding ookinete surface proteins (OSP) have been cloned from *Plasmodium* species of rodent, human and avian malaria parasites. To investigate whether mucosal vaccines against malaria para-

site OSPs are feasible strategy for induction of systemic transmission-blocking immunity, the yeast-synthesized *P. yoelii* Pys25 protein was administered to DBA/2 mice through oral or nasal route in combination with cholera toxin (CT). Intranasal administrations of Pys25 in the presence of CT induced storong systemic humoral immune responses evidenced by high levels of serum IgG1 with low but detectable levels of IgG2a antibodies, suggesting that Th-2 type immune responses were predominantly induced against Pys25 protein. Howeber, in the absence of CT the intranasal immunization failed to induce any detectable levels of serum antibodies. Oral administrations of Pys25 even in the presence of CT did not induce antibody responses. When *Anopheles stephensi* mosquitoes were allowed to feed on mice infected with *Plasmodium yoelii* 17X, which previously vaccinated intranasally with Pys25/CT, oocyst formation in mosquito midgut was completely blocked in every engorged mosquitoes examined. Further, passivc transfer of immunesera to naïve mice conferred complete transmission-blocking immunity. Similarly, intranasal immunization of mice with *Plasmodium vivax* ookinete surface protein, Pys25 induced significant serum IgG with high IgG1/IgG2a ratio. Feeding *Anopheles dirus* mosquitoes with mixtures of immunesera and gametocytemic blood derived from vivax volunteer patients in Thailand significantly reduced both the number of midgut oocyst as well as the percentage of infected mosquitoes. The observed transmission-blocking effect was dependent on immunesera dilution. These studies demonstrate for the first time that the mucosally induced mouse immunesera against malaria ookinete surface proteins can completely block parasite transmission to vector mosquitoes, suggesting the possibility of developing non-invasive mucosal vaccines against malaria.

W－III－2) HIGH EXPRESSION OF CHOLERA TOXIN B SUBUNIT PENTAMER IN METHANOPHILIC YEAST *PICHIA PASTORIS* AND ITS ORAL IMMUNOGENICITY IN MICE

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Cholera toxin B subunit (CTB) has been expressed in bacterial gene expression systems and successfully used for the induction of mucosal and systemic immune responses to chemically or genetically conjugeted antigens. In the present study we tested the possibility for yeast to express CTB gene and evaluated the feasibility of recombinant yeast-based oral immunization strategy. We introduced into *Pichia pastoris* chromosome 1) the authentic CTB gene (CTB^{ori}, 2) CTB gene deleted with 22-amino acid leader peptide coding sequence ($CTB^{\Delta 1}$), and 3) CTB gene fused at the C' terminus with yeast microsomal retention signal (HDEL)-encoding sequence (CTB^{HDEL}). We found significant levels of CTB^{ori} and CTB^{HDEL} pentamers retaining G_{M1}-ganglioside affinity were produced and secreted into the medium (30 μg/ml). We detected $CTB^{\Delta 1}$ transcript by RT-PCR but the protein was not detected. Yeast-synthesized recombinant CTB was found heat-labile, quickly dissociated into monomers when cells were heat-treated at 100 ℃, but the protein was almost completely resistant to desiccation. Next, to evaluate oral immunogenicity of yeast-produced CTB, yeast or culture supernatant calculated to contain 8-16 μg of pentameric CTB was given to mice by oral gavage once a week for 6 weeks. Mice fed yeast cells, but not with culture supernatant, developed CTB-specific serum IgG (predominantly IgG1) and intestinal S-IgA antibodies. Feeding 10 μg of commercially available CTB protein mixed with untransformed yeast cells induced lower level of CTB-specific serum IgG, indicating that recombinant CTB accumulated within yeast cells has higher oral immunogenicity than naked CTB, probably due to increased resistance to digestive degradation in gastrointestinal tract. The induced antibody response was maintained at least for six months when mice were given extra booster feedings of yeast cells. Taken togegher, these results suggest that yeast can be used as an efficient vaccine production system as well as an oral delivery system for vaccination.

W－III－3) EXPRESSIONS OF CYTOKINES AND MONOSACCHARIDE TRANSPORTERS IN THE DUODENAL MUCOSA OF GASTRO-INTESTINAL SYMPTOMATIC PATIENTS IN THE RURAL AREA OF THAILAND

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Expression levels of cytokines and GLUT family-monosaccharide transporters in the duodenal mucosa were examined in patients from Nongkhai, Thailand, who had received gastrofiberscopy because of the gastrointestinal problems. Duodenal biopsy specimens were collected from a total of 33 patients (24 males and 9 females, 45.0 +/- 13.5 years old): 10 patients showed present or recent intestinal helminth infections, including strongyloidiasis, taeniasis and ascariasis (group A), 7 were urea-test positive, indicating *Helicobacter pylori* infection (group B), and 16 had neither helminth infection nor urea test positivity (group C). Total RNA was extracted from the biopsied specimens and semi-quantitative RT-PCR was performed. Positivities of IL-13, IL-5 and IFN-γ mRNA expressions in all patients were 24.2, 60.6 and 100%, respectively, with the highest IL-13 and IL-5 positivities in group A followed by group C and B. IL-5 positive rate was also significantly higher among patients with high peripheral blood eosinophil counts (>4%) than in patients with low peripheral blood eosinophil counts. GLUT-1 and GLUT-5 were detectable in all patients. Although GLUT-1 expressions did not differ between group A, B and C, GLUT-5 expressions were significantly lower in group B than in group C. These results indicate that helminth and *H.pylori* infections result in different immunopathological responses in the duodenal mucosa: lower expressions of type 2 cytokines and monosaccharide transporters in *H.pylori* infection than in helminth infections.

W－III－4) STUDY OF *ASCARIS* AND ULCERATIVE COLITIS-GENETIC ANALYSIS OF CROSS-REACTIVE ANTIGENICITY BETWEEN *ASCARIS LUMBRICOIDES HOMEINIS* ANTIGEN AND HUMAN COLON MUCOSA

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It is known that the antibody against the *Ascaris* is produced in patient's serum of ulccrative colitis (UC). We have already reported the cross-reactive antigens between *A. lumbricoides* and human colon mucosa are presented. In this study, to determine the genes related with cross-reactive antigens between *A. lumbricoides* and human colon mucosa, we analysed the screening method at the DNA levels using the human colon cDNA library (Clontech laboratories Inc., CA,USA). λ phage was infected to *E. coli*. XL-1 Blue, and IPTG induced-protein derived from each plaques were transferred to the membrane and they were investigated by immuno-staining method using the anti-*A.lumbricoides* rabbit serum. We analyzed 50,000 plaques approximately, 3 clones were reacted positively as a result of secondary screening. To determine the sequences of these clones, each cDNA was analyzed by the DNA sequencer ABI PRISM 310 Genetic analyzer. As the result, one was considered to be a transmembrane mucin 12 homolog. Other two genes were similar to actin, gamma 2 of enteric smooth muscle and beta-casein-like-protein. Mucin exists in the digestive organs, especially in the colon and was reported that the antibody against colonic mucin might contribute to the epitherial cell injury of colonic mucosa through antibody-dependent cell-mediated cytotoxicity (ADCC) and this autoantibody levels are high in some patients for UC. Mucin12 gene contains in the coding regions that are homologous to mucin3, mucin4, mucin11 and mucin17 with high frequency. In these results, *A. lumbricoides* was likely to harbor in the human intestine, because mucin3 exists richly in the intestine, and it was guessed to be a suitable environment for *A. lumbricoides*.

W－IV－1) FUSION DNA VACCINE AGAINST INFECTION WITH HIGH VIRULENT *TOXOPLASMA*

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For most obligate intracellular protozoa such as *Toxoplasma gondii* (*T. gondii*) and *Trypanosoma cruzi*, available vaccines have not yet been developed, despite that those kinds of pathogen are resistant or less effective for chemotherapy in general. Cellular immunity especially CD 8^+ T cell response plays essential roles for the protection against those kinds of pathogens. Effective protective immunity against those infections is, however, hardly inducible by conventional vaccination using proteins/peptides derived from target pathogens. It is a well-established consensus that protein antigens must be ubiquinated and then processed by proteasome within antigen-presenting cells when MHC class I-restricted $CD8^+$ T cell response is preferentially induced. From these bases, we fused naked DNA of SAG-1 (major surface antigen of *Toxoplasma gondii*) with that encodinig ubiquitin. Thereafter, BALB/c mice were subcutaneously injected with this fusion DNA using gene gun before the infection with a high virulent RH strain of *T. gondii*. Strikingly, most mice vaccinated with the fusion DNA acquired strong protective immunity and controlled the infection, although DNA vaccine with eigher DNA encoding SAG-1 gene alone or that encoding ubiquitin gene alone was not effective. Furthermore, the effect of ubiquitination was confirmed in vitro using a specific inhibitor for proteasome, MG-132. Mice immunized with the fusion DNA showed a potend cytotoxic activity of CD^+ T cells specific for SAG-1 transfected target cells. This kind of vaccine strategy is expected to be usuful for infections with other type intracellular pathogens including viruses and bacteria.

W－IV－2) AN INTERLEUKIN-18 EXPRESSION PLASMID ADMINISTRATION INITIATES A PROTECTIVE T HELPER TYPE 1 IMMUNE RESPONSE AND RESOLVES CUTANEOUS LEISHMANIASIS

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We investigated the therapeutic effect of an IL-18 expression plasmid administration on susceptible BALB/c mice infected with *Leishmania major*. BALB/c mice were subcutaneously challenged with *L. major* into hind footpad. Simultaneously, they were weekly treated with 4 μg IL-18 and/or IL-12 expression plasmid DNA with the gene gun. All of the treated mice showed similarly decreased footpad swelling and less parasite burden compared with those treated with empty vector (control mice). IFN-γ was signigicantly increased at 3 weeks after infection in all treated mice but not in control mice. Antibody administration demonstrated that CD4+ T cells and IFN-γ played critical roles in the protection against *L. major* induced by IL-18 expression plasmid administration. Moreover, TNF-α was also increased in the treated mice. In TNF-α-deficient BALB/c mice, which were also susceptible and rapidlly succumbed to progressive disease after *L. major* infection, IL-18 expression plasmid administration did not lead to the resistance as in the wild type mice, despite of the high level of *L. major*-specific IFN-γ production. This demonstrated the critical role for TNF-α in *L. major* infection. These results suggest that administration of an IL-18 expression plasmid with gene gun is able to protect susceptible mice against an intracellular protozoan infection, with shifting of immune response towards Th1 direction.

W－IV－3) PROTECTIVE ROLE OF IgE IN MICE INFECTED WITH *PLASMODIUM BERGHEI* ANKA

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Recently, it is has been reported that IgE is related to the pathogenesis in severe malaria infection, particularly in cerebral malaria. IgE levels in the sera of individuals living in high *P. falciparum* endemic areas were investigated, approximately 85% of people from these areas had significantly elevated level of total IgE. However, the role and biological significance of IgE in the protection against *P. falciparum* and pathogenesis of malaria are not known. For these reasons, we decided to examine the possible roles of IgE responses using IL-5 transgenic and SJA/9 mice, which are genetically producing high levels of IgE under normal condition or deficient of IgE production. 1×10^3 *P. berghei* ANKA-infected erythrocytes were injected i.v. into IL-5 transgenic and the background mice, and the parasitemia was measured every day. After the infection of *P. berghei*, although the parasitemia was incresed gradually in both groups of mice, the parasitemia in IL-5 transgenic mice was always lower than that in background mice, and the IL-5 transgenic mice showed longer survival. We examined the protective role of IgE in *P. berghei* infection using congenitally selective IgE-deficient SJA/9 and the background SJL/J mice. After *P. berghei* infection, the parasitcmia in SJA/9 mice was significantly higher than that of SJL/J mice, and the mortality of SJA/9 mice was also higher than that of SJL/J mice. These results indicated the protective roles of IgE for *P. berghei* infection. The production of anti-*P. berghei* specific IgE antibody in the *P. berghei* ANKA-infected mice was examined by Western blot analysis, the results showed that anti-*P. berghei* IgE antibody was also produced in the sera from these mice. Since IgE has both protective and pathogenic effects in infectious diseases via two major receptors, the high-affinity receptor FcεRI and low-affinity FcεRII or CD23, we examined the possibility that those two kind of receptors involved with IgE-mediated resistance against *P. berghei* infection using WBB6F1-W/W^v mice, which are congenitally deficient mast cells bearing FcεRI receptor and CD23 Ko mice. After *P. berghei* infection in W/W^v and CD23 Ko mice, the parasitemia in W/W^v and CD 23 was always higher than those in the background mice, suggesting that the protective effect of IgE on *P. berghei* infection was mediated via FcεRI and FcεRII. In conclusion, IgE and anti-*P. berghei* ANKA IgE antibody have important roles for the protection of *P. berghei* ANKA infection mediated through FcεRI and RII.

W－IV－4) THE ROLE OF ERYTHROCYTES ON SUPPRESSION OF IMMUNE RESPONSE IN MURINE MALARIA

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It is well known that malaria is associated with hepatosplenomegaly, anemia and myeloid hyperplasia. The increased levels of hemopoiesis and the phagocyte system are thought to be implicated in this pathogenesis. Immune depression during malaria is also a well-recognized phenomenon that likely contributes on the host-parasite relationship. Previous studies have shown that suppressor T cells or macrophages are involved this phenomenon. Recently we found that RBC from *Plasmodium*-infected mice showed increased levels of affinity to some FITC-labelled plant lectins.

In the present study, we examined the possible role of RBC of *Plasmodium*-infected mice on the regulation immune response upon *Plasmodium* infection. Proliferative response of splenocytes upon stimulation with Con A or allogenic APC increased in the presence of RBC from normal mice, whereas depressed in the presence of RBC from *P. yoelii*-infected mice. Supernatant of RBC lysate showed similar effect on the proliferative response with the RBC. On the other hand, RBC lysate from *P. yoelii*-infected mice induced superoxide production from neutrophils. Time course study revealed that the stimulatory activity of the

RBC lysate coincided with the increase of parasitemia during *P. yoelii* 17X infection. The stimulatory activity of the RBC lysate was partially lost after the treatment with heparin-agarose. HPLC analysis equipped with G3000SW column indicate that the stimulatory factor possesses a molecular weight mass of around 14kd. The relationship between the depression of the immune response by RBC of *P. yoelii*-infected mice and superoxide production from neutrophils was discussed.

W－V－1) APPLICATION OF QUESTIONNAIRES FOR MONITORING *SCHISTOSOMA MANSONI* INFECTION AFTER MASS-TREATMENT

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The purpose of the study is to determine if adding some questions about water-contact behavior on signs and symptoms improve the efficacy of questionnaires for the monitoring of *Schistosoma mansoni* infection.

<Subjects and methods>

A total number of 1033 schoolchildren aged 7-17 years in four primary schools in Moshi Tanzania were involved in the study. February 2001, they received mass treatment with praziquantel and mebendazol for the deworming of schistosome and intestinal parasites. Before the treatment, the children were requested to answer a questionnaire. They were examined for the ova of schistosome and intestinal parasites in stool before the treatment and one month later. A year later in February 2002, the examination and questionnaire were carried out again. Only 267 children completed the examination and questionnaire. The questionnaire consists of the questions on the subjective symptoms in the previous two weeks and frequency and nature of their water-contact behaviors. The answers of questions recorded as 'yes', 'no' or 'I don't know'. Stool examination was conducted with Kato-Katz procedure. The association between infection and each questionnaire variable was calculated by using a chi-square test and a logistic binomial regression analysis.

<Results>

1) The prevalence of *S. mansoni* infection was 67.4% before the treatment and decreased to 6.4% after treatment. In 2002, one year after the treatment, the prevalence increased to 27.0%. The mean intensities were 29.8 eggs/gram, 0.3 eggs/gram and 2.5 eggs/gram respectively.
2) Before treatment, constipation (OR=2.91, 95% C.I.; 1.29-6.58, P=0.010) and net fishing (OR=2.30, 95% C.I.; 1.29-4.11, P=0.005) were significantly associated with infection. Sensitivities and specificities were 20.6%, 89.7% and 43.5%, 70.6% respectively. One year after treatment, only diarrhea (OR=1.91, 95% C.I.; 1.10-3.32, P=0.021) had significant association with infection. The sensitivity and specificity were 61.1%, 54.9% respectively.
3) No water contact behavior had significant association with infection in this study.
4) When the factor of the previous infection before treatment was introduced to a logistic binomial regression analysis, both previous infection (OR=1.96, 95% C.I.; 1.04-3.70, P=0.037) and diarrhea (OR=1.88, 95% C.I.; 1.08-3.27, P=0.026) were significantly associated with the current infection.

In the study area, questions about water-contact behavior added on signs and symptoms did not improve the efficacy of questionnaires for the monitoring of *Schistosoma mansoni* infection.

W－V－2) RAPID SCHOOL HEALTH QUESTIONNAIRE SURVEYS ON URINARY SCHISTOSOMIASIS IN COASTAL KENYA AND ZANZIBAR

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The WHO's resolution on schistosomiasis has set the goal of attaining a minimum target of regular administration of chemotherapy to at least 75% of all school-age children at risk of morbidity by 2010. However, systematic data on the prevalence of infection, the coverage of administration of chemotherapy and school health education have not been collected in Sub-Saharan African countries. Therefore, we conducted rapid school health questionnaire surverys in Kwale District of Coast Province of Kenya (K), and in the two islands of Zanzibar; Unguja (U) and Pemba (P) in 2001. In total, 264 schools (113 in K, 84 in U, and 67 in P) were participated in the study. Ten boys and ten girls in class 4 were asked to answer questions in these surveys. In Zanzibar trained health staffs visited schools and conducted the survey. In Kenya the procedure of survey was explained to teachers in a district meeting, and teachers themselves conducted the survey in their schools. The results were collected by mail. Questions asked were knowledge on schistosomiasis, presence or absence of self-diagnosed urinary schistosomiasis, subjective symptoms such as blood in urine, contact with river water in the previous two weeks, and experiences of urine examination, treatment, and health education. Mean percentages of children answering "yes" to questions in K, P, and U were as follows (sex combined): Knowledge on what schistosomiasis is, 73%, 50%, 31%; Self-diagnosed urinary schistosomiasis, 39%, 21%, 6%; Blood in urine, 33%, 19%, 6%; Contact with river water in the previous two weeks, 82%, 57%, 16%; Health education, 34%, 24%, 31%; Urine examination, 26%, 19%, 8%; Treatment, 26%, 35%, 11%. These results indicate the needs of sysytematic efforts to achieve the WHO's goal. The governments must annually conduct this kind of rapid monitoring survey on health status of school children, coverage of school health servies, and coverage of health education to accumulate the basic information on school health.

W－V－3) WATER CONTACT BEHAVIORS AMONG SCHOOLCHILDREN IN AN ENDEMIC AREA OF SCHISTOSOMIASIS, TANZANIA: A COMPARATIVE STUDY OF DIRECT OBSERVAITON AND 24-HOUR RECALL METHOD

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Estimation of exposure to cercarial infested water is critical for epidemiological and immunological studies in a schistosomiasis endemic area. Questionnaire surveys have been used to estimate water exposures, addressing frequency of water contact only, due to its cost-effectiveness and less labor-intensiveness. Direct observation needs more cost and time, but it provides more accurate exposure information. We compared the results of 24-hour recall method with direct observation method for clarifying whether 104 schoolchildren living in Lower Moshi area, a *Schistosoma mansoni* endemic area, of Tanzania could accurately recall their water exposures. Individual tracing method was applied to obtain detailed direct observation data for all water contact behaviors, and, on the following day, an interview was carried out to collect corresponding information on all contact behaviors. Exposure index (EI: %min/day) was calculated by contact duration and intensity of exposure (percent body surface area) using Lund and Browder burn chart. The most critical water contact behavior for the infection was swimming/playing in the river and canal among schoolchildren. They used safe water, in most cases, from the tap or deep well for bathing purpose. We found a significantly smaller frequency (4.5 vs. 6.4, $p<0.001$), but longer duration (29.7 vs. 24.6 min, $p<0.01$), of water contact in the re-

call data than directly observed data. For the risk water contacts, the EIs of water contacts obtained by recall method were significantly higher than those of direct observation ($p<0.001$). The EIs of 24-hour recall were significantly correlated with those of direct observation ($\rho=0.885$, $p<0.01$); more strongly among boys ($\rho=0.931$, $p<0.01$) than girls ($\rho=0.750$, $p<0.01$). Although the values of the EI are significantly higher in the recall data and should be corrected in practice, these results show a possibility of replacing direct observation data by detailed recall data. The 24-hour recall method is a practical procedure to accurately quantify water exposure in Lower Moshi area where *S. mansoni* has been endemic.

W－V－4) SCHOOL-BASED AND RESIDENCE-BASED ANALYSES ON INFECTIONS WITH *S. HAEMATOBIUM* AND *S. MANSONI* AMONG CHILDREN IN MOSHI, TANZANIA

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Schistosomiasis in Africa is not widely and evenly spread along river lines, but is scattered in clusters in some particular areas. The areas vary in seasons and years, which seems to have influence upon the infection with the disease to human bodies. This paper is to report the characteristics of distributions for two kinds of schistosomes, *S. haematobium* and *S. mansoni*, both existing in the same area, one from the view of school districts and another from that of dwelling places.

Subjects for our survey were the children in grades four and five from Chekereni primary school in Chekerini, and those from Oria primary school in Oria school district, the number of which was 284 in total, the former 199, and the latter 85. Whether inflection was positive or negative was determined by examining existence of the eggs in subjects' feces and urine. Kato-Katz method for *S. mansoni*, and nuclepore filter paper method for *S. haematobium* diseases were adopted.

Presence of *S. mansoni* eggs was positive among 50.8% of children from Chekereni primary school whereas 31.2% in Oria primary school. *S. haematobium* eggs were observed among 20% of children in Oria primary school, while only few children from Chekereni primary school were infected. No significant difference was observed on the prevalence of *S. mansoni* between Chekereni children coming from Chekereni area and those coming to the same school from Oria area ($P>0.05$). While for that of *S. haematobium*, a significant difference was observed ($P<0.05$).

Epidemiological surverys related to the infection with the parasaites have often been carried out on the basis of school districts. The distripution of infection is often biased by endemic area. We conclude that location of subjects' inhabitation should also be considered for epidemiological surveys on parasitic diseases.

W－VI－1) CHARACTERIZATION OF THE cDNA OF THE MIRACIDIAL ANTIGEN FAMILY OF *SCHISTOSOMA JAPONICUM* (CHINESE MAINLAND STRAIN).

W－VI－2) MOLECULAR CLONING OF A NOVEL PROCYCLIC FORM-SPECIFIC GENE (CDNA) FROM *TRYPANOSOMA BRUCEI BRUCEI*

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Trypanosoma brucei brucei protozoa spreads between the mammal host and the tsetse fly vector in its lifecycle. In order to control *Trypanosoma*, we analyzed gene-expression profiles of blood stream and procyclic (insect) forms of *T. b. brucei* using the method of fluorescence diffierential display. About 12000 cDNAs were surveyed, and a total of 39 differentially regulated cDNA bands were identified. One markedly differential expressed cDAN, which was up-regulated in procyclic from, was selected and full length of which was obtained by 5'RACE method. The result showed that the gene is 3304bp encoding an ORF of 813 amino acids. Genbank database search revealed that this gene has 38% identity with *Trypanosoma cruzi* (*T. cruzi*) Cyclin 5 gene. *T. cruzi* Cyclin 5 was known to be bound to TzCRK1 (*T. cruzi* Cdc2p-related kinase 1) which is one of Cdks (cyclin dependent kinase). The function of *T. cruzi* Cyclin5 is still uncertain but presumed to play an important role(s) in controlling the cell. It is thus suggested that the novel procyclic form-specific gene (cDNA) from *T. b. brucei* also plays an important role in the process of the cell cycle similar to *T. cruzi* Cyclin5.

The result of RT-PCR demonstrated that the gene was not expressed in long slender blood stream form, while it was expressed in short stumpy (pre-adapted) form, which can change to procyclic form when the blood is sucked by tsetse fly. Therefore, it seems likely that this gene has an important function in lifecycle change of *T. b. brucei* from blood stream to procyclic forms. In order to clarify the function of the gene, RNAi-mediated gene knock down analysis is under way.

W－VI－3) ANALYSIS OF *LEISHMANIA* AMASTIGOTE STAGE-SPECIFIC MOLECULES

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A *Leishmania* protozoan differentiates from the promastigote to the amastiogote during its digenetic life cycle between invertebrate and vertbrate hosts respectively. The promastigote can be easily cultivated *in vitro*. This has resulted in *Leishmania* promastigotes being investigated in a wide range of basic biological sciences and immunology on leishmaniasis. In contrast, *in vitro* cultivation of the amastigote stage is still a challenge. As a result, relatively little is known about amastigote stage-specific molecules. The proliferation of amastigotes in macrophages results in cell rupture and tissue damage, giving rise to the various symptoms associated with leishmaniasis. Thus, detailed investigation of amastigotes in teams of protein levels is crucial to understand the pathogenesis of leishmaniasis. Moreover, these studies will help to understand the survial mechanisms of amastigotes during infection within the mammalian host. The identification of amastigote stage-specific proteins was addressed by proteomic approaches using the technique of two-dimensional electrophoresis. The protein expression patterns of *Leishmania major* amastigotes purified from cutaneous lesions in experimentally infected Rag2 knock out mice (BALB/c) and cultured promastigotes were compared. The majority of protein spots occurred between 28 kDa and 94 kDa; at least 32 spots from amastigotes and 43 spots from promastigotes were clearly visualized on a Coomassie Brilliant Blue -stained gel. although 11 protein spots were found to be expressed by both amastigotes and promastigotes, and the remaining 21 protein spots were specific to amastigotes and 32 spots were promastigote specific. These results demonstrated that *L. major* expresses stage-specific proteins. Sequentially, to detect amastigote stage-specific antigens, proteins from promastigotes and amastigotes were analyzed by western blotting using the polyclonal antibodies from a rabbit immunized with homogenates of purified *L. major* amastigotes. Three specific spots of amastigotes were detected. Determination of amino acid sequences of these amastigote stage-specific antigens is now on going. These studies will contribute to the develop-

ment of imunological diagnosis or treatment, or effective vaccine against leishmaniasis with the new concept that amastigote stage-specific antigents should be targets for control measures.

W－VI－4) THE QUANTIFICATION OF THE GENES INVOLVED NO PRODUCTION IN LEISHMANIA-INFECTED MACROPHAGE BY REAL-TIME PCR

YAMAMOTO, Y.

W－VII－1) CHEMOTHERAPEUTIC APPROACH TO AFRICAN TRYPANOSOMIASIS: THE USE OF DIORGANOTIN AND ITS DERIVATIVES

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African trypanosomiasis is an endemic disease in nature with recurrent outbreaks, affecting both human and his domestic livestock; with no vaccine at near sight. The disease is fatal if untreated and chemotherapy remains the most powerful strategy to date, albeit narrow therapeutic index. Despite the tremendous progresses made in the identification and characterisation of biochemical and molecular properties of trypanosomes, potential drug target to produce an “ideal trypanocide” proves elusive. Current drugs that are available are old, scarce, less effective, toxic and encounter parasite resistance. Therefore there is an urgent need for new drugs for the treatment of this disease. The development of *in vitro* drug screening assays has improved the search for compounds against pathogenic trypanosomes. The proliferation of trypanosomes is rapid and has been likened to some types of cancer cells and all the currently used drugs against human African trypanosomiasis (HAT) have anti-cancer effect or *vice versa*. DFMO was first developed as anti-cancer drug, but now has therapeutic activity against Gambian sleeping sickness, even in the late CNS-involved stage. Organometallics have also been reported to have both anti-cancer and anti-trypanosomal effects. To date only organoarsenic melarsoprol that cures all forms of HAT albeit undesirable and often fatal side effects. African Trypanosome are partially auxotrophic for lipids and are salvaged from the host environment for the synthesis of conventional membrance and GPI anchor.

In an attempt to explore the chemotherapeutic potential of organotin and to broaden the search for much needed trypanocide an approach was made against three species of *trypanosoma*, using organometallic dibutyltin dichloride and its fatty acid derivatives as antitrypanosomal agents *in vitro*. The compounds mediated the killing of the parasites with minimum inhibitory concentration (MIC) values ranging from 76.0-597.0 ng/ml, 53.2-597.0 ng/ml and 106.4-600.0 ng/ml for *Trypanosoma brucei brucei, T. b. gambiense* and *T. b. rhodesiense*, respectively. Our observed selectivity indices of these compounds against murine-derived fibroblasts are not as high as the currently used trypanocides. These observations suggest the chemotherapeutic potentials of organotins and the approach could be useful in either developing new lead compounds or improve the use of the current trypanocide in use.

W－VII－2) THE INDUCTION OF TNF-α AND iNOS EXPRESSION BY MACROPHAGE CELLS AGAINST ONO-4007 (A SYNTHETIC LIPID A DERIVATIVE), IFN-γ AND MEGLUMINE ANTIMONIATE IN VITRO STUDY

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Introduction: In leishmaniasis, TNF-α induces a lytic mechanism in protecting against *Leishmania* parasites through stimulating inducible nitric oxide synthase (iNOS), which catalyzes to nitric oxide (NO) by activated macrophage cells. The purpose of this study to clarify the antileishmanial action of ONO-4007 (a LPS derivative) or IFN-γ or meglumine antimoniate as a single or alternately in combination of ONO-4007 plus IFN-γ, induced by TNF-α and iNOS expression.

Material and Methods: 1) Promastigtes of *L. major* (MHOM/SU/73/5ASKH) and murine macrophage cell line, J774 were cultured in RPMI and DMEM medium respectively. 2) *ELISA for TNF*-α: Macrophages were cultured and infected with *L. major* promastigotes for 24 hrs. The drugs were administered as a single regime: ONO-4007 (0.01, 0.10 and 1.0 mg/ml), IFN-γ (1, 10 and 100U/ml), meglumine antimoniate (0.085, 8.5 and 850 μg/ml) and also in combination regime: ONO-4007+IFN-γ for 24 hrs. TNF-α in the supernatant was assessed at 6-24 hrs incubation by ELISA. 3) *Immuno-histochemical study*: Macrophages were cultured and incubated for 24 hrs with ONO-4007, IFN-γ, and also in combination of ONO-4007+IFN-γ. After 6, 12 and 24 hrs of incubation with these drugs, fixation of the cells were done with 100% ethanol alcohol and immunostaining performed by using primary antibody against iNOS (Wako, Japan).

Results and Conclusion: ONO-4007 alone and in combination of ONO-4007 plus IFN-γ, induced the significant higher level of TNF-α following 6-24 hrs of incubation at three different concentrations as compared to control. TNF-α production by infected-Mϕ cells in response to IFN-γ and meglumine antimoniate as a single regime did not induce the significant higher level at three different concentrations as compared to contorol. Immunohistochemical observations revealed that the positive reactivity for iNOS expression were observed at 6 hrs of incubation and the intensity of the staining pattern gradually increased following 12 hrs and 24 hrs of incubation, in response to ONO-4007 and IFN-γ as a single and also in combination regime of ONO-4007+IFN-γ. From these observations, we suggest that ONO-4007 either alone or in combination of IFN-γ might act as an antileishmanial agent by inducing TNF-α and iNOS production by macrophage cells in vitro study. Meglumine antimoniate exerts its antileishmanial action through different mechanism but not involving the cytokine-immune pathway.

W－VII－3) MARINE SPONGES; HOPEFUL SOURCES OF NEW ANTI-PROTOZOAN DRUGS

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In recent marine sponge has received much attention as a source of unique chemicals, which affect eukaryotic cells. Those chemicals include mycalolide B and jasplakinolide, which shows anti-tumor activities and effects on the structure of actin filaments, respectively. In this study we examined the anti-protozoan activities of extracts from various marine sponges. The lipophilic and hydrophilic layers from marine sponges were examined on the point of inhibition activities against *Plasmodium falciparum* and *Leishmania amazonensis*. Inhibition activities against *P. falciparum*

erythrocytic stages in vitro were examined by direct counting on Giemsa stained smears. For anti-leishmanial activities, *L. amazonensis* LV78 promastigotes transfected with enhanced green fluorescent protein (EGFP) gene were used. The fluorescence intensity of transfected promastigotes was in proportion to the number of promastigotes, indicating that this method could be the high throughput screening method. Among extracts from 93 marine sponges tested against *P. falciparum*, 8 lipophilic and 4 hydrophilic layers showed > 50% inhibition at the concentration of 1 μg/ml. For anti-leishmanial activites 5 lipophilic layers among those from 61 marine sponges showed > 50% inhibition at the concentration of μg/ml. Further fractionation of these lipophilic layers by HPLC, the 50% inhibition concentration (IC_{50}) values of 4 fractions against *P. falciparum* and 4 fractions against *L. amazonensis* were < 100 ng/ml. These results suggest that marine sponges are hopeful sources of new anti-protozoan drugs.

W－VII－4) COMBINATION EFFECTS OF CHLOROQUINE WITH FEBRIFUGINE / ISOFEBRIFUGINE MIXTURE AGAINST CHLOROQUINE-RESISTANT *PLASMODIUM BERGHEI* NK65 IN ICR MICE

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Of strategies on controlling malaria which is one of the major health problems in the world, chemotherapy is the primary defense against malaria. Under world-wide emergence of chloroquine-or multiple drug-resistant variants of *Plasmodium falciparum*, new approaches to treatment of malaria are urgently required as well as a development of new effective drugs. Recently, several reports have been published in combination therapy. In this study, we report our results on combination of chloroquine (CQ) with febrifugine/ isofebrifugine mixture (FI) in ICR-*P. berghei* NK65 model. The alkaloid fraction was prepared from the dried leaves of *H. macrophylla* var. *Otaksa* using a porous polymer gel (Mitsubishi Diaion HP-20) column and a cation exchanger (Amberlyst 15) column. Outbred male ICR mice, 7 weeks old, were infected intraperitoneally with$1x10^5$ *P. berghei* NK65-parasitized erythrocytes. 1) From day 4 after injection of parasitized erythrocytes, mice were treated orally with a single dose of 40 mg/kg of CQ base, a two-day dosage of 20 or 40 mg/kg of CQ base or a three-day dosage of 20 mg/kg of CQ base in the treated group. Distilled water was given in the untreated, infected mice. Mice in the untreated control showed a progressively increasing parasitemia leading to death. A treatment of CQ alone showed little effect against *P. berghei* NK65 infection, and all mice died up to day 17 with an increasing parasitemia. 2) A four-day dosage of 1 mg/kg, twice a day, of FI alone showed a little effect, but all mice died up to day 27. All mice treated with FI showed low parasitemia levels during a drug administration and following a few days, but then malaria parasites increased in the bloodstream of the treated mice until death. On the other hand, mice treated with CQ plus FI survived during the experiment. Malaria parasites in the mice given CQ plus FI decreased on day 6 and then could not be detected by a microscopic examination during observation period. Studies are in progress to clarify the mechanisms of combination effects of CQ plus FI against the parasite.

B－1) CLINICAL FEATURES OF MUCOCUATANEOUS LEISHMANIASIS IN THE AMAZONIAN REGION OF DCUADOR

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PURPOSE: To determine in an active survey the presence of mucocutaneous leishmaniasis (MCL) in persons living at north east of the Amazonian region of Ecuador.

INTRODUCTION: MCL is the most serious and destructive clinical form of American cutaneous leishmaniasis. Figures of skin lesions facial deformities have been represented on pre-Inca pottery from Ecuador, Peru and Colombia. Although MCL was reported since 1924 and is believed to be endemic in the Amazonian region, relatively little active research has been done. Data reported in Ecuadorian medical journals during 1920 to 1987 showed 260 cases, of these 18 (6.9%) were MCL.

STUDY AREA, PATIENTS & METHODS; The study was carried out in the provinces of Sucumbios and Orellana. Individuals suspected to have MCL were clinically and parasitologically examined using Giemsa-stained smears, culture of aspirated, PCR and histopathologic study of skin biopsy. Sandfly collections were done by protected human bait. Itraconazole was the drug used to treat all patients.

RESULTS: 13 patients were found positive. The age ranged from 22 to 67 years. All patients have got lesions in nasal mucosa, septum and turbinate, 5 in upper lip and 4 in pharynx. Lesions in early stages encountered were erithema, bleeding and crusts, nasal infiltration, nodules and granulomas in the septum. Large evolution determined septal perforation, swelling of upper lip, protuberant nose and disphonia. 12 of 13 had evidence of previous scars. 7 antrophophilic *Lutzomyia* could be identified.

DISCUSSION & CONCLUSIONS The progressive inflammatory destruction seen in the more advanced cases resulted in septal perforation (7 cases), and invasion of the larynx causing dysphonia (4 cases). 7 (53.8%) were classified as having severe MCL. Severe disease might follow a long period of evolultion and/or be attributed to the virulence of *L.(V.) braziliensis*. None of the subjects had evidence of active CL. These findings are in agreement with previous observations but contrasts with those reported from Colombia. These differences may reflect the parasite species and ecological location. This active search favors the description of the lesions in early and late stages. This report emphasises the importance of an active search in the endemic communities for early diagnosis and treatment to prevent irreversible disfigurement.

B－2) MALARIA EPIDEMIOLOGY IN LOMBOK ISLAND, INDONESIA

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We had carried out the malaria epidemiological survey at Sokotong in Lombok island and at Stowe Brang, Penyaring and Labanka IV in Sumbawa island from 1991 to 2000. The result revealed that malaria endemics in surveyed areas were restricted to coastal areas where vector mosquitoes, Anopheles subpictus bred. Therefore, we had the preconception that malaria endemic in Lombok island should be restricted to coastal areas before starting the JICA partnership projekt “Malaria control in Lombok and Sumbawa island”. The Muninting Health Center covering area was selected as the area for malaria contorol in Lombok. Immediately after the preliminary survey that examined the spleen size of schoolchildren at grade 1 and 2 of all the primary schools in the area, we noticed that malaria endemic in this area was distributed not only in the coastal area but also in the forested and hilly area. Thereafter, we have been conducting parasitological surveys and entomological surveys. Parasitological surveys were done by the regular monitoring survey at four subvillages four times a year and by case finding and treatment that was done by three teams on the

occation of circulating visit to each subvillage, and revealed that most of malaria cases appeared in forested and hilly areas and a few cases in coastal subvillages located at the northern part of the project area. In forested, hilly areas malaria casea were found constantly through the year. Since in coastal areas malaria usually shows a seasonal epidemic, there still remains a possibility of a sudden occurrence of malaria epidemic although only a few cases were found at the moment. On the map of two subvillages for the regular survey, malaria cases were plotted. No biased distrbution was not demonstrated. The entomological team demonstrated that the main vector in the forested area was *Anopheles balabacensis* and that in the coastal was *An. sundaicus*. The parasitological team examined cerebral malaria cases at the Mataram hospital and found that most of the cases were from forested and hilly areas. This indicated that malaria information which we had obtained previously was biased by the old preconception. Although we began the first trial of distribution of impregnated mosquito nets and residual indoor spraying in selected subvillages, we suppose that these trials would be effective in the coast but not in the forest judging from the results of the entomological survey.

B－3) *BABESIA* SP. AND OTHER HEMOPARASITES IDENTIFIED IN WILD RATS IN CHIANG MAI AND MAE HONG SON PROVINCE, THAILAND

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Wild rodents are thought to be the most important reservoir hosts of zoonotic infectious diseases especially when a tick is involved as a vector. Human babesiosis is a typical case of emerging tick-transmitted parasitic diseases induced by wild rodent reservoirs. Thus, human babesiosis, which is mostly caused by rodent babesias, *Babesia microti*, has occurred mainly in the United States, and a few sporadic cases have recently emerged in the other countries including Japan. In order to infer the possibility of the emergence or latent existence of human babesiosis in the malaria endemic area, we started in 2000 to investigate the prevalence of *Babesia* infection in wild rodents in Chiang Mai and Mae Hong Son Province, Thailand and partly reported elsewhere. Here, we report the results of the consecutive investigation and analysis regarding *Babesia* infection as well as the detection of other hemoparasites in wild rats in the area.

During 2000 to 2002, the epizootiologic surveys for wild rats were carried out at several sites in Chiang Mai and Mae Hong Son Province, Thailand. In total 47 wild rats, including 30 *Bandicota indica* and 17 *Rattus exulans*, were caught. The blood smears showed that 17 of 30 *B. indica were* parasitized by *Babesia* sp. (parasitemia 0.03-11.02%) but not in any of 17 *R. exulans.* All of *Babesia*-positive *B. indica* were captured in Chiang Mai but not in Mae Hong Son Province. Twelve of 17 *Babesia*-positive *B. indica* were infested with ticks of *Haemaphysalis* spp.. No other kinds of ticks were identified on any of captured wild rats. All of the ss-rDNA sequences of 6 *Babesia* isolates from different rats were completely identical without regard to the site and the year for capture of rats. The phylogenetic analysis based on the sequence of ss-rDNA showed that the Thai *Babesia* sp. is most closely related with canine babesias, *B. canis* and *B. gibsoni*, but not so with *B. microti*. Two dogs kept near the site where the prevalence of *Babesia* infection in wild rats was high were found negative with *Babesia* parasites by PCR as well as blood smears. Regarding other hemoparasites, two *Babesia*-positive *B. indica* showed the simultaneous infection of *Hepatozoon*-like parasites (gamonts) in leukocytes. *Hepatozoon* is also known to be transmitted by ticks. The ss-rDNA sequence analysis supported that the parasite is most closely related with *Hepatozoon* sp.. On the other hand, *Trypanosoma* sp. was identified only in *R. exulans* (3/17), but not any of *B. indica*. Although human babesial infection has not been reported in Thailand, the risk of zoonotic babesial infection cannot be denied at present. Indeed, many ticks bites are noticed for human as well as for domestic dogs in the area. Serologic survey using the Thai babesial infection is hold among human population in the area.

B－4) ADULT BLACK FLIES ATTRACTED TO HUMANS AND WATER BUFFALOES, AND NATURAL INFECTIONS WITH *ONCHOCERCA* LARVAE IN CHIANG MAI, IN NORTHERN THAILAND

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Several *Simulium* species were investigated as to their biting habits and natural infections with filarial larvae at Ban Pan Fan, Chiang Mai Province, in northern Thailand. Female adult flies landing on or flighting around a human and a water buffalo were collected during the daytime from 06.00 to 19.00 hours on 22 June 2001. As a result, 217 *S. nodosum*, 86 *S. asakoae* and 2 *S. nigrogilvum* were obtained from a human attractant, and 416 *S. nodosum*, 25 *S. nakhonense*, 16 *S. asakoae*, 4 *S. fenestratum* and 2 *S. nigrogilvum*, from a water buffalo. The blood-feeding was confirmed only for *S. nodosum* and *S. nigrogilvum* on humans, and for *S. nodosum* and *S. nakhonense* on water buffalos. Dissections of these simuliids showed that *S. nodosum* was naturally infected with developing filarial larvae. Two types of microfilariae were distinguished but only one type of infective larvae. These larvae resembled *Onchocerca suzukii*, a parasite from a wild Japanese bovid, suggesting that an unknown *Onchocerca* species from ruminants was transmitted in Thailand. Infection rates with all stages of larvae and third-stage larvae were 2.3% (14/608) and 1.0% (6/608), respectively. This is the first report of natural infections of black flies with *Onchocerca* larvae in Southeast Asia, and the involved black-fly species is anthropo and zoophilic.

B－5) SEASONAL CHANGES OF WILD ANOPHELINE MOSQUITOES AROUND JICHI MEDICAL SCHOOL

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Abstract

We have collected wild mosquitoes around Jichi Medical School, Tochigi Prefecture, for two years (2001-2002). A light trap was hanged overnightly in a cattle hut 2 km far from Jichi Medical School, and the number of mosquitoes collected was counted next day. Mosquito species collected were *Anopheles sinensis, Culex tritaeniorhynchus, Culex pipiens pallens* etc... *An. sinensis*, which can transmit vivax malaria, was collected from June to September, in particular, middle July to beginning of August. On the peak day, more than 1800 of *An. sinensis* female mosquitoes were collected. Almost all *An. sinensis* had fed blood. *Cx. tritaeniorhynchus*, which can transmit Japanese encephalitis, made a peak, more than 1600 per night, in August. Three weeks interval was observed between peaks of *An. sinensis* and *Cx. tritaeniorhynchus*. Both mosquito species might feed on cattle and oviposit in paddy fields several hundred meters far from the cattle hut. In winter, no adult mosquitoes were collected from the cattle hut. We should notice that these vector mosquitoes still live together with us in Japan. We will follow these mosquitoes' number as the information for the future.

B－6) A NOVEL GT-MISMATCH BINDING PROTEIN POSSIBLY INVOLVED IN BASE EXCISION REPAIR OF THERMALLY INDUCED MATAION

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Watson-Crick-type base pairs are extremely strict in genomic DNA because of high specificity of polymerase activities and error-correcting machineries. Even nonreplicating DNA is exposed to error-prone stresses such as reactive oxygen species, radiation and high temperature that modify bases or cut backbone DNA strands. Among mismatches found in DNA, a G/T mispairing is most common because cytosine is frequently deaminated after an enzymatic methylation. We report here a serendipitously discovered novel G-to-T-mismatch binding protein (nGTBP).

This protein bound to a CYBB promoter-originated 23 -mer heteroduplex with a G/T mismatch at bp-177. None of heteroduplexes with the rest 15 possible base pairs at bp-177 bound to nGTBP. Similar heteroduplexes with G/T or T/G at different bp positions failed to recruit nGTBP. DNA heterodupolex-nGTBP comlex was not disturbed by an MSH6 consensus oligmer nor anti-MSH6 antibody on an electrophoresis mobility shift assay. In additon, the nGTBP was detected in MSH6 deficient cell lines. The protein minimally required a middle 14-mer heteroduplex of the original 23-mer and TRTGNB as the core sequence with the G mispairing with T. Neither oxidized T [thymineglycol] nor oxidized G [8-oxoguanine] at bp-177 of 14-mer homoduplex made the duplex recruit the nGTBP. Deaminated A [hypoxanthine] and deaminated C [uracil], however, made the duplex bind to the nGTBP. These results suggest that the nGTBP primarily recognizes deaminated "matched" pairs. Deaminations occur by spontaneous hydrolysis that is increased by high temperature. Accordingly this nGTBP may be involved in the repair of thermally injured deoxynucleotides.

B－7) MITOCHONDRIAL QUINOL-FUMARATE REDUCTASE (COMPLEX Ⅱ) FROM *ASCARIS SUUM* AS A TARGET FOR CHEMOTHERAPY

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Ascaris suum, a parasitic nematode, lives in host small intestine, where oxygen tension is limited and develops a unique anaerobic energy generation system. In this system called NADH fumarate reductase system, mitochondrial complex Ⅱ catalyzes fumarate reduction and functions as a terminal oxidase. The difference between *A. suum* and mammalian complex Ⅱ that functions as succinate dehydrogenase gives us an idea that complex Ⅱ must be a promising target for chemotherapy. In this study, we purified *A. suum* complex Ⅱ to almost homogeneity and performed enzymatical studies and crystallization.

First, we established a new method of mitochondria preparation from *A. suum* muscle and we could prepare 500 mg protein of mitochondria from 500 g (about 400 nematodes) muscle. Second, we performed 2 kinds of anion-exchange chromatography and could purify about 7.5 mg of complex Ⅱ from 1000 mg protein of mitochondria.

In enzymatical analylsis, we showed that *A. suum* complex Ⅱ had higher affinity to fumarate than bovine complex Ⅱ and that *A. suum* complex Ⅱ could function as fumarate reductase by this high affinity to fumarate. Some inhibitors were tested and we showed that TTFA and carboxin, the inhibitors of bovine complex Ⅱ, did not inhibit *A. suum* complex Ⅱ and this suggests that the structure of inhibition site must be different between *A. suum* and bovine complex Ⅱ. So flutolanil can be a promising lead compound of anthelmintics.

In trials of crystallization, we could obtain the crystal with the size of 30 micrometer reproducibly. Stabilization of *A. suum* complex Ⅱ activity by additon of 1 mM malo-

nate and screening of wide range of detergents led to the crystal growth of *A. suum* complex Ⅱ. Now the X-ray diffraction study is undergoing.

This study shows that complex Ⅱ is a promising target for chemotherapy and is a first step for development of new anti-parasitic drugs.

B－8) CHEMOTACTIC FACTORS FOR LEUKOCYTES FROM EGGS OF *SCHISTOSOMA MEKONGI*

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We previously showed that soluble extract of eggs (SEA) of *Schistosoma-japonicum* possesses intense chemotactic activities for eosinophils or neutrophils, and pointed out a contribution of the granulomatous lesions around deposited eggs in the liver. *Schistosoma mekongi* is one of the newly recognized species of *Schistosoma* that is closely related to *Schistosoma japonicum*. However, little information is available about the pathogenesis of *S. mekongi* infection.

In the present study, we examined leukocyte chemotactic activity of SEA of *S.. mekongi* using multi-well microchemotaxis chambers equipped with Millipore membrane filters with a pore size of 3 μm. SEA of *S. japonicum* was used as a positive control. *S. mekongi* SEA showed lower eosinophil chemotactic activity than *S. japonicum* SEA. In contrast, *S. mekongi* SEA showed more intense neutrophil chemotactic activity than *S. japonicum* SEA. Western blot analysis revealed that *S. mecongi* SEA generally showed a similar antigenecity with *S. japonicum* SEA, whereas *S. mecongi* SEA showed lower affinity to an antibody against the high-molecular weight ECF purified from *S. japonicum* SEA. Toluene-extractable fraction of *S. mekongi* SEA showed comparable chemotactic activities with that of *S. japonicum* SEA, whereas toluene-nonextractable fraction of *S. mekongi* SEA showed lower chemotactic activity than that of *S. japonicum* SEA did. These results indicate that a defect of glycosilation of the high molecular weight components of SEA resulted in the lower grade of eosinophil chemotactic activity of *S. mekongi* SEA than *S. japonicum* SEA.

B－9) CHARACTERIZATION OF RECOMBINANT FRAGMENTS OF THE 150-KDA LECTIN (IGL) OF *ENTAMOEBA HISTOLYTICA*

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Carbohydrate-protein interactions initiate the contact-dependent cytotoxicity for which *Entamoeba histolytica* was named. It is well known that the 260-kDa galactose (gal)- and *N*-acetyl-D-galactosamine (GalNAc)-inhibitable surface lectin of the ameba mediates adherence to human colonic mucins, colonic epithelial cells, neutrophils, and erythrocytes. Recently, we identified another Gal/GalNAc lectin, a 150-kDa surface antigen recognized by monoclonal antibodies (mAbs). The mAbs inhibited amebic adherence to erythrocytes and Chinese hamster ovary (CHO) cells, erythrophagocytosis, and cytotoxicity to CHO cells. The 150-kDa lectin, named as intermediate subunit (Igl), is a cysteine-rich protein consisting of 1101 amino acids and containing multiple CXXC motifs in amino acid sequences. In the present study, various lengths of recombinant fragments of Igl were prepared in *Escherichia coli* and the reactivity of the mAbs to the fragments was examined for epitope localization. Two of four adherence-inhibitory mAbs reacted with the recombinant protein of full length but with none of the partial fragments, demonstrating that conformation of the protein is important. The epitopes of the two other inhibitory mAbs were localized in the parts of amino acids 603-753 and amino acids 989-1088. These parts may have the carbohydrate recognition sites and could be vac-

cine candidates.

B－10) INVOLVEMENT OF PROTEASOME FUNCTION IN *ENTAMOEBA* GROWTH AND DIFFERENTIATION

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Protein degradation by ubiquitin-proteasome pathway plays an essential role for a wide variety of cellular processes in eukaryotic cells. Studies with selective proteasome inhibitors such as lactacystin have demonstrated possible contribution of protein degradation by this pathway in cellular processes. Therefore, we examined whether proteasome inhibitors affect growth and encystation as well as excystation of *Entamoeba*. Three proteasome inhibitors, lactacystin, *clasto*-lactacystin β-lactone, and MG-132, were used for *Entamoeba histolytica* and *E.invadens*. All these drugs inhibited *E. histolytica* growth in a concentration-dependent manner, while lactanystin was most potent for the inhibition and MG-132 showed the inhibitory effect only at higher concentrations. *E. invadens* was more resistant to these drugs than *E. histolytica*. Encystation of *E. invadens* was also inhibited by these inhibitors and was more sensitive to the drugs than the growth, where the β-lactone was most potent for inhibition of encystation. The inhibitory effect of lactacystin and the β-lactone on encystation was slightly or little abrogated by the removal of the drug, respectively. Multinucleation occurred in *E. histolytica* trophzoites treated with these drugs, being most marked by lactacystin. Electron microscopy revealed that treatment of *E. histolytica* trophozoites with lactacystin led to an increase in the number of cells with many glycogen granules in the cytoplasm. Lactacystin, β-lactone and MG-132 had no or little effect on the excystation and metacystic development of *E. invadens*. These results show that proteasome function plays an important role for *Entamoeba* growth and encystation, but has no obvious effect on the excystation and metacystic development.

B－11) MOLECULAR CLONING AND CHARACTERIZATION OF FARNESYLTRANSFERASE OF *ENTAMOEBA HISTOLYTICA*

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Protein farnesylation, which is catalysed by farnesyltransferase (FT), is one of the post-translational lipid modification of proteins such as low molecular weight GTPase Ras, and thereby enables Ras to attach membranes and interact with other proteins. To elucidate significance of protein farnesylation in *Entamoeba histolytica* and to evaluate its FT as a target for chemotherapy, we have started biochemical characterization of FT of this parasite. We obtained cDNA encoding α-and β-subunits of FT (FT α and β) of *E. histolytica* by PCR amplification using primers designed on the fragments of corresponding genes found in the TIGR *E. histolytica* genome database. The genes of *E. histolytica* FTα and FTβ encoded 298 and 375 amino acids, respectively, and showed 24-36% positional identity with those of human, *Arabidopsis thaliana*, *Saccharomyces cereviside*, and *Trypanosoma brucei*. The coding regions and the ribosome binding sites between them were cloned in tandem in pQE31, and expressed in *E.coli*. The 38 kDa and 43 kDa proteins corresponding FTα and FTβ, respectively, were identified by SDS-PAGE. The recombinant *E.histo-*

lytica FT showed a FT activity against wild type human recombinant Ras (-CVLS) but not against mutant human Ras (-CVLL). We then prepared 4 recombinant *E.histolytica* Ras homologues (-CIMF, -CELL, -CSVM, -CVVA) to examine their substrate activities. Among them, only Ras (-CVVA) showed the substrate activity against the recombinant *E. histolyca* FT, suggesting a difference in substrate specificity between human and *E. histolytica* FT. These results suggest that *E. histolytica* FT may become a possible target for chemotherapy.

B－12) RECENT SITUATION OF DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER IN THE PHILIPPINES (1995－2001)

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[**Introduction**] Dengue fever (DF) and dengue hemorrhagic fever (DHF) cases were reported approximately 13,000 every year and its mortality rate was 1% among children and adolescent in the Philippines. The Research and Biotechnology Division, St. Luke's Medical Center and the Institute of Tropical Medicine, Nagasaki University have done collaborative study on dengue for 7 years (1995-2001) in Metro Manila. To know the impact of DF and DHF in the Philippines, we conducted virological, serological and clinical surveillance using blood samples and clinical records of dengue suspected / clinically diagnosed patients.

[Materials and Methods] Using 3,564 blood samples, virus isolation using C6/36 mosquito cells and RT-PCR were performed as viroloical examinations. Hemagglutination inhibition test (HI test), IgM-capture ELISA and IgG indirect ELISA were done as serological examinations.

[Results and Discussion] The peak season of dengue virus infection was from July to November. The dengue outbreak occurred every three years that were in 1995, 1998 and 2001. Dengue confirmed cases were 53% of the examined cases and approximately 80% of them were dengue secondary infection examined by HI test and IgG indirect ELISA. The major serotypes of isolates were DEN2 and 3 in 1995 but it gradually changed to DEN 2 and 1 by 2001. The phylogenetic analysis of DNE2 envelope region indicated the DEN2 virus local strains circulate within the Philippines. The severity of clinical symptoms based on WHO criteria could classify dengue as DHF grade 2 (38%), DF (31%) and Grade 3 (10%) among 863 dengue laboratory confirmed cases.

B－13) A MECHANISM OF THROMBOCYTOPENIA DURING THE ACUTE PHASE OF DENGUE VIRUS INFECTION: INVOLVEMENT OF PLATELET-ASSOCIATED IGG AND ANTI-DENGUE VIRUS IGG

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Although the public health impact of dengue is rapidly increasing, the mechanism of thrombocytopenia in this disease remains unknown. To elucidate this mechanism, the relationship between platelet-associated IgG (PAIgG) and platelet count in 83 patients with secondary dengue virus infection was investigated in a prospective-hospital-based study. A significant increase in PAIgG levels as well as a significant decrease in platelet count were found in all cases of secondary infection, compared with 57 healthy volunteers. Only two of the 83 patients with secondary dengue

virus infection were positive for anti-platelet IgG in plasma. The PAIgG levels in 53 patients with a platelet count of less than $100x10^3/\mu L$ were significantly higher than those in 30 patiets with platelet counts greater than $100x10^3\mu L$ or those from healthy volunteers. A significant inverse correlation between the two parameters was found. The low baseline platelet counts during the acute phase in 12 patients significantly increased during the convalescent phase, while the increased PAIgG levels of the acute phase in these patients significantly decreased during the convalescent phase. Involvement of anti-dengue virus IgG was also shown in platelets from 8 patients.

These findings suggest a pivotal role of PAIgG formation, involving anti-dengue virus IgG, in the inducement of transient thrombocytopenia during the acute phase of secondary dengue virus infection.

B－14) EVALUATION OF A HEPATITIS B VACCINATION IN CHIANG MAI, THAILAND

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In 1992, the Government of Thailand started a nationwide vaccination program for mother-to-infant transmission of hepatitis B virus (HBV), following a pilot program in Chiang Mai and Chon Buri. We report the long time efficacy of this vaccination program in Chiang Mai. Ninety three percent of the urban and 60% of rural children had received adequate vaccination in the 4 to 6 year-old age group. The prevalence of HBsAg and anti-HBs positivity was 0.9 and 42.8% in the children who were adequately vaccinated, compared to 7.2 and 24.6% in the children who were not or inadequately vaccinated, respectively. The prevalence of HBsAg and anti-HBs positivity of the adequately vaccinated children was 1.1 and 33.8% in urban areas and 0.5 and 28.2% in rural areas, respectively. These findings indicated that the program has a certain level of efficacy, however it can be hardly said to be sufficient, comparing with the result of Japan. Several factors such as the commercial source of the vaccines, improper transportation and storage systems and insufficient methods of the administration might reduce the effectiveness of vaccination.

B－15) ACUTE RESPIRATORY INFECTIONS (ARI) AMONG CHILDREN IN VIETNAM: DETERMINATION OF BACTERIAL ETIOLOGY AND ANTIBIOTIC RESISTANCE OF PATHOGENS

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Acute respiratory infections (ARI) are common causes of morbidity and mortality in tropical and developing countries. This study was designed to determine whether a quantitative culture method employing bronchial secretion obtained by nasopharyngeal swab is useful for determination of causative bacterial pathogens of ARI among children, and to determine the antibiotic susceptibility of major bacterial pathogens.

We enrolled 102 patients with ARI and 43 control patients without ARI. A chest radiograph was taken and bronchial secretion was obtained from each patient by nasopharygeal swab, and the quantitative culture of bronchial secretion was done.

The isolation rate of bacterial pathogens was 51.0% (25/49) in patients with bacterial pneumonia (BP), 30.2% (16/53) in patients with acute bronchitis (AB) and 18.6% (8/43) in control (CT) patients, respectively. The isolation rate in BP, but not in AB, was significantly higher than that in CT. Major pathogens isolated were *S.pneumoniae* (n=12) and *H.influenzae* (n=11). Major bacterial pathogens of 102 patients of ARI were *S.pn* (45.5%) and *H.inf* (36.3%), respectively. We determined the MICs of them and also ex-

amined drug resistant genes of these strains by PCR. A very high rate of penicillin-resistant *S.pn* (85%) and β-lactamase producing *H.inf* (70%). Most of PRSP strains had penicillin-binding protein (PBP) mutations in all of *pbpla*, *pbp 2x* and *pbp2b*, and were belong to serotype 19F or 23F.

Our data suggest a quantitative culture method using bronchial secretion is a useful tool for the bacterial diagnosis of BP in pediatric patients, and demonstrate a high rate of β-lactam antibiotic resistance in major bacterial pathogens of pediatric patients of ARI in Vietnem.

B－16) A TRIAL OF NOSOCOMIAL INFECTION CONTROL IN THE BACH MAI HOSPITAL PROJECT FOR FUNTIONAL ENHANCEMENT IN VIETNAM

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A technical cooperation project has been implemented in Bach Mai Hospital in Vietnam since January 2000 with the purpose of upgrading the quality of medical care service in the hospital and disseminating the benefit of better medical care to wide areas. In this project, nosocomial infection control was integrated in the program under the recognition that effective control is indispensable to improve the quality of medical care. This is the first trial as a hospital project in developing countries. In this study, the process of the introduction of nosocomial infection control was summarized aiming to realize better control in the developing countries.

The activities of nosocomial infection control in the project are as follows:

1. Setting up of Nosocomial Infection Control Department and allocation of staff
2. Supply of essential equipment and training of the staff
3. Inspection of current situation of nosocomial infection control in some hospitals
4. Making out a manual, teaching materials, pamphlets, posters, etc.
5. Organizing training courses and evaluation
6. Carrying out a fact-finding study for 1,266 in-patients in Bach Mai Hospital

After the start of the project consciousness of nosocomial infection control among hospital staff is increasing and control system is being created. The average score of the tests conducted after the training courses showed the increase of 29.4 compared with pre-tests, and the trainees had high satisfaction. The fact-finding study revealed infection rate with 5.77% (73/1,266). As causative agents, Pseudomonas aeruginosa, Acinetobacter baumanii, Escherichia coli, Enterococcus, Klebsiella pneumoniae revealed high frequency. 19 out of 73 cases were regarded as infections associated with surgical procedures.

Nosocomial infection rate in developing countries is high and effective control method has been increasingly requested. It should be stressed the importance of basic sanitary procedures such as hand washing. Enlightenment using manuals, teaching materials, and conducting training courses are also effective. In addition, appropriate use of antibiotics based on the results of monitoring causative agents is desired.

B－17) EXPERIMENTAL INFECTION INTO SCID MICE WITH MICROSPORIDIA ISOLATED FROM AN AIDS PATIENT

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We found out a case of keratitis was caused by a species of microsporidium which was first isolated from the case. We successfully established an *in vitro* culture technique for the microsporidium, and we gathered from the morphology by transmission electron microscopy (TEM) that the species was *Trachipleistophora anthropophthera* in all likelihood.

The specimen of corneal biopsy was obtained from a 42 years old HIV-infected patient at King Chulalongkon Memorial Hospital, in Thailand. The man suffered from chronic keratitis in his left eye for more than 6 months. Several corneal scrapes stained for bacterial, fungal and viral agents were all negative. The corneal specimen was inoculated into a 25 cm^2 culture flask with fibroblasts derived from newborn mouse brain, and maintained with MEM culture medium supplemented with 5% fetal bovine serum and 5% newborn calf serum at 26C, 29C or 37C. The microorganism could multiply at the same proliferation speed at the three different temperatures. Many an oval-shaped spore, which was compatible with microsporidium, was detected under a light microscope after the smear of foci in fibroblasts was stained with chromotrope 2R or Calcofluor White M2R. The cell cultures were periodically scraped, fixed in glutaraldehyde, and examined with transmission and scanning electron microscope. Bisporous and multisporous vacuole-like sporophorous vesicles were observed simultaneously in foci of the microsporidia under TEM. They proved the species that was identified as *T. anthropophthera*, not as *T. hominis*.

To investigate the course of the infection, the spores from cultures were inoculated into SCID mice intraperitoneally (ip) or intramuscularly (ip). The mice were sacrificed to look for which tissue and organs were infected with the microsporodia. We initiated the tissue cultures of the SCID mice to isolate spores from each cultivation. We examined the stump preparation and pathological sections, and detected DNA of the microsporidium by PCR in each organ. In the im mice, the infection site was limited in the original part. In the ip mice, the spores became spread into the general body except the lung, probably through lymphatic circulation. These findings clearly indicate that microsporidia can be infectious into almost organs of a SCID mouse. Immuno-histological assay will be helpful to confirm accurately further which tissue and organs are infected. Since intraperitoneal and intramuscular inoculation is artificial, we will infect by different transmission routes (ocular, intranasal and oral inoculation) into SCID mice in the near future.

C－01) ANALYSIS OF ANTIGEN-SPECIFIC IGG SUBCLASSES IN SERUM AND URINE SAMPLES FROM BANCROFTIAN FILARIASIS PATIENTS

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A sensitive and specific urine ELISA, which detects filarial antigen-specific antibodies, is suitable for field studies. However, basic information on the immunoglobulins and total protein in urine and their correlation to those in serum has not been well studied. In this study, antigen-specific IgG subclasses are compared using paired serum and urine samples from bancroftian filariasis patients.

Seventy-six paired serum and urine samples from the antigen positives were used in this study. They were collected from residents in Matara, Sri Lanka, an endemic area of bancroftian filariasis. Blood sample, 100 μl, was taken on a filter paper strip. Collected urine samples were added with sodium azide. Bancroftian filaria-derived antigen was detected in serum samples eluted from the filter paper using an antigen detection kit (Trop-Ag *W. bancrofti*). Anti-*Brugia pahangi* female adult worm specific IgG subclasses were measured by ELISA. Serially diluted standard serum samples were applied to each ELISA plate to make a standard curve for each subclass. Each IgG subclass was shown with unit calculated from the standard curve. Urine samples were used directly and serum samples were diluted 5000 times for IgG1 and IgG4, and 100 times for IgG2 and IgG3. Protein levels in urine samples were measured using Protein Assay (Bio Rad).

Both IgG1 and IgG4 to *B. pahangi* antigens were detected in serum and urine samples: their levels in serum and urine samples were correlated well ($r=0.67$, $P<0.0001$ and $r=0.73$, $P<0.0001$, respectively). Strong correlation between the IgG1 and IgG4 levels both in serum and urine samples were also observed ($r=0.71$, $P<0.0001$ and $r=0.70$, $P<0.0001$, respectively). Both IgG2 and IgG3 subclasses to the antigens in urine samples were low and some of them were not detectable. Very few part (0.1～0.001%) of serum IgG1 and IgG4 were found in urine samples. Serum/urine ratio of IgG1 and IgG4 correlated well ($r=0.73$, $P<0.0001$). Although 5 out of 75 urine samples showed moderately high protein levels (30～55mg/dl) and their anti-*B. pahangi* IgG4 levels were high, the remaining 70 urine samples were normal (<30mg/dl). These observations suggest that presence of anti-*B. pahangi* IgG4 in urine, which is utilized for diagnosis, is not due to leak caused by abnormality of kidney function.

C－02) DIRECT AGGLUTINATION TEST (DAT) WITH URINE SAMPLES FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Due to high sensitivity, specificity and ease to perform, direct agglutination test (DAT) has been widely accepted for the diagnosis of visceral leishmaniasis (VL) especially in the field. However this method requires blood, which hinders the sample collection in the field particularly with pediatric subjects. In this study we applied urine, as a sample for DAT instead of blood.

We prepared trypsin treated, formalin fixed, and Coomassie brilliant blue stained *Leishmania donovani* promastigotes as DAT antigen. The quality of the antigen was standardized with the commercially available antigen produced by Koninklijk Instituut voor de Tropen, Amsterdam. Fifty microliter of urine sample was applied without concentration to a well of a V-shaped 96-well microtiter plate, to which 50 μl DAT antigen was added. Then the plate was incubated overnight at room temperature. The results were judged with standard positive and negative samples.

The sensitivity of the assay was 66.7% (38 positives/ 57 VL samples). The specificity of the assay, determined with 137 non-VL samples, including 59 non-endemic

healthy controls, 58 malaria, 13 tuberculosis and 7 other diseases, was 94.1% (129 negatives/137 non-VL samples).

We previously reported a urine-based ELISA for the diagnosis of VL, which showed 95.0% (57 positives/60 VL samples) sensitivity and 95.3% (204 negatives/214 non-VL samples) specificity. One parasitologically confirmed sample became negative with urine-based ELISA, where as, in urine-based DAT that sample became positive. As the sensitivity of the urine-based DAT is still inferior, we are trying to improve it. Urine-based DAT has several advantages over conventional DAT. (1) Urine collection is non-invasive. (2) The assay system can process more number of samples in one plate, which will reduce the amount of antigen, the cost, time and labour/test.

C－03) DETECTION OF *PLASMODIUM BERGHEI* AND *P. GALLINACEUM* BY ICT *P.F./P.V.* IMMUNOCHROMATOGRAPHIC TEST

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ICT *P.f./P.v.* is an immunochromatographic dipstick test which detects histidine-rich protein, HRP-2 of *Plasmodium falciparum.* ICT *P.f./P.v.* can also detect *P. vivax* and other non-*P. falciparum* parasites with a pan-specific antibody. Before employing ICT *P.f./P.v.* for routine screening, extensive evaluation of its usefulness must be made. It is very important to study at which extent of parasitaemia a newly-infected patient shows a positive result by ICT *P.f./P.v.,* and how its reactivity are affected during clinical course. Several groups have already reported such information. However, under field conditions, difficulty in confirming 'newly-infected' among the malaria-positive subjects, persistent post-treatment antigenaemia, cross reactivity with rheumatoid factor, and poor skills of local microscopists, should be taken into account. Recently, we found the pan-malarial antibody of ICT *P.f./P.v.* recognises non-human parasites, *P. berghei* and *P. gallinaceum.* In this report *P. berghei*-irfected mice and *P. gallinaceum*-infected chickens which had been infected by either merozoite injection or mosquito bite, were tested for microscopic examination and ICT *P.f./P.v.* test. Lowest numbers of parasite in the blood containing *P. berghei* or *P. gallinaceum* detected by ICT *P.f./P.v.* were 8,400 and 10,000 parasites, respectively. Reportedly the pan-specific antibody shows good detection sensitivity on blood samples containing parasites more than 500/μl (7,500 parasites in 15 μl), which is close to our result on *P. berghei.* Thus the mouse model seems to be useful for the study of detection sensitivity on onset parasitaemia after mosquito bite. Further study on persistent post-treatment antigenaemia is underway. *P. berghei*-mouse system may provide a good model for evaluation of ICT *P.f./P.v.* and other rapid diagnostic tests for malaria.

C－04) ANTI-SERA ANTIBODY PRODUCTION AS A PARAMETER OF ANTI-MALARIA IMMUNITY IN INDIVIDUALS IN THE SOLOMON ISLANDS

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Serine-rich antigen (SERA) of *Plasmodium falciparum* is one of the most promising vaccine candidates against erythrocytic stage of the parasite in humans. Based on the recent in vitro observations showing effective SERA-mediated protective immunity, the present study was conducted to uncover the SERA-mediated protective immunity in individuals in endemic areas of falciparum malaria through the sero-epidemiological approach. People in Guadalcanal, where transmission of falciparum malaria is meso or hyper endemic, were divided into three categories;

slide(-)/symptom(-), slide(+)/symptom(-), and slide(+)/symptom(+). Number of each group was 70, 19 and 26, respectively. The N-terminal portion of SERA (SE36') and the N-terminal 6 blocks of MSP1 (Ml/6) were used for ELISA. We observed that total IgG production specific for both SE36' and M1/6 was significantly higher in slide(-)/symptom(-) group than in slide(+)/symptom(-) group ($p < 0.01$). The difference was much more apparent in SERA-specific IgG levels than that in M1/6-specific IgG. When we compare IgG_3 levels in the two groups, no symptomatic individuals of 15 yr or more showed detectable level of SE 36'-specific IgG_3. On the other hand, we did not observe any difference in polio virus-specific antibodies between tbe two groups. Out of 70 slide(-)/symptom(-) group, 14 were negative for SERA-specific IgG, while all of the group showed positive IgG to M1/6. Strong association was observed for degree of parasitemia and SE36'-specific IgG. No individuals with parasite density of 100 and more had detectable level of SE36'-specific IgG. Together with those, we concluded that SERA is a promising vaccine candidade for human falciparum malaria in our seroepidemiological study in the Solomon Islands.

C－05) SERODIAGNOSIS OF TUBERCULOSIS USING MPB64 DERIVED FROM *MYCOBACTERIUM BOVIS*

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It is known that the number of patients for tuberculosis is gradually increased in the worldwide, including in Japan. Especially in the developing countries, it's important to identify the patients for active tuberculosis. In the previous study, the MPB64 antigen was efficient by the patch test for the diagnosis of active tuberculosis. In addition, there was another report for MPB64 in which PCR analysis was useful to detect the diagnosis of tuberculous pleuritis and meningitis. In this study, we try to find MPB64 has the high specificity and sensitivity for the diagnosis of the active tuberculosis. *mpb64* cDNA was kindly donated from Dr. Matsuo (Nagasaki University) and cloned into the expression vector by the GATEWAY technology system (invitrogen Inc.,). The recombinant protein MPB64 was expressed as a fusion protein with His-tag for the C-terminus and the M. W. was guessed about 30 kDa. Western blot analysis was done using a patient's sera for active tuberculosis and healthy subjects as a control. The result was shown that a specific band for 30 kDa was detected with the patient but not for the control. Therefore this band was also detected by the immunoreactive with anti-His Tag antibody. So this was MPB64-specific reactivity for the patient's serum. Then MPB64 protein was affinity-purified using His-tag and the IgG titers in the sera were measured by ELISA method. The mean value of patients was higher than that of healthy subject. And, there is a tendency to decrease the IgG titers time-dependently. It is considered that IgG titers specific for MPB64 were decreased by the effect of the treatment progress in sera. These results suggested that MPB64 might to be a valid antigen for the patient of active tuberculosis and there is a possibility to use the screening method of the patient for the active tuberculosis in the field of developing country.

C－06) A METHOD OF BLOOD EXAMINATIONS IN OVERSEAS MOBILE CLINIC —ITS CLINICAL APPLICATION TO THE REMOTE AREAS IN LAO PDR—

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In this study, we carried out overseas mobile clinic in Lao PDR from April to May (for 21 days) in 2002, and examined the serum biochemical tests and blood examinations using newly developed filter paper plasma skimming film which is easy to separate without centrifuge.

The sera were collected from the inhabitants of DakEuy village (168 subjects) at SeKong Province and NonTao village (68 subjects) at OudomXay Province. In this time, we were able to examine by the skimming filter for GOT (AST), GPT (ALT), γ-GTP, BUN, Creatinine, Total cholesterol, HDL-cholesterol, Triglyceride, Amylase and Uric acid by the filter paper for Hemoglobin, HBs Ag and HCV Ab by the rapid blood sugar analyzer for Blood Sugar.

The results of these examinations were GOT 35.5± 16.7 IU/l, GPT 21.3±5.7 IU/l, γ-GTP 35.5±l4.1 IU/l, BUN 11.5±3.4 mg/dl, Cr. 0.4±0.1 mg/dl, T—CHO 135.0 ±21.2 mg/dl, HDL-CHO 31.7±6.4 mg/dl, TG 107.9± 45.6 mg/dl, AMY 113.4±82.0 U/l, UA 3.3±0.9 mg/dl, Hb 16.1±1.8 g/dl, BS 88.6±l3.6 mg/dl, in DakEuy village. In NonTao village, GOT 20.1±12.1, GPT 21.3±5.7, γ-GTP 33.9±15.4, UA 5.4±1.0, Hb 13.3±2.1, BS 94.8±l8.3. In the 4 cases of liver disfunction, we have a chance to measure HBs Ag and HCV Ab. Two cases were positive for HCV Ab, but no case was positive for HBs Ag.

There was no person who showed higher than upper range of normal limit of Total cholesterol, Uric acid and renal function. And, the patient of diabetes mellitus was not diagnosed in these inhabitants. In the DakEuy village, hemoglobin values of the inhabitants were higher, because it might be influence that this village located in the highland over 1,000m altitude.

Therefore, it was concluded that these methods (plasma skimming film and filter paper), which were developed as screening test in Japan, were suitable for the mobile clinic of rural areas in the developing country.

C－07) COMPARISON OF ULTRASONOGRAPHIC IMAGES BETWEEN *SCHISTOSOMA JAPONICUM* AND *S. MEKONGI* INFECTIONS

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In order to estimate liver fibrosis due to *Schistosoma japonicum* and *S. mekongi* infections, ultrasonographic (US) examination were used in the schistosomiasis endemic areas of Philippines and Cambodia. Typical liver damage due to schistosomiasis japonica can be detected by US, as “network pattern” or “fish scale pattern”. Advanced bridging fibrosis reveals these patterns, and periportal fibrosis shows other changes such as thickening of portal vein wall. Severe thickening of portal vein wall and collateral vessels are frequently found in elder cases with network pattern. But in young cases, we find no relationship between network pattern and periportal fibrosis detected as thickening of portal vein wall.

In *S. mansoni* infections, based on the degree of periportal fibrosis, liver fibrotic changes are classified into some patterns and these patterns do not include network pattern. In *S. mekongi* infections, typical network pattern has not be found. And except for the pattern like irregular fatty liver,

most of the patterns of liver fibrosis are similar to those of schistosomiasis mansoni.

US examination is broadly used in developing countries as one of the appropriate techniques, because this technique is easy and cheap. US has been used for morbidity studies of schistosomiasis. In *S. mansoni* infections, for morbidity studies, we have a standard criteria of US diagnosis. In *S. japonicum* and *S. mekongi*, infections, standard criteria of US diagnosis is expected.

C－08) COMPARISON OF ULTRASONOGRAPHIC IMAGES OF LIVER AND SPLEEN BY AGE GROUP AND THE RELATIONSHIP BETWEEN FREQUENCY OF PRAZIQUANTEL TREATMENT AND ULTRASONOGRAPHIC IMAGES OF PATIENTS SUFFERING FROM *SCHISTOSOMA JAPONICUM* AND *S. MEKONGI* INFECTION IN THE PHILIPPINES AND IN CAMBODIA

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We conducted studies in Oriental Mindoro, Philippines for schistosomiasis japonica (Sj) during July and August 2002 and in Kratie, Cambodia for schistosomiasis mekongi (Smek) during May and June 2002. The patients were examined by ultrasonography (US) and questioned concerning the frequency of praziquantel (PZQ) treatment since 1995, in which the drug were given at a dosage of 40mg/kg body weight in Cambodia and 40-50mg/kg body weight in the Philippines. The number of patients examined were 234 (170 males: 64 females) in the Philippines and 382 (214 males: 168 females) in Cambodia.

The patients were classified into two groups, *i.e.* 19 years of age or younger and those at least 20 years old. The frequency of PZQ treatment since 1995 was classified as A) 0 to 2 times, B) 3 to 5 times and C) more than 6 times. Spleen size was estimated by both US and palpation and was classified as A) within normal limits (WNL), B) less than 15 cm from the costal arch (less than 15) and C) over 15 cm from the costal arch (over 15). Periportal thickening of portal vein and collateral circulation of the splenic vein were also observed and recorded by US.

Percentages of patients with splenomegaly in groups A) WNL, B) less than 15 and C) over 15 were 96.0%, 4.0% and 0% in the Philippines and 80.2%, 19.8% and 0% in Cambodia, respectively among the younger patients, and 91.8%, 8.2% and 0% in the Philippines and 35.3%, 57.4% and 7.4% in Cambodia, respectively among the older patients. Periportal thickening of the portal vein was observed in 54.0% (Sj) and 50.5% (Smek) of younger patients and 53.3% (Sj) and 66.3% (Smek) of those over 20 years of age. Collateral circulation of the splenic vein were observed by US in 0% (Sj) and 4.2% (Smek), respectively in patients of the younger age group but in 2.2% (Sj) and 30.5% (Smek), respectively of the older age group.

As shown in the results, frequent PZQ treatment reduced the incidence of periportal thickening of the portal vein but not those of hard enlarged spleen and collateral circulation of the splenic vein. These observations indicate that early diagnosis and PZQ treatment of schistosomiasis japonica and mekongi is important.

C－09) THE INFLUENCE OF *SCHISTOSOMA HAEMATOBIUM* INFECTION ON VOIDING FUNCTION

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To estimate the influence of the *Schistosoma haematobium* infection on the voiding function quantitatively, urinary flow velocity and residual urine volume after urination in primary school boys were measured using portable uroflow meter (RMS-100, Tyouryou-systems, Japan) and ultrasound machine (Bladder Scan BVI3000, Alcare, US.).

This study was done in two areas in Kenya during February, 2002. One area was Kiambu district near Nairobi, the capital of Kenya. *Schistosoma haematobium* infeciton was not endemic in this area. The other study area was Kwale district, Coast Province, which lay in the east of the Kenya. The *S. haematobium* infection was endemic in this area. Study subjects were mainly primary school boys because the uroflow meter was designed to measure the male voiding function only. Before examination, 500 ml of juice was given to each students. When they felt bladder full, they started to pass the urine into the uroflow meter. The uroflow meter measured the maximum flow velocity, average flow velocity, total voiding time, total voiding volume. After urination, the students were examined about residual urine volumes. The *S. haematobium* eggs number in 10 ml of urine was also examined using Nucleo-pore filer.

The eggs count revealed that the boys in Kiambu were not infected with *S. haematobium* but 50% of the boys in Kwale were infected. The uroflow meter analysis showed no difference in the proportion of the maximun uroflow velocity to the total voiding volume between boys in Kiambu and in Kwale. Furthermore the residual urine volumes were also not different between the boys. The same kind of examination to the adults showed that the proportion of the maximum uroflow velocity to the total voiding volume in Kwale adult males was lower than that in Kiambu adult males. From these results, we concluded that *S. haematobium* infection did not cause the voiding dysfunction in the children but it caused the dysfunction in adults.

C－10) EPIDEMIOLOGICAL SURVEILLANCE ON SCHISTOSOMIASIS IN MOUNTAINOUS REGIONS IN SICHUAN

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The area infested and the population infected with *Schistosoma japonicum* in China, reduced after the control program. The infested area and the infection rate, however, appear to have increased in some highlands in Sichuan and Yunnan. The primary objective was to study the epidemiology and the factors impeding the control in the areas.

Surveyed were five villages near Qionghai Lake, Xichang City, Sichuan Province, in which the fecal examination of villagers in 2000 had revealed the positive rate for *S. japonicum* ova of over 30%, of around 10% or of 3-5%: Two sections from each village were investigated.

Results: Cercaria-positive *Oncomelania* snails were found in a section of Xinmin Village (7.7%); that of Zhanglin Village (0.9%) and that of Gucheng Village (0.7%). The trapped and dissected field mice were free of schistosomes.

In Xinmin Village where the egg-positive rate of over 30% had been found in the previous examination, the fecal samples from the villagers one year after the treatment revealed the positive rate of 33.9% in Sections 3 and that of 65.6% in Section 7 for miracidia.

Discussion: The villagers cultivate vegetables using human excreta as manure and working bare-footed. They wash in irrigation ditches. The snail inhabits in the irrigation canal near the hamlet and at the bottom and side walls of the irrigation ditches in the farms. More positive snails were found near the houses. The dominant species of field mice is *Apodemus chevreri*, which seems to supply ova

poorly in spite of its frequent contact with water. Although the infected human and domestic animals were treated, the infection rate has not reduced in a village. The sanitary life-style has to be changed and the household water has to be independent of the farming water to solve the problem.

C－11) PARASITOLOGICAL SURVEY AND RELATED HEALTH AWARENESS INVESTIGATION BY QUESTIONNAIRE AT A VILLAGE IN THE CENTRAL REGION IN GHANA

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Background: In Ghana, as well as in other sub-Saharan countries, many parasitic infections affect people. In such situation, irrigation projects have been introduced to increase agricultural productivity and income. It has been also pointed out that irrigation often results in expansion of mosquito-borne and/or water-borne parasitic infections. Therefore, continuous efforts to keep health of residents living around the area are important. We conducted health education on endemic parasitic diseases in a village (Okyereko, located in the coastal area in Ghana) in which a small-scale irrigated agriculture promotion project (SSIAPP) by JICA was under implementation. Here we show the results of a baseline survey of endemic parasitic diseases and health awareness investigation in the village. **Methods:** Urine and stools have been collected from residents in the village for parasitological assay. Eggs in the stools were detected by Kato-Katz method and eggs in the urine were detected by Nucleopore membrane filtration. Questionnaires on awareness of parasitic diseases present in the village were administered to residents aged more than or equal to 10 years old. **Results and Discussion:** *Schistosoma haematobium* infection was the most prevalent disease among those we examined. Approximately 50% of the residents aged 10 to 29 years old were infected. Second highly prevalent helminth infection was hookworm infection, which affects 23% of the residents. *Schistosoma mansoni*, *Trichuris trichiura* and *Ascaris lumbricoides* infections were also observed. Additionally, in a previous study, lymphatic filariasis (*Wuchereria bancrofti* infection) was shown to be endemic in this village (Dzodzomenyo, *et al*., 1999). Thus, as for helminthiasis, we can conclude that urinary schistosomiasis, hookworm infection and lymphatic filariasis are highly endemic in this village. Analysis of awareness and behavior of residents on such parasitic diseases-related symptoms revealed that they had a tendency to seek medicines from chemists or peddlers rather than to see doctors. Only approximately 20% and 10% of the persons who had disease-related symptoms tend to see doctors, in the case of malaria and urinary schistosomiasis, respectively. Analysis of their knowledge on transmission route of parasitic diseases showed that they had a relatively correct knowledge of transmission of malaria, urinary schistosomiasis and intestinal infections. In contrast, they did not know the relationship of mosquito bite and lymphatic filariasis. This fact may be due to the long time progression of the chronic symptoms of the disease.

C－12) DAMAGE IN CERCARIAE OF *SCHISTOSOMA MANSONI* INDUCED BY PRESSURE JAR IN WATER

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We experimented about influence of the water pressure vibration for a cercariae of *Schistosoma manson.* Cercaria comes out of a shell of intermediator into water by light, and cercaria has a form peculiar which connection department of head and tail is narrow. Therefore, mechanical energy of the water pressure vibration may be influenced to cercaria with strong damage.

In the first, we were examined some following load to cercaria of each group with 50 cercaria in water. The ratios of tail loss were 10.0% by magnetic rotation field for 30 minute, 13.9% by static water pressure for 30 minute, 27.0% by magnetic rotation field and low speed stream for 30 minute, 87.2% high-speed stream for 10 minute, respectively. Then control group was 0% at ratio of tail loss.

In the next, cercaria were exposed mechanical energy by 46 kHz of ultrasonic wave generator was load for 1 minute, 2 minutes, 3 minutes and 5 minutes in water. The connection department of head and tail was amputated by the mechanical energy, and the ratio of tail loss reached 100% for less than 4 minutes as a result of regression line in statistical analysis.

Furthermore, we were examined the ultrasonic wave load to infected shell of intermediate host for one minute. Body of the shell took shelter in the depths of conch during ultrasonic wave exposure. Cercaria without tail was 99.2% in which cercaria flowed out of the shell after ultrasonic wave exposure only one minute.

In this study, it was suggested that following. The tail of cercaria which appeared from a shell evolved for a purpose to arrive at final host in a short time. But the small part between head and tail of cercariae was weak against high-speed stream or water pressure vibration induced by ultrasonic wave generator. Interference pattern reflected of ultrasonic wave at inside wall of a shell was very effective for amputation of cercariae, in which configuration of the conch that the depths narrow progressively. Therefore, it is most effective to expose shell to pressure jar in water for amputation of cercariae tail.

C－13) COMPARISON OF INTESTINAL PARASITIC INFECTIONS AND MALNUTRITION IN SCHOOLCHILDREN IN URBAN AND RURAL AREAS IN HANOI, VIETNAM

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Surveys on parasitic infections and nutritious status in schoolchildren were conducted in urban(U) and rural(R) areas of Hanoi, Vietnam. Data of both parameters obtained from 75 and 104 children in U and R respectively were analysed and compared for the prevalence and intensity of parasitic infections and nutritious status between the two areas. The school children were examined for parasites in their fecal samples, their daily food intake, weight and height, and anthropometric indices following the Kato-Katz technique, '24 hour recall' method and the standard US National Center for Health Statistics charts and tables, respectively. *Results*: 1) Overall prevalence of parasitic infections was significantly higher in R (96.2%) than that in U (83.0%). In both areas *Trichuris trichiura* was the most dominant parasite. 2) The geometric mean of EPG (egg counts per gram feces) of Ascaris and Trichuris was significantly higher in R than that in U. 3) Weight and height were significantly greater in U than those in R. 4) Weight-for-age analysis showed that 13 (18.1%) children in U and 59 (56.7%) in R were malnutritioned. 5) Height-for-age analysis showed that 16 (21.3%) children in U and 51 (49.0%) in R were malnutritioned. 6) A stratified analysis by Mantel-Haenszel chi-square test showed no relationship between the prevalence of infections, malnutrition and the

sites examined. 7) No significant differences in total calorie intake was observed between the normal and malnutritional groups. *Conclusions*: Schoolchildren in the rural area in Hanoi, Vietnam had a higher prevalence and intensity of parasitic infections, and a higher rate of malnutrition than those in the urban area. There was no significant difference between nutrition status and the prevalence of parasitic infections with the same calorie intake in normal and malnutritional groups. (*Supported partly by Grants-in-Aid (No. 11691211) from the Ministry. Educ. Sci. Sport. & Cult, Japan).

C－14) DETECTION OF MICROORGANISMS IN TAP WATER IN INDONESIA AND THAILAND

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Water samples were directly collected from house faucets at each collection site and filtered through membranes. The membranes were brought to Japan and examined for protozoan parasites by immunomagnetic separation. Coliform and *Escherichia coli* were examined at each sample collection site using commercially available kits. A total 115 water samples were examined and 37 (32%) were revealed positive for four types of microorganisms. These microorganisms were two kinds of bacteria (coliform and *Escherichia coli*) and two species of protozoa (*Giardia intestinalis* and *Cryptosporidium parvum*). Of those detected, coliform was the most common and was found in all three areas with a mean detection rate of 30%. *G. intestinalis* and *C. parvum* were found at the rates of 9% and 1%, respectively. Bacterial contamination was closely related to the concentration of residual chlorine but not protozoan contamination. The water samples that were positive for any of the four microorganisms showed a tendency to have lower residual chlorine concentrations and higher turbidities compared with negative samples. It is important to supply safe water in order to maintain people's health because most of the people surveyed ordinarily drank tap water without treating it. Continued efforts are needed to maintain and improve drinking water quality.

C－15) EPIDEMIOLOGICAL ANALYSIS OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* INFECTION IN INDONESIA

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Enterohemorrhagic *Escherichia coli* (EHEC), which can occasionally cause big outbreaks and sporadic cases in developed countries, is rarely isolated as causative agents of diarrheal diseases in developing countries. In this study, we perform an etiological investigation on diarrheal cases and a carrier rate of enteropathogens among non-diarrheal cases from two medical facilities in Surabaya, focusing on EHEC, and the carrier rate of EHEC among domestic animals such as cattles, pigs and goats. In addition, serological analysis on the serum IgG level of anti-VT1 and anti-O157 LPS antibodies among Indonesian and Japanese pediatric cases was performed to clarify real situation of EHEC infection in Indonesia.

It was revealed that six strains of *Vibrio cholerae* O1,

six strains of *Shigella*, three stains of *Salmonella*, one strain of enteroinvasive *Escherichia coli*, 19 strains of enteropathogenic *Escherichia coli* and only one strain of EHEC strain (O8:H4) were isolated from diarrheal and non-diarrheal patients. Thirteen strains of EHEC were isolated but no EHEC O157 was isolated from cattle stool samples. Three strains of EHEC O157 were isolated from goats. No EHEC was isolated from pigs. The production pattern of verotoxin was completely identical to that of toxin genes detected by PCR. The fingerprinting pattern of genomic DNA by PFGE was almost identical to those of strains isolated from sporadic cases in Nagasaki prefecture.

The serum levels of anti-VT1 and anti-O157 antibody determined by ELISA among pediatric cases in Indonesia increase by age since neonatal period, while those in Japan seem to decrease once after birth until they begin to increase at four to five years of age. These findings suggest that previous exposure to EHEC or VT1, or VT1 related antigens could evoke and sustain high levels of serum antibody against VT1 and O157 LPS.

C－16) THE PROBLEM ON MALARIA TREATMENT —ABOUT THE DELAY OF DIAGNOSIS AND TREATMENT—

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In our experience to treatment for five cases of malaria patients, we had great deal of serious situation. That was lateness to the diagnosis and treatnent.

Case 1: A middle school boy of 15 years old caused by ovale-malaria; His diagnosis was two days behind the admission and treatment was five days lingered because of difficulties to obtain anti-malaria medicine.

Case 2: A trading company employee of 44 years old caused by falciparm-malaria; He made a long stay in Lagos in Nigeria from 1973 to 1975 and had arisen a complication of cerebral symptoms, but Chloroquine and Primaquine were very good efficacious to him nevertheless.

Case 3: A woman of 40 yeas old university lecturer in English caused by vivax-malaria; She olso started to apply the medicine after six days of the diagnosis. The reason was one and the same as case 1.

Case 4: A Vietnamese seaman of 33 years old caused by vivax-malaria; He had not been comfirmed his recovery perfectly. Because his cargo boat had left Osaka port to Pusan in Korea just after the diagnosis on this evening.

Case 5: A male patient, 41 years old American, who teaches English at the Berlitz school in Osaka; He had recovered before becoming encephalopathy, although he was a case of Chloroquine-resistant falciparum-malaria. He had taken instant effect on Quinine-injection in spite of renal disorder.

Fortunately, we had not experienced miserable cases.

To countermeasure the delay of diagnosis

1. Precise sampling and exact observation (Giemsa stain; Buffer solution, not pH6.4 but pH7.2～7.4).
2. Enlightenment of Polymerase chain reaction technique, Fluorescent antibody technique etc..

To countermeasure the delay of treatment

1. Vid. Today's Therapy. Vol. 44. 2002 (Edit. Sachio Takasu M.D. Igaku-shoin Co. Ltd). In this textbook, the name of Organization in Japan which keep anti-malaria medicine is printed with telephone number.
2. Qinghaus; ask Guilin No2 Pharmaceutical Factory Guangxi, China Republic.

C－17) A SEVERE CASE OF *PLASMODIUM FALCIPARUM* INFECTION COMPLICATED WITH BLACK WATER FEVER.

KOYAMA, K.

C－18) CORRELATION BETWEEN THE RECOGNITION BY OBSTETRICIANS ABOUT CONGENITAL TOXOPLASMOSIS AND THEIR CLINICAL ACTIVITIES

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In the present study, correlation between the recognition held by obstetricians about congenital toxoplasmosis (CT) and their clinical activities was investigated with a questionnaire for 15 subjects, or obstetricians in Tokachi-Obihiro area, Hokkaido, Japan. Besides their attributes, obstetricians were asked about their clinical activities on CT, such as serum antibody tests and explanation for pregnant women, as well as their recognition about the risk of CT. According to the present study, obstetricians who are negative for the explanation are relatively older with longer clinical experience. Obstetricians who have experienced some cases of CT are more positive for the explanation than those who have not. For serum antibody test to *T. gondii,* on the other hand, no correlation is observed with statistical significance. On the recognition about the risk of CT, self-employed obstetricians have lower estimates on the risk of infection for pregnant women (RIW) as well as that for fetus (RIF) than employed obstetricians. Obstetricians who have not experienced any cases of CT also hold lower estimates on RIW and RIF than those who have.

Obstetricians for medical clinics indicate their lower estimates on the damage to fetus (DF) than those for private general hospitals. It is also indicated, the older and with the longer clinical experience, the lower estimates on RlW, RIF and DF. The number of cases of CT, experienced by obstetricians, positively correlates with their estimates on RIF.

The present study implies that relatively older self-employed obstetricians for medical clinic, with longer clinical experience but few cases of CT, are typically negative for the explanation, backed by their lower estimates on the risk of CT. The results of the present study, we believe, reflect a tragedy that CT has been paid less attention here in Japan. As we showed before, the lower risk estimates on CT are not in line with recent epidemiological evidence. Comprehensive epidemiological research is required to inspect their recognition from the view point of EBM (Evidence Based Medicine).

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