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GENOTYPE IDENTIFICATION OF HEPATITIS C VIRUS (HCV) ISOLATED FROM A SINGLE JAPANESE CARRIER IN NAGASAKI PREFECTURE AND GENOME ANALYSIS OF E1 AND E2/NS1 ENVELOPE GLYCOPROTEIN REGIONS

WEI-YUN ZHENG

Received September 20, 1994/Accepted October 30, 1994

Abstract: The nucleotide (nt) sequence of a Hepatitis C virus genome (HCV-N) which was derived from a single Japanese patient's serum in Nagasaki Prefecture has been determined by multiple clones covering 22 overlapping regions of the HCV geneme. The sequenced region consisted of 9295 nt, including 248 nt of 5'-untranslated region (UTR), a single large open reading frame (ORF) encoding a polyprotein of 3010 amino acids (aa) and a 17 nt of 3'-UTR. Phylogenetic analysis indicated that HCV-N belongs to II/1b genotype of group 1. Two other Nagasaki HCV strains (HCV-N1 and HCV-N2) were also sequenced in the E1 and N-terminus of the E2/NS1 regions. Two hypervariable regions (HVR 1 and HVR 2) were found in the N-terminus of E2/NS1 region among 3 Nagasaki strains and 7 other HCV strains with published sequences. Two well-conserved aa sequences were also identified among 10 HCV strains in the E1 and N-terminus of the E2/NS1 regions. The results will be useful for future understanding on the pathogenesis, virological diagnosis and development of vaccine for HCV.

INTRODUCTION

Hepatitis C virus (HCV) is the major cause of nonA-nonB (NANB) hepatitis, and chronic infection with HCV has been linked to the development of liver cirrhosis and hepatocellular carcinoma (Plagemann, 1991). Many entire and partial HCV sequence informations published so far have shown that HCV genome consisted of about 10 Kb single-stranded positive-sense RNA with 5'UTR, a single long ORF followed by 3'UTR. The viral genome organization resembles that of the flaviviruses and pestiviruses (Kato *et al.*, 1990; Plagemann, 1991).

Since Choo et al. (1989) cloned the genome of an RNA virus from the plasma of a chimpanzee inoculated with plasma from patient with NANB hepatitis and designated it as HCV, entire sequences have been reported for at least 15 HCV strains. There are: HCV-J (Kato *et al.*, 1990), HCV-1 (Choo *et al.*, 1991), HCV-H (Inchauspe *et al.*, 1991), HCV-BK (Takamizawa *et al.*, 1991), HC-J6 (Okamoto *et al.*, 1991), HC-J1 (Okamoto *et al.*, 1992a), HC-J8 (Okamoto *et al.*, 1992b), HC-J4/83, HC-J4/91 (Okamoto *et al.*, 1992c), HCV-JT (Tanaka *et al.*, 1992), HCV-T (Chen *et al.*, 1992), HCV-JK1 (Honda *et al.*, 1993), HC-C2 (Wang *et al.*, 1993), HC-G9 (Okamoto *et al.*, 1994) and NZL1 (Sakamoto *et al.*, 1994). Depending on the HCV sequence similarity, HCV genome can be classified into 6 genotypes: I, II, III, IV, V and VI as reviewed by Sakamoto et al. (1994), or into 6 major genotypes: 1 (a, b, c), 2 (a, b, c), 3 (a, b), 4, 5 and 6 (Simmonds *et al.*, 1933b).

Recently, the newest classification has been summarized by Sakamoto et al. (1994) who classified HCV genome into 3 major groups with entire published sequences. The entire HCV sequence of Japanese strains have been assigned either to group 1 (I/1a genotype: HC-J1; II/1b genotype: HCV-J, HCV-BK, HC-J4/83, HC-J4/91, HCV-JT and HCV-JK1) or group 2 (III/2a genotype: HC-J6; IV/2b genotype: HC-J8) according to Sakamoto et al. (1994). In order to know whether there is particular HCV in the local area of Nagasaki, Japan, HCV patient serum which came from Nagasaki Prefecture was obtained and the genome sequence of the HCV-N in this serum was determined by multiple overlapping clones.

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In order to see the genetic variability of HCV in the Nagasaki area, HCV genome sequence in 2 more HCV patient sera in Nagasaki Prefecture (HCV-N1 and HCV-N2) were also analyzed similarly in the E1 and N -terminus of the E2/NS1 envelope glycoprotein regions. Two hypervariable regions have be found among these 3 Nagasaki HCV strains using the sequence diversity comparison with published sequence data of 7 HCV strains.

MATERIALS AND METHODS

RNA extraction from HCV patient serum

Serum samples were obtained from NANB patients in Nagasaki Prefecture which were kindly provided by the Second Department of Internal Medicine of Nagasaki University Hospital. These patients were confirmed to be infected with HCV by anti-C100 HCV ELISA Kit (Ortho Diagnostic Systems, Tokyo, Japan) and HCV reverse-transcription polymerase chain reaction (RT -PCR) as published by Kurihara (1992). One hundred microliters of HCV patient serum was mixed with 20 μl of 10% sodium dodecyl sulfate (SDS) and 80 μl of sterile distilled H_2O at room temperature for 5 min. HCV RNA was extracted with phenol / chloroform and precipitated with 3 volumes of ice-cold absolute ethanol. After storage at −80°C for 1hr, HCV RNA was pelleted in an Eppendolf centrifuge with 15,000xg at 4°C for 30 min. The pellet was washed once in 70% ethanol, vacuum dried and dissolved in 100 μl sterile distilled water. RNA solution was stored at −80°C.

Selection and synthesis of oligonucleotide primers

Oligonucleotide primer sequences were selected based on the published sequence data (Choo *et al.*, 1991, Takamizawa *et al.*, 1991) and gene walking method. Oligonucleotide primers were synthesized by Applied Biosystems Model 392 DNA / RNA Synthesizer and confirmed for their purity by ion-exchange gel chromatography (Gen-pack; Waters).

RT-PCR

Ten microliters of RNA solution were added with 90 μl of RT-PCR mixture [100 pmol of each primers, 0.2 mM deoxynucleoside triphosphate, 10 mM Tris (PH 8. 9), 1.5 mM $MgCl_2$, 80 mM KCl, 0.5 mg of bovine serum albumin per ml, 0.1% sodium cholate, 0.1% Triton X -100, 10 U of reverse transcriptase (Life Science Inc.) and 2U of Tth DNA polymerase, a thermostable DNA polymerase (Toyobo Co.)]. The reaction mixture was covered by 2 drops of mineral oil and incubated for 10 min at 53°C for RT. PCR amplification (94°C for 60 sec, 53°C for 90 sec and 72°C for 120 sec by thermal cycler; Iwaki Co.) was started immediately after RT and repeated 35 times. cDNA product was subjected to agarose gel electrophoresis and visualized by ethidium bromide staining.

Cloning and sequencing of HCV cDNA product

The amplified HCV cDNA was excised from agarose gel, phosphorylated with T4 polynucleotide kinase (Nippon Gene Co.) and blunted with T4 DNA polymerase (Takara Co.). The modified cDNA fragment was ligated into SmaI site of pUC19 and transformed into *Escherichia coli* JM 109 strain. The recombinant pUC19 carrying inserted cDNA fragment was purified with Wizard™ Minipreps DNA Purification System (U.S. A.). The cDNA fragment sequence was determined in both directions with sense and antisense primers by dideoxy chain termination method using both ^{35}S radioisotope-labeling (DNA Sequencing Kit Version 2.0, U.S. A.) and fluorescent dye-labeled DNA sequencing system (373A DNA Sequencer, Applied Biosystems). To avoid sequence variability, 3 colones from each RT-PCR product were isolated independently for nucleotide sequence determination.

Analysis and homology comparison in nucleotide and deduced amino acid sequences were carried out for HCV-N and 7 other HCV strains with published entire sequence, using a computer system with DNASIS Mac Version 2.2, NEW CD2 system (Hitachi Software Engineering Co., Ltd, 1992).

RESULTS

The genome characterization of HCV-N

Multiple cDNA clones were isolated from altogether 22 overlapping regions which covered almost entire genome of HCV-N, except extreme 5' and 3' terminals (Fig. 1). For each of the overlapping region, 3 independent cDNA clones were isolated from the RT -PCR product and sequenced. The cleavage site of the polyprotein coded by the ORF of the HCV-N genome was assigned according to the publications by Okamoto et al. (1992b) and Honda et al. (1993). Total length of the sequenced region of HCV-N consisted of 9295 nt and 3010 aa, respectively (Fig. 2). The sequenced region can be divided into 248 nt in the 5'UTR, 9030 nt in a single ORF and 17 nt in the 3'UTR, respectvely. The ORF was considered to be translated into C (191 aa), E1 (192 aa), E2/NS1 (346 aa), NS2 (277 aa), NS3 (609 aa), NS4 (398 aa) and NS5 (997 aa) proteins.

Genotype classification and genome homology comparison among HCV-N and 7 other HCV strains with published sequences

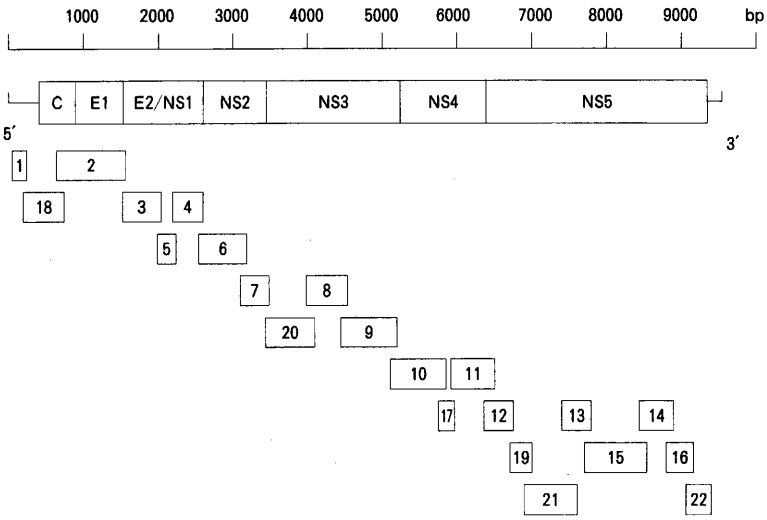

Figure 1 Physical map of the HCV genome and locations of the cDNA clones derived from HCV-N strain. Nucleotides are numbered from putative 5'end as indicated on the top. The genome organization of HCV is according to Okamoto et al. (1994), showing coding region from structural proteins: core (C), envevop (E1 and E2) to nonstructural proteins (NS1-NS5) by blocks, untranslated region (UTR) (5' and 3') by bars. The order of clone number is based on the cloning process.

Entire sequence informations have been published for the following 7 HCV strains: HCV-1 (Choo *et al.*, 1991), HCV-BK (Takamizawa *et al.*, 1991), HCV-J (Kato *et al.*, 1990), HC-J6 (Okamoto *et al.*, 1991), HC-J8 (Okamoto *et al.*, 1992b), HCV-JT (Tanaka *et al.*, 1992) and HCV-T (Chen *et al.*, 1992). Homology comparison between HCV-N and these 7 HCV strains was shown in Table 1. The most conserved region is the 5 UTR which showed nt homology more than 91.5% among 8 HCV isolates. The sequence homology of the C protein region was also highly conserved (nt > 81.1%, aa > 88.5%). In the putative E1 and E2/NS1 regions, the HCV-N showed high homology with HCV-BK, HCV-J, HCV-JT and HCV-T (nt > 84.5%, aa > 85.8%). Whereas HCV-N showed low homology with HC-J6 and HC-J8 (nt < 68.8%, aa < 72.3%). Nt sequence homology between HCV-N and HCV-1 in the E1 and E2/NS1 regions was 73.7%, 74.5% and aa sequence homology was 76.0%, 81.2%, respectively. From NS2 to NS5 regions, it was evident that HCV-N is closer to HCV-BK, HCV-J, HCV-JT and HCV-T and remote from HC-J6 and HC-J8, while HCV-1 seems to be located intermediate. The total sequence homology comparison among 8 HCV isolates gave us a conclusion that HCV-N belongs to HCV-BK/HCV-J/HCV-JT/HCV-T genotype. In contrast, HCV-N is remote from J6 and J8 genotypes,

Table 1 Homology comparison of nucleotide and deduced amino acid sequence among 8 HCV strains (HCV-N, HCV-1, HCV-BK, HCV-J, HCV-JT, HCV-T, HC-J6 and HC-J8). The homology is indicated by %. The nucleotide sequence homology is shown in upper and amino acid sequence homology is shown in lower with parenthesis, respectively.

Region	HCV-N & HCV-1	HCV-N & HCV-BK	HCV-N & HCV-J	HCV-N & HC-J6	HCV-N & HC-J8	HCV-N & HCV-JT	HCV-N & HCV-T
Total	77.9 (84.5)	92.0 (94.1)	91.5 (93.7)	62.4 (70.7)	61.7 (69.2)	91.7 (93.9)	90.0 (92.1)
5'UTR	98.8	99.2	98.4	94.0	91.5	99.2	99.2
C	90.7 (96.3)	96.0 (97.4)	95.8 (97.9)	83.0 (90.1)	81.1 (88.5)	95.1 (96.3)	93.9 (93.7)
E1	73.7 (76.0)	89.0 (89.6)	91.7 (93.2)	60.1 (52.9)	56.7 (51.8)	92.2 (92.2)	91.1 (93.8)
E2/NS1	74.5 (81.2)	85.7 (88.1)	84.5 (86.1)	68.8 (71.1)	66.8 (72.3)	84.7 (85.8)	85.3 (87.0)
NS2	71.0 (74.4)	92.1 (91.7)	92.7 (94.9)	59.8 (58.7)	58.2 (56.2)	89.0 (92.4)	87.8 (91.3)
NS3	78.1 (89.0)	90.7 (93.4)	90.4 (91.8)	69.9 (79.3)	69.8 (78.8)	91.1 (94.9)	89.4 (92.8)
NS4	78.9 (88.2)	93.1 (97.5)	92.7 (97.2)	66.8 (74.1)	67.0 (72.9)	93.5 (97.0)	89.9 (94.0)
NS5	78.8 (83.7)	93.5 (96.3)	92.7 (95.2)	59.5 (71.8)	59.2 (71.5)	93.1 (95.2)	91.8 (92.5)

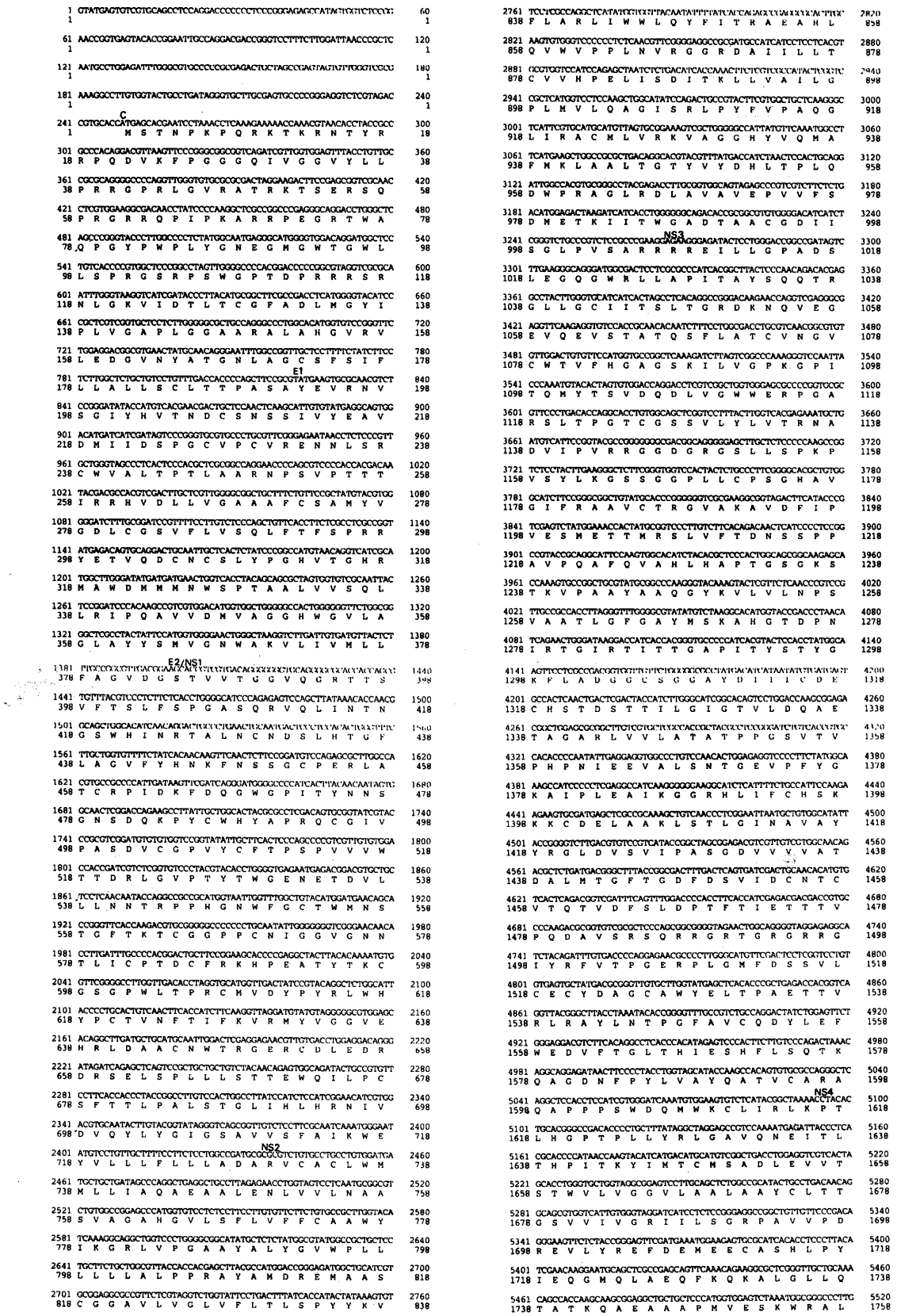

Figure 2 Nucleotide and deduced amino acid sequence of HCV-N strain. The nucleotide sequence of HCV-N is shown in the upper line and deduced amino acid sequence of HCV-N is shown in the lower line, respectively.

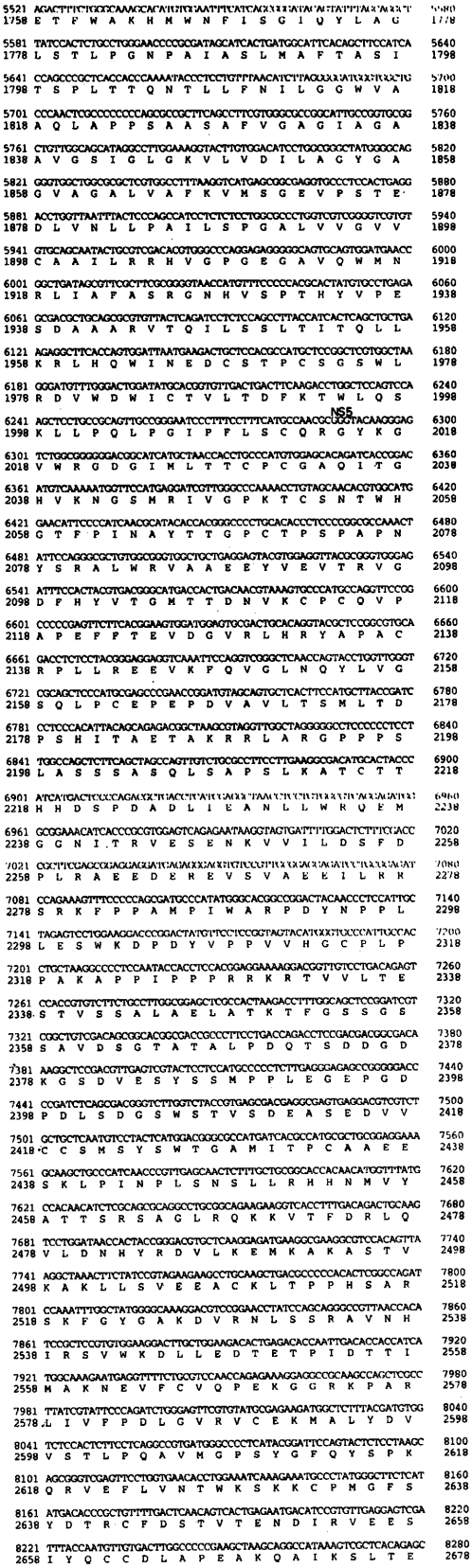

Figure 2

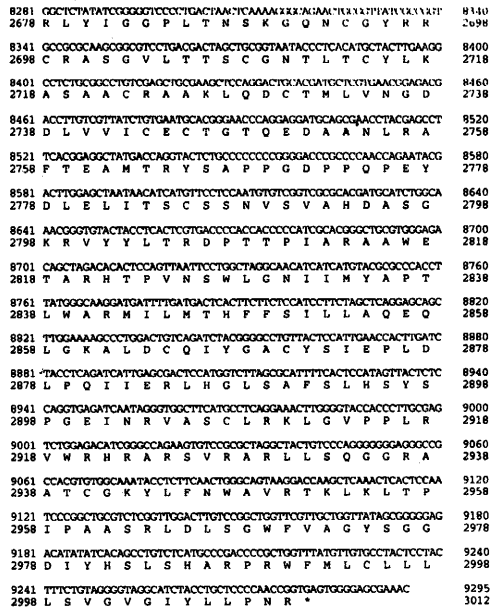

Figure 2

respectively. The sequence homology of HCV-N also showed its closer relationship to HCV-1 than to HC-J6 and HC-J8 isolates (Table 1). HC-J8 possessed 23% divergence with HC-J6 and has been clearly identified as a separate genotype from HC-J6 (Okamoto *et al.*, 1992b). In order to confirm this conclusion, phylogenetic trees were constructed base on nucleotide

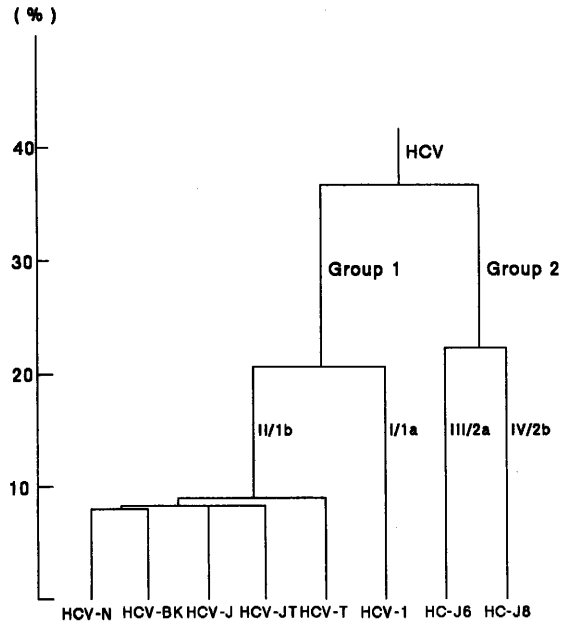

Figure 3 Phylogenetic trees are based on nucleotide divergence (%). Sequence of all 8 HCV strains were campared and analyzed by the nearest neighbor method.

divergence throughout entire sequence using nearest neighbor method (Williams and Lance 1977) (Fig. 3). This result indicated that 8 HCV strains can be classified into 2 groups: the group 1 contains genotype II/1b (HCV-N, HCV-BK, HCV-J, HCV-JT, HCV-T) and genotype I/1a (HCV-1); while group 2 contains genotype III/2a (HC-J6) and genotype IV/2b (HC-J8).

Sequence variation in the E1 and N-terminus of E2/NS1 envelope glycoprotein regions among 3 HCV Nagasaki strains and 7 other HCV strains with published sequences

Recently, many papers reported that hypervariable regions existed in the N-terminus of E2/NS1 envelope glycoprotein region of HCV genome (Hijikata *et al.*, 1991; Weiner *et al.*, 1991; Honda *et al.*, 1993). In order to know the HCV sequence diversity of the envelope glycoprotein in local area of Nagasaki, 2 other HCV Nagasaki strains (HCV-N1 and HCV-N2) were also cloned and sequenced for the region of nt 623-1988, aa 126-580 (nt and aa base on the HCV-N number). Alignment comparison of deduced aa sequence among 3 Nagasaki strains and other 7 HCV strains with published sequences were shown in Fig. 4. Two hypervariable regions (HVR) were discovered in the N-terminus of E2/NS1 region. HVR 1 (aa 384-411) was located directly downstream at the beginning of the E2/NS1, whereas HVR 2 (aa 475-480) was observed 64 aa downstream from the HVR 1. In the HVR 1 consisting of 28 aa, 10 aa residues were well-conserved among 3 Nagasaki strains. Whereas only 3 aa were conserved in this

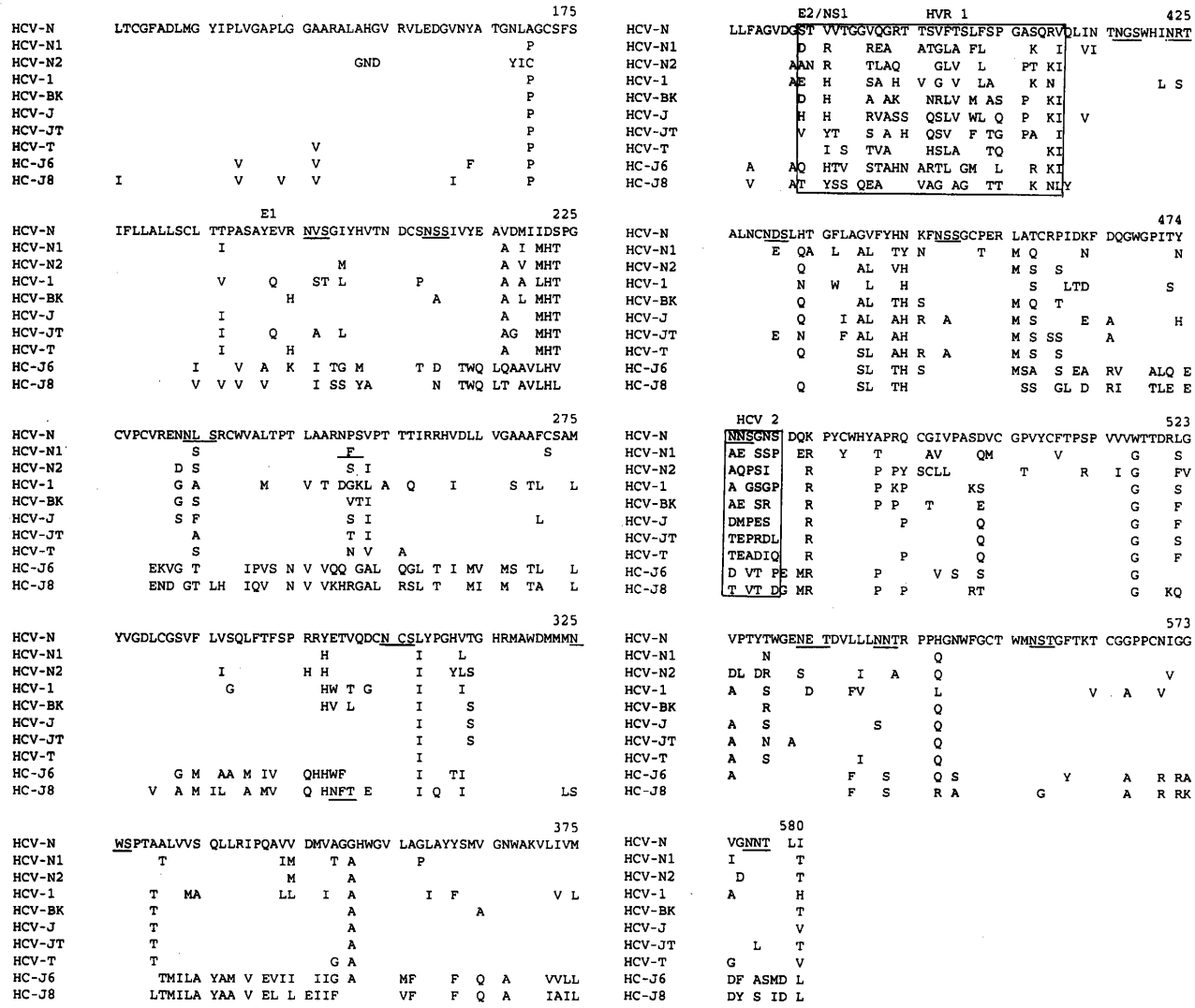

Figure 4 Alignment of amino acid sequence among 10 HCV strains (HCV-N, HCV-N1, HCV-N2, HCV-1, HCV-BK, HCV-J, HCV-JT, HCV-T, HC-J6 and HC-J8) in the E1 and N-terminus of E2/NS1 regions (aa 126-580). Two hypervariable regions (HVR 1 and HVR 2) are shown by boxes. Potential N-glycosylation sites (N-X-S/T) are underlined.

Table 2 HCV genotype classification including HCV-N from this study and 15 entire HCV strains which have been reported by Sakamoto et al. (1994).

Group	Group 1			Group 2		Group 3
Genotype	I/1a	I c	II/1b	III/2a	IV/2b	V/3a
HCV strains	HCV-1 HCV-H HC-J1	HC-G9	HCV-J HCV-BK HC-J4/83 HC-J4/91 HCV-JT HCV-T HCV-JK1 HC-C2 HCV-N	HC-J6	HC-J8	NZL1
Geographic Area	U.S.A. Japan	Indonesian	Japan China Taiwan	Japan	Japan	New Zealand

region when a total 10 HCV strains were compared. In the HVR 2 consisting of 6 aa, none of the aa were conserved among 3 Nagasaki strains. This hypervariability was also observed when a total 10 HCV strains were compared simultaneously. In the E1 and N-terminus of the E2/NS1 regions, 2 well conserved aa sequences were observed among 10 HCV isolates: G-H-R-M-A-W-D-M-M (aa 315-323) and W-F-G-C-T-W-M-N (aa 549-556). Although 14 N-glycosylation sites (N-X-S/T) were identified in HCV-N, one of them (NNS at aa No. 475-477) was unique to this strain and not present in 2 other Nagasaki strains and 7 other HCV strains with published sequences. Instead, HCV-N1 and HCV-N2 possessed another N-glycosylation site (NFS or NSS at aa No. 250-252). This glycosylation site is found also in HCV-BK, HCV-J, HCV-JT, and HCV-T (Fig. 4). Altogether, only 9 N-glycosylation sites were conserved in the E1 and N-terminal of the E2/NS1 regions among 10 HCV strains shown in Fig. 4.

Discussion

Relationship between genotype and geographic area of HCV strains including HCV-N was shown by their sequence comparison using the classification by Sakamoto et al. (1994) (Table 2). The sequence homology indicated that HCV-N strain did not possess its local genotypic character, and similar to HCV-BK, HCV-J and HCV-JT strain which were isolated from other areas of Japan. All these strains apparently belong to the same genotype II/1b of group 1. This genotype also included HC-J4/83; HC-J4/91; HCV-JK1 of Japanese HCV strains, HC-C2 of Chinese HCV strain and HCV-T of Taiwan strain. The genotype 1c of group 1 includes HC-G9 which was derived from Indonesian strain. While, the genotype I/1a of group 1 includes HCV-1 and HCV-H which were derived from American strains. Although another HC-J1 was Japanese strain, it was supposed to have originated from the US, because HC-J1 was derived from a Japanese haemophiliac who developed hepatitis C after receiving US-made factor VIII (Okamoto *et al.*, 1992a). In group 2, HC-J6 and HC-J8 belong to the III/2a and the IV/2b genotype, respectively. Both of them were Japanese strains. In group 3, NZL1 that came from New Zealand belongs to V/3a genotype (Table 2). Simmonds et al. (1993b) have classified 6 major genotypes of HCV from 76 HCV isolates, which were almost worldwide collection, using phylogenetic analysis of the NS5 region. The 76 HCV isolates contained entire sequence of HCV-1, HCV-H, HCV-J, HCV-BK, HCV-T, HCV-JT, HC-J6 and HC-J8 strains and other partial sequence of HCV strains. From this classification, only genotype 4, 5 and 6 showed highly restricted geographical distributions, being apparently confined to Egypt, South Africa and Hong Kong respectively (Simmonds *et al.*, 1993b).

Two hypervariable regions have already been observed in the N-terminus of the E2/NS1 region. The number of conserved aa in the HVR 1 among 3 Nagasaki strains were higher than among a total 10 strains including 3 Nagasaki strains and 7 other HCV strains with published sequences. The numbers of conserved aa (5 aa/28 aa) among HCV strains of genogroup 1 (HCV-N/HCV-1/HCV-BK/HCV-J/HCV-JT/HCV-T) were higher than those (3 aa/28 aa) among 10 HCV strains which included genogroup 2 (HC-J6 and HC-J8). This result may give us an idea that aa conservation in the HVR 1 of the same genotype or genogroup of HCV are

higher than among the different genotype or genogroup of HCV strains. Therefore, the 3 Nagasaki HCV strains may have been originated from the same ancestor.

In contrast, none of the aa was conserved among 3 Nagasaki strains nor among a total 10 HCV strains in the HVR 2 which showed higher variability than the HVR 1. The reason why mutations occur so frequently in only limited regions such as HVR is not known (Tanaka *et al.*, 1992). The higher degree of divergence in HCV E2/NS1 region might reflect the immune selection and suggest that this region cannot probably be an ideal target for future vaccine development.

Regarding the N-glycosylation sites in the E1 and N-terminus of the E2/NS1 region, only 9 sites were conserved out of the 14 sites seen in HCV-N. Some of the glycosylation sites, therefore, would not be essential for the survival, transmission and maintenance of HCV in nature. On the other hand, 2 well-conserved aa sequences were found in the E1 and N-terminus of the E2/NS1 regions among the 10 HCV strains compared. These conserved sequences may be better targets of vaccine development if they were related with protective immunity.

The nt sequence homology of HCV-N in the 5'UTR shows high conservation comparing with other 7 HCV isolates (nt > 91.5%) and was used as an ideal target for PCR amplification to detect HCV RNA (Okamoto *et al.*, 1990). But recently, some papers reported that several HCV strains have sequence variation in the 5' UTR (Lee *et al.*, 1992; Bukh *et al.*, 1992; Simmonds *et al.*, 1993a). Therefore additional informations would be required to select optimal primer for PCR diagnosis on HCV. The functional motifs of the putative encoded proteins of HCV (Plagemann *et al.*, 1991; Tanaka *et al.*, 1992) have also been found in HCV-N. There are consensus sequences of RNA helicase in the NS3 region: Gly-Ser-Gly-Lys-Ser-Thr (aa 1233-1238) and Gln-Arg-Arg-Gly-Arg-Thr-Gly-Arg (aa 1486-1493), while NS5 region possessed consensus sequence of RNA-dependent RNA polymerase: Gly-Asp-Asp (aa 2736-2738). The sequence heterogeneity in putative structural proteins and nostructural proteins of HCV provided significant evidence for genotype classification. The genetic informations of HCV-N genome obtained in this study will be useful in future understanding on the pathogenesis, diagnosis and development of vaccine for HCV.

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SEROEPIDEMIOLOGICAL SURVEYS FOR LEPROSY IN ECUADOR

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Abstract: Serological examination of leprosy in endemic areas of cutaneous leishmaniasis were carried out using the sera collected during a survey for cutaneous leishmaniasis and several parasitic diseases in Ecuador. There was no correlation between prevalence rates for leprosy and seropositive rates of the antibodies (anti-PGL-I and LAM-B antibodies) in the subjects living in several provinces in Ecuador. Seropositive rates of anti-PGL-I antibodies of the leprosy patients and their families in Los Ranchos, Department of Manabi, were relatively high (84.6%, 11/13) in comparison with the average seropositive rates (42.4%, 154/365) of the subjects from other areas of Ecuador. It was suggested that serological survey of families of leprosy patients might be useful for screening of household contacts in a low endemic areas, such as Department of Manabi, Ecuador.

INTRODUCTION

Leishmaniasis and leprosy are etiologically completely different diseases, but it has been known that the two diseases cause immunologically similar responses in their hosts (Bryceson, 1981). Therefore, it may be important to know seroepidemiological features of leprosy in endemic areas of cutaneous leishmaniasis. Two types of skin tests, Lepromin test for leprosy and Leishmania (Montenegro) skin test for leishmaniasis, were made on several leprosy patients and thier families. Using sera which were collected during surveys for cuteneous leishmaniasis and other parasitic diseases inculuding leprosy, the value of anti-PGL-I (phenolic glycolipid-I) antibodies and anti-LAM-B (Lipoarabinomannan-B) antibodies were measured for the serological studies of leprosy. PGL-I is a major secretory product of *Mycobacterium leprae* (Hunter, *et al.*, 1982). LAM-B is a complex glycolipid found in large amounts (15 mg per g of bacilli) within the cell walls of *M. leprae* and *M. tuberculosis* (Gaylord and Brennan, 1987). The present paper reports the result of preliminary serological examinations of leprosy. Furthermore, based on the results obtained, a brief comment was also made on the screening method to detect leprosy patients in early stage of the disease.

MATERIALS AND METHOD

Subjects examined

In this preliminary epidemiological survey of leprosy, an evaluation was made on the serodiagnosis of the subjects from the following areas of Ecuador: Los Ranchos, Portoviejo and Guayabales, Department of Manabi; Echeandia, Department of Bolivar; Antepara, Machala, Pinas, Portovelo and Zaruma, Department of El Oro; Pedro Carbo, Guayaquil and Olon, Department of Guayas; Selva Alegre, Department of Esmeraldas; and other areas of Ecuador. A total of 365 subjects (153 males, 184 females and 28 unknown), with 3 to 73 years

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old, were examined for anti-PGL-I antibodies (IgG and IgM) and LAM-B antibodies (IgG and IgM). The mean age of subjects was 26.2-year-old in male, 25.4-year-old in female and 25.8-year old in total. In the present subjects from different areas, the following underlying diseases were reported: Department of Bolivar, cutaneous leishmaniasis, 15; El oro, Chagas' disease, 15 and gnathostomiasis, 1; Guayas, cutaneous leishmaniasis, 13, Chagas' disease, 9, gnathostomiasis, 6, toxoplasmosis, 4; Pichincha, cuteneous leishmaniasis, 17, Chagas' disease, 1; Esmeraldas, cuteneous leishmaniasis, 25.

Sera

Serum samples from 365 subjects were examined. These sera were collected during the surveys for several infectious diseases, such as cutaneous leishmaniasis, gnathostomiasis and Chagas' disease, including leprosy.

Skin test

The lepromin tests were performed in eight subjects suspected for leprosy. An amount (0.1 ml) of Mitsuda lepromin solution was injected intradermally on the flexor surface of the forearm using the small needle (a disposable needle; size 26G), and the skin test area was observed for erythema and induration (induration/erythema in mm) at 48 hours later. Erythema size of more than 11 mm in diameter at the injection site was considered positive reaction, and reaction with 7 × 10 mm was considered an undetermined (+/−) Mitsuda early reaction.

Leishmania skin tests (Furuya, *et al.*, 1989) were made in 13 subjects suspected of leprosy (file number G-1 - G-13). An amount (0.1 ml) of *Leishmania* promastigotes antigen solution was injected intradermally on the flexor surface of the forearm. The skin test area was observed for erythema and induration (induration/erythema in mm) at 48 hours later. Induration size of more than 5 mm in diameter at the injection site was considered as a positive reaction.

Serological examination

Blood samples were collected by venipuncture. The sera was separated with a centrifuge at several field laboratories in Ecuador. The sera were stocked in a freezer at the temperature of −20 °C. The value of anti-PGL-I antibodies and anti-LAM-B antibodies were measured by enzyme-linked immunosorbent assey (ELISA) in a laboratory at the National Institute for Leprosy Research in Japan (Jzumi *et al.*, 1993). Cut-off levels are as follows: PGL-I-IgG 0.08 OD (optical density) units; PGL-I-IgM 0.38 OD units; LAM-B-IgG 0.25 OD units; LAM-B-IgM 0.05 OD units. A criterion for considering the diagnosis was made as follows: PGL(+) and LAM(−), suspected leprosy; PGL(−) and LAM(+), suspected acid-fast bacteria infection including leprosy; PGL-IgG(+) and IgM(−), suspected leprosy (old leprosy and spontaneousry healing subjects); PGL-IgG(−) and IgM(+), suspected leprosy.

The diagnosis of leprosy was made on clinical, bacteriological and immunological grounds, according to the Rjdley-Jopling classification (Ridley and Jopling, 1996) by the doctors from the Welfare Ministry of Ecuador.

RESULTS

The results obtained are summarized as shown in Tables 1 to 6. There was no correlation between prevalence rates for leprosy and seropositive rates of the

Table 1 Prevalence rates of leprosy and anti-PGL-I* antibody positive subjects from different areas of Ecuador.

Department**	Prevalence rate (×1000)	No. examined	Positive for PGL-I Total (%)	Male	Female
1. Bolivar	0.63—0.91	15	3 (20.0%)	1	2
2. El oro	0.63—0.91	16	3 (18.7%)	3	0
3. Guayas	0.27—0.38	198	61 (30.8%)	16***	32***
4. Pichincha	0.10—0.16	18	10 (55.6%)	5	5
5. Manabi	0.10—0.16	15	12 (80.0%)	5	6
6. Esmeraldas	0.10—0.16	98	64 (61.2%)	23	41
7. Other areas		5	1 (20.0%)	0	1
Total		365	154 (42.2%)	53 (37.9%)	87 (62.1%)

*, PGL-I: phenolic glycolipid-I; **, 1. Bolivar, Echeandia; 2. El Oro, five resions; 3. Guayas, Pedro Carbo and Guayaquil; 4. Pichincha, Puerto Quito and Quito; 5. Manabi, Los Ranchos; 6. Esmeraldas, Selva Alegre; ***, Sex of 13 subjects were unknown.

Table 2 Correlation between anti-PGL-I* (IgG) and anti-PGL-I(IgM) antibodies of subjects from differnt areas of Ecuador.

Departments and cities for villages	No. and (%) of subjects in each category				Total
	PGL(IgG)+ PGL(IgM)+	PGL(IgG)+ PGL(IgM)−	PGL(IgG)− PGL(IgM)+	PGL(IgG)− PGL(IgM)−	
1. Bolivar	0 (0.0)	1 (6.7)	2 (13.3)	12 (80.0)	15
Echeandia	0	1	1	7	9
San Francisco	0	0	1	5	6
2. El Oro	0 (0.0)	2 (12.5)	1 (6.3)	13 (81.2)	16
Antepara	0	1	0	0	1
Machala	0	0	0	2	2
Piñas	0	0	1	4	5
Portovelo	0	1	0	4	5
Zaruma	0	0	0	2	2
?	0	0	0	1	1
3. Guayas	4 (2.0)	8 (4.1)	49 (24.7)	137 (69.2)	198
Pedro Carbo	3	6	41	115	165
Guayaquil	0	0	2	13	15
Olon	1	1	0	2	4
Others	0	1	6	6	13
4. Pichincha	2 (11.1)	2 (11.1)	6 (33.3)	8 (44.5)	18
Puerto Quito	2	1	6	8	17
Quito	0	1	0	0	1
5. Manabi	4 (26.7)	1 (6.6)	7 (46.7)	3 (20.0)	15
Los Ranchos	4	0	7	2	13
Portoviejo	0	0	0	1	1
Guale	0	1	0	0	1
6. Esmeraldas	12 (12.3)	11 (11.2)	41 (41.8)	34 (34.7)	98
Selva Alegre	12	11	41	34	98
7. Other areas	0	0	1	4	5
Total	22 (6.0)	25 (6.9)	107 (29.3)	211 (57.8)	365

*, PGL-I: phenolic glycolipid-I.

antibodies (anti-PGL-I- and LAM-B antibodies) in the subjects living in several provinces in Ecuador. The seropositive rates of anti-PGL-I antibodies in Ecuador was 42.2%. The seropositive rates for PGL-I was higher in female than in male. Correlation between anti-PGL-I-IgG antibodies (PGL-IgG) and anti-PGL-I-IgM antibodies (PGL-IgM) was summerized in Table 2. The distribution of anti-LAM-B antibodies in the subjects with negative anti-PGl-I antibodies in the patients with cutaneous leishmaniasis, gnathostomiasis, Chagas' disease and others was summarized in Table 3. In 211 PGL-I seronegative persons, 128 (60.7%) were found to be LAM-B seropositive; 16 (7.7%), IgG-positive and IgM-positive; 16 (7.7%), IgG-positive and IgM-negative; 96 (46.10%), IgG-negative and IgM-positive; and 80 (38.5%), IgG-negative and IgM-negative. The subjects of file number EH57 and EH119 from Esmeraldas and G-7 from Manabi showed positive reaction to all the anti-PGL-I and anti-LAM antibodies examined. The number of anti-LAM-B-IgG antibody positive nonleprous subjects in Ecuador was 69 (19.6%) out of 352.

The results of examinations of leprosy patients and their family in Los Ranchos, Department of Manabi were summarized in Tables 4 to 6. The number of subjects examined for serological tests of leprosy was 13 (7 males and 6 females). The results of skin tests were shown as follows. Only one subject, G-1, showed undetermined (+/−) Mitsuda early reaction. Seven other subjects proved to be Mitsuda negative. Leprosy patients (G-7, G-10 and G-13) except G-1 showed positive raection to the Leishmania skin test, while 10 other subjects showed negative reaction to the skin test (Table 4). A subject, G-7, showed all positive reaction against PGL-IgG and -IgM, and LAM-IgG- and -IgM. He was a borderline lepromatous leprosy patient who still had active symptoms, such as infiltrated erythema, ulcerative lesions of feet and neuralgia on the four extremities. The patient was treated with multi-drug therapy (MDT). Other two leprosy patients, G-10 and G-13, were positive for PGL-IgM, and LAM-IgG and

Table 3 Distribution of anti-LAM-B* antibodies in the subjects, negative for anti-PGL-I antibodies, from differnt areas of Ecuador.

Departments	No. and (%) of subjects in each category				Total
	LAM(IgG)+ LAM(IgM)+	LAM(IgG)+ LAM(IgM)−	LAM(IgG)− LAM(IgM)+	LAM(IgG)− LAM(IgM)−	
1. Bolivar	1 (8.3)	1 (8.3)	6 (50.0)	4 (33.4)	12
Echeandia	0	1	3	3	7
San Francisco	1	0	3	1	5
2. El Oro	1 (7.7)	2 (15.4)	4 (30.8)	6 (46.1)	13
Antepara	0	0	0	0	0
Machala	0	0	1	1	2
Piñas	1	1	0	3	5
Portovelo	0	1	3	0	4
Zaruma	0	0	0	1	1
Others	0	0	0	1	1
3. Guayas	8 (5.9)	9 (6.6)	65 (47.8)	54 (39.7)	136
Pedro Carbo	7	8	58	42	115
Guayaquil	0	1	4	8	13
Olon	0	0	2	0	2
Others	1	0	1	4	6
4. Pichincha	3 (37.5)	2 (25.0)	0 (0.0)	3 (37.5)	8
Puerto Quito	3	2	0	3	8
Quito	0	0	0	0	0
5. Manabi	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)	3
Los Ranchos	0	0	1	1	2
Portoviejo	0	0	0	1	1
Guayabales	0	0	0	0	0
6. Esmeraldas	3 (8.8)	2 (5.9)	20 (58.8)	9 (26.5)	34
Selva Alegre	3	2	20	9	34
7. Others	0	0	0	2(100.0)	2
Total (%)	16 (7.7)	16 (7.7)	96 (46.1)	80 (38.5)	208

*, LAM-B: lipoarabinomannan-B; **, PGL-I: phenolic glycolipid-I.

-IgM. A leprosy patient, G-10, with borderline tuberculoid leprosy had relatively deep ulcers on both soles. The type of leprosy in G-13 was unknown and the subject had no specific dermatological findings of leprosy. All the leprosy patients were LAM-IgM-positive, and the subjects, G-1, G-7 and G-10 except G-13, were strongly positive for LAM-IgM, showing more than 0.190 OD units. Correlation between anti-PGL-I-IgG and anti-PGL-I-IgM antibodies in the patients with leprosy and their families was summarized in Table 5. Two leprosy patients, G-1 and G-7, were positive for PGL-IgG and -IgM. Other two leprosy patients, G-10 and G-13, were PGL-IgG-negative and PGL-IgM-positive. Correlation between anti-PGL-I antibodies and anti-LAM-B antibodies in the patients and their families was summarized in Table 6. Three patients with leprosy, G-7, G-10 and G-13, were PGL-IgM-positive and LAM-B-IgG-positive. Indeterminate leprosy patient, G-1, was negative for only LAM-IgG. There were no subjects who were PGL-IgM-negative and LAM-IgG-positive.

COMMENT

Leprosy has a wide range distribution in the world. The disease is included among the six most important infectious diseases which the World Health Organization (WHO) planned to stop from being an endemic. In some regions of Ecuador, the prevalence rates are very high. For example, the rate in Department of Los Rios showed 1.17 per 1,000 habitants. Persons living in the regions, where the prevalence rate is over 1.0 per 1,000, would be exposed to serious danger of infection whether they have or not contact with leprosy patients. The immediate counter plan for chronic infectious disease should be considered.

Recently, serodiagnosis of leprosy was considered as one of the useful methods for early diagnosis (Buchanan, *et al.*, 1983). Although, we could not examine large numbers of leprosy patients during the current

Table 4 Leishmania skin test and Lepromin test in three leprosy families in an endemic area of cutaneous leishmaniasis, Los Ranchos, Department of Manabi, Ecuador, in 1992.

No. (File no.)	Age	Sex	Lepromin test*	Leishmania skin test	Symptoms	Type of leprosy
1 (G-1)	12	F	(+/−)**	(−)	hypopigmented freckle with anesthesia	indeterminate leprosy
2 (G-2)	10	F	(−)	(−)	none	
3 (G-3)	9	F	(−)	(−)	none	
4 (G-4)	4	F	(−)	(−)	none	
5 (G-5)	11	F	(−)	(−)	none	
6 (G-6)	38	F	(−)	(−)	none	
7 (G-7)	41	M	(−)	(+)	annular and/or infiltrated erythemata, nodules with anesthesia	borderline-lepromatous
8 (G-8)	8	M	(−)	(−)	none	
9 (G-9)	15	M		(−)	none	
10 (G-10)	31	M		(+)	neuralgia, ulcers with anesthesia	borderline-tuberculoid
11 (G-11)	6	M		(−)	none	
12 (G-12)	3	M		(−)	none	
13 (G-13)	56	M		(+)	neuralgia, anesthesia	leprosy***

* Mitsuda early reaction; **, Mitsuda early reaction is undetermined with 5.7×7.5mm(+/−); *** leprosy type is unknown.

Table 5 Correlation between anti-PGL-I* (IgG) and anti-PGL-I (IgM) antibodies in the leprosy patients and their household contacts in Los Ranchos, Department of Manabi, Ecuador, in 1992.

Category	PGL (IgG) + PGL (IgM) +	PGL (IgG) − PGL (IgM) +	PGL (IgG) + PGL (IgM) −	PGL (IgG) − PGL (IgM) −
File no.	G-1, G-5 G-7, G-12	G-2, G-3, G-4 G-6, G-8, G-10 G-13	none	G-9, G-11
No. of leprosy	2	2	0	0
Total	4	7	0	2

* PGL-I: phenolic glycolipid-I;

Table 6 Correlation between anti-PGL-I* and anti-LAM-B** antibodies in leprosy patients and their household contacts in Los Ranchos, Department of Manabi, Ecuador, in 1992.

Category	PGL (IgM) + LAM (IgG) +	PGL (IgM) + LAM (IgG) −	PGL (IgG) + LAM (IgM) −	PGL (IgM) − LAM (IgM) −
File no.	G-4, G-7, G-8 G-10, G-13	G-1	G-2, G-3 G-5, G-6, G-12	G-9, G-11
No. of leprosy	3	1	0	0
Total	5	1	5	2

* PGL-I: phenolic glycolipid-I; ** LAM-B: lipoarabinomannan-B;

survey, anti-PGL-I and anti-LAM-B antibodies were examined by using accepted sera from different areas of Ecuador. The seropositive rates of anti-PGL-I antibodies in Ecuador was high, showing a rate of 42.2%. Recently, anti-LAM-B antibodies have been measured to detect the subjects with multibacillary leprosy whose values of anti-PGL-I antibodies are very low (Izumi *et al.*, 1993). In 215 PGL-I seronegative persons, 132 (61.4%) were found to be LAM-B seropositive. Some of them have the possibility of showing symptoms of lep-

rosy. Anti-PGL- and anti-LAM-B antibody positive subjects would need detailed medical examination to rule out the disease by acid-fast bacteria. A total of 154 subjects (42.2%) were serologicaly suspected of suffering from *M. leprae* and 128 subjects (35.1%) were suspected of diseases caused by acid-fast bacteria including *M. leprae*. In the examination, tuberculosis, tuberculosis cutis and its related diseases such as lupus vulgaris, tuberculosis verrucosa cutis, scrofuloderma, erythema induratum Bazin and infectious diseases of atypical mycobacteria should be taken into consideration.

Izumi *et al.*, (1993) reported the distribution of anti-LAM-B antibodies in non-leprous sera in Japan and South Sulawesi, Indonesia. According to their studies, the numbers of anti-LAM-B-IgG- antibody positive non-leprous subjects in Japan and Indonesia were 18 (4.9%) out of 367 and 20 (12.4%) out of 161, respectively. In comparison, the positive rate of anti-LAM-B-IgG antibodies in Ecuador was relatively high, showing a rate of 19.6%. From these results, we suspected that there might be large numbers of inhabitants infected subclinically by the bacillus and some of them have the possibility of showing symptoms of the disease.

In the present study, no correlation between positive rates of antibodies (anti-PGL-I and anti-LAM-B antibodies) and prevalence rates of leprosy was observed in each area of Ecuador. In Manabi, Ecuador, the prevalence rate of leprosy was 0.10-0.16 per 1,000 inhabitants, showing a relatively low rate. But the anti-PGL-I-seropositive rates of the patients and their family in Los Ranchos, Manabi (84.6%) were higher than the average positive rate (42.2%) of all subject in several areas in Ecuador. From the data shown in Table 5, it was considered that PGL -IgG and IgM- positive cases were bacteriologically and immunologically active patients.

In general, the values of anti-PGL-IgM and LAM-B-IgG antibodies were used for serological diagnosis of leprosy because the combination thought to be clinically useful for the diagnosis in early stage of the disease. As shown in Table 6, three leprosy patients were positive for PGL-IgM and LAM-IgG. The value of anti-LAM-B-IgM antibody was thought to be unreliable, because of the low of cut off value (>0.19 OD unit). But a strong positivity of LAM-IgM might be an indicator for the diagnosis of leprosy, because three out of four leprosy patients, were strongly positive for LAM-IgM (data not shown). As the subjects, G-5 and G-12, were positive for PGL-IgG and -IgM, they should be watched for the development of the disease symptoms, though no clinical findings of leprosy were observed in the present examination.

Correlation of the skin tests between cutaneous leishmaniasis and leprosy was partly found; three out of four leprosy patients showed positive reaction to *Leishmania* promastigotes antigen. From this result, it would be speculated that the specific defect of cell-mediated immunity for *M. leprae* might be covered by other activated cellular immunity.

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マラリア予防薬としてメフロキンを長期投与した際の副作用について

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はじめに

自衛隊はペルシャ湾，カンボジアに引き続きモザンビーク共和国に48名の自衛隊員を輸送調整中隊として1993年5月から派遣している。ペルシャ湾に始まる自衛隊の海外への部隊派遣ではいずれも自衛隊医官が同行しておりモザンビークにおいても医官2名が同行し隊員の健康管理，及び衛生環境整備を行っている。カンボジア，モザンビーク共和国ともにマラリアの汚染地域であるため，カンボジア施設大隊ではマラリア予防薬としてドキシサイクリンを使用していたが，今回，著者等はモザンビーク共和国でメフロキンを使用した。メフロキンの有効性，及びその内服期間を通じて自衛隊員が訴えた体調の変化，及び血液学的変化でマラリアの副作用と思われる点について述べる。

対　象

今回，対象としたのは第2次モザンビーク派遣輸送調整中隊としてモザンビーク共和国に1993年11月22日から1994年6月18日の間，派遣されていた48名である。全員男性で平均年齢は32.0歳（SD-5.5）であった。陸・海・空の内訳は陸上自衛官38名，海上・航空自衛官各5名であった。48名の既往歴としては1名が約10年前に水腎症の為，片腎摘出術を受けていた。又，日本出国前に全員がB型及びC型肝炎の検査を受けており，1名がB型肝炎キャリアであった以外は肝炎は認めなかった。

モザンビークの地理的，気候的背景

モザンビーク共和国はアフリカ大陸支部に位置し（Fig. 1）南緯10度から27度，西経30度から41度内に存在する[1]。標高は平均200mと低く，南北に国が延びている為，気候は北中部は熱帯気候に南部は亜熱帯気候に属する。乾季の時期を除いて高温多湿である。Fig. 2に代表的4都市（ナンプラ，テテ，ベイラ，ナマーシャ）の月別平均気温及び降水量を示す[2]。季節は11月から4月までの雨期と5月から10月までの乾季に分けられる。標高が低く降水量が多い為，湿地帯が多く，それに加えて高温多湿である為，マラリアを媒介するハマダラカの温床になっている。モザンビーク共和国は南北に国が延びている為，国連モザンビーク活動（以下ONUMOZ）も北部，中部，南部に分けて活動が行われていた。当第2次モザンビーク派遣輸送調整中隊48名の内38名は首都マプトから約8km離れたマトラに宿営（南部地域の輸送調整業務を担当）し，10名はベイラから約30km離れたドントに宿営（中部地域の輸送調整業務を担当）していた（Fig. 2）。派遣隊員数は業務を行いうる最少人数であった為，マトラ，ドント共にポルトガル軍の宿営地内に宿営し食事，シャワー等の後方支援はポルトガル軍に依存していた。

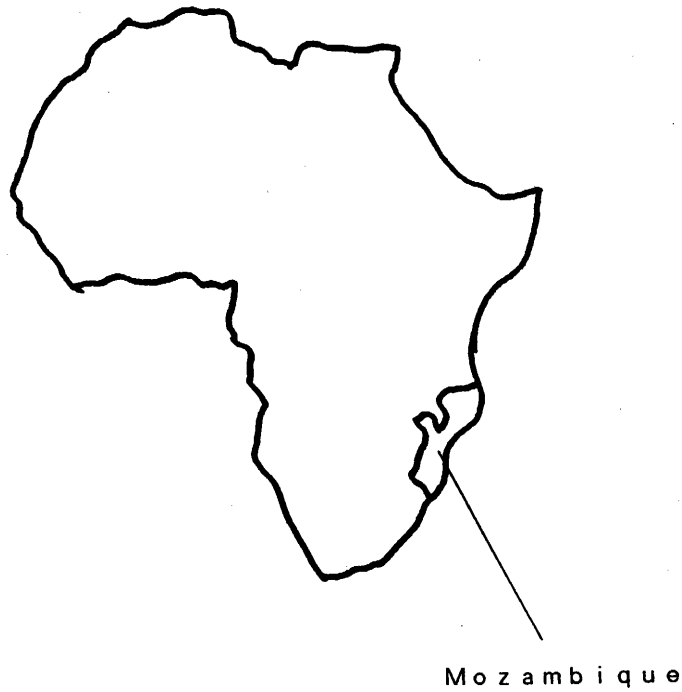

Figure 1 The location of Mozambique in Africa. Mozambique lies between 10°～27°S and 30°～41°W.

自衛隊札幌病院　外科
札幌市豊平区平岸1条12－1　自衛隊札幌病院　外科

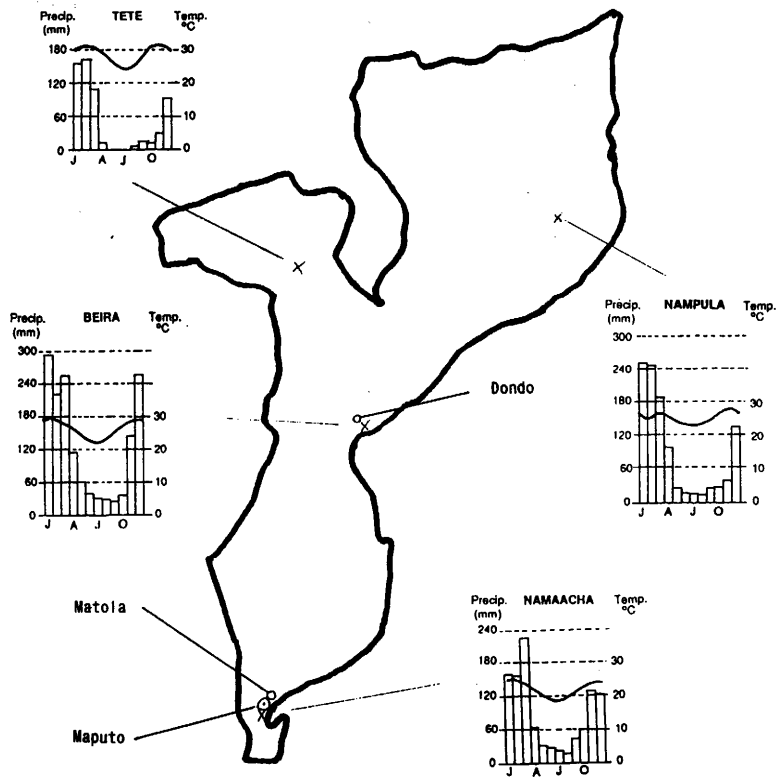

Figure 2 The average temperature and rainfall of main cities in Mozambique. Japanese 2nd Movement Control Company stayed in Matola (38 members) and Dondo (10 members).

第2次モザンビーク派遣輸送調整中隊のマラリアに対する予防衛生

ONUMOZの総司令部には公衆衛生幹部がいたが組織的な防疫活動，駐屯地における衛生指導は行われておらず不定期に殺虫剤が配付されるのみであった。当中隊では以下の4点についてマラリアに対する予防衛生活動を行った。

1）殺虫剤（殺虫スプレー，蚊取り線香，防虫スプレー，殺虫燻煙剤）の配付と使用勧告。

当初は日本からすべての殺虫剤を追送していたが，殺虫スプレー，殺虫燻煙剤については代用品が現地調達できるようになり追送品は蚊取り線香，防虫スプレーのみとなった。蚊取り線香は各テント毎に配付し毎晩使用するように指導した。防虫スプレーは夜間の外出，旅行時には必ず携行させた。殺虫燻煙剤は2～3カ月に1回の割合で蚊，その他の病害動物に対して各テント，プレハブ毎炊かせた。

2）駐屯地内の防疫活動

1次隊から駐屯地区の防疫活動は行われており，当初はダイアジノン，スミチオンを野外トイレ，テント周囲及び駐屯地周辺の水たまりに散布していたが，効果が弱い為，1994年2月からペルメトリンに変更し効果を上げた。

3）メフロキンの予防内服

ONUMOZの勧告と同様の250mg/wを出国前1週間から開始した。熱帯熱マラリアの潜伏期間が7日～30日を考慮して帰国後4週間内服を継続した。

4）蚊帳に対するリペラント処置及びその使用指導

蚊帳に対するリペラント処置の効果は数々の文献で証明されている[3)4)5)]。1次隊ではリペラント処置をされていない蚊帳を使っていた為，2次隊ではペルメトリンによるリペラント処置を施行した。現在リペラント処置を施行された蚊帳が市販されており今後，マラリア流行地域に部隊が派遣される場合にはこれを購入するのが適切であると考えている。

マラリア予防薬メフロキンの投与量，投与方法及び副作用調査方法

我々は1）クロロキン耐性熱帯熱マラリアがアフリカ大陸にも多く認められること[6)]，2）WHO，フランス厚生省の推奨するプロトコール[7)8)]がONUMOZが推奨するプロトコールと一致する事，からマラリア予防薬としてメフロキン250mg/wのプロトコールを採用した。メフロキンの内服はモザンビーク着国時，既に有効血中濃度に達しているように出国前1週間から始めた。内服開始前には全隊員に服用の必要性，服用方法，副作用等についてパンフレットを配付し，説明した。帰国後は熱帯熱マラリアの潜伏期間7日～30日を考慮し4週間まで続けた。内服は必ず食後とし多めの水と共に服用するように指導した。メフロキンは現地での休暇期間中も必ず持参させ確実に毎週服用させた。ただし，後で述べる4名についてはモザンビーク入国4カ月で副作用と思われる症状が徐々に増強した為，服用間隔を1週間から2週間に延長している。この4名はメフロキンを計29錠服用した事となるがその他の隊員は設36錠内服した事になる。モザンビークの宿営地内には医務室を設け体調不良時には常時受診できるよう配慮した。又，派遣後1カ月目，3カ月目にアンケートを用いて全隊員の体調をチェックし4カ月目，帰国直前に副作用についてのアンケートを行った。

血液学的検査としては日本出国前，帰国後に血液算球検査，生化学的血液検査を施行しており又，モザンビーク着国後3カ月には遠心分離後凍結した血清を日本へ航空機輸送，日本で生化学的血液検査を施行している。

〈結果1—隊員の体調の変化について〉

症状としての副作用はすべてアンケートを用いて調査した。それぞれの時期に施行したアンケートの回収率はすべて100%であった。

内服期間を通して重篤な症状は認めなかったが1名に嘔吐（内服し3週間目から1回/月，内服日夜間）が定期的に

認められた。この隊員に対しては消化剤，制吐剤の適宜投与を行うことにより4カ月目には症状は消失し予防内服を250mg/wで続ける事ができた。モザンビーク到着後4カ月目に施行したアンケートの結果をTable 1に示す。全身倦怠感，頭痛の内，各2名は重複している。計16名（33.3%）に副作用があった事となる。いずれも軽度の副作用であったが頭痛2名，全身倦怠感2名が投与間隔の延長を希望した為，この4名に対してのみ以後，帰国後4週間までの投与間隔を1週間から2週間に延長した。残りの14名に対しては症状の強さと本人の希望を考え合わせ従来通りの250mg/wのままとした。帰国前に再度施行したアンケートでは頭痛，全身倦怠感を訴えていた4名は症状は残っていたが軽快していた。帰国後，メフロキン内服を中止して約2カ月半経過した現在，新たな副作用の出現，重篤な副作用の出現は認めていない。

Table 1 Side effects with mefloquin (48 persons) Following symptoms were gotten by 4 month questionnaire. Two of the general fatigue cases are also part of the headache group.

Side effects	Number of cases	Beginning of side effects			
		1W	1M	2M	3M
General fatigue	8 (16.7%)	2	2	1	3
Headache	6 (12.5%)	1	1	2	2
Nausea・Vomiting	2 (4.2%)		1	1	
Vertigo	1 (2.1%)	1			
Diarrhea	1 (2.1%)			1	

〈結果2－血液検査データの変化〉

日本出国前，モザンビーク到着後3カ月目，そして日本帰国後に生化学的血液検査（3カ月目は遠心分離し日本へ凍結輸送した）を施行している。

血液算球検査については日本出国前と日本帰国後のみの比較になるが，Fig. 3に示す通りである。亜熱帯地域での勤務，生活による脱水の影響を受けた為か赤血球は上昇が激しい。これについてはカンボジアにおける派遣でも認められている。白血球も有意に上昇しているがメフロキンとの因果関係については不明である。尚，白血球の上昇に関してはカンボジアでは認められていない。

3カ月目の血液検査では3名（6.3%）の肝機能軽度異常者，6名（12.5%）のガンマGTP異常者，15名（31.3%）のTTT異常者が認められた。帰国後の白血検査では8名（16.7%）の軽度肝機能異常，7名(14.6%)のガンマGTP異常者，16名（33.3%）のTTT異常者が認められた。ZTT，ビリルビン，LDH，LAP，BUNについては異常を認める者はなかった。肝機能異常を示した隊員と全身倦怠感を訴えた隊員は必ずしも一致していなかった。

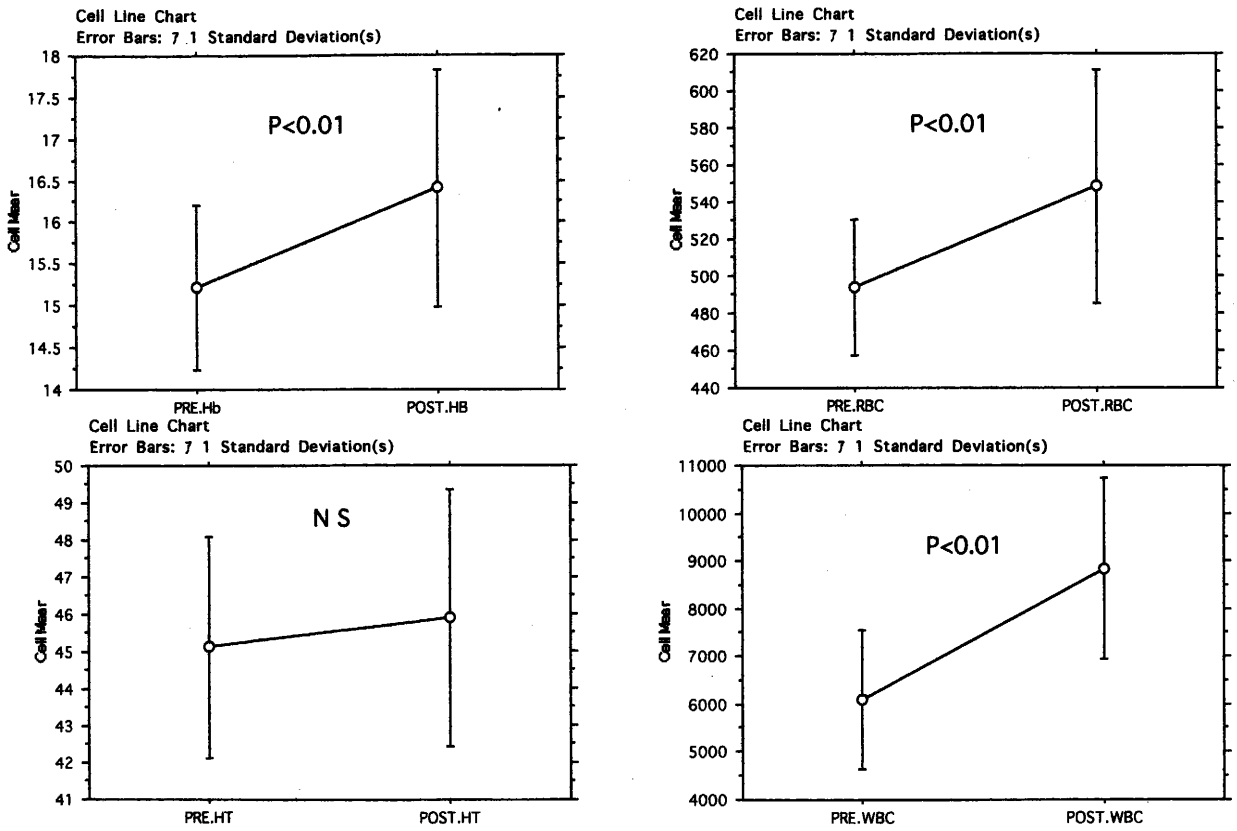

Figure 3 Comparison of CBC test between Pre-departure and Post-return

Table 2 Eight members had abnormal liver functions in post-return test. This shows the change of liver functions among Pre-departure, 3 month and Post-return testing periods.

No.	Liver function	Pre-departure	3 Month	Post-return
A	GOT	27	19	48
	GPT	46	47	62
B	GOT	16	23	35
	GPT	21	27	61
C	GOT	14	10	21
	GPT	27	14	38
D	GOT	37	36	43
	GPT	39	51	53
E	GOT	18	31	25
	GPT	41	41	36
F	GOT	26	23	44
	GPT	40	24	43
G	GOT	34	19	42
	GPT	40	22	35
H	GOT	20	25	42
	GPT	17	19	36

帰国後の血液検査では肝機能異常を認めた8名の隊員の検査値の推移をTable 2に示す。モザンビーク到着後明らかに肝機能が上昇した者はA．B．D．Cの4名（8.3%）であった。

尚，B型肝炎キャリアの隊員はいずれの時点での血液検査でも肝機能には異常は認めなかった。

肝機能異常がメフロキンの副作用である可能性は十分にあるが，脂肪肝，アルコール性肝障害の除外診断を施行していない為，厳密には全てが副作用であるとは言いきれない。

メフロキンの予防薬としての有効性について

モザンビークに派遣された自衛隊員ではマラリア患者は1994年8月1日現在，一人も出ていない。しかし，メフロキンの予防内服が推奨されているONUMOZ内では多数のマラリア患者の発生が認められている（Fig. 4）。勤務地域もしくは，居住地域によって罹患率が違ってくる事は当然予想されるしメフロキン耐性マラリアについても考える必要が出てくる。

自衛隊は48名中38名は南部地域に，10名は中部地域に，いずれもポルトガル軍と同じ駐屯地内に居住していた。自衛隊が南部，中部業共に一緒に居住していたポルトガル軍のマラリア患者数を見ると（Fig. 5）必ずしも自衛隊員にマ

	Jun-93	Jul-93	Aug-93	Sep-93	Oct-93	Nov-93	Dec-93	Jan-94	Feb-94	Mar-94	Apr-94	May-94
North 1271	32	42	27	8	12	11	30	35	43	40	40	55
Central 1228	3	29	15	7	2	7	4	2	7	5	8	13
SOUTH 2452	5	18	13	4	1	12	9	3	167	227	200	68
Total 4951	40	89	55	19	15	30	43	40	217	272	248	136

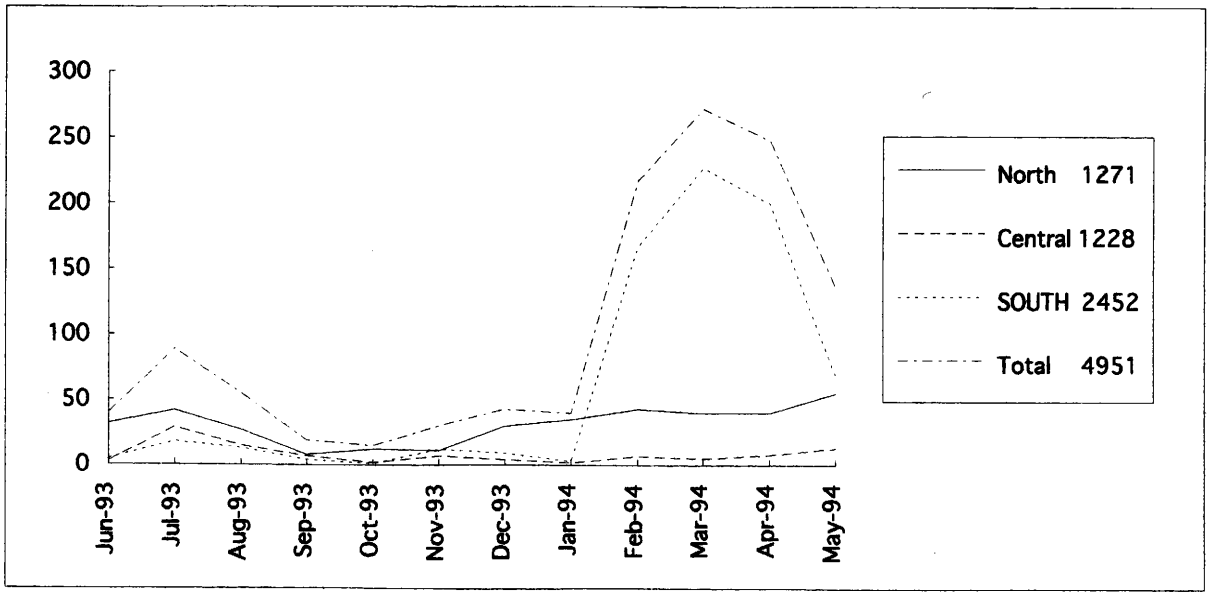

Figure 4 ONUMOZ Malaria

ラリア患者が発生していないのは居住地域による違いではないと言える。ポルトガル軍の衛生兵からは，ポルトガル軍マラリア患者はメフロキンを飲み忘れていた兵士か，副作用の発生を恐れて飲んでいなかった兵士であると情報を得ている。又，ONUMOZ内ではマラリア治療にはほとんどのケースでメフロキンを使用しているがメフロキン耐性マラリアについても1994年8月1日現在報告がない。

以上の事よりメフロキンの予防薬としての効果は今のところモザンビークにおいては非常に高いと考えられる。

考　察

我々が勤務したモザンビーク共和国は亜熱帯地域に位置し標高も低く降水量が多い[1)2)]ためマラリアを媒介するハマダラカの温床となっていた。又，モザンビークは11月から4月までの雨期と5月から10月までの乾季に分けられていたが我々，第2次モザンビーク派遣輸送調整中隊が派遣された時期は最高気温47度という雨期の真只中であった事も加わり，特にマラリアには注意が必要であった。Fig. 4に示すようにONUMOZ内でも2月から4月までは200人/月以上のマラリア患者発生が認められていた。我々が調査した現地の4つの医療施設でもいずれもマラリアを疾患別患者数のトップにあげていた。又，現地新聞（NOTICIAS 5 April 1994）でもマラリアによる死亡数が第1位であると報道していた。

当中隊では派遣間そして後も一人のマラリア患者を出すことなく任務を遂行できたが，これはメフロキンの予防内服を含めた予防衛生活動によるものと考えている。なぜならばONUMOZでのマラリア発生患者はそのほとんどが予防薬を服用していなかったという情報があり，また駐屯地における防疫活動も著者が知る限りではイタリア軍野戦病院，アルゼンチン軍野戦病院，そして日本隊のみであったからである。

サハラ砂漠以南のアフリカでは85%以上が熱帯熱マラリアと従来より言われてきた[9)]。3つあるONUMOZの野戦病院の1つであるバングラデッシュ野戦病院は熱帯熱マラリアがほぼ100%だと言っていた。また，モザンビーク厚生省に我々が直接質問状を提出したところ種類別マラリアの割合を熱帯熱マラリアが95～99%，4日熱マラリアが1～5%，卵形マラリアが0.1～0.5%で3日間マラリアはないと返答を受けた。モザンビークにおいてマラリアの死亡数が多いのは，そのほとんどが致死的となる重症マラリアを引き起こす熱帯熱マラリアであると考えられる。

メフロキンの予防内服はこれまで3カ月を限度として使用するように勧告されていた[10)]が，今回我々の約8カ月に渡る予防内服では4名の投与間隔延長者はいたものの，いずれも軽度の副作用を33.3%の隊員に認めるだけで済んだ。しかし，メフロキン250mg/wを3カ月以上投与するとそれ

	Jun-93	Jul-93	Aug-93	Sep-93	Oct-93	Nov-93	Dec-93	Jan-94	Feb-94	Mar-94	Apr-94	May-94
North 63	6	8	2	2	1	3	0	1	5	1	4	2
Central 77	1	0	0	0	1	0	0	2	5	2	0	0
South 133	1	0	0	0	1	0	0	1	3	1	3	2
Total 273	8	8	2	2	3	3	0	4	13	4	7	4

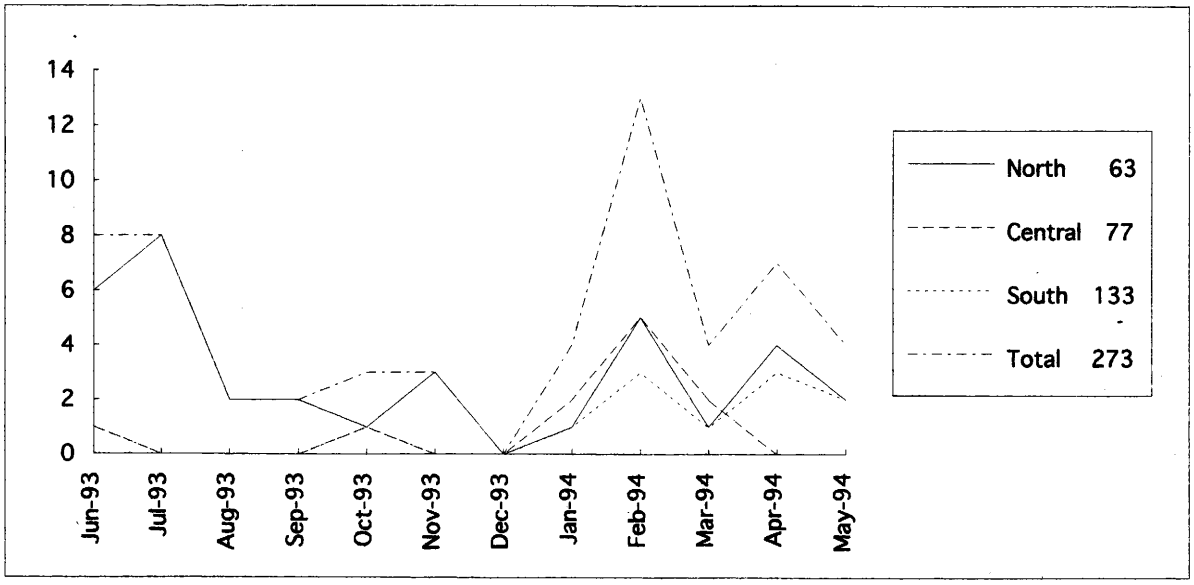

Figure 5 Portugal malaria

ま.で出ていた軽度の副作用が増強したり，肝機能障害が出現する可能性がある為，今後長期に渡る内服が必要な場合は血中濃度をモニタリングし，どのような服用方法が最適であるかを調査する必要があると思われる。投与間隔を延長した4名の隊員については血中濃度分析を行ってはいないがメフロキンの最高血中濃度到達時間が2—12時間，半減期が15—33日[10)11)]である事より薬剤の体内蓄積を考慮し投与間隔を2週間に延長している。アメリカ保健省はメフロキンの体内蓄積を考慮してか，予防内服開始後4週目までは毎週250mgの内服としその後は250mgを隔週で内服する事を推奨している[12)]。しかし，西アフリカでこの通り内服したアメリカ平和協力隊では予防内服を隔週に切り替えた後にマラリア患者の発生を認めている為，当中隊では250mg/wを長期に渡り継続した。

血液検査では全員の白血球数が有意に上昇していたがHarinasuta等[13)]の報告では治療量では白血球の減少は認められているものの増加は認められていない。数名に肝機能の上昇を認めた事とあわせて，今後の追加研究が必要と思われる。

今回の副作用の内訳を従来から言われてきている副作用報告[3)]（Table 3）と較べると全身倦怠感が8名（16.7%）と多数であるが他の4項目については従来の報告と一致する。従来から指摘されている平衡感覚障害[14)15)]については1名が眩暈を訴えてはいたものの全員，日常生活そして輸送調整業務上も全く支障はなかった。今回は副作用でけいれん等の神経症状の出現[16)17)]は認めず，メフロキンを中止して他剤へ変更する事もなかった。

カンボジア派遣海上輸送補給部隊として参加した菅沼等[18)]の報告によればメフロキンを389名に対して12週間，250mg/wで投与した副作用出現率は延べ12.8%，副作用のために予防内服をメフロキンからドキシサイクリンに変更したのは6名1.5%であった。アンケート調査でのSteffen等の報告[19)]の21.2%，Lobel等[20)]の47.2%と較べても低率であったがこれは投与期間による差とも考えられる。今回の我々の長期投与による副作用出現率は全体で33.3%でアメリカ平和部隊を対象とした調査（Table 3）の39%に較べると若干低かった。

1993年5月にONUMOZが始まってからONUMOZはメフロキンを予防薬として推奨し全部門にこれを配付，またONUMOZの3つの野戦病院の内，アルゼンチン軍，イタリア軍野戦病院の2つはマラリア治療薬の第1選択にメフロキンを採用，残るバングラデッシュ野戦病院についても第2選択にメフロキンを採用していたが1994年8月現在，メフロキン耐性熱帯熱マラリアについてはONUMOZ内でも報告がない。ちなみに，モザンビークにおけるクロロキン耐性マラリアについては30～50%というデータをモザンビーク厚生省から得ている。

長期にわたりマラリア予防薬としてメフロキンを使用する場合の副作用についての報告は稀であったが，以上よりメフロキンはマラリア予防薬としては，その耐性は認めない地域においては副作用も軽く効果の高い薬剤であると思われる。また，従来より3カ月を限度とした予防内服が推奨されていたが耐性獲得を考慮しなければ，注意して使用すれば6カ月を越える予防内服も可能であると思われた。

Table 3 Reports on side effects with mefloquin

1. Survey of European staying in east Africa: Nausea (10.7%) Vertigo (6.7%) Headache (6.1%)
2. Survey of German travelers: occurrence of psychic symptoms 1/13,000 (prophylactic)
3. Report from C.D.C.: hospitalization rate (3/201, 284) convulsion (2 cases) mental disorder (1 case)
4. Survey of Spanish travelers: mental disturbance (vertigo, headache, euphoria, depression, uneasy, insomnia, unstable emotions) (11 cases) eruption (3 cases)
5. Survey of American peace keeping force: incidents of minor side effects mefloquin group 39% chloroquin group 50%

quoted from reference 3)

結　語

第2次モザンビーク派遣輸送調整中隊員48名を対象にマラリアに対するメフロキン250mg/wの予防投与を約8カ月に渡り行った。全身倦怠感16.7%，頭痛12.5%，嘔気・嘔吐4.2%，眩暈2.1%，下痢2.1%といったいずれも軽度の副作用を認めた。血液学的検査では全隊員の白血球が有意に上昇しており，また4名の隊員に軽度ではあるが明らかに肝機能の悪化を認めた。投与開始後4カ月後に副作用増強のため投与間隔を1週間から2週間に延長した隊員が4名いたが投与を中止し薬剤を変更した者はなかった。今回はONUMOZの推奨に近いメフロキンの予防投与を長期に渡り行ったが幸いモザンビークではメフロキン耐性マラリアの発生については1994年8月現在報告はない。耐性獲得を考慮しなければメフロキンの長期予防投与は可能と思われた。

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Original Article

SIDE EFFECTS WITH MEFLOQUIN FOR LONG-TERM MALARIA PROPHYLAXIS

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Key words: malaria/mefloquin/side effects/Mozambique/ONUMOZ (Operation des Nations Unies en Mozambique)

Forty eight members belonged to Japan Self Defense Force took part in ONUMOZ (Operation des Nations Unisis en Mozambique) as the second Movement Control Company from Nov. 1993 to Jun. 1994. I belonged to the company and stayed in Mozambique during the period. As malaria is rampant in Mozambique, I used mefloquin 250mg/week as prophylaxis follwing the recommendations of ONUMOZ, W.H.O. and the Ministry of Health in France. In view of the necessary concentration of the drug and the incubation period of Plasmodium falciparum, we took the drug from the day one week before we left Japan to the day four weeks after returning. The total tablets each members took was 36 (except for four who dropped out). We investigated the symptomatic and laboratory side effects of this prophylactic treatment.

The following symptoms were observed as side effects of mefloquin: general fatigue 16.7%, headache 12.5%, nausea & vomiting 4.2%, vertigo 2.1%, diarrhea 2.1% (two cases each of general fatigue and headache overlapped). Mean WBC was evevated post-return in comparison to pre-departure. Liver function of four cases deteriorated slightly in comparison to predeparture, three months after arriving, and post-return. Two each members who suffered general fatigue and headache prolonged the period of taking the drug for one week to two weeks from 4 months after arriving to 4 weeks after returning because the symptoms had worsened. But there were no serious side effects during the prophylactic period.

Over 200 members of ONUMOZ contracted malaria from Feb. 1994 to Apr. 1994, but I had no malaria outbreak in the Japanese contingent.

ONUMOZ provided mefloquin to all members for prophylaxis and two out of three field hospital used mefloquin for malaria treatment as the first choice, but no malaria resistant to mefloquin had been reported as of the beginning of August 1994.

We conclude that mefloquin is effective for malaria prophylaxis in Mozambique, and minor side effects are observed in only 33.3% of subjects in good health.

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日本における輸血マラリア

——血小板輸血により感染したと考えられる熱帯熱マラリア1症例を中心に——

狩野繁之，鈴木　守

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はじめに

1993年の日本人年間海外渡航者数は11,933,620人，また外国人の新規入国者数は3,040,719人であり（法務省入国管理局統計），その数は年々増加の傾向を示し，それに伴って日本国内における輸入マラリアの症例数も増加してきている。現在年間罹患者数は報告されるだけでも100名を越え（大友ら，1993），マラリアはもはや珍しい病気ではなくなってきている。しかし，日本においてはマラリアを媒介するハマダラカの密度が非常に低いため，輸入マラリア（imported malaria）が土着性（indigenous）とならないばかりか(大鶴，1958)，マラリア患者からハマダラカを媒介して偶然に感染する導入マラリア（introduced malaria）の危険性もほとんどない(大友ら，1973)。ところが近年の輸血製剤の需要の高さより，全国における献血者数も1991年には800万人を越え，いわゆる誘発マラリア（induced malaria）の範疇にある輸血マラリア（transfused malaria）の危険性が論じられるようになってきている。日本における輸血マラリアの報告は酒井（1935）が初めてであり，第二次世界大戦前後に集中しているが，その後散発的に報告され，1985年の矢野ら（1985）が最後である。今回われわれは，血小板減少症の治療に濃厚血小板を輸血し，それにより感染したと考えられる熱帯熱マラリアを経験した。わが国における輸血マラリアの文献的考察を加えて報告する。

症　例

患者：70歳，日本人女性。千葉県船橋市在住。海外渡航歴なし。麻薬，覚醒剤等の常用歴なし。

既往歴：1984年6月，A型肝炎で某院入院(20日間)。この時初めて血小板減少症（8万/mm³）が認められた。1988年4月，鼻出血が止まらず入院（1カ月間)。血小板数4.7万/mm³，軽度の肝肥大，胆石，右腎嚢胞が認められた。1989年3月，腰痛を訴えて入院（1カ月間)。白血球数（3400/mm³)，血小板数（6.6万/mm³）の減少が認められた。1991年2月，腰痛のため再度入院（1カ月間)。

現病歴および経過：1991年3月30日，腰痛の悪化を主訴として入院した。単純X線写真上第3腰椎に圧迫骨折像が認められ，骨粗鬆症と診断された。身長152cm，体重45kg。現症として右上肢手拳大皮下出血，白血球数の減少，血小板数の減少が認められた。血小板減少症の治療のため，4月4日，5日，6日，11日，12日，13日に10単位ずつ合計60単位濃厚血小板輸血を行ったが，改善は認められなかった（Figure 1)。4月23日，全身状態が良好であったため，一時帰宅して入浴をした。

4月24日，突然の悪寒戦慄，発汗を伴う39°Cの発熱を示した。氷枕するも効果なく，ボルタレンサポ™50mgを投与し一旦下熱をみた。血液の一般細菌培養は陰性の結果を得た。25日，悪寒を伴う37.6°Cの発熱が再度出現し，翌朝ボルタレンサポ™50mgで下熱した。4月26日，27日，28日，29日と全身倦怠感を訴えるも37°C以下の平熱を維持した。29日19：00，顔色優れず，21：00より酸素吸入を開始した。翌30日，食欲低下，全身倦怠感，悪寒を訴えた。また，傾眠傾向を認めた。

5月1日03：00，嘔気の訴えがあった。06：00，再度嘔気，倦怠感を訴えるも朦朧状態。また，患者は尿閉に陥った。10：00，顔色不良，皮膚の黄染が認められた。眼球運動が認められなくなったが，呼鳴には反応した。中脳における出血を疑い，脳CTスキャンを行うが異常を認めなかった。血糖値14mg/dl，インスリン値3.3μU/mlを示し，低血糖による脳機能低下を疑いブドウ糖を投与するも効果が認められなかった。14：00，呼鳴に反応せず，四肢の硬直を認めた。睫毛反射・対光反射は正常であった。18：00，瞳孔の散大を認めた。20：40，顔色悪く，貧血も高度になり，新鮮血3単位を輸血した。22：15，37.1°Cの発熱。瞳孔は縮小したが，顔面に痙攣が認められ，意識は消失に至った。5月2日01：00，37.9°Cの発熱。06：00，38.1°Cに上昇。顔面，胸部，腹部黄疸著明。意識消失状態。徐々に血圧，呼吸数，脈拍数が低下し，07：50，心停止をきたし，07：55，永眠した（Figure 1)。

血液検査所見：経時的な血液細胞学的検査所見，ギムザ染色した血液塗抹標本の顕微鏡下に観察したマラリア原虫寄生赤血球数，間接螢光抗体法（IFAT）による血清中のマラリア抗体価（抗熱帯熱マラリア原虫抗体価：*P.f.* titer，抗三日熱マラリア原虫抗体価：*P.v.* titer）をFigure 1に，入院時，初回発熱時，死亡直前の主な血液生化学的所見を

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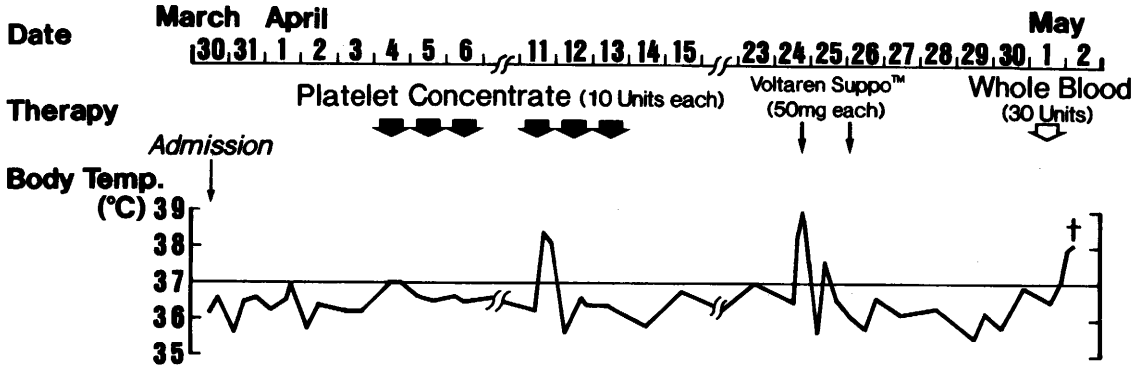

WBC (/mm³)	3000	2800	3500	3000	17300	20400
RBC (x10⁴/mm³)	364	364	374	368	223	233
Hb (g/dl)	12.2	12.2	12.6	12.4	7.2	7.6
Ht (%)	36.1	35.9	36.4	35.6	22.1	22.9
Plt (x10⁴/mm³)	5.8	5.8	4.7	4.1	2.7	2.1
ESR 1h (mm)	32			50	60	
No. of *P.f.* / 10⁴ RBCs	0		(Apr. 17) 0	6	550	190
P.f. titer				<1:4	1:256	1:256
P.v. titer				<1:4	1:16	1:4

Figure 1 Clinical course of the patient.

Table 1に，そして5月1日の血液薄層塗抹標本像をPhoto. 1に示す。

考　　察

日本における最初の輸血マラリアの報告は，酒井(1935)が細菌性赤痢後'ヂストロヒー'の1歳6カ月の男児と，百日咳肺炎の1歳1カ月の男児への輸血療法後に誘発した三日熱マラリアの2例を記載したものである。その後1948年までの輸血マラリアの報告一覧として，貴田，中山（1948）が諸家の症例29例をまとめている。それらの供血者は，マラリア既往を有するもの11例，マラリア流行地に滞在の事実のある者5例とあり，明らかに輸血マラリアの危険の高い供血者を含んでいる。また，父，母，兄弟からの供血が多いが，6人の職業供血者からいわゆる売血により供給された血液材料が提供されている。第二次世界大戦終戦前後においては，この職業供血者からのマラリア感染の危険性が強く憂慮されていた(山内，長野，1956)。戦後輸血材料は主に保存血となり，一回の輸血に複数の供血者の血液が投与されるため，マラリアの感染の危険性も高くなった。この保存血輸血により誘発されたマラリアの本邦における第1症例が高田（1955）により報告された。保存血中のマラリア原虫の生存期間に関しては，金森（1944）が7日，村瀬ら（1956）が6～18日，加藤ら（1956）が4～6°Cで14日と記載している。また1960年当時の厚生省基準では4～6°Cで96時間保存後に輸血材料として使用するとあるが，増田（1960）はこの時間以後における血液材料からのマラリアの感染性を実験的に証明している。現在血小板製剤は，分離後常温で保存され72時間以内に使用されることになっており，実際にはもっと早急に半日ほどで輸血に供されることが多い。万一輸血材料にマラリア原虫感染赤血球が含まれていれば，原虫の死滅する可能性は低いと考えられる。実際にわれわれの日常業務で，熱帯熱マラリア患者から採取した血液を12～24時間室温に置いた後でも，それを培養系に移し原虫を増殖させている。戦後の輸血マラリアの報告例は，1956年までの22例を伊藤ら（1985）が一覧にまとめているが，戦前の報告例を合わせて，日本における輸血マラリアの総数を57例，うち熱帯熱1例，三日熱41例，卵形1例，不明14例と記載している。その他にわれわれが文献上で調べた限りにおいては，熱帯熱1例（飛田，1952），三日熱11例（神田，1955；日野，1955；阿部，花輪，1956；若林，上原，1956；古寺，1956；宮本，1952；矢野ら，1985），四日熱1例（花田ら，1966），種不明4例（大鶴，加茂，1954；土屋，1957）の報告がある。即ち，今般報告する症例は日本における輸血マラリアの第75例目，熱帯熱マラリアとしては第3例目，そして初めての死亡例と考えられる。その他に輸血マラリアとは異なるが，誘発マラリアの症例としては，「覚醒剤常用者の間に特発した不潔な注射器による人工接種例」9例を大鶴，加茂（1953）が詳細に報告し，森下（1959）が文献上346例（1945～1958年）をまとめている。また近年になって天野ら（1976）は針事故によって感染したと最も考えられる熱帯熱マラリア

Table 1 Laboratory findings

Date	April 2	April 25	May 2
TP (g/dl)	7.0	7.1	4.0
ALB (g/dl)	3.4	3.5	2.2
TTT (U)	8.7	8.5	7.9
ZTT (U)	22.9	23.3	12.0
T-Bil (mg/dl)	0.7	1.2	11.0
D-Bil (mg/dl)	0.2	0.4	5.8
GOT (IU)	69	81	7710
GPT (IU)	75	57	1425
LDH (IU)	531	553	15105
ALP (K-U)	17.2	14.3	36.5
γ-GTP (IU)	35	31	14
LAP (IU)	73	79	91
ChE (IU)	197	232	138
UN (mg/dl)	7.7	29.5	83.4
Creatinin (mg/dl)	0.8	1.3	4.7
Na (mEq/l)	135	138	145
K (mEq/l)	3.5	3.3	6.8
Cl (mEq/l)	102	102	95
Ca (mg/dl)	8.6	8.1	6.4
B-Glu (mg/dl)	93	82	367
T-cho (mg/dl)	172	149	50
TG (mg/dl)	84	130	110
CRP			6.9

症例（21歳，看護婦）を報告し，医療従事者におけるマラリアの二次感染の危険性を強く提起している。なおこの看護婦の例は，治療の甲斐なく脳性マラリアを合併し死亡している。

われわれの今回報告する症例が不幸な転帰をたどったのは，医師をしてマラリアを疑わしめるにはあまりに非定型的な例であったためであると考えられる。現在マラリアの流行のない日本において，麻薬や覚醒剤の常用歴がなく，海外渡航歴もない70歳のいわゆる普通の老女に，その発熱をみてマラリアを第一に疑うことは医師においては困難と言うよりむしろ突飛でさえある。また，マラリアの典型的な症状を示してからの進行も著しく早かった。その理由としてはまず，本患者が従来より重症マラリアに陥った際の一つの指標でさえある血小板減少症に悩まされていたことがあろう。死亡時は，出血性症状，FDP 値40μg/ml，plt 2.1万/mm^3，PT 25.2秒を示し，播種性血管内凝固症候群の基準を充分満たしていた。また，白血球の減少症も認められており，免疫学的にも何らかの不全があったことが疑われ，発熱の程度が感染初期に著しく高く示されなかったのも単なる高齢によるためだけではなかったと考えられる。さらには，慢性肝炎の素因による肝不全への移行の早さが，血清学的データや，黄疸などの症状より推察される。血清学的データからはさらに，高度の腎機能障害もうかがわれる（Table 1）。死亡直前に至っては，傾眠傾向，眼球運動障害，意識消失などの脳症状を合併した脳マラリアに陥ったと診断できる。本患者はマラリアによる急速な多臓器不全により死亡したと考えるのが最も妥当であると考えられる。

しかるに，マラリアの治療は本患者には施されなかった。5月1日に検査室が血液像検査のために作製した薄層塗抹標本中にマラリア原虫を検出するのが，翌早朝7：55の死亡時より遅れたためである。原虫種の確定診断のために群馬大学医学部寄生虫学教室に標本が送付され，本患者は熱帯熱マラリアに感染していたことが判明した（Photo. 1）。さらにわれわれの問い合わせの結果，4月2日，17日，25

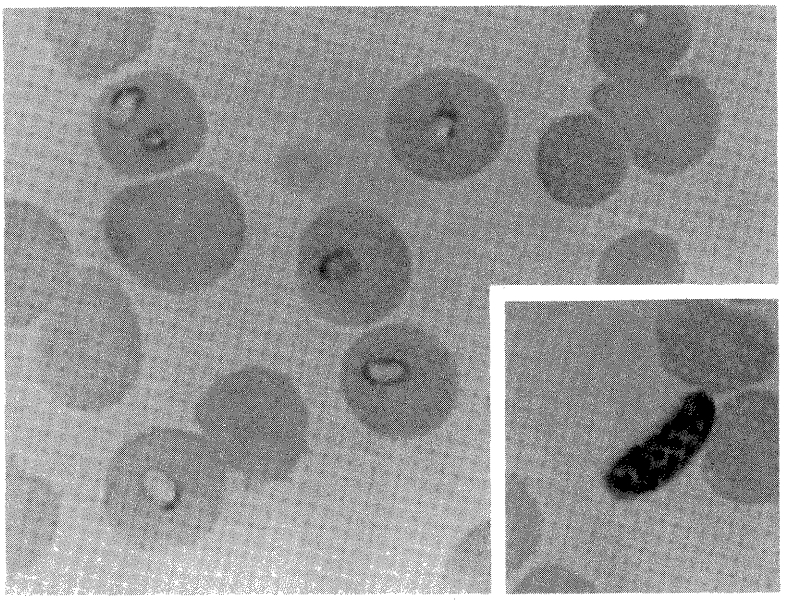

Photo. 1 Giemsa-stained blood smear of the patient showing ring forms, and a gametocyte of *Plasmodium falciparum*.

日の標本が保存されていることがわかり，それらを注意深く検鏡したところ，4月2日，17日の標本には原虫が確認されなかったが，4月25日の標本には赤血球1万個に対して6個という低い感染率の熱帯熱マラリア原虫が検出された（Figure 1）。その標本中の原虫は形態的に定型的でなく，また感染率も低いことより，一般病院の検査室におけるスクリーニング作業では診断が極めて困難であり，この標本からの虫体の検出は一般検査技師・医師に要求される能力を越えると考えられた。また，群馬大学医学部寄生虫学教室において後日行ったIFATによるマラリア抗体検査により，本患者の5月1日，2日の血清は*P.f.* titerが1：256と高値を示し，血清学的にも熱帯熱マラリアとの診断が下された（Kano *et al.*, 1990）。また4月25日の血清は，*P.f.* titer, *P.v.* titerともに陰性であった。一般に原虫血症を示してから血清中の抗体価が上昇するまでには数日かかり（Voller, Draper, 1982），4月25日は感染のごく初期であったと思料される。以上標本観察とIFATとの所見により，本患者のマラリア感染の経過は，4月4日以来一連にわたって行われた濃厚血小板の輸血によりマラリア原虫が本患者に接種された結果と考えられた。

当該血小板製剤の供給のあった血液センターの協力を得て，カルテに記載された輸血Lot番号の供血者データを調べたところ，供血者は述べ40人（男30人，女10人。日本人39人，外国人1人。年齢22～64歳）であることが判明した。しかしながら個別の感染源調査は行わなかった。血液センターでの献血時には，献血申込書（診療録）に付随して問診表が供血者に手渡され，供血者の現症，既往歴が尋ねられる。肝炎，結核などの感染症，糖尿病，腎臓病などの代謝疾患，出血傾向に関する血液疾患，さらに心臓疾患の既往に関して聞かれるが，残念なことにマラリアを個別に挙げて尋ねる項目が無い。しかし献血時の医師の問診において，マラリア既往者でその後3年以上経過していない人からは採血しないこと，またWHOが指定している流行地からの帰国者については，行き先，生活状態，期間などを詳細に尋ね，感染が疑わしい人からも3年間は採血しないことを日赤の血液センター業務基準としている。マラリア流行地に旅行する日本人や，流行地から訪日する外国人の数も増えてきている現在，今後医師の問診を強化し，さらに質問表にそれを尋ねる項目を加え，マラリアの二次感染の危険性に備えてゆく必要があるものと考えられる。日赤では輸血用製剤の安全性の確保のため，血液による感染の危険が高いB型肝炎，C型肝炎，エイズ，成人T細胞白血病，梅毒の5つの感染症について検査を実施している。献血申込書には「エイズ検査目的の献血はお断り致します。」と記してあるが，現在においては前述のような金銭目的で供血を行う者こそなくなったものの，熱帯地への渡航帰りに，いわゆる上記の性行為感染症を調べたい目的で供血を行う者も少なからずあるとされている。その中にはマラリア既往者，さらには感染ごく初期の患者も含まれてくる可能性が一般の善意による献血者に比べ高いであろうことが予測され，このような状況にあっては問診票にマラリアに関する質問項目を用意して，供血者全員に対してその質問がなされてしかるべきであろう。さらに供血前1年くらいの間にマラリア流行地への渡航経験があり，なおかつ発熱の既往がある供血者には，血液塗抹標本観察による原虫検出作業を検査に取り入れる必要があろう。また前述のIFATによるマラリアの抗体検査も，マラリアの既往を検査するための確定的な診断法となる（Kano *et al.*, 1990）。

血小板輸血によって誘発されたマラリアの記載は，Center for Disease Control (CDC), US Department of Health, Education and Welfare, Public Health Service (1978)の年次報告に，急性白血病の女性に血小板製剤の投与を行って感染したと考えられる熱帯熱マラリア症例がある。また本邦の伊藤ら（1985）の症例では，濃厚赤血球と同時に血小板製剤の輸血を行っており，その可能性が著者らによって論じられている。Fajardo, Tallent（1974）は三日熱マラリア患者の血液中の血小板にマラリア原虫が能動的に進入し，そこで生存していると考えられる像を電子顕微鏡で観察しており，血小板自体によってもマラリアの誘発が起こる可能性を強く示唆している（Perkash *et al.*, 1984）。また，血小板製剤に混入した原虫感染赤血球によってマラリアが誘発される可能性も否定できない。濃厚血小板製剤への赤血球の混入率は上限2万個/m*l*との基準があるが，本患者に投与された血液製剤の供給元である血液センターにおいては，その数は1000個/m*l*以下程度との結果を得ている。成分輸血の血小板製剤1単位20m*l*で，マラリア原虫寄生赤血球率が1万個の赤血球に1個程度であるとすれば，一人の供血者からの血液材料中には2個の感染赤血球が混入する可能性があることになる。よって顕微鏡観察によって原虫血症が検出できるマラリア患者からの血液供給であれば，たとえ血小板製剤であっても混入した赤血球によってマラリアが誘発されうる。本症例は血小板輸血で誘発されたと考えられるマラリアの日本国内第一例であるが，上記のいずれの機作によって感染が成立したかは断言できない。

大鶴（1958）は，わが国において熱帯熱マラリアの国内感染が終戦直後にあっても非常に少なかった理由について，日本国内に分布する最も有力なマラリア伝搬者はシナハマダラカであって，主に三日熱マラリア伝搬性が強いこと，そして熱帯熱マラリアの伝搬に関与するであろうオオツルハマダラカの密度は非常に低いことをあげている。本患者は千葉県船橋市に在住していたが，居住地付近は一般住宅地であって，蚊の密度を上げる可能性のある家畜等を飼育する農家も見あたらない。また成田国際空港からの直線距離も40kmあり，万一マラリア感染蚊が外国から運び込まれたとしても，その飛翔距離内には入っていないと考えられ，本患者が蚊によって媒介されて自然感染を起こしたマラリアであった可能性は無いものと考えられる。しかしながら国内において土着マラリアは消退し，導入マラリアの可能性もほとんど無いとは言え，大鶴（1952）が終戦直後に「何時の日か大陸，南方方面に交を求めることが出来るようになれば，必ずやマラリアの問題は日にそう影のよう

につきまとうことになるであろう」と予言したとおり，現在においては輸入マラリアがその存在を無視できないほど数を増やしており，それに伴ってもたらされるであろうあらゆる危険性に対して，医療従事者においては特に注意を払わなければならない。本報告ではその輸入マラリア患者から血小板の供血を受けて感染したと考えられ，しかも不幸な転機をとった熱帯熱マラリアの症例を紹介したが，最後に輸血マラリア国内初報告において酒井（1935）が記載し，現在においても繰り返し強調されるべき文章を引用し，本報告を締めくくる。「輸血時給血者の撰擇に當って，既往症に'マラリア'の有無を確かめる必要がある。尚給血者の健康狀態を吟味する事も大切である。」

結　　語

血小板減少症の治療のために濃厚血小板を60単位投与され，それによって熱帯熱マラリアを誘発し，脳マラリアおよび多臓器不全を合併し死亡した70歳の日本人女性の症例を報告した。わが国における輸血マラリアの文献的考察を含め，今後の血液製剤の安全性確保に関しての検討を行った。

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A CASE OF ACCIDENTAL TRANSMISSION OF *PLASMODIUM FALCIPARUM* THROUGH PLATELET TRANSFUSION

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[Case] The patient was a Japanese female, 70 years of age, living in Funabashi, Chiba, and had never been abroad. The woman was suffering from thrombocytopenia and received a total of 60 units of platelet concentrate during the period, 4-13 April, 1991. On April 24, she manifested a fever of 39°C which was successfully treated with Voltaren Suppo™, a non-steroidal anti-inflammatory drug. Her body temperature remained normal until May 1, when she suddenly developed symptoms of cerebral malaria, liver and renal dysfunctions. In the early morning of May 2, she lost consciousness and went into cardiac arrest. Giemsa stained thin blood smears obtained for blood cytological examination which were kept at the laboratory of the hospital, later revealed that she had contracted *Plasmodium falciparum* malaria. The parasitemia from examination of the slides were 0% on April 2, 0% on April 17, 0.06% on April 25, 5.5% on May 1 and 1.9% just before her death on May 2. Antibody titers against *P.f.* antigen were <1:4 on April 25, 1:256 on May 1 and May 2. These parasitological and serological results confirmed that she accidentally received transmission of *P. f.* through platelet transfusion.

The possibility of transmission of malaria through platelet transfusion has been discussed by Fajardo and Tallent (1974), who showed the existence of the parasite in a platelet of a *P.v.* infected patient by electron microscopy. Nevertheless, since platelet packs may contain some red blood cells, parasites could be carried by erythrocytes, platelets or both. Still, this report is the first case of induced malaria resulting from platelet transfusion, and the 75th case of transfused malaria in Japan since 1935.

As the number of Japanese who went abroad and of foreigners who entered Japan increased, so did the number of imported malaria cases, which were reported to be not less than 100 in 1993. Therefore malaria is no longer recognized as a very rare disease in Japan, and the risk of transfused malaria from donors who have come back from malaria endemic places has to be taken into careful consideration. To date, routine examinations on blood supplied at Blood Centers are for hepatitis B and C, syphilis, adult T cell leukemia, and AIDS, but not for malaria. The questionnaire which is used in obtaining the medical history of donors rarely includes inquiries on a past history of malaria. Special attention has to be made for the safety of blood transfusion, reminding the danger of malaria transmission.

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COMPARISON OF POPULATION OF VECTOR MOSQUITOES OF *DIROFILARIA IMMITIS* AND THEIR NATURAL INFECTION RATES IN SOUTHERN AND NORTHERN PARTS OF NAGASAKI CITY, JAPAN

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Abstract: Female mosquitoes including *Culex pipiens pallens*, the main vector of *Dirofilaria immitis*, and some secondary vectors, were collected at 4 to 10 sites in Nagasaki City from 1983 to 1989 using light traps, and the number of the mosquitoes and the natural infection rate with *D. immitis* were compared between the southern and the northern parts of the city. In 1983, *Cx. p. pallens* was extremely prevalent in the southern part but not in the northern part. After 1986, the prevalence of *Cx. p. pallens* became very low in both the parts. The rapid decrease of prevalence of this species may be attributable to the decrease in breeding sites by improvement of roads and open roadside ditches in parallel with spread of sewage systems. On the basis of the number of infected mosquitoes in Nagasaki City, it was suggested that, *Cx. tritaeniorhynchus* and *Ae. albopictus* are important vectors in addition to *Cx. p. pallens*, but *Ae. togoi* does not play a significant role in the transmission of *D. immitis*.

INTRODUCTION

We previously reported that the percentage of house dogs having the larvae of *Dirofilaria immitis* increased from 1968 to 1983 in the eastern, the western and the southern parts of Nagasaki City, but this percentage decreased during the same period in the northern part of the city (Oda *et al.*, 1993). We also assumed that the decrease of the infection rate of house dogs in the northern part be due to the spread of public sewage systems contributing to the reduction of breeding sites of the main vector mosquito, *Culex pipiens pallens*. To test the validity of this assumption, we compared the number and the natural infection rate of *Cx. p. pallens*, caught by using light traps, between the southern and the northern parts of this city during the period from 1983 to 1989, and analyzed the data in relation to the development of public sewage systems. Furthermore, we attempted to clarify the role of some secondary vector mosquitoes in the transmission by comparing the population densities and the natural infection rates.

PLACES AND METHODS

Between 1983 and 1989, we collected mosquitoes including *Cx. p. pallens*, which is the main vector mos quito, and three secondary vectors (*Cx. tritaeniorhynchus, Ae. albopictus* and *Ae. togoi*), using light traps (20 watt black light), in the southern part of Nagasaki City where the microfilaria positive rate was high and in the northern part where the positive rate was low. In 1983, we placed a trap in each of two southern districts

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Table 1 Period and place of mosquito collection in Nagasaki City

Date and (times) of collection	Southern part			Northern part		
	Trap. No.	District No. and name	Place of collection	Trap. No.	District No. and name	Place of collection
1983 Jul. 28-Oct. 21 (13)	S 1	28 Fukahori	Building	N 1	39 Nishiurakami	Building
	S 2	27 Doinokubi	Building	N 2	34 Sakamoto	Building
1986 Aug. 18-Sep. 27 (24)	S 3	25 Tomachi	Building	N 3	35 Takao	Building
	S 4	25 Tomachi	Building	N 4	35 Takao	Building
1987 Jul. 1-Sep. 1 (24)	S 5	25 Tomachi	House	N 5	36 Yamazato	House
1988 Jun. 10-Aug. 11 (27)	S 6	25 Tomachi	House	N 6	34 Sakamoto	House
	S 7	25 Tomachi	House	N 7	34 Sakamoto	House
1989 Jun. 5-Aug. 10 (20)	S 8	25 Tomachi	House	N 8	34 Sakamoto	House
	S 9	25 Tomachi	House	N 9	34 Sakamoto	House

S and N show southern and northern parts, respectively.

(Doinokubi and Fukahori) and two northern districts (Nishiurakami and Sakamoto). These four light traps were, as a rule, lit for operation twice weekly. In 1986, we placed 2 traps in a southern district (Tomachi) and 2 in a northern district (Takao), and collected mosquitoes three or four times weekly. In 1987 through 1989, we placed a trap in 5 houses which had microfilaria -positive dogs in each of the southern and the northern parts of the city. These light traps were lit three or four times weekly. Table 1 shows the survey sites and periods. Fig. 1 shows the locations where light traps were placed. The names and lot numbers for Nagasaki City districts were derived from the residence list of the parts used by Suenaga *et al.* (1971). The mosquitoes collected were identified and kept at −12°C. Under a stereomicroscope, female mosquitoes were dissected in physiological saline and examined for larvae of *D. immitis*. The developmental stages of larvae were decided using the classification of Suenaga (1972).

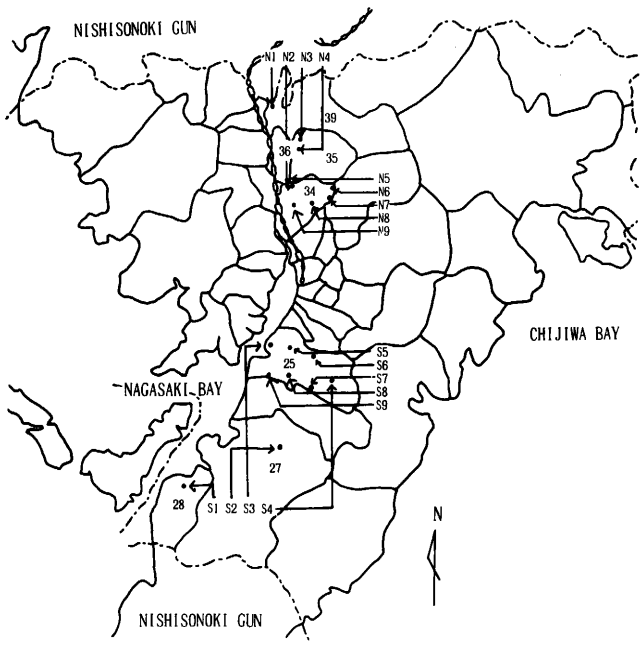

Figure 1 A map* showing district No. and place of light traps for collection of vector mosquitoes in sourthern and northern parts of Nagasaki City (see Table 1 for district No. and trap No.).

*Cited from Suenaga *et al.*, 1971.

RESULTS

I. Population and natural infection rate of the main vector mosquito, *Cx. p. pallens*, in the southern and the northern parts

1. Number of *Cx. p. pallens*

Table 2 shows the monthly numbers of female *Cx. p. pallens* in the southern and the northern parts in 1983. In two southern districts with considerably high microfilaria positive rate in dogs where Trap S1 and S2 were operated, larger numbers of mosquitoes were caught in July through October, compared with two northern districts with low positive rate where Trap N1 and N2 were operated. A comparison of the mean numbers revealed that the number of *Cx. p. pallens* caught in the southern part was about three times larger than that in the northern part.

Table 3 shows the data obtained in 1986 in Tomachi (a southern district with high microfilaria positive rate) and in Takao (a northern district with a little high

Table 2 Number of *Culex pipiens pallens* females collected by a light trap in southern and northern parts in Nagasaki City, 1983

Month and (times) of collection	No. of females collected in the indicated trap* in southern part						No. of females collected in the indicated trap* in northern part					
	S 1*		S 2*		Total		N 1*		N 2*		Total	
	No.	(Mean)	No.	(Mean)	No.	(Mean**)	No.	(Mean)	No.	(Mean)	No.	(Mean**)
Jul. (2)	7	(3.5)	5	(2.5)	12	(3.0)	2	(1.0)	5	(2.5)	7	(1.75)
Aug. (5)	41	(8.2)	34	(6.8)	75	(7.5)	4	(0.8)	12	(2.4)	16	(1.6)
Sep. (4)	8	(2.0)	4	(1.0)	12	(1.5)	2	(0.5)	7	(1.75)	9	(1.13)
Oct. (2)	11	(5.5)	2	(1.0)	13	(3.25)	2	(1.0)	3	(1.5)	5	(1.25)
Total (13)	67	(5.15)	45	(3.46)	112	(4.31)	10	(0.77)	27	(2.08)	37	(1.43)

* Trap No. is shown in Table 1.
**Mean number of females indicates mean per trap per night.

Table 3 Number of *Culex pipiens pallens* females collected by a light trap in southern and northern parts in Nagasaki City, 1986

Month and (times) of collection	No. of females collected in the indicated trap* in southern part						No. of females collected in the indicated trap* in northern part					
	S 3*		S 4*		Total		N 3*		N 4*		Total	
	No.	(Mean)	No.	(Mean)	No.	(Mean**)	No.	(Mean)	No.	(Mean)	No.	(Mean**)
Aug. (2)	1	(0.5)	0	(0.0)	1	(0.25)	0	(0.0)	0	(0.0)	0	(0.0)
Sep. (4)	2	(0.5)	0	(0.0)	2	(0.25)	1	(0.25)	1	(0.25)	2	(0.25)
Total (6)	3	(0.5)	0	(0.0)	3	(0.25)	1	(0.17)	1	(0.17)	2	(0.17)

* Trap No. is shown in Table 1.
**Mean number of females indicates mean per trap per night.

microfilaria positive rate). In contrast to the data in 1983, only a few *Cx. p. pallens* were caught in the both districts in 1986 (Table 3). Then we placed a light trap in 5 households keeping dogs positive for microfilariae in each of three districts (Tomachi in the southern part, and Sakamoto and Yamazato in the northern part), and examined the number of *Cx. p. pallens* caught during the three-year period from 1987 to 1989. As shown in Table

Table 4 Number of *Culex pipiens pallens* females collected by a light trap in southern and northern parts in Nagasaki City, 1987-1989

Year and (time) of collection	No. of females collected in the indicated trap* in southern part											
	S 5*		S 6*		S 7*		S 8*		S 9*		Total	
	No.	(Mean)	No.	(Mean)	No.	(Mean)	No.	(Mean)	No.	(Mean)	No.	(Mean**)
1987 (24)	0	(0.0)	5	(0.21)	0	(0.0)	2	(0.08)	4	(0.17)	11	(0.09)
1988 (27)	3	(0.11)	3	(0.11)	1	(0.04)	0	(0.0)	0	(0.0)	7	(0.05)
1989 (20)	1	(0.05)	1	(0.05)	2	(0.1)	1	(0.05)	2	(0.1)	7	(0.07)
Year and (time) of collection	No. of females collected in the indicated trap* in northern part											
	N 5*		N 6*		N 7*		N 8*		N 9*		Total	
	No.	(Mean)	No.	(Mean)	No.	(Mean)	No.	(Mean)	No.	(Mean)	No.	(Mean**)
1987 (24)	0	(0.0)	6	(0.25)	12	(0.5)	7	(0.29)	1	(0.04)	26	(0.22)
1988 (27)	0	(0.0)	2	(0.07)	5	(0.19)	3	(0.11)	0	(0.0)	10	(0.07)
1989 (20)	0	(0.0)	2	(0.1)	3	(0.15)	1	(0.05)	4	(0.2)	10	(0.10)

* Trap No. is shown in Table 1.
**Mean number of females indicates mean per trap per night.

Table 5-1 Annùal changes in rate of population using a sewage system in southern and northern parts in Nagasaki City, 1983-1986

Part	District	Year											
		1983			1984			1985			1986		
	No. Name	Total population (A)	Population using SS* (B)	RPSS** (B/A×100)	Total population (A)	Population using SS* (B)	RPSS** (B/A×100)	Total population (A)	Population using SS* (B)	RPSS** (B/A×100)	Total population (A)	Population using SS* (B)	RPSS** (B/A×100)
Southern	20 Sako	6,934	4,802	69.3	6,781	5,035	74.3	6,877	5,705	83.0	6,912	5,900	85.4
	21 Nita	5,144	1,410	27.4	5,022	1,671	33.3	5,052	2,552	50.5	5,138	3,218	62.6
	22 Kitaohura	12,548	1,379	11.0	12,410	1,503	12.1	12,185	1,751	14.4	11,927	2,059	17.3
	23 Minamiohura	6,999	0	0.0	6,848	0	0.0	6,698	0	0.0	6,598	0	0.0
	24 Naminohira	3,531	0	0.0	3,415	0	0.0	3,318	0	0.0	3,122	0	0.0
	25 Tomachi	15,496	0	0.0	15,408	0	0.0	15,459	4,719	30.5	15,490	5,190	33.5
	26 Kogakura	5,715	0	0.0	5,609	0	0.0	6,167	4,805	77.9	6,869	5,335	77.7
	27 Doinokubi	15,034	0	0.0	15,289	0	0.0	15,382	0	0.0	15,329	0	0.0
	28 Fukahori	11,275	0	0.0	11,452	0	0.0	11,241	0	0.0	10,920	0	0.0
	29 Minami	993	0	0.0	988	0	0.0	986	0	0.0	990	0	0.0
	30 Mogi	7,504	1,490	19.9	7,515	1,410	18.8	7,615	1,402	18.4	7,787	1,330	17.1
	31 Hayasaka	5,846	0	0.0	5,832	0	0.0	5,908	0	0.0	5,821	199	3.4
	32 Hiyoshi	1,615	0	0.0	1,612	0	0.0	1,575	0	0.0	1,565	0	0.0
	Total	98,634	9,081	9.2	98,181	9,619	9.8	98,463	20,934	21.3	98,468	23,231	23.6
Northern	33 Zenza	6,449	4,869	75.5	6,413	4,932	76.9	6,236	5,035	80.7	6,194	5,242	84.6
	34 Sakamoto	10,874	6,621	60.9	10,848	6,616	61.0	10,724	6,767	63.1	10,737	7,271	67.7
	35 Takao	18,620	0	0.0	18,984	0	0.0	18,860	0	0.0	18,694	0	0.0
	36 Yamazato	11,754	1,820	15.5	11,438	1,752	15.3	11,531	1,799	15.6	11,650	1,748	15.0
	37 Nishishiroyama	13,352	0	0.0	13,665	0	0.0	13,982	0	0.0	14,126	0	0.0
	38 Shiroyama	6,153	0	0.0	6,132	0	0.0	6,001	0	0.0	6,030	0	0.0
	39 Nishiurakami	23,790	3,063	12.9	19,744	995	5.0	19,649	964	4.9	19,590	1,064	5.4
	40 Nameshi	39,120	38,622	98.7	39,336	38,584	98.1	39,663	40,474	102.0	39,758	40,974	103.1
	41 Nishikita	12,179	404	3.3	12,108	564	4.7	12,222	551	4.5	12,386	551	4.4
	42 Nishimachi	17,228	0	0.0	16,973	0	0.0	16,841	0	0.0	16,878	0	0.0
	43 Kawabira	6,926	0	0.0	4,478	0	0.0	4,401	0	0.0	4,392	0	0.0
	Total	166,445	55,399	33.3	160,119	53,443	33.4	160,110	55,590	34.7	160,435	56,850	35.4

* SS: sewage system
**RPSS: the rate of population utilizing a sewage system.

4, the number of *Cx. p. pallens* caught was again very small in each district.

2. Relationship between *Cx. p. pallens* population and development of sewage system

The sharp decrease in number of *Cx. p. pallens* in the southern and the northern parts of Nagasaki City during the 1986-1989 period seems to be associated with the improvement of public sewage systems. Tables 5-1 and 5-2 show annual changes in the rate of human population utilizing sewage systems (RPSS) in the total population of the southern and the northern parts. The RPSS values were not so high in Nagasaki City as those in other cities in Japan, owing to a characteristic topography with a lot of steep slopes, which made difficult to establish the sewage system. In 1983 and 1984, only 4 of the 13 southern districts had sewage systems. After 1985, sewage systems were found in about half of all southern districts. The RPSS for the southern part was low in 1983 (9.2%) and 1984 (9.8%). It was doubled in 1985 (21.3%) and further rose to 37.0% in 1989. In the northern part, sewage systems were already present in 1983 and 1984 in 6 of the 11 districts. The RPSS was about 33.0% in 1983 and 1984, which was much higher than the rate for the southern part. It is therefore evident that sewage systems had been better developed in the northern part than in the southern part of this city around 1983.

Fig. 2 shows the relationship between the RPSS and the number of *Cx. p. pallens* caught in individual districts. The values of RPSS used in this figure were derived from Tables 5-1 and 5-2. The RPSS value in 1983 for the southern part is the average for Fukahori and Doinokubi, and the value for the northern part is the average for Nishiurakami and Sakamoto. Because Takao, Sakamoto and Yamazato were located close to each other, the RPSS values in 1984 through 1989 for these three districts in the northern part were averaged.

Table 5-2 Annual changes in rate of population using a sewage system in southern and northern parts in Nagasaki City, 1987-1989

Part	District No.	Name	Year 1987 Total population (A)	1987 Population using SS* (B)	1987 RPSS** (B/A×100)	1988 Total population (A)	1988 Population using SS* (B)	1988 RPSS** (B/A×100)	1989 Total population (A)	1989 Population using SS* (B)	1989 RPSS** (B/A×100)
Southern	20	Sako	6,744	5,959	88.4	6,554	6,217	94.9	6,352	5,940	93.5
	21	Nita	5,330	4,097	76.9	5,216	428	8.2	5,071	4,313	85.1
	22	Kitaohura	11,753	2,354	20.0	11,392	2,809	24.7	11,137	2,848	25.6
	23	Minamiohura	6,408	0	0.0	6,361	0	0.0	6,213	0	0.0
	24	Naminohira	3,110	0	0.0	3,036	0	0.0	2,979	0	0.0
	25	Tomachi	15,206	6,085	40.0	14,852	6,577	44.3	14,670	7,855	53.5
	26	Kogakura	7,508	5,918	78.8	8,203	6,349	77.4	8,471	6,825	80.6
	27	Doinokubi	16,064	550	3.4	16,480	1,277	7.8	16,902	1,737	10.3
	28	Fukahori	10,603	0	0.0	10,353	0	0.0	10,007	0	0.0
	29	Minami	978	0	0.0	957	0	0.0	950	0	0.0
	30	Mogi	7,997	1,311	16.4	8,083	1,307	16.2	8,239	998	12.1
	31	Hayasaka	5,803	567	9.8	5,831	1,014	17.4	5,679	1,844	32.5
	32	Hiyoshi	1,550	0	0.0	1,544	0	0.0	1,549	0	0.0
		Total	99,054	26,841	27.1	98,862	29,818	30.2	98,219	32,360	33.0
Northern	33	Zenza	6,045	5,206	86.1	5,984	5,176	86.5	5,757	5,029	87.4
	34	Sakamoto	10,679	7,260	68.0	10,390	7,105	68.4	10,161	6,868	67.6
	35	Takao	18,396	0	0.0	18,424	228	1.2	18,054	839	4.6
	36	Yamazato	12,005	1,760	14.7	11,715	2,727	23.3	11,428	3,283	28.7
	37	Nishishiroyama	11,594	0	0.0	11,358	0	0.0	11,020	0	0.0
	38	Shiroyama	5,869	0	0.0	5,813	0	0.0	5,775	0	0.0
	39	Nishiurakami	19,647	1,116	5.7	19,466	1,291	6.6	19,437	1,337	6.9
	40	Nameshi	40,188	41,493	103.2	30,088	40,985	102.2	39,240	39,240	100.0
	41	Nishikita	12,246	583	4.8	12,205	600	4.9	12,008	609	5.1
	42	Nishimachi	16,826	0	0.0	16,468	0	0.0	16,374	0	0.0
	43	Kawabira	4,340	0	0.0	4,249	0	0.0	4,151	0	0.0
		Total	57,835	57,418	36.4	166,160	58,112	37.2	153,405	57,205	37.3

* SS: sewage system
**RPSS: the rate of population utilizing a sewage system.

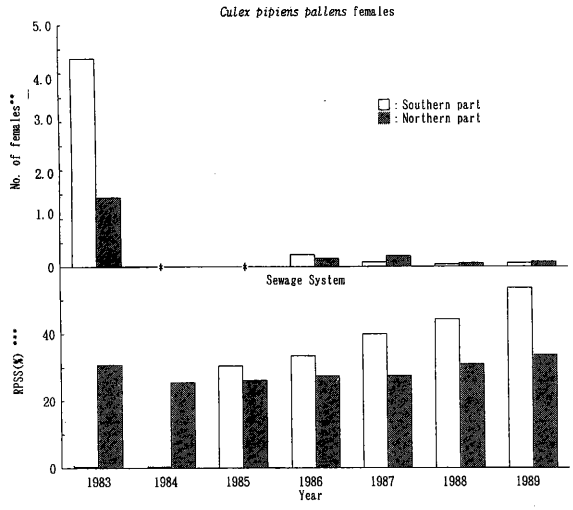

Figure 2 Annual changes in number of *Culex pipiens pallens* females and rate of population using a sewage system in southern and northern parts in Nagasaki City, 1983-1989 (see Tables 1 to 5-2).

* Collection was not made.
** Mean number of females/trap/night.
***RPSS shows the rate of population utilizing a sewage system.

The number of *Cx. p. pallens* was expressed by the mean per trap per night. As seen in Fig. 2, the southern part had a high density of *Cx. p. pallens* and inadequate public sewage systems in 1983, but the sewage systems became to be spread widely from 1986, resulting in a sharp decrease in *Cx. p. pallens*. Fig. 2 also indicates that the northern part had less *Cx. p. pallens* and more extensive public sewage systems already around 1983, compared with the southern part. Such sharp decrease of this mosquito in 1986 on seems to be due to a decrease in the breeding sites, by improvement of roads and open roadside ditches in pararell with the spread of sewage system.

3. Natural infection rates

Table 6 shows the natural infection rate in *Cx. p. pallens*. The percentage of larvae-having mosquitoes in 1983 was zero in the northern part, but it was 0.9% in the southern part. Similar results were also obtained in 1986 and 1988. These results suggest that *Cx. p. pallens* were more abundant and the natural infection rate was higher

Table 6 Number and percentage of *Culex pipiens pallens* females with *D. immitis* larvae in southern and northern parts in Nagasaki City, 1983-1989

Year	Southern part		Northern part	
	No. females dissected (A)	No. (B) and (%)* of females with larvae	No. females dissected (A)	No. (B) and (%)* of females with larvae
1983	112	1**(0.9)	37	0 (0.0)
1986	3	1**(33.3)	2	0 (0.0)
1987	11	0 (0.0)	26	2**(7.7)
1988	7	1**(14.3)	10	0 (0.0)
1989	7	0 (0.0)	10	0 (0.0)

* Natural infection rate (B/A×100)
**1st stage larvae

in the southern part than in the northern part.

II. Population and natural infection rates of three secondary vector mosquitoes in the southern and the northern parts

For the 1983-1989 period, we analyzed the number (the mean per trap per night) of *Cx. tritaeniorhynchus*, *Ae. albopictus* and *Ae. togoi* and their natural infection rate (the percentage of *D. immitis* larvae-carrying mosquitoes) in the southern and the northern parts of Nagasaki City.

Table 7 shows the annual number of *Cx. tritaeniorhynchus* for the southern and the northern parts. There was no significant difference in the annual number of this mosquito between the southern and the northern parts. The natural infection rate for this species also did not differ significantly between the two parts of the city.

The number of *Ae. albopictus* did not differ significantly in any year between the southern and the northern parts (Table 8). The natural infection rate for this species tended to be little different between the northern and the southern parts.

The number and the infection rate of *Ae. togoi* are shown in Table 9. Any *Ae. togoi* were not caught in the northern part, and the number was very small in the southern part. Only one female was found to be infected in the southern part.

Table 7 Number of *Culex tritaeniorhynchus* females collected in light traps and natural infection rate in southern and northern parts in Nagasaki City, 1983-1989

Year and (times) of collection	Southern part			Northern part		
	Total No. females (A) collected and dissected	Mean No.*	Females with larvae No. (B) (%)***	Total No. females (A) collected and dissected	Mean No.*	Females with larvae** No. (B) (%)***
1983 (13)	115	4.43	1** (0.9)	200	7.69	1** (0.5)
1986 (6)	19	1.59	0 (0.0)	26	2.17	0 (0.0)
1987 (24)	300	2.50	14** (4.7)	150	1.25	11** (7.3)
1988 (27)	593	4.39	2** (0.3)	695	5.15	6** (0.9)
1989 (20)	152	1.52	0 (0.0)	159	1.59	0 (0.0)

* Mean number of females/trap/night.
** 1st stage larvae
***Natural infection rate (B/A×100)

Table 8 Number of *Aedes albopictus* females collected in light traps and natural infection rate in southern and northern parts in Nagasaki City, 1983-1989

Year and (times) of collection	Southern part			Northern part		
	Total No. females (A) collected and dissected	Mean No.*	Females with larvae No. (B) (%)***	Total No. females (A) collected and dissected	Mean No.*	Females with larvae No. (B) (%)***
1983 (13)	9	0.35	1** (11.1)	10	0.39	0 (0.0)
1986 (6)	1	0.09	0 (0.0)	4	0.34	0 (0.0)
1987 (24)	25	0.21	1** (4.0)	51	0.43	1** (2.0)
1988 (27)	63	0.47	0 (0.0)	39	0.29	3** (7.7)
1989 (20)	27	0.27	0 (0.0)	16	0.16	5** (31.3)

* Mean number of females/trap/night.
** 1st stage larvae
***Natural infection rate (B/A×100)

Table 9 Number of *Aedes togoi* females collected in light traps and natural infection rate in southern and northern parts in Nagasaki City, 1983-1989

Year and (times) of collection	Southern part			Northern part		
	Total No. females (A) collected and dissected	Mean No.*	Females with larvae No. (B) (%)***	Total No. females (A) collected and dissected	Mean No.*	Females with larvae No. (B) (%)***
1983 (13)	4	0.16	1** (25.0)	0	—	—
1986 (6)	1	0.09	0 (0.0)	0	—	—
1987 (24)	2	0.02	0 (0.0)	0	—	—
1988 (27)	3	0.02	0 (0.0)	0	—	—
1989 (20)	2	0.02	0 (0.0)	0	—	—

* Mean number of females/trap/night.
** 1st stage larvae
***Natural infection rate (B/A×100)

Table 10 Annual changes in mean number of 3 species of vector mosquitoes and natural infection rate in Nagasaki City, 1983-1989

Year	*Cx. p. pallens*			*Cx. tritaeniorhynchus*			*Ae. albopictus*		
	Mean No.* (A)	Natural Inf.** (B)	Infected No. (A×B)	Mean No.* (A)	Natural Inf.** (B)	Infected No. (A×B)	Mean No.* (A)	Natural Inf.** (B)	Infected No. (A×B)
1983	2.87	0.45	1.2915	6.06	0.70	4.2420	0.37	5.55	2.0535
1986	0.21	16.65	3.4965	1.88	0.00	0.0000	0.22	0.00	0.0000
1987	0.16	3.85	0.6160	1.88	6.00	11.2800	0.32	3.00	0.9600
1988	0.06	7.15	0.4290	4.77	0.60	2.8620	0.38	3.85	1.4630
1989	0.09	0.00	0.0000	1.56	0.00	0.0000	0.22	15.65	3.4430

* Mean number of females/trap/night.
**Natural Inf. (Natural infection rate in %)

III. Comparison of the role in the transmission among *Cx. p. pallens, Cx. tritaeniorhynchus* and *Ae. albopictus*

From the number and the natural infection rate of female mosquitoes of three dominant species collected to date, the number of infected females was determined annually as shown in Table 10. Generally speaking, the natural infection rate of *Cx. tritaeniorhynchus* was lower and the number of the females was much greater than that of *Cx. p. pallens*, but the number of infected females was about as same as that in *Cx. p. pallens*. On the other hand, the number of *Ae. albopictus*, the natural infection rate and the number of infected females all were similar to that in *Cx. p. pallens*.

DISCUSSION

Before 1980, the number of *Cx. p. pallens* caught in Tomachi (a southern district) was considerably large (Oda *et al.*, 1993). From 1986 on, however, the number has been almost zero. This change in Tomachi may be attributable to a decrease in the sites where the mosquitoes can breed, following environmental changes, i. e., construction of large-scale apartments after about 1982, completion of southern Nagasaki sewage disposal plants in 1984, establishment of a sewage system for Tomachi in 1985 (Tables 5-1 and 5-2), and accompanying changes in roads and open roadside ditches. The number of *Cx. p. pallens* in Nagasaki City will further decrease as the current plan to expand sewage systems is put into practice.

Suenaga *et al.* (1973) suggested the principal role of *Cx. p. pallens* in the transmission of *D. immitis* in Nagasaki City, on the grounds that the number was larger and the natural infection rate was higher in *Cx. p. pallens* than in *Cx. tritaeniorhynchus* and *Ae. albopictus*. In the present study, the infection rate of *Cx. tritaeniorhynchus* was lower but the number was larger than that of *Cx. p. pallens*, resulting in similar numbers of infected mosquitoes in the both species. This may indicate that the relative importance of *Cx. tritaeniorhynchus* in the transmission has recently become a little higher in Nagasaki City as Konishi (1989) reported that this mosquito is important in transmission of *D. immitis* in Kobe. Although we can not estimate exactly the population size of *Ae. albopictus* by a light trap because they are active in daytime, our data showed that the number

of infected females of *Ae. albopictus* was not very different from that of *Cx. p. pallens*. Therefore the ability of transmission of *Ae. albopictus* seems to be quite high in the city. *Ae. togoi* was caught only in the southern part and its number was very small, but the natural infection rate was high. Keegan (1967) was the first to report *D. immitis*-carrying *Ae. togoi*. Prior to the present paper, however, no survey of *D. immitis*-carrying *Ae. togoi* in Nagasaki City has been published. Since the number of *Ae. togoi* caught was very small, this seems to have little relationship with the transmission of *D. immitis*, as suggested by Suenaga *et al.* (1973).

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A NEW BLACKFLY SPECIES OF *SIMULIUM* (*MOROPS*) FROM SOLOMON ISLANDS, SOUTH PACIFIC (DIPTERA: SIMULIIDAE)

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Abstract: A new blackfly species, *Simulium* (*Morops*) *kerei* sp. nov. is described based on male and pupal specimens collected from the Solomon Islands, South Pacific. This species is placed in the *farciminis*-group by the pupal gill consisting of 1 inflated, horn-like filament and 4 slender filaments. This species is unique in possessing the parameral hooks in the male genitalia which are absent in all the Papua New Guinean *Morops* species. From the known species of the *farciminis*-group, this species is easily distinguished by the absence of the distinct hair crest on the male fore basitarsus, and in the pupa by the plate-like terminal hooks, all terga well-sclerotized, and the normal, wall-pocket-shaped cocoon covering whole of the thorax and abdomen. This is the first record of the *farciminis*-group of the subgenus *Morops* from the Solomon Islands.

In the Solomon Islands, South Pacific, Stone and Maffi (1971) reported two species of the subgenus *Morops* Enderlein (i. e., *Simulium* (*M.*) *sherwoodi* Stone and Maffi, and *S.* (*M.*) sp. nr. *avilae* Smart and Clifford) from Guadalcanal Island. In 1992 and 1993 one of us (H. S.) collected larvae, pupae and adults of blackflies in the Solomon Islands. One new species belonging to the subgenus *Gomphostilbia* Enderlein was already described (Takaoka, 1994). In this paper we describe one new species which is placed in the *farciminis*-group of the subgenus *Morops*, defined by Crosskey (1967). The uniqueness of this species within the species-group, as well as within the subgenus is indicated.

DESCRIPTION

Simulium (*Morops*) *kerei* sp. nov.

Male. Body length ca. 3.0 mm. *Head*. Width somewhat wider than thorax. Upper eye consisting of large facets in 12 horizontal rows and in 11 vertical columns. Clypeus black, densely covered with hairs (except sparsely in the center). Antenna composed of 2 + 9 segments; 1st flagellomere somewhat elongated, ca. 1.3 × as long as 2nd flagellomere. Maxillary palp composed of 5 segments with proportional length of 3rd, 4th and 5th segments 1.0: 0.9: 2.1; 3rd segment (Fig. 1) of normal size with small, elliptical sensory vesicle which is 0.22 × length of 3rd segment, and bears small, rounded opening medially. *Thorax*. Scutum brown uniformly and densely covered with yellow pubescence. Scutellum with long dark hairs and yellow pubescence. Postscutellum brown, without hairs. Pleural membrane with ca. 56 yellow pubescence. Katepisternum longer than deep, with ca. 30 dark hairs on each side. *Legs*. Fore basitarsus slightly dilated, ca. 6.0 × as long as wide, with usual dorsal hair crest. Hind basitarsus (Fig. 2) parallel-sided, much narrower than tibia. Calcipala (Fig. 2) well developed, ca. 1.4 × as long as wide and ca. 0.8 × as wide as basitarsus. *Wing*. Costa with spinules and hairs; subcosta bare; basal section of vein R fully haired; hairs at base of stem vein dark brown; basal cell absent. *Abdomen*. Basal scale dark with fringe of long yellow hairs. Abdominal segments 2-9 blackish except segment 2 pale on basal 1/2; tergite 2 broadly shiny when illuminated, and tergites 5-7 each with pair of large dorsolateral shiny areas. *Genitalia* (Figs. 3-7). Coxite rectangular in ventral view, ca. 1.5 × as long as wide. Style short, ca. 3/4 × length of coxite, curved inwards, tapered apically, with terminal spine. Ventral plate (Fig. 3) transverse, narrowed posteriorly, with posterior margin nearly straight or undulate or concave (different by angles) in ventral view, moderately setose on ventral and posterior surface, with short arms converged; ventral plate much produced ventrally as

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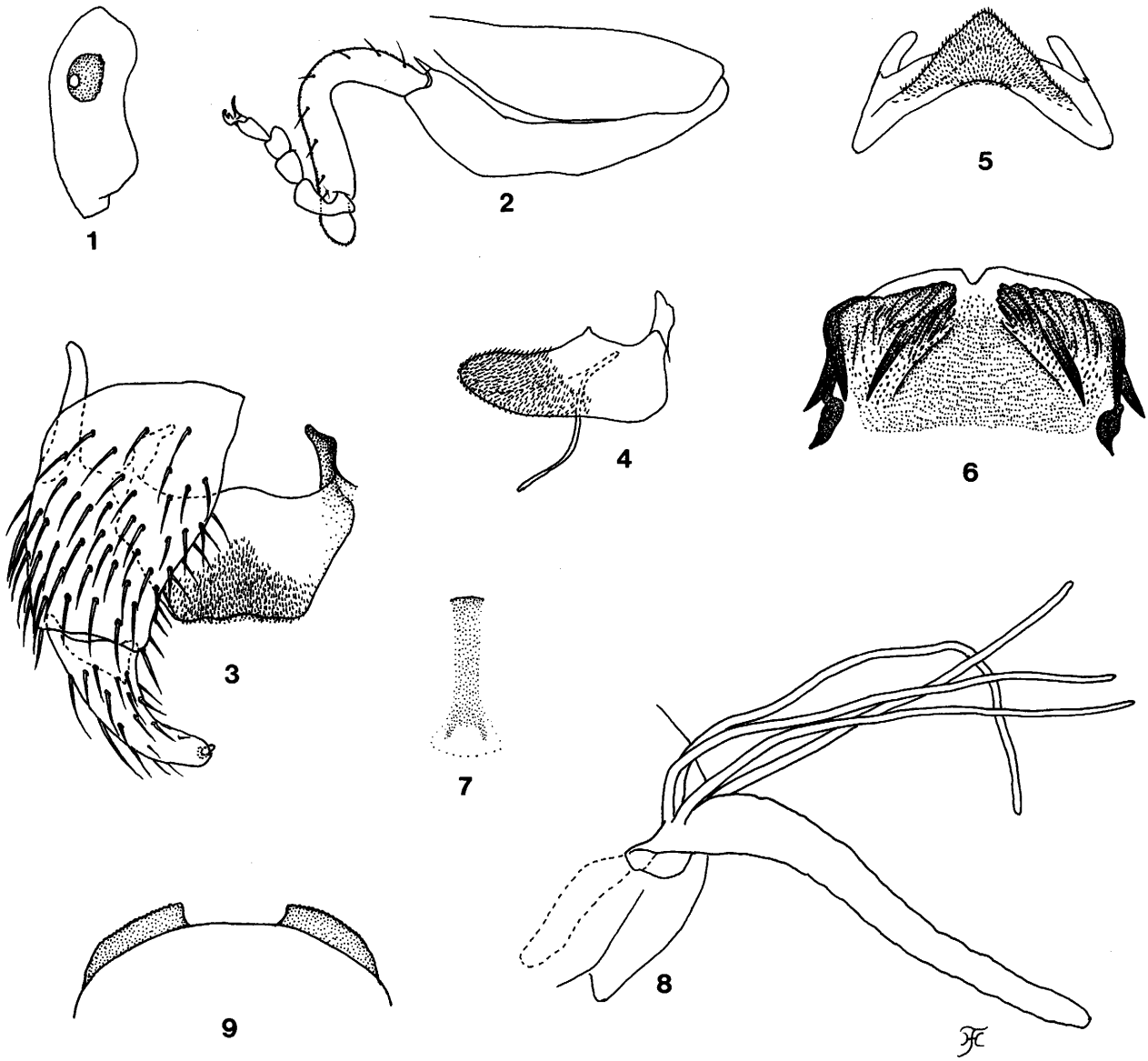

Figs. 1-9. Male and pupa of *Simulium* (*Morops*) *kerei* sp. nov. 1, 3rd segment of male maxillary palp showing sensory vesicle; 2, male hind leg (coxa and trochanter omitted); 3, coxite, style (right side only) and ventral plate in ventral view; 4, ventral plate and median sclerite in lateral view; 5, ventral plate in end view; 6, parameres showing distinct parameral hooks in posterodorsal view; 7, median sclerite; 8, pupal gill (right side only) in lateral view; 9, terminal hooks of pupal abdomen in end view.

seen in Figs. 4 & 5. Parameres (Fig. 6) each with 3 distinct parameral hooks and several undeveloped ones. Median sclerite (Fig. 7) plate-like, moderately sclerotized except distal portion transparent.

Pupa. Body length (excluding gill filaments) ca. 3.0 mm. *Head* and *thorax*. Integument brown, bare except posterior 1/2 moderately covered with small tubercles; antennal sheath of usual form. Head with 1 facial pair of long, bifid trichomes and 3 frontal pairs of long, simple trichomes. Thoracic trichomes 6 pairs (5 anteriorly, 1 posterolaterally), all long and simple except 3 or 4 anterodorsal pairs bifid. Gill (Fig. 8) with 1 inflated, horn-like filament (0.8-1.0 mm long) and 4 slender filaments (1.0-1.4 mm long) arising at base from dorsal and interolateral surface of horn-like filament; all filaments brown except base of horn-like filament transparent ventrally, and covered uniformly and densely with

minute tubercles; horn-like filament gradually tapered toward apex, thick-walled, irregularly with several annular constrictions; 4 slender filaments very slightly tapered toward apex, with numerous annular furrows. *Abdomen.* All terga brown, sclerotized, with intersegmental transparent space; terga 1 and 2 without tubercles; tergum 1 with long seta on each side; tergum 2 with 6 simple setae on each side, 1 seta much longer than others; terga 3 and 4 each with 4 hooked spines directed forward along posterior margin on each side; terga 6-9 each with spine-combs in transverse row and comb-like groups of minute spines on each side; tergum 5 devoid of spine-comb; tergum 9 with a pair of plate-like terminal hooks which are serrated on ourter margins (Fig. 9). Sternum 4 with a simple hook and a few minute setae on each side; sternum 5 with a pair of bifid hooks on each side; sterna 6 and 7 each with a pair of bifid inner and bifid or simple outer hooks; comb-like groups of minute spines present medially and laterally on sternite 4, submedially each on sterna 5-7 and medially on sternite 8; grapnel-like hooklets absent on each side of last segment. *Cocoon.* Simple, wall-pocket-shaped, tightly woven, extending ventrolaterally in some cocoons but not in others, covering whole of thorax and abdomen; anterior margin strongly woven and well defined; floor formed on posterior 1/2.

Female and larva. Unknown.

Type Specimens. Holotype ♂, dissected out of pupa, slide mounted together with pupal exuvia, collected at Noro, New Georgia Island, New Georgia Islands, Western Province, Solomon Islands, 5. IX. 1992, H. Suzuki. Paratypes 1 pharate ♂, 3 pupal exuviae, in alcohol, same data as holotype. Holotype and one of paratypes will be deposited at the Natural History Museum, London, U. K. and other paratypes will be deposited at Bishop Museum, Honolulu, U. S. A., and at the Carnegie Museum Natural History, Pittsburgh, U. S. A.

Ecological notes. The pupae were collected from trailing grass leaves in a small, fast-flowing stream with width of 1-2 m, running in a natural forest, midpoint between Munda and Noro, together with *S.* (*G.*) *hiroshii* Takaoka, *S.* (*M.*) *sherwoodi* and *S.* (*M.*) sp.

Etymology. This new species is named after Dr. N. Kere, Permanent Secretary, Ministry of Health and Medical Services, Solomon Islands, for his cooperation.

Remarks. *Simulium* (*M.*) *kerei* is assigned to the subgenus *Morops* by having hairs on the katepisternum and pleural membrane of the male adult, although female adult and mature larva are not available. This species is further placed in the *farciminis*-group of the subgenus, defined by Crosskey (1967), by the form of the pupal gill which consists of 1 inflated, horn-like filament and 4 slender filaments. However, this species is unique in possessing the distinct parameral hooks in the male genitalia which are absent in all *Morops* species reported from Papua New Guinea and Australia. This species has also a few characters which depart from the diagnoses for the *farciminis*-group: i. e., the absence of distinct dorsal hair crest on the fore basitarsus, the cocoon of no short form but of normal form covering whole of the thorax and abdomen, the pupal terga well sclerotized, and terminal hooks not cone-shaped but plate-like with serrate outer margin. Either of these characters easily separates *S.* (*M.*) *kerei* from the known species of this species-group. The pupal gill itself most closely resembles that of *S.* (*M.*) *wantoatense* Smart and Clifford from Papua New Guinea (Smart and Clifford, 1965), although in the latter species there are distinct tubercles on the surface of horn-like filament, and 2 of 4 slender filaments arise a little distant from the base of the other 2 filaments. Apart from those characters mentioned above, this species also differs from the latter species by the narrow male hind basitarsus, and the absence of saw-like projections on the pupal antennal sheaths. The male hind basitarsus is enlarged, and pupal antennal sheaths are provided with saw-like projections in *S.* (*M.*) *wantoatense* and 4 other related species of the *farciminis*-group (the presence of the latter character has been confirmed in the specimens held in the Natural History Nuseum, London by H. T.).

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Research Note

GROWTH INHIBITION OF CULTURED *PLASMODIUM FALCIPARUM* BY IMMUNE SERUM FROM TANZANIA.

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Received April 22, 1994/Accepted September 15, 1994

Abstract: The inhibition titers of sera from adult residents in Dar es Salaam, Tanzania to intraerythrocytic growth of *Plasmodium falciparum* were not correlated to their antibody titers by immunofluorescence and enzyme immunoassay, and did not exhibit specificity to the strain with a certain MSA-1 gene. Another inhibitory factor(s) was found in a low molecular fraction with molecular weight less than 30,000 that was separated from a high molecular fraction by centrifugation on a membrane filter (centriprep-30, Amicon). This factor induced morphological changes of intraerythrocytic parasites and was thought to correspond to crisis form factor (CFF) by Jensen *et al.* (1982). The factor was shown to remain in the serum of an adult Tanzanian who had lived in a non-malarious area six months prior to the commencement of this study and to be contained in a hyperimmune serum from a Japanese patient even though on a low level.

INTRODUCTION

Various humoral factors in immune sera to inhibit malaria parasite growth were described by Mcgregor and Wilson (1988). Although intraerythrocytic growth inhibition has been considered to be associated mostly with antibodies, Jensen *et al.* (1982, 1983 & 1984) have shown that sera from Sudanese living in malaria endemic areas contained factors independent of antibodies which induced crisis forms of erythrocytic stages of *P. falciparum* and which were absent in Indonesian immune sera with high immunofluorescence antibody (IFA) titer. These results suggested the presence of two different and ethnogenetically biased humoral mechanism for elimination of asexual blood forms. In the present work we examined the inhibitory pattern of Tanzanian sera to cultured *P. falciparum* in relation to anti-malaria antibodies. Furthermore serum samples were devided into a high (HMF) and a low molecular fraction (LMF) by centrifugation on a cut off membrane filter for Mr 30,000 to specify the growth inhibitory factors.

MATERIALS AND METHODS

Serum samples and parasite strains.

Twenty five serum samples were collected from adult malaria patients in Muhimbili Hospital in Dar es Salaam, Tanzania where malaria is hyperendemic. The samples were collected in sterile tubes and preserved with 0.05% sodium azide prior to transportation. A serum sample T was obtained from a Dar es Salaam resident, a 39-year-old man who had lived in a nonendemic area, Japan for six months prior to this study and another sampe M was from a Japanese malaria patient, a 43-year-old man who had contracted the disease while on visit to Mozambique four months before and repeated recrudescence till the serum collection. Before use the Tanzanian serum samples were freed of sodium azide by Sephadex G-25 column chromatography, and were screened for chloroquine (CHQ) according to the modified Hanskins method (Hanskins MM II) as described by Mount *et al.* (1987). Control samples were obtained from voluntary persons in Japan. Part of the serum samples were diluted 2.5 fold with serum free RPMI 1640 media, devided into two fractions by centrifugation at 3,500 rpm for about 55 minutes using

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centriprep-30 concentrator (Amicon, USA) at a cut-off of Mr 30,000. The filtered low molecular fraction (LMF) was kept alone and the remaining high molecular fraction (HMF) was adjusted to the original volume by adding serum free RPMI 1640 media and kept at 4°C till further use. Three *P. falciparum* strains FCR, K1 and Thai 836 were used in the inhibition assays. Briefly the strain FCR was used due to its easiness of culture and the latter two strains were because they had the distinct genotype of the precursor of the major merozoite surface antigen (MSA-1) (Jongwutiwes *et al.*, 1991). *P. falciparum* parasites were usually cultured in group O red blood cells adjusted to 5% hematocrit in a RPMI 1640 medium supplemented with 10% human serum as described by Tragar and Jensen (1976) at humidified conditions of 5% carbon dioxide, 5% oxygen, 90% nitrogen and then overlaid media were changed with fresh ones everyday.

Enzyme linked immunosorbent assay (ELISA).

FCR strain infected red blood cells with 13% parasitemia were processed for antigen preparation according to the method by Spencer *et al.* (1979). Protein estimate by the method of Lowry in the final supernatant which was the source of antigen was 3.2 mg/ml. The antigen was aliquoted and stored at -80°C till further use. Microplates (ELISA Plate, MS-3496, Sumitomo-Bakelite) were coated with 0.2 ml antigen diluted in 0.06 M carbonate buffer, pH 9.6 at 1 : 300 overnight at 4°C, washed 3 times with PBS, sealed and kept at 4°C till further use. Prior to use in the next step the microplates were blocked by Block Ace solution (Dainippon Pharmaceuticals) according to the manufacturer's instruction. This solution was also used as a diluent for the following steps. Test serum was diluted at 1 : 200. 0.2 ml of diluted serum was run in duplicate on the antigen coated microtiter plates, incubated at 37°C for two hours. The plates were washed three times in PBS-Tween and incubated with 0.2 ml of dilute (1 : 500) horseradish peroxidase-conjugated goat IgG fraction to human IgG (Organon Teknika Co.) for two hours at room temperature. The plates were read at 400 nm after 30 min incubation with 0.3 ml of substrate (o -Phenylenediamine) at room temperature. The ELISA titer was expressed as the ratio of OD value of each sample to that of control.

Immunofluorescence assay (IFA).

Dried thin layer blood slides of parasitised cells were treated with various dilutions of immune sera and incubated in moist chamber at 37°C for one hour. The slides were then washed 3 times with PBS and incubated with 100 times dilute fluorescein conjugated anti-human IgG (Fc fragment) (Organon Teknika Co.) in PBS at 37°C for half an hour. The slides were then 3 times washed with PBS and mounted with 5% glycerol in PBS for fluoroscopy. The IFA titer was expressed as the highest dilution that showed positive.

Growth inhibition assays.

Sorbitol synchronized parasites were prepared as described by Lambros and Vandergerg (1979). After an overnight culture the synchronous culture with more than 95% trophozoites was adjusted to 0.5 to 2.5% parasitemia depending on the experiment and to a hematocrit of 5% by freshly washed group O red blood cells. Assays containing final volume of 0.2 ml with ten times dilute immune serum or LMF or HMF (at final concentration of 10%) were prepared in flat bottomed 96 well culture plates. Where immune serum was used together with the normal serum, the latter was adjusted to give the filal concentration of 10%. In one experiment the immune sera were adjusted to final dilutions from 5 times to 160 times. Culture conditions were as described earlier and the overlying media were changed daily for fresh media. Giemsa stained blood films were examined under the light microscope to ascertain the level of parasitemia and possible morphological changes. The inhibition rate was expressed as the ratio of the difference of parasitemia between control (C) and sample (S) to control; (C-S/C)x100.

Table 1. IFA titer and growth inhibition rate after 72h incubation to three *P. falciparum* strains by four Tanzanian immune sera.

Sample No.	FCR		K1		Thai 836	
	IFA	Inhibition (%)	IFA	Inhibition (%)	IFA	Inhibition (%)
8	≧3,200	57	1,600	79	1,600	65
34	≧3,200	58	≧3,200	81	≧3,200	79
5	400	46	400	78	200	74
7	400	41	400	71	400	65

Table 2. CHQ levels and serum antibody titers to FCR strain of immune sera and their inhibition rates after 72h incubation.

Sample No.	CHQ (μM)	ELISA	IFA	Inhibition (%)
11	0	2.7	100	54
10	0	3.0	800	45
8	1.5	8.1	≧3,200	74
34	61.5	18.3	≧3,200	80
15	2.3	5.6	100	61
5	56.3	4.1	400	53
7	0	5.9	400	68
3	62.3	7.5	1,600	65
4	61.0	3.3	800	65
T[a]	0	4.1	400	73
M[b]	0	>18.3	≧3,200	57

[a] A serum from a Dar es Salaam resident living in Japan for 6 months.
[b] A serum from a Japanese patient with chronic infection for 4 months.

Results and Discussion

Using the preliminary ELISA 9 samples were selected from 25 for further examination. They had IFA titers ranging from 100 to more than 3,200 that well corresponded to ELISA titers from 2.7 to 18.3 (Table 2). Of these samples four were used in the growth inhibition assay of *P. falciparum* strain FCR, K1 and Thai 836 (Table 1).

The 72 hours inhibition pattern of the immune sera did not correspond well with their respective antibody titers (Table 1 and 2). Although the Thai 836 (MAD 20 type) is slightly less susceptible to the inhibition assay than the K1 type, the least susceptibility was seen in the FCR. This we attributed to the fact that FCR strain is better adapted to in vitro culture conditions than the other two strains. Eleven serum samples were analysed for a widely used anti-malaria drug CHQ and its metabolite desethylchloroquine and used in the inhibition assay of synchronous culture of *P. falciparum* FCR strain (Table 2). Levels of CHQ and its metabolites above 2.0 μM were considered significant. However, although serum samples 34, 5, 3 and 4 exhibited high drug levels close to the 75 μM considered to be the maximum therapeutic serum levels in the treatment of malaria (Staiger *et al.*, 1981) they did not influence the growth inhibition by the immune serum. We consider this to be due to the low CHQ concentration levels in the final culture volumes because the immune sera were 10 times dilute in the final inhibition assays. This could also have been augmented by possible CHQ resistance by the parasite strains used.

Table 3. Growth inhibition after 72h incubation to *P. falciparum* FCR strain by LMFs and HMFs of different immune sear.

Serum sample		Average inhibition (%)
CONTROL	LMF	10
	HMF	0
M	LMF	27
	HMF	37
T	LMF	57
	HMF	16
34	LMF	39
	HMF	33

The analysis of the inhibitory capacity of the LMFs and HMFs of the serum M, T and 34 on synchronously growing *P. falciparum* FCR strain revealed a stronger inhibition tendency of LMF than HMF except the Japanese serum M (Table 3), although the ELISA titer by the dilution method (Spencer *et al.*, 1979) for the Japanese serum M was two times higher than the serum sample 34 which was the highest value 10,240 in Tanzanian samples. Surprisingly the sample T from a Tanzanian who had lived in Japan for six months still maintained high inhibitory level of LMF despite his low antibody titer. This finding may partly explain reported findings by various groups that *P. falciparum* infection does not cause the severe complication of acquired immunodeficiency syndrome, AIDS (Wabwire-Mangen *et al.*, 1989, Lucas, 1990 and Simooya *et al.*, 1991).

Further analysis of the LMF and HMF inhibition using the serum T showed that the inhibition was dose -dependent in either case. Inhibition capacity of the

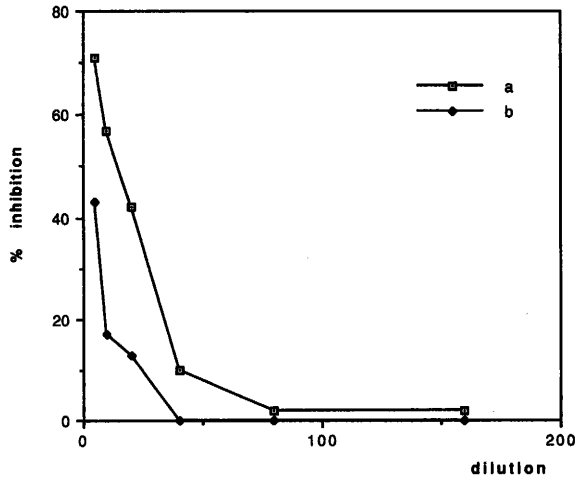
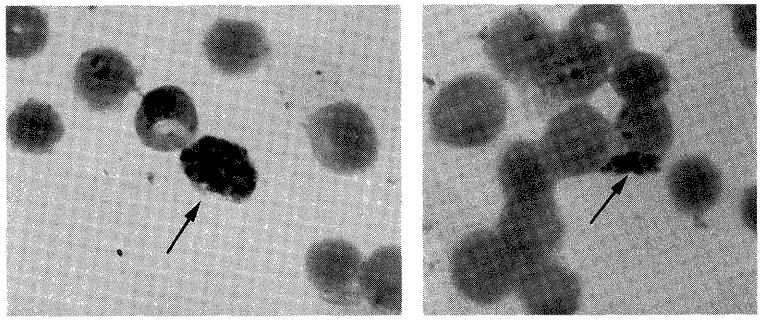


Figure 1 The effect of concentration on the inhibition capacity of LMF (a) and HMF (b) from the serum T on synchronously growing *P. falciparum* FCR strain in 48 hours.



a b

Figure 3 Giemsa stained thin blood films of synchronous culture after 72h culture with a nonimmune serum (a) and with a immune serum T(b). Note the number of produced merozoites (→).

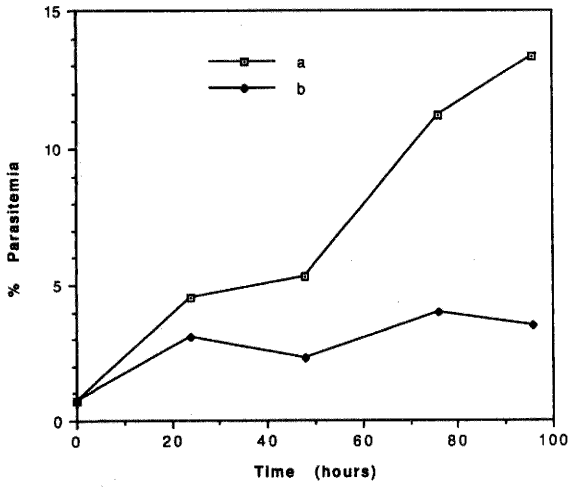


Figure 2 Growth of *P. falciparum*, FCR strain in the presence of LMF of T serum (b) and in the absence (a). At time points 0, 48, and 96h the predominant forms were trophozoites while at time points 24 and 76h ring forms predominated.

serum fractions was almost absent after 40 times dilution (Figure 1). To determine the life stage of the parasite that was affected by the immune sera, the growth pattern was monitored everyday. Synchronization was observed to start disappearing after 48 hours. Crisis forms were noted with typical characteristics of bulky ring appearance, poorly staining IRBC, denaturated schizonts and mature schizonts showing remarkably fewer merozoites (between 4 and 7) as described by Jensen *et al.* (1983) instead of Laverania characteristic of the number of merozoites between 12 and 16 (Figure 3). Inhibition was observed at all stages as characterised by low parasitemia and a negative gradient in the immune serum, implicating intracellular growth impairment (Figure 2).

We have shown here that the LMF with a molecular weight less than 30,000 was a more potent inhibitor than the HMF which contained the immunogloblins. Infected red blood cells cultured with the LMF, washed and incubated with fluorescein conjugated anti-human IgG could not show malaria antibodies, while with the HMF we could detect antibodies on the surface of the infected red blood cells (results not shown). We thus report presence in the Tanzanian immune sera, of an anti-malaria factor(s) that is capable of growth inhibition of intracellular parasites and formation of crisis forms, which support previous findings where it was refered to as crisis form factor (CFF) by Jensen *et al.* (1982), originally by Taliaferro and Taliaferro (1944). We could not induce crisis forms by the HMF of the immune serum. This mechanism of malaria defense is possibly a parallel mechanism to antibody defence and our findings suggest that they do occur together in an individual at varying degrees of potency. Further studies are necessary to define the nature of the factor.

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SURVEY OF INTESTINAL PARASITE INFECTIONS IN JAPANESE LIVING IN INDONESIA

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More than ten million Japanese travelers and workers visit various foreign countries including tropics each year. Intestinal parasitic infections are one of public health problems in developing countries today (Minakami et al). Unfortunately there are few reliable information on the risk of parasitic infection in developing countries. It is because most of the studies on prevalence of parasitic infection in developing countries were done in selected individuals, such as those in the hospital or others administered medical treatment for the infection. The true prevalence of parasitic infection should be derived from data on unselected populations (Chacin-Bonilla et al 1992).

Our objective was to investigate the incidence of intestinal infection caused by protozoa and other parasites in Japanese living in Indonesia, and to identify factors that may play a role in the dissemination of the infection.

This investigation was conducted in 9 cities of Indonesia in 1993: [Djakarta, Barongang, Semarang, Surabaja, Probalinggo, Ujung Pandang, Merak, Menado, and Luwuk]. Samples of stool and blood collected from 231 Japanese and 25 natives of Indonesia. Each stool specimen was fixed in 10% formalin and centrifuged at 2500 rpm for 3 minutes. Aliquots of slurries were microscopically examined by the formalin -ether method. The remaining aliquots were mixed with a drop of Lugoul's solution (5% iodine, 10% KI in 100ml of distilled water). and examined for the presence of protozoa and helminths. A cellophane tape method was used in collecting specimens to be examined for *Enterobius vermiculuris* in 76 Japanese residents.

We assessed the prevalence of protozoa and other parasites in stool of Japanese residents of Indonesia and among native population. Five species of protozoa and five of helminths were identified in stool samples of 256 individuals (Table 1). The rate of infectioin was highest with *Blastocystis hominis*, 35 of the 256 (13.7%). *Giardia lamblia, Endolimax nana*, and *Trichuris trichiura* were observed in 4 (one Japanese and 3 native Indonesians) of 256 (1.6%) stool samples. *Ascaris lumblicoides, Iodomoeba buetschlii* and hookworm were detected in stools of 5 native Indonesians only. *Enterobius vermicularis* was found in 2 of the 76 Japaneses so tested. A community-based study of *Blastocystis* and other intestinal parasites in the Asaro Valley of Papua, New Guinea, showed an extraordinarity high prevalence (from 0.4% to 58.4%) and variety of protozoan infections. The age -specific prevalence of *Blastocystis* infection resembled that of *Entamoeba coli* and *Endolimax nana*, indicating similarity in their mode of transmission (Ashford and Atkinson 1992). Zierdt et al (1967) proposed that *Blastocystis hominis* is not a yeast, as had been thought for many years, but rather a protozoan, and suggested

Table 1 Intestinal parasitic infections in Indonesia

	Japanese	Indonesians
Blastocystis hominis	25/231	10/25
Endolimax nana	2/231	3/25
Giardia lamblia	3/231	1/25
Trichuris trichiura	1/231	3/25
Hookworm	0/231	3/25
Enterobius vermiculuris *	2/76	n. t.**
Entamoeba coli	1/231	0/25
Heterrophyes heterophyes	1/231	0/25
Asaris lumbricoides	0/231	1/25
Iodomoeba buetschelii	0/231	1/25

positive for parasites / tested residents
* : by cellophane tape method
** : not tested

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this organism might be pathogenic. Two patients holding *Blastocystis hominis* were sometimes suffering from diarrhea, and the other residents did not show any symptoms. We cannot consider that *Blastocystis hominis* may be pathogenic. Both Sheehan et al (1986) and Pikula (1987) reported a positive association between *B. hominis* and *E. histolytica*. *E. histolytica* was not found in Japanese and Indonesian residents.

Serological evidence of parasitic infection was difficult to evaluate, as we could not differenciate an active infection from that of a prior infection by *Entamoeba histolytica* (Ximenez et al 1993). While stool samples were negative for *E. histolytica* in all 256 stool samples, 14 subjects were positive for anti-*E. histolytica* antibodies in blood by using ELISA. It must also be careful to infect *E. hystolytica* in Indonesia as it is considered that at least there were past histories infected with *E. histolytica*. Five native indonesians had *Blastocyctis hominis* and antibodies to *E. histolyticla*. Japanese residents, who kept *Blastocystis hominis*, had not antibodies to *E. histolytica*. (data not shown)

In this study, the incidence of parasitic infection in the Japanese residents with less than one year stay was 0%. As the parasitic infection rate of Japanese with more than one year stay was more than 10%, there may be a risk for Japanese to infect parasites in Indonesia. Meanwhile residents of three Japanese communities [Luwuk, Merak, and Menado] showed a zero infection rate, the parasitic infection may be blocked if Japanese apart from Indonesian society and rural environments.

These results suggest that there is a risk for visitors to infect intestinal parasites in Indonesia.

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Case report

CASE REPORT OF LEPROSY AND A TRIAL OF SCREENINGS FOR THE FAMILY MEMBERS IN ECUADOR

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Abstract: Four cases of patients with leprosy were seen in an area endemic for cutaneous leishmaniasis, Los Ranchos, Department of Manabi, Ecuador. Two cases of them (borderline lepromatous leprosy and indeterminate one) in a single family and result of screenings for the family members were reported. It was suggested that family examination of leprosy patient might be useful for early detection of leprosy in a low endemic areas for leprosy, such as Department of Manabi. A nine banded armadillo kept by the family was examined, but no acid-fast bacilli was observed in the liver materials.

INTRODUCTION

Leprosy and leishmaniasis are etiologically completely different diseases but it has been known that the two diseases have similar cutaneous manifestations (Jopling, 1984; Butto *et al.*, 1994). Therefore, both diseases should be differentiated in the endemic areas. During a survey for cutaneous leishmaniasis in Ecuador, leprosy patients were also examined. The current paper deals with the two cases in a single family in detail and preliminary screenings for the family members of leprosy. Furthermore, based on the result obtained a brief comment was also made on the screening methods to detect leprosy patients in early stage of the disease.

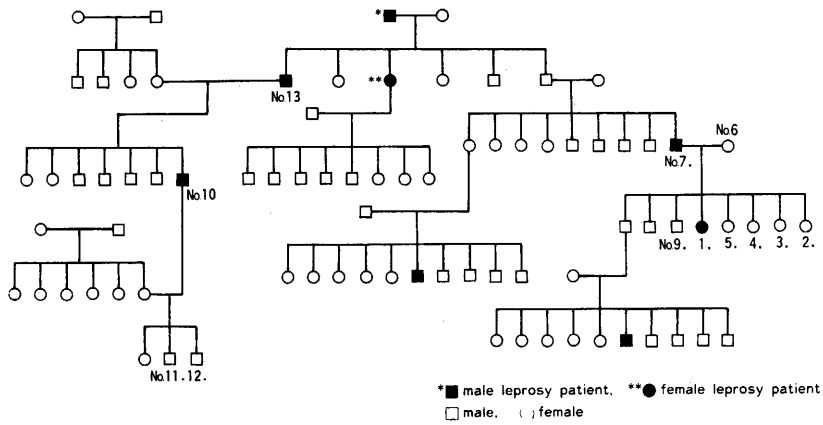

Figure 1 The pedigree of leprosy patients in Los Ranchos, Department of Manabi, Ecuador.

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CASE 1

The patient was a 41-year-old-male (No-7).

Family history. His grand father, an uncle, an aunt, a nephew, a cousin, and a daughter were reported as suffering from leprosy by interview to the family (Fig. 1).

Present history. About 15 years ago, erythemata with anesthesia appeared on the abdomen. The eruption gradually increased in number on the face, the trunk and the four extremities. At the same time, there was a rising in body temperature, and neuralgia appeared in both exteremities. In 1982, a doctor from the Welfare Ministry diagnosed him as leprosy.

Treatment. A 100mg per day of DDS (4, 4' -diaminodiphenylsulfone) was regularly prescribed for two years. In 1990, multidrug therapy (MDT) was started according to MDT for multi-bacillary leprosy recommended by the World Health Organization (WHO) (Noodeen, 1991). His prescription was as follows; DDS (100mg/day) and B-663 (50mg/day), every day; B-663 (300mg/day) and Rifampicin (600mg/day), once a month.

Present illness. Infiltrated erythemata on the face, destruction of nasal septum and deformity of the nose were observed (Fig. 2). Pea sized reddish nodules on the earlobes were soft and the induration of the lesions were not palpated (Fig. 3). Annular and infiltrated erythemata were scattered on the trunk. Anesthesia was observed on all these eruptions. The body surface was dry except for the axillary, the epigastric, the lower abdomen, the inguinal, the perineal and the anal region. The ichthyosis-like change was seen on the region of the waist, the back, the extensor aspect of the legs and the feet (Fig. 4). Although the deep ulcers on the soles had the sharply demarcated bank, it did not have the induration at the margin of the ulcer that was observed in cutaneous leishmaniasis. The patient had anesthesia at the ulcers and could walk without pain. The big toes were mutilated and remaining toes were shortened (Fig. 5). Bilateral apehands and claw-hands were seen and fissures caused by trauma on the right palm were marked (Fig. 6). There was no loss of hair at any region of the body. Hypertrophy of the ulnar nerves were palpated at the elbows.

Laboratory examination.

1) The lepromin test: negative.

2) Value of anti-phenolic glycolipid (PGL)-I and anti-lipoarabinomannan (LAM)-B antibody: Positive result were PGL-I (IgG) 0.089 OD unit, PGL-I (IgM) 1.693 OD unit, LAM-B (IgG) 0.545 OD unit and LAM-B (IgM) 0.281 OD unit.

3) Examination of sensory function: Anesthesia

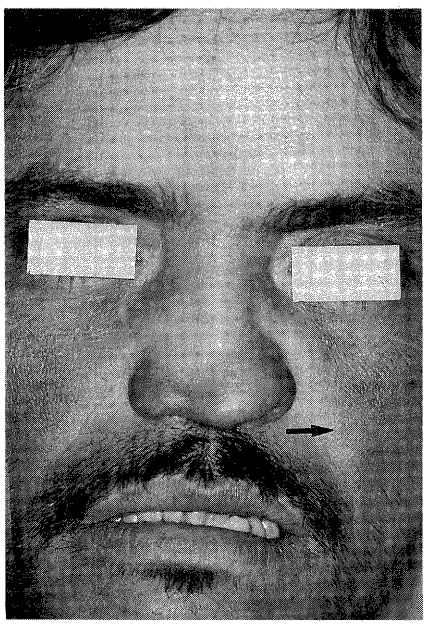

Figure 2 Deformity of the nose because of destruction of nasal septum and infiltrated erythema with sensory loss on the face (⟶) of Case 1 (No. 7: 41-year-old male).

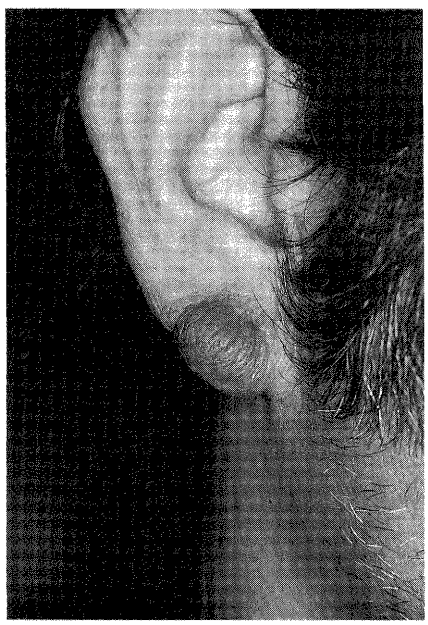

Figure 3 Pea sized nodule with sensory loss on the earlobe of Case 1.

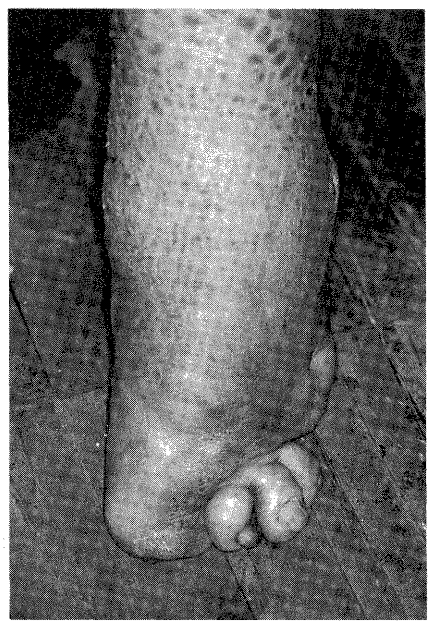

Figure 4 The ichthyosis-like change on the extensor aspect of leg and foot. Deformity of toes are also obseved.

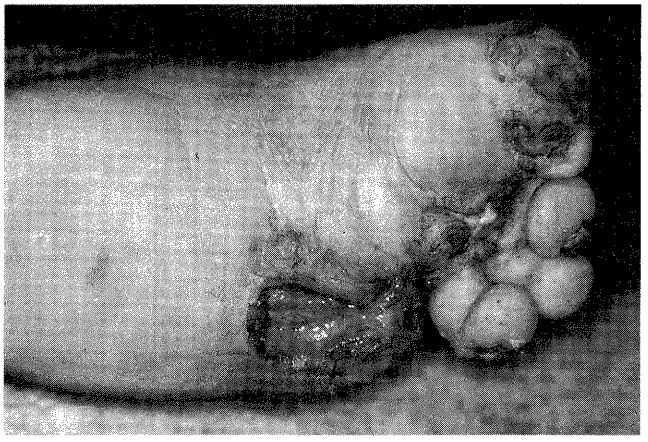

Figure 5 Deep ulcer on the sole and shortened and mutilated toes on the foot of Case 1.

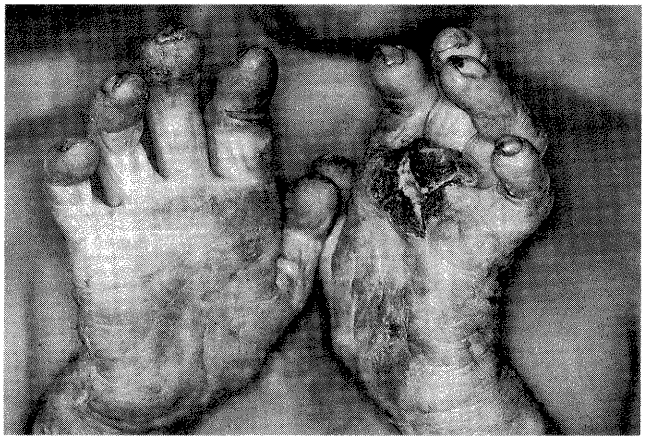

Figure 6 Ape-hand and claw-hand of both hands of Case 1.

was observed on all over the body surface except for the scalp, the axillary, the epigastric, the inguinal, the perineal and the anal region.

4) Histological findings of the specimen taken from the infiltrated erythema on the cheek: Rete peg disappeared. Clear subepidermal zone was observed (Fig. 7). Dermis was edematous. Though relatively large number of epithelioid cells and lymphocytes infiltrated in the dermis, epithelioid cell granuloma was not observed. By acid-fast staining (The Fite-Feraco staining Method), acid fast bacilli were observed (Fig. 8). Biopsy index: 3+, SFG index, 5; SFG value, 1-2-1. By skin slit smear of the left earlobe, acid fast bacilli stained by Ziehl-Neelsen's staining were found. Bacterial index showed 3+; SFG index, 4; and SFG value, 1-2-2. Nasal scraping was negative. The type of leprosy was borderline lepromatous.

CASE 2

The patient was a 12-year-old female (No-1). A daughter of Case 1 (Fig. 1).

Present history. When we examined the family of the Case 1 patient, hypopignented fleckle on the extensor aspect of the left thigh was noticed. The patient had been living in the house at the village of Los Ranchos, Depatment of Manabi since her birth.

Present illness. Palm sized hypopigmented freckle with anesthesia on the extensor aspect of the left thigh (Fig. 9). Hair loss was not observed at any region of the body surface. Hypertrophy of peripheral nerve was not palpated.

Laboratory examination;

1) Mitsuda eary reaction (48 hrs): 7.5mm×7.5mm/7.5mm×7.5mm, undeterminable (+/−).

2) Value of anti-PGL-I and LAM-B antibodies: Positive results were PGL-I (IgG) 0.117 OD unit, PGL-I (IgM) 0.804 OD unit and LAM-B (IgM) 0.191 OD

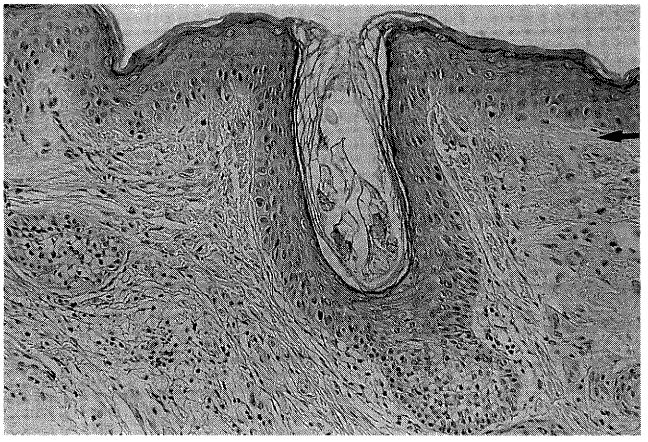

Figure 7 Case 1, clear subepidermal zone is presented (⟶), and typical epithelioid cell granuloma is not presented (HE, ×100).

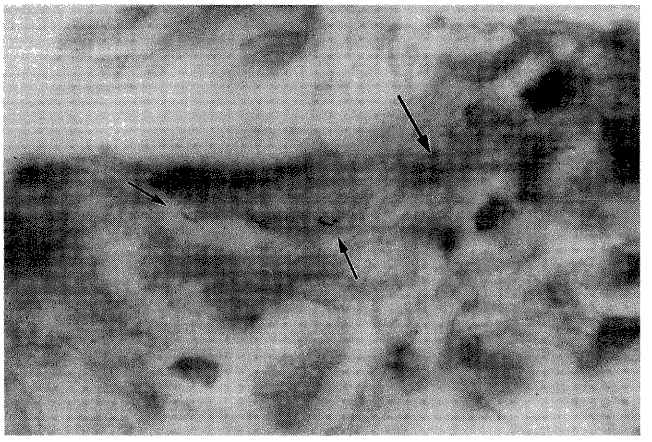

Figure 8 Case 1, *Mycobacterium leprae* (⟶) observed in dermis (Fite-Feraco staining Method, ×1,000).

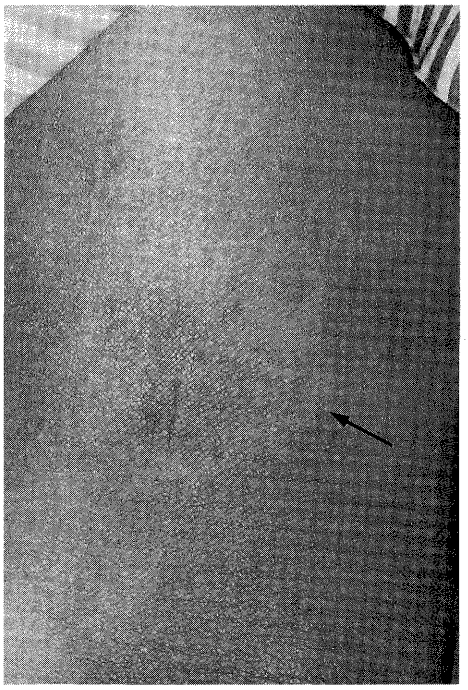

Figure 9 Palm sized hypopigmented freckle with anesthesia on the extensor aspect of thigh of Case 2 (⟶).

unit. Negative resut was LAM-B (IgG) 0.060 OD unit.

3) Examination of sensory function: Anesthesia was observed only at the lesion of the left thigh.

4) Histological findings of the specimen taken from the lesion; small number of lymphocytes infiltrate around the capillaries and the appendages. There was no epithelioid cell granuloma in the dermis. No acid fast bacilli was observed in the skin tissue section stained by acid-fast staining (Ziehl-Neelsen and the Fite-Feraco staining Methods). The patient was diagnosed as indeterminate leprosy according to the histological and clinical criteria of Ridley and Jopling (1966).

Besides the two cases mentioned above, other two leprosy patients (one with borderline tuberculoid and other with unknown type) were examined in the current study. In addition to these four cases, the existence of other four patients with leprosy in this village was determined. Besed on these examinations and interview, the figure of the pedigree was depicted, including relationships among leprosy patients in the endemic area for cutaneous leishmaniasis of Los Ranchos, Department of Manabi (Fig. 1). A nine banded armadillo kept by the family was examined, but no acid-fast bacilli was observed in the liver materials.

Comments;

Leprosy (Hansen's disease) is a chronic mycobacterial disease (infectious in some cases), primarily affecting the peripheral nervous system and secondarily involving skin and certain other tissues (Jopling, 1984). Leprosy has a wide range distribution in the world. Leprosy is included among the six most important infectious diseases, as well as leishmaniasis, which the WHO planned to stop from being an endemic, and is still a public health problem in developing countries including Ecuador. In Ecuador, 110 patients with leprosy were newly deagnosed in 1991; the prevalence rate of leprosy in Ecuador was relatively low (0.25 per 1,000 habitants in total) in comparison with that of endemic areas such as South east Asia and Africa, 0.1-4.9 and 0.1-6.9 per 1,000 hab. respectively (McDougall *et al.*, 1989). It was relatively low (0.10-0.16 per 1,000 hab.) in Department of Manabi, from where the present cases were reported. As to future group examinations for leprosy, in a low endemic area, such as Department of Manabi, the screening of leprosy family would be effective and useful in consideration of a program for the early detection. The deformities of Case 1 could be prevented by early detection and adequate treatment. Case 2 was fortunately detected in early stafge of the disease by a doctor during the present examination.

In Okinawa, Japan, for example, group leprosy examinations had been done in almost all regions of the prefecture during about 20 years by specialists who were appointed by the prefectural goverer (Saikawa, 1989). The examination was called as a general medical check-up and not as a mass examination for leprosy, because in Okinawa, many of the inhabitants have been prejudiced against leprosy patients as well as the inhabitants in Ecuador. Members of the group examinations were consisted of a leprologist, public health nurse from the public health center of each district, manager of leprosy and tuberculosis from the prefectural office, a dermatologist, an internist and a clinical technologist etc. In the group examination, therefore, various examinations were done to find skin diseases, infectious diseases inncluding leprosy and tuberculosis, circulatory diseases such as hypertension, diabetes mellitus and other internal disorders. The data from this medical check-up were reported to each subject by the public health center. If some pathological result was observed, the subjects were examined in detail at the nearest hospital or public health center. Leprosy patients detected in the general medical check-up were treated by the leprologists. Their family members were also examined in detail and some of them received preventive medication.

Recently, serodiagnosis of leprosy was considered

as one of the useful methods for early diagnosis (Bchanan, *et al.*, 1983). The serodiagnosis have also been carried out by leprologists in Okinawa (Abe *et al.*, 1991). During the current family examination, serological examination for 13 subjects was also carried out. Three leprosy patients were positive for PGL-I (IgM) and LAM-B (IgG), and three out of four leprosy patients were strongly positive for LAM-B (IgM) (data not shown). Based on the results, it was suggested that serological examination of families of leprosy might be useful for screening of house hold contacts in a low endemic area for leprosy.

These systems of group leprosy examination in Okinawa might be a good guide for a future program for the early detection in Ecuador. In consideration of economical and man-power resources, some modifications will be needed to implement this system of group leprosy examination into Ecuador.

Cutaneous manifestations in leprosy sometimes showed a similarity to those of cutaneous leishmaniasis. Early nodular lesions of cutaneous leishmaniasis are similar to those of lepromatuous leprosy (LL) and the type of cutaneous leishmaniasis most likely to be confused with LL is the disseminated anergic form. In post kala azar dermal leishmaniasis, hypopigmented flekle appear on trunk and limbs, which should be distinguished from those observed in indeterminated leprosy (Jopling, 1984). In the present preliminary examination for leprosy, anestesia at the lesions was the most important symptom for the diagnosis.

The patients (Cases 1 and 2) kept a nine banded armadillo for their food. In the smear specimens of the liver, no acid-fast bacilli and *Leishmania* amastigotes were observed. Wild armadillo with leprosy were found in U. S. (Smith *et al.*, 1978; Marchiondo *et al.*, 1980). Although no such a report was described in Ecuador, the examination should be continued to ascertain whether leprosy was a zoonosis or not, and whether the nine banded armadillos were natural resorvoirs of the pathogen in Ecuador.

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