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CHANGES IN HTLV-I POSITIVE RATES AMONG PREGNANT WOMEN IN OKINAWA PRIOR TO THE EFFECTS OF MEASURES INTRODUCED TO PREVENT VERTICAL TRANSMISSION THROUGH BREAST MILK FEEDING

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Abstract: Objectives: Human T cell leukemia virus type-I (HTLV-I) is a causative agent of human T-cell leukemia and HTLV-I associated myelopathy (HAM/TSP). HTLV-I carriers are often infected vertically, especially via mother's milk. Since 1985, clinical measures have been adopted at a hospital in Okinawa to prevent vertical infections.

Methods: We examined HTLV-I antibodies in all of the women (total 11,506) who gave birth after 24 gestational weeks at a hospital on the Okinawa main island from January 1985 to December 1999.

Results: The positive rate among all pregnant women was always higher than that among primipara alone. Both figures decreased over the period studied, but the primiparity rate (36-39%) did not change significantly. The percentage of HTLV-I positive primipara pregnant women among the HTLV-I positive total was close to the primiparity rate from 1985 to 1988, but it was considerably lower than the overall primiparity rate thereafter (22-26%).

Conclusions: Preventive measures against HTLV-I infection did not contribute to the decrease in HTLV-I positive mothers before 1999 because these measures were adopted from 1985, and so there must be other reasons for the decrease in HTLV-I positive rate. Further studies on social factors and by year of birth are needed to identify factors influencing HTLV-carrier ratios among pregnant women.

Key words: HTLV-I, Okinawa, pregnancy, ATL

INTRODUCTION

Adult T cell leukemia (ATL), which develops in human T cell leukemia virus I (HTLV-I) carriers [1], is endemic in several regions of the world, including southwestern Japan [2,3]. Routes of infection are vertical, the main one, and horizontal [4]. Because there is no possibility of developing ATL after horizontal infection of HTLV-I by sexual intercourse, one of the best ways to reduce the morbidity rate of ATL is to prevent vertical infection.

Vertical infection is transmitted primarily via mother's milk [5,6]. Thus, to prevent HTLV-I infection via this route, freeze-thawing mother's breast milk and other measures for breast milk feeding have been instituted [7,8]. It is known that these methods can prevent mother-infant infection, but it is still too early to draw a conclusion if these preventive

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measures have contributed to a decrease in the carrier rates in areas that instituted these measures.

The above measures have been applied clinically at a hospital in Okinawa since 1985. Hospital records showed that the youngest pregnant woman during the period of this study, 1985-1999, was born in 1983. Hence, none of the pregnant women were affected by the preventive measures. An examination of the changes in HTLV-I carrier rates from 1985 to 1999 will help to shed light on the impact of the clinical preventive effects of the feeding of freeze-thawed mother's breast milk and other measures, because the effects of these measures would have become apparent after 2000 when a baby born in 1985 became 15 years old. Thus we examined HTLV-I antibodies in all of the pregnant women who since 1985 gave birth at over 24 gestational weeks at a hospital on the main island of Okinawa in southwestern Japan, and we obtained interesting findings.

MATERIALS AND METHODS

Location and Population

Subjects were patients at the Toyama Obstetrical Gynecological Clinic in Ginowan City on the Okinawa main island in southwestern Japan (Fig 1). From April1 985 to March 2000, a total of 29,385 babies were born in this city, and from January1985to December 1999, a total of 11,506 pregnant women gave birth at over 24 gestational weeks at the hospital from which the data was collected.

Assay of serum antibodies to HTLV-I

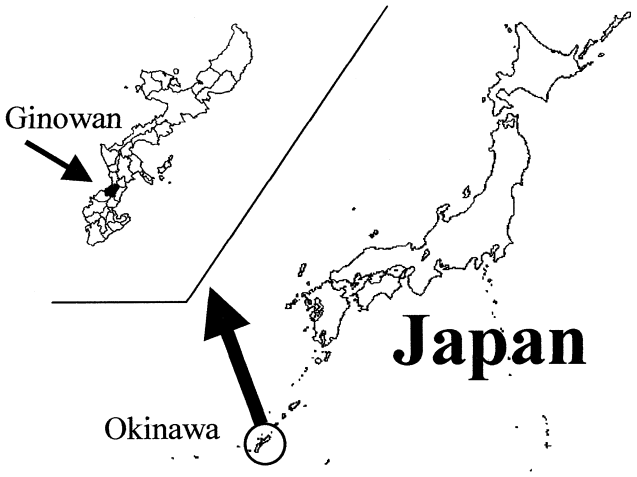

Figure 1. The location of Ginowan City on the Okinawa main island in southwestern Japan.

For particle agglutination (PA) assay and screening of HTLV-I, peripheral blood was drawn from all pregnant women after obtaining informed consent, and the results were judged as either positive or negative. Samples judged positive by PA were confirmed by the immuno-fluorescence method using HTLV-I infected cell line MT-1 [9] originated by Hinuma et al. [10] All HTLV-I positive pregnant women were informed of the result, and before delivery they were advised to institute feeding by artificial milk or freeze-thawing of mother's milk to their newborns.

RESULTS

From January 1985 to December 1999, a total of 11,506 pregnant women (4,259 primiparas) gave birth at a gestational age of over 24 weeks at our hospital. Patient characteristics are shown in Table I. The average age of primipara pregnant women was 25.9±4.6, and that of all pregnant women was 28.6±5.0. The HTLV-I positive rate among all the pregnant women and among the primipara pregnant women alone was studied (Figure II). Though both rates became lower over the term of the study, the positive rate among all pregnant women was always higher than that among primipara pregnant women.

Table II shows the analysis of HTLV-I positive rates by birth years. The positive rate gradually decreased by birth year, especially in primipara pregnant women.

Figure III shows the changes in the overall primiparity

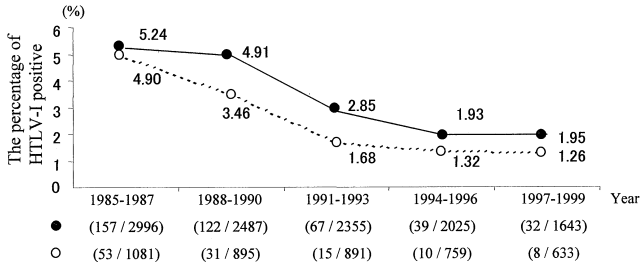

Figure 2. HTLV-I positive rate among all pregnant women and primipara pregnant women who delivered at over 24 gestational weeks. ●, total pregnant women, ○, primipara pregnant women. (numbers of positive women / all women)

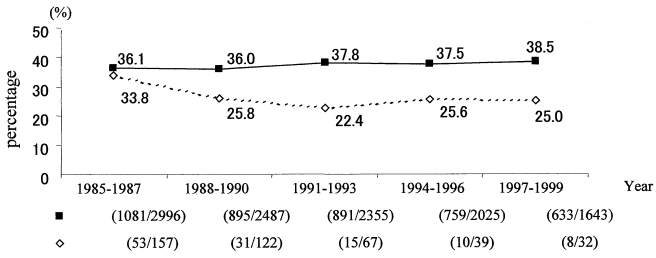

Figure 3. Primipara rate among the total of all pregnant women (■) and primipara HTLV-I positive pregnant rate among the total of all HTLV-I positive pregnant women (◇).

Table Ⅰ. Characteristics of pregnant women.

years		1985～1987	1988～1990	1991～1993	1994～1996	1997～1999
Primipara	No.	1081	895	891	759	633
	Age±SD	26.23±4.56	25.84±4.56	25.75±4.51	25.73±4.56	26.11±4.69
Multipara	No.	2996	2487	2355	2025	1643
	Age±SD	30.11±4.66	30.11±4.67	30.11±4.68	30.11±4.69	30.11±4.70

All women delivered at over 24 gestational weeks from January 1985 to December 1999

Table Ⅱ. HTLV-I positive rate among pregnant women by year of birth.

birth years		～1954	1955～1959	1960～1964	1965～1969	1970～1974	1975～
Primipara	% of positive	4.81	4.32	3.45	2.57	1.40	0.32
	positive/total	9/187	24/555	40/1159	32/1246	11/785	1/312
Multipara	% of positive	5.30	5.34	4.18	2.67	1.23	2.78
	positive/total	55/1037	3/104	3/105	3/106	3/107	3/108

rate and the HTLV-I positive primiparity rate. The primiparity rate tended to increase slightly within a range of 36.1-38.5%. The primiparity rate of HTLV-I positive pregnant women was close to the overall primiparity rate from 1985 to 1988, and then stabilized within 25.8-22.4%, considerably lower than the primiparity rate, thereafter.

DISCUSSION

We reported the world's first preventive measure against HTLV-I, i.e. feeding by freeze-thawing of mother's milk, and this measure and other prevention measures have been adopted in Okinawa Prefecture since 1985 [8]. Now, the preventive measures are evaluated [11,12], but that the changes in HTLV-I positive rates among pregnant women who were born before 1985 were not affected the preventive measures for mother-infant infection.

In the present study, we examined the changes in HTLV-I positive rate among pregnant women from 1985 to 1999. The positive rate among all pregnant women is clinically, but, since the same mother might deliver more than once at the same hospital, primipara pregnant women were also examined to avoid any overlapping of positive individuals.

The positive rate among primipara pregnant women and among all pregnant women decreased over time. The positive rate among primipara pregnant women decreased dramatically between 1985 and 1993, while the total positive rate began to fall rapidly about 3 years later, over the period from 1988 to 1993.

The preventive measures against HTLV-I infection, i.e. modified milk feeding and feeding by freeze-thawing of mother's breast milk, did not contribute to the decrease in the HTLV-I positive rate among mothers who delivered before 1999 because these measures have been adopted only since 1985. However, it is possible that the studies actually affected the number of children born from women who were HTLV-I positive, because women whose first child was HTLV-I positive may have decided to avoid delivering another child so as not to worry about further HTLV-I infection. Hence, we looked for changes in the primiparity rate and its correlation with the percentage of primipara pregnant women among all HTLV-I positive pregnant women.

The percentage of primipara women among all HTLV-I positive pregnant women was close to the primiparity rate from 1985 to 1988 but was considerably lower than the primiparity rate thereafter (22-26%), indicating a difference between the pregnant women who delivered around 1988 and thereafter and those who delivered prior to this date. The cohort effect does not significantly influence the decrease in positive rate, because if HTLV-I positive women avoid delivering a second child, the primiparity rate should increase year by year, and the percentage of primipara pregnant women among the HTLV-I positive pregnant women should also increase remarkably. However, there are many possible reasons for a spontaneous decrease in the positive rate.

Kashiwagi K. et al. [13] pointed to a decrease in the number of mothers breast feeding and a shortening of the breast-feeding period.

Socioeconomic changes in Okinawa and the improvement of the health environment can also be sited as reasons for decrease in HTLV-I positive rate among pregnant women.

The aim of this study was to ascertain the changes in HTLV-I positive rates among pregnant women in Okinawa prior to any effects of the introduction of measures to prevent vertical transmission through breast milk feeding. We found a decrease in HTLV-I positive rates among pregnant women. In the further, we intend to evaluate the effects of the measures introduced to prevent vertical transmission through breast milk feeding in light of the results shown in

this study. Further studies regarding social factors, and rates by the year of birth, are needed to identify factors influencing HTLV-carrier rates among pregnant women.

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INHIBITION OF *TRYPANOSOMA CRUZI* GROWTH IN MAMMALIAN CELLS BY NIMODIPINE, WITH LOW CYTOTOXICITY TO HOST CELLS

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Accepted 30, April, 2004

ABSTRACT: An in vitro infection system of *Trypanosoma cruzi* and HeLa cells was used to measure the anti-*T. cruzi* activities of various calcium antagonists classified into dihydropyridines, diphenylalkylamines, and benzothiazepines and of allopurinol and benznidazole as medium and highly effective reference compounds, respectively. Six dihydropyridines (10 μM each), i.e. nifedipine, nicardipine, nimodipine, nisoldipine, nitrendipine, and amlodipine, decreased the rates of infection of HeLa cells from 11.7% (control) to 5.8, 0.9, 1.2, 3.6, 5.9, and 1.7%, respectively. Nicardipine and amlodipine were highly toxic to HeLa cells, causing detachment of cells from coverslips. Nimodipine was thus the most effective inhibitor tested against *T. cruzi* infection in HeLa cells. Verapamil and gallopamil (diphenylalkylamines), diltiazem and midazolam (benzothiazepines), and allopurinol (positive control) were less effective than nimodipine. IC_{50} values, the concentrations of compounds that elicited a 50% reduction in the infection rates of HeLa cells, were 2.5, 2.6, 1.3, 2.1, and 1.7 μM for nicardipine, nimodipine, amlodipine, verapamil, and benznidazole, respectively, while the values for nifedipine, diltiazem, and allopurinol were much higher. Nicardipine, amlodipine, and verapamil again showed significant cytotoxicities to HeLa cells. When Swiss 3T3 fibroblasts replaced HeLa cells, nimodipine markedly lowered the host-cell-infection rate, with an IC_{50} value of 8.3 nM. Thus, nimodipine is expected to be a highly effective anti-*T. cruzi* lead compound, with low cytotoxicity to mammalian cells. Structural formulas of nimodipine and nicardipine in relation to their low and high cytotoxicities, respectively, against HeLa cells are discussed.

Key Words: *Trypanosoma cruzi*, calcium antagonist, nimodipine, dihydropyridine, growth inhibition

INTRODUCTION

Chagas' disease, the causative agent of which is a parasitic protozoan *Trypanosoma cruzi*, affects about 17 million people, and 25% of the population of Latin America is at risk of acquiring this protozoan infection [1]. *T. cruzi* exhibits two different pathogenic forms in mammalian hosts [2,3]. The nondividing and infective trypomastigote form, which possesses a flagellum, circulates in the bloodstream. After invasion into host cells, the trypomastigote form transforms into the amastigote form, which has no free flagellum. The amastigote multiplies by binary fission in the host cell cytoplasm and eventually transforms back to the trypomastigotes, resulting in the breakdown of infected cells and re-emergence in the circulation.

Nifurtimox and benznidazole are currently used for chemotherapy of Chagas' disease [1], but these drugs are highly toxic, often leading to the discontinuation of the therapy. An effective therapeutic agent with a low toxicity is thus needed. To this end, we previously established a culture system of the host HeLa cells infected with *T. cruzi* and, using this system, determined quantitatively the time courses of the protozoan infection and proliferation [4,5]. This in vitro system also enabled us to examine purine and pyrimidine analogs, including allopurinol, for their efficacies in inhibiting the rate of host cell infection by *T. cruzi* and the parasite growth inside the host cells [4].

Invasion into mammalian cells by *T. cruzi* requires the activation of signaling pathways and Ca^{2+} response both in the parasite and in the host cell [6,7]. Application of this knowledge to chemotherapy against experimental murine Chagas' disease resulted in the amelioration of the acute and chronic stages of *T. cruzi* infection by verapamil, a Ca^{2+} antagonist of the diphenylalkylamine class [8,9], with no effect on the protozoan calcium homeostasis by nifedipine [10], another Ca^{2+} antagonist of the dihydropyridine class.

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We report here that the in vitro HeLa and *T. cruzi* infection system is also useful for examination of various Ca^{2+} antagonists for anti-*T. cruzi* activities, and that nimodipine, a second generation calcium antagonist of the dihydropyridine class, markedly reduced the parasite infection and proliferation in mammalian cells, with lower cytotoxicity against the host cells. The effects of these (nifedipine-like) dihydropyridines on parasite infection and proliferation in other mammalian cells, i.e. fibrosarcoma HT 1080 and Swiss 3T3 fibroblasts, were also examined in this study.

MATERIALS AND METHODS

Media and chemicals

Eagle's Minimum Essential Medium (MEM) and Dulbecco Modified Eagle's Medium (DMEM) were obtained from Sigma-Aldrich Japan. Fetal bovine serum (FBS) was obtained from Daiichi Pure Chemicals, Tokyo, Japan. Diff-Quick and HSR solutions (International Reagents Corp., Kobe, Japan) were used for staining cells infected with *T. cruzi* and for embedding the stained specimens, respectively. The following Ca^{2+} antagonists were obtained from Wako Pure Chemical Industries, Tokyo: nifedipine, nicardipine hydrochloride, nisoldipine, nitrendipine, amlodipine besilate, gallopamil hydrochloride, and diltiazem hydrochloride. Other Ca^{2+} antagonists, i.e. nimodipine and verapamil hydrochloride (Sigma-Aldrich) and midazolam (F. Hoffmann-La Roche, Basel, Switzerland), were also used. Allopurinol was obtained from Sigma-Aldrich and benznidazole was kindly provided by F. Hoffmann-La Roche. These compounds were dissolved in 50% ethanol prior to the examination for anti-*T. cruzi* activity; the solvent, the final concentration of which was adjusted to 1.0%, did not significantly affect the protozoan infection in HeLa cells. Other chemicals were commercial products of the highest grade.

Parasite and mammalian cells

The Tulahuen strain of *Trypanosoma cruzi* and HeLa cells, used as an in vitro host, were maintained and passaged in cultures as described previously [4,5]. Briefly, the human cancer cell line, HeLa, and the infection complex of the HeLa cells and *T. cruzi* [11]were successively subcultured every 3 to 4 days at cell densities of 3-5 x 10^5HeLa cells per ml (total volume of 5 ml) in 25-cm^2 plastic flasks. Swiss 3T3 fibroblasts were provided by F. Hanaoka, Graduate School of Frontier Biosciences, Osaka University. This cell line was subcultured every 3 days at an initial cell density of 3 x 10^5 per ml (total volume of 5 ml) in DMEM containing 10% FBS in 25-cm^2 plastic flasks [4]. HT 1080 cells, a human fibrosarcoma cell line, were obtained from the Japan Health Sciences Foundation (Tokyo, Japan) and subcultured as in the case of Swiss 3T3 cells [12]. Throughout the present study, cell cultures were maintained in a humidified incubator at 37℃ and at 5% CO_2 in air.

In vitro infection and addition of test compounds

HeLa cells were infected in vitro with *T. cruzi* trypomastigotes using a modification of the method described previously [4,5]. A round coverslip (12 mm) was placed in each well of the 24-well plate. Into these wells were added the logarithmically growing HeLa cells (5 x 10^3/ml/well), harvested from the preceding subcultures. After incubation at 37℃ for 2 days, the cells were infected with *T. cruzi* trypomastigotes (3 x 10^6 parasites/well) as described previously [4]. Within 24 hours after the infection the parasite invasion appeared to be accomplished. The trypomastigotes remaining in the medium were not removed in this study.

Each chemical compound tested for its inhibitory effect on the parasite infection and proliferation was added immediately after the infection and was left in the medium during the experiment. Similarly, Swiss 3T3 and HT 1080 cells were also infected with *T. cruzi* and examined effects of the added compounds. Cytotoxicities caused by test compounds against normal control host cells were estimated on the basis of the decrease in cell number per microscopic image (54 μm x 112 μm), using the above system of host cells +*T. cruzi*+test compound with an omission of *T. cruzi* infection.

Determination of the rate of host-cell infection and parasite proliferation

The method for determining the rate of infection by *T. cruzi* of host cells was described in detail [4]. Briefly, host cells attached onto the coverslip were fixed and stained with Diff-Quick in the well of a 24-well plate, then the coverslip was transferred upside down onto a slide glass, and the cells were finally embedded in the HSR solution for observation under a light microscope. The microscopic images of 54 μm x 112 μm (see Fig. 3), taken by a conventional digital camera, were used for counting the number of total cells, infected cells, and amastigotes per image. The percentage of infected host cells containing more than one amastigote and the mean number of amastigotes per infected cell were determined by analyzing more than 200 host cells distributed in four randomly chosen microscopic fields. The results were statistically evaluated by the *t* test for small samples. Differences between means giving a probability of less than 5% were considered significant.

RESULTS

Time course of effects of nimodipine on the rate of infection of HeLa cells and on the average number of amastigotes per infected HeLa cell

In a preliminary examination, nimodipine, a second generation calcium antagonist of the dihydropyridine class, markedly decreased the *T. cruzi* infection and proliferation in HeLa cells in vitro (data not shown). Accordingly, we determined the effect of this compound on the time course of the host-cell-infection rate and the average parasite (amastigote) number per infected HeLa cell, using allopurinol and benznidazole as a medium and a highly effective reference compound, respectively (Fig. 1). In the control wells with no added compound and with 1.0% ethanol as the solvent control, the infection rate (Fig. 1A) and the average amastigote number (Fig. 1B) were increased time-dependently by day 5 and day 6, respectively. Addition of allopurinol resulted in gradual increases, although generally to a lesser extent than the control, in the infection rate and the amastigote number by day 6 and day 7, respectively. Nimodipine did not appear to significantly lower the rate of *T. cruzi* infection of HeLa cells by day 2, with the decreased average number of amastigotes per infected HeLa cell. Subsequently, however, particularly on day 4, we clearly observed the differences in the rate of host cell infection among the control, allopurinol-, nimodipine- and benznidazole-added wells (Fig. 1A). Benznidazole, a drug currently used for treatment of Chagas' disease in Latin America, appeared to be the strongest inhibitor for the infection rate of HeLa cells and the average amastigote number per infected cell. Technically, the infection rate and the parasite number on day 1 and 2 were sometimes variable, while on day 6 the infected HeLa cells were often detached from the coverslips during the staining procedure. Unless otherwise stated, therefore, the infection rate and the parasite growth were determined on day 4 after the *T. cruzi* infection and the addition of test compounds.

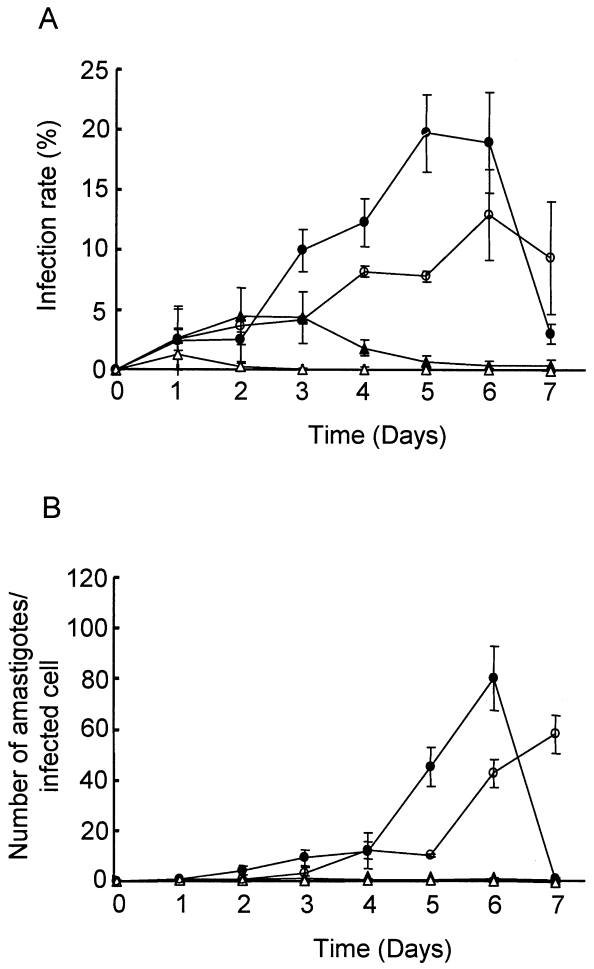


Figure 1. Time course of effects of nimodipine on the rate of *Trypanosoma cruzi* infection of HeLa cells and on the average number of amastigotes per infected cell. Exponentially growing HeLa cells (5 x 10^3/well) were inoculated in each well of 24-well plates, cultured for 2 days, and infected with 3 x 10^6 trypomastigotes of the *T. cruzi* Tulahuen strain. Immediately after the infection, 10 μM nimodipine, allopurinol or benznidazole was added. The host-cell-infection rate (**A**) and the average amastigote number per infected cell (**B**) were determined every 24 hours as described in Materials and Methods. Closed circle, control (no compound added); open circle, 10 μM allopurinol; closed triangle, 10 μM nimodipine; open triangle, 10 μM benznidazole. Data shown are the means$\pm$ 1 SD of 3 separate determinations.

Effects of various Ca^{2+} antagonists on the rate of infection of HeLa cells and on the average number of amastigotes per infected HeLa cell

Figures 2A and 2B show the effects of various calcium antagonists on the rate of infection of HeLa cells and on the average number of amastigotes per infected cell, respectively. Nifedipine, nicardipine, nimodipine, nisoldipine, nitrendipine, and amlodipine markedly lowered the infection rate from 11.7% (control) to 5.8, 0.9, 1.2, 3.6, 5.9, and 1.7%, respectively. Decreases in the average amastigote number per infected HeLa cell, elicited by these dihydropyridines, roughly paralleled the decreases in the host-cell-infection rates by corresponding dihydropyridines. Nicardipine and amlodipine were markedly cytotoxic, often resulting in the detachment of infected HeLa cells from coverslips and thus probably yielding apparently decreased infection rate and protozoan growth. Nifedipine and nisoldipine were also somewhat cytotoxic. Taken together, the

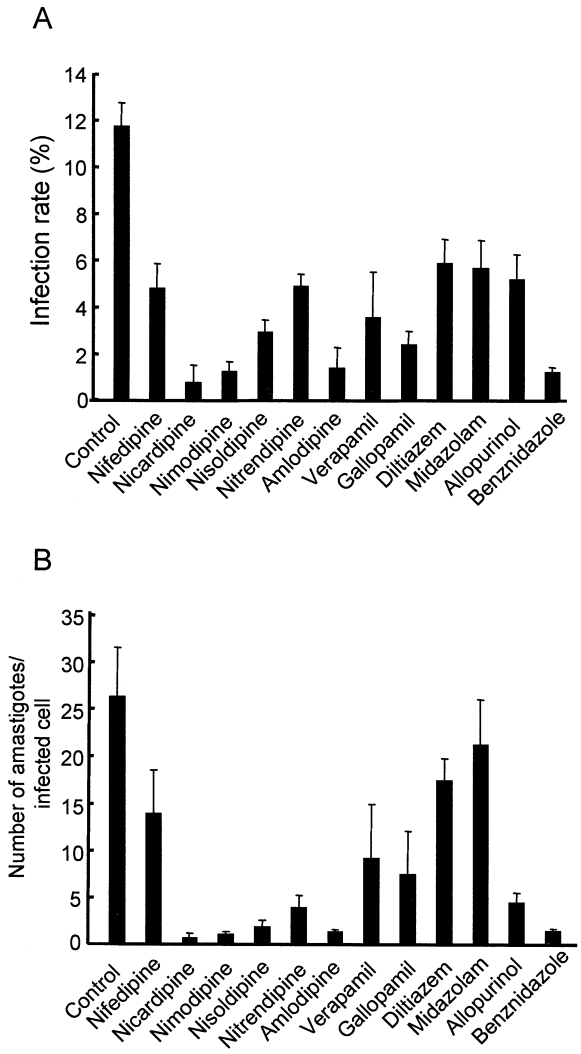


Figure 2. Effect of various calcium antagonists on the rate of *Trypanosoma cruzi* infection of HeLa cells and on the average number of amastigotes per infected cell. Inoculation of HeLa cells, infection by *T. cruzi* trypomastigotes, and addition of test compounds (10 μM each) were as in Fig. 1. On day 4 after the infection, the rate of HeLa cell infection (**A**) and the average amastigote number per infected cell (**B**) were determined as described in Materials and Methods. Data shown are the means +1 SD of 3 separate determinations.

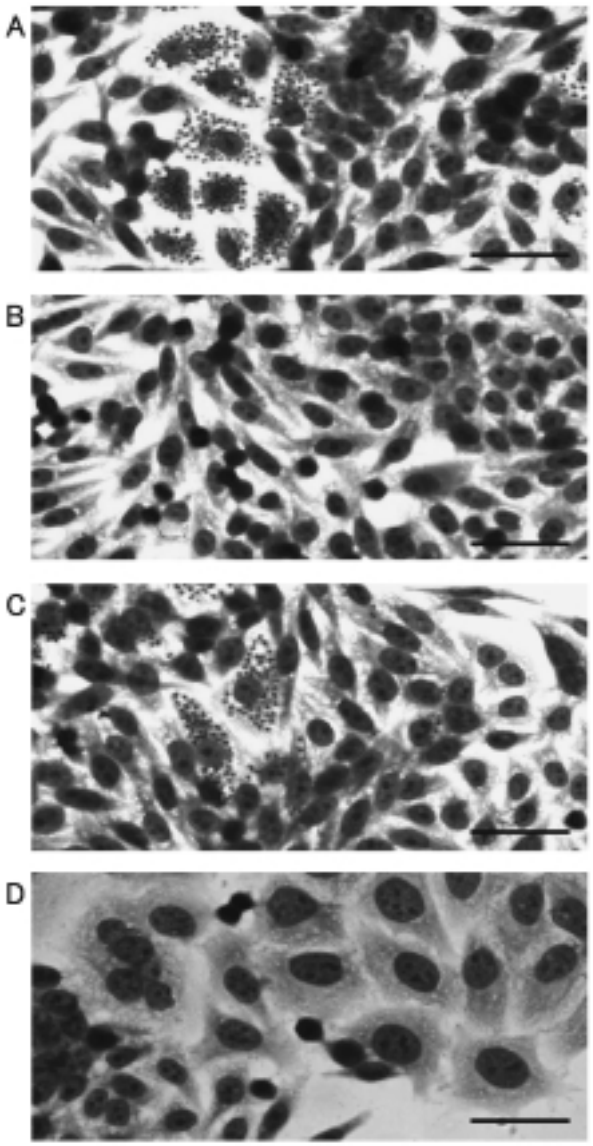


Figure 3. Microscopic images of HeLa cells infected by *Trypanosoma cruzi* and treated with nimodipine, allopurinol or benznidazole. HeLa cells (5 x 10^3/ well) were seeded in each well of 24-well plates, cultured for 2 days, and infected by *T. cruzi* trypomastigotes (3 x 10^6 parasites/well). Immediately after the infection, ethanol (final concentration of 1%) as control (A) or 10 μM each of nimodipine (B), allopurinol (C) or benznidazole (D) was added to each well, followed by further incubation for 4 days. The specimens were fixed, stained, and photographed as described in Materials and Methods. Bar: 20 μm.

results indicate that nimodipine is the most potent dihydropyridine against *T. cruzi* infection and proliferation in HeLa cells in vitro, with the lowest cytotoxicity to the host HeLa cells under the conditions examined. Verapamil and gallopamil (the diphenylalkylamine class), diltiazem and midazolam (the benzothiazepine class), and allopurinol were less effective than nimodipine and benznidazole in decreasing the infection rate and the amastigote proliferation (Fig. 2).

Benznidazole also seemed to be one of the strongest inhibitors on the average amastigote number per infected cell (Fig. 2B). Figure 3 shows the typical microscopic images of HeLa cells infected by *T. cruzi* and treated with ni-

modipine, allopurinol or benznidazole. In nimodipine- and benznidazole-treated cultures, few *T. cruzi* amastigotes were recognized, while allopurinol appeared to cause a somewhat reduced amastigote number, when compared to the control culture. The microscopic images of nimodipine- and allopurinol-treated HeLa cells resembled those of the control cells, but surprisingly, benznidazole-treated HeLa cells often showed enlarged nuclei and cytoplasm (Fig. 3D) and also shrinking, probably necrotic, figures (data not shown). We measured the individual areas of 700 randomly chosen nimodipine- and 700 benznidazole-treated HeLa cells and those of 770 normal control HeLa cells, using the Image-Pro Plus software (Media Cybernetics) (data not shown). A statistical analysis revealed that the χ square value for nimodipine versus control was 51.9 ($P<0.005$) while that for benznidazole versus control was 1617.9 ($P< 0.005$), a significant difference indicating that benznidazole yielded a much stronger cytotoxicity to HeLa cells than nimodipine.

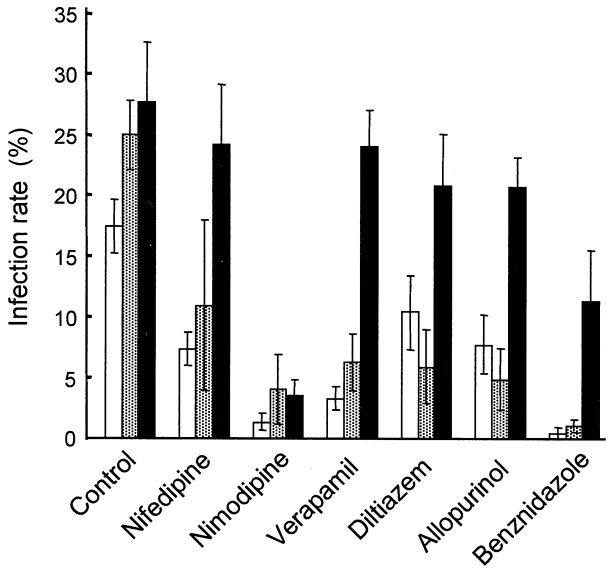


Figure 4. Effect of different mammalian cell lines as in vitro hosts on the rate of *Trypanosoma cruzi* infection. HeLa cells (open bar), human fibrosarcoma HT 1080 cells (dotted bar), and mouse fibroblast Swiss 3T3 cells (closed bar) (5 x 10^3/well) were inoculated in each well of 24-well plates and incubated for 2 days. Infection of these cells by *T. cruzi* (3 x 10^6 trypomastigotes/well), addition of calcium antagonists (10 μM each), and determination of the rate of host cell infection were as in Fig. 1. Data shown are the means$\pm$1 SD of 3 separate determinations.

Effect of different mammalian cell lines, used as in vitro hosts, on the rate of Trypanosoma cruzi infection and on the average number of amastigotes per infected cell

To determine whether or not other mammalian cells could serve as hosts in our in vitro infection and drug-screening system, the infection rates of HeLa, HT 1080, and Swiss 3T3 cells were compared on day 4 after the *T. cruzi* infection and addition of various calcium antagonists (Fig. 4). Swiss 3T3 cells demonstrated generally high infection rates, i.e. 24.1, 24.1, 20.8, 20.7, and11.4%, with the addition of nifedipine, verapamil, diltiazem, allopurinol, and benznidazole, respectively, as compared to the control (28.4%). Nimodipine had the lowest infection rate of 3.3%. HT 1080 cells seemed to resemble HeLa cells in that benznidazole and nimodipine brought about the largest reduction in the rates of *T. cruzi* infection of these cell lines.

Effect of varying concentrations of various Ca^{2+} antagonists on the rate of Trypanosoma cruzi infection of mammalian cells and on the growth of host cells

Effects of varying concentrations (0.1-100 μM) of Ca^{2+} antagonists on the rates of HeLa cell infection were examined and IC_{50} values (the concentrations of compounds that elicited a 50% reduction in the infection rate) were calculated (Table 1). The values for nicardipine, nimodipine, amlodipine, verapamil, and benznidazole ranged from 1.3 to 2.6 μM, while the values for nifedipine, diltiazem, and allopurinol were much higher. Nicardipine, amlodipine, and verapamil were again cytotoxic to HeLa cells, causing their detachment from the coverslips. We examined the direct effects of varying concentrations of calcium antagonists on non-infected, normal HeLa cells (Table 1). IC_{50} values for nifedipine, nicardipine, nimodipine, amlodipine, verapamil, diltiazem, alloprinol, and benznidazole were 51.7, 10.0, 66.2, 8.8, 22.0, 46.8, approximately 100, and 100 μM, respectively. The relative selective cytotoxicities (the ratios of the latter IC_{50} values divided by the former IC_{50} values) were highest for nimodipine (25.5) and benznidazole (58.8). When HeLa cells were replaced with Swiss 3T3 cells in our in vitro infection system, the IC_{50} value for nimodipine was extremely low (8.3 nM), yielding a selective cytotoxicity of 5,750, approximately 40-fold greater that that for benznidazole. This seems to be related to the different origin of these host cells, that is, HeLa was established as a cancer cell line, while Swiss 3T3 originated from normal mouse fibroblasts. However, further studies are needed before any such conclusion can be reached. The results again indicated that nimodipine is the most potent dihydropyridine against *T. cruzi* infection and proliferation in mammalian cells in vitro, with relatively low cytotoxicity to the host cells.

DISCUSSION

Previous studies by Nunez-Vergara et al. and by Morris et al. [10,13,14] showed that, in *T. cruzi* epimastigotes, verapamil (the prototype diphenylalkylamine) inhibited the uptake of Ca^{2+} but did not inhibit the protozoan growth in vitro; nifedipine (the prototype dihydropyridine) and diltiazem (the prototype benzothiazepine) also exerted no inhibitory effect on the parasite proliferation. In an apparently related finding, verapamil reportedly exerted a cardioprotective effect and ameliorated experimental murine Chagas' disease [8,9]. However, further searches for an anti-*T. cruzi* calcium antagonist that brings about protozoan growth inhibition have not been successful, probably, at least in part, because of a lack of a suitable screening system.

On the basis of the recent, rapid development of calcium channel blockers in cardiovascular and cerebrovascular disorders [15-20], we attempted to examine the anti-*T. cruzi* activities of various calcium antagonists, particularly the second and third generation dihydropyridines, using an in vitro infection system of *T. cruzi* and HeLa cells [4]. To our knowledge, this is the first report that nimodipine, a second generation dihydropyridine calcium antagonist widely used for treatment for cerebrovascular disorders, is the strongest inhibitor against *T. cruzi* infection and proliferation in mammalian cells in vitro, with relatively low cytotoxicity to the host cells (Figs. 2 and 4; Table 1). Likewise, benznidazole, used in this study as a positive control, strongly inhibited the parasite infection and growth in HeLa cells, but, unexpectedly, it caused abnormal enlargement of nuclei and cytoplasm of the host cells (Fig. 3), a possible indicator of cytotoxicity in an in vitro culture [21] consistent with the statistically evaluated difference between the nimodipine- and benznidazole-treated-cell areas.

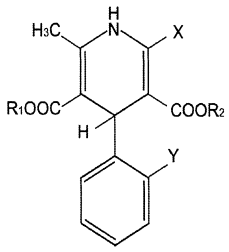
Five dihydropyridines, i.e. nifedipine, nicardipine, nimodipine, nisoldipine, and nitrendipine, possess a common structural formula (Fig. 5) [19,20]. Interestingly, highly anti-trypanosomal but cytotoxic nicardipine bears $(CH_2)_2N(CH_3)CH_2$-phenyl group as the R_2 group in Fig. 5A, while nimodipine, the strongest anti-*T. cruzi* calcium antagonist with the lowest cytotoxicity, has $(CH_2)_2OCH_3$ at the same position. It is surprising that these differences might be behind the great cytotoxicity or almost no cytotoxicity to the host HeLa cells. The second and third generation calcium antagonists act more slowly than the prototype cal-

Table 1. Effect of varying concentrations of calcium antagonists on the rate of *Trypanosoma cruzi* infection of mammalian cells and on the growth of host cells

Host cells	Compound added	IC_{50} values (μM)	
		Rate of infection of host cells	Growth of host cells
HeLa cells			
	Nifedipine	22.6±4.4	51.7±7.7
	Nicardipine	2.5±0.4	10.0±0.8
	Nimodipine	2.6±0.5	66.2±13.9
	Amlodipine	1.3±0.5	8.8±0.6
	Verapamil	2.1±0.9	22.0±6.0
	Diltiazem	42.8±25.2	46.8±10.2
	Allopurinol	18.2±2.3	100*
	Benznidazole	1.7±0.4	100*
Swiss 3T3 cells			
	Nimodipine	0.0083±0.010	47.7±2.5
	Benznidazole	1.9±0.21	296.7±32.1

Values are the means±1SD of 3 separate determinations. *Approximate average values of 3 separate determinations.

A



B

Dihydropyridine	X	R_1	R_2	Y
Nifedipine	CH_3	CH_3	CH_3	NO_2
Nicardipine	CH_3	CH_3	$(CH_2)_2N(CH_3)CH_2Ph$	NO_2
Nimodipine	CH_3	$CH(CH_3)_2$	$(CH_2)_2OCH_3$	NO_2
Nisoldipine	CH_3	CH_3	$CH_2CH(CH_3)_2$	NO_2
Nitrendipine	CH_3	(CH_3)	CH_2CH_3	NO_2
Amlodipine	$CH_2O(CH_2)_2NH_2$	CH_3	CH_2CH_3	Cl

Figure 5. Structural formulas of various dihydropyridines. **A**, the common structural fomula of dihydropyridines. Chemical structure of nifedipine, the prototype dihydropyridine, is 1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester. **B**, Structural differences of side chain groups in dihydropyridines. Note that nicardipine and nimodipine possess $-CH_2CH_2N(CH_3)CHPh$ (phenyl group) and $-CH_2CH_2OCH_3$, respectively, in place of a methyl group of the two methyl esters of nifedipine. These differences may have caused a marked cytotoxicity or almost no toxicity on the host HeLa cells. Amlodipine, a third generation dihydropyridine calcium antagonist, bears distinct side chain groups.

cium channel blockers [15-17, 19,20]. This is probably expressed as slowly acting nimodipine in a time-course experiment (Fig. 1A). It may be worthwhile, therefore, to examine the therapeutic efficacies of the slow acting nimodipine and other dihydropyridines against the chronic stage of murine model Chagas' disease.

In this study, we measured the anti-*T. cruzi* activities of various calcium channel blockers of dihydropyridine, diphenylalkylamine, and benzothiazepine classes. The target of these compounds is the L-type calcium channel [15, 19, 20, 22]. It was reported, however, that HeLa cells and Swiss 3T3 fibrobrasts appeared to carry no L-type channel [23,24], with no report concerning the L-type channel in HT 1080 cells. This is consistent with the IC_{50} value for nimodipine, which is as high as 2 μM, in the inhibition of the host-cell-infection rate (Table 1), a value that may not be low enough to claim the presence of a high affinity L-type channel [15,19,20]. Whether other types of calcium channels and/or calcium binding proteins are the possible targets of the calcium antagonists, especially nimodipine, used in the present study is an important topic for future research. Another important question is whether the key target of nimodipine exists in the mammalian host cells or in the *T. cruzi* trypomastigotes. Studies are underway using the parasites pre-incubated with nimodipine.

ACKNOWLEDGMENTS

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A LIST OF AND KEYS TO BLACK FLIES (DIPTERA: SIMULIIDAE) IN THAILAND

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Abstract: Forty-five known species of *Simulium* Latreille s. l. in Thailand are listed, and keys to subgenera and species within each subgenus are provided for adults, pupae and mature larvae.
Key words: *Simulium*, black fly, Thailand, key, identification

Takaoka and Suzuki [1] provided the first keys to identify all the 19 species of *Simulium* Latreille s. l. so far recorded from Thailand. During the last two decades, the number of species newly described or recorded from this country has increased dramatically to 45 [2]-[9]. New keys to identify all the known species are essential for further taxonomic and ecological studies on Simuliidae in Thailand.

We present a list of the species of *Simulium* s. l. and keys to subgenera and species for adult females, males, pupae and mature larvae. The definitions of the subgenera and species-groups refer to those of Takaoka [10], and terms of morphological features used in the keys follow Takaoka [10].

LIST OF THE SPECIES OF SIMULIIDAE IN THAILAND

Genus *Simulium* Latreille s. l.

Subgenus *Daviesellum* Takaoka and Adler

1) *courtneyi* Takaoka and Adler, 1997
2) *pahangense* Takaoka and Davies, 1995

Subgenus *Gomphostilbia* Enderlein

(A) *batoense* species-group
3) *angulistylum* Takaoka and Davies, 1995
4) *decuplum* Takaoka and Davies, 1995
5) *dentistylum* Takaoka and Davies, 1995
6) *gombakense* Takaoka and Davies, 1995
7) *parahiyangum* Takaoka and Sigit, 1992
8) *siamense* Takaoka and Suzuki, 1984

(B) *ceylonicum* species-group
9) *asakoae* Takaoka and Davies, 1995
10) *inthanonense* Takaoka and Suzuki, 1984
11) *sheilae* Takaoka and Davies, 1995

(C) *varicorne* species-group
12) *burtoni* Takaoka and Davies, 1995
13) *chumpornense* Takaoka and Kuvangkadilok, 2000

Subgenus *Montisimulium* Rubtsov

14) sp. G

Subgenus *Nevermannia* Enderlein

(A) *feuerborni* species-group
15) *feuerborni* Edwards, 1934

(B) *ruficorne* species-group
16) *aureohirtum* Brunetti, 1911

(C) *vernum* species-group
17) *caudisclerum* Takaoka and Davies, 1995

Subgenus *Simulium* Latreille s. str.

(A) *griseifrons* species-group
18) *choochotei* Takaoka, 2002
19) *digrammicum* Edwards, 1928
20) *grossifilum* Takaoka and Davies, 1995
21) *maenoi* Takaoka and Choochote, 2002
22) *nigrogilvum* Summers, 1911
23) *rudnicki* Takaoka and Davies, 1995
24) *suchariti* Takaoka and Choochote, 2004
25) *yongi* Takaoka and Davies, 1997

(B) *malyschevi* species-group
26) *siripoomense* Takaoka and Saito, 1996

(C) *multistriatum* species-group
27) *chainarongi* Kuvangkadilok and Takaoka, 1999
28) *chaliowae* Takaoka and Boonkemtong, 1999
29) *fenestratum* Edwards, 1934
30) *malayense* Takaoka and Davies, 1995
31) *triglobus* Takaoka and Kuvangkadilok, 1999

(D) *nobile* species-group
32) *nobile* De Meijere, 1907
33) *nodosum* Puri, 1933

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(E) *striatum* species-group
34) *chiangmaiense* Takaoka and Suzuki, 1984
35) *nakhonense* Takaoka and Suzuki, 1984
36) *quinquestriatum* (Shiraki, 1935)
37) *thailandicum* Takaoka and Suzuki, 1984
(F) *tuberosum* species-group
38) *brevipar* Takaoka and Davies, 1995
39) *rufibasis* Brunetti, 1911
40) *setsukoae* Takaoka and Choochote, 2004
41) *tani* Takaoka and Davies, 1995
42) *weji* Takaoka, 2001
(G) *variegatum* species-group
43) *barnesi* Takaoka and Suzuki, 1984
44) *chamlongi* Takaoka and Suzuki, 1984
(H) *Simulium* s. str. unplaced to group
45) *baimaii* Kuvangkadilok and Takaoka, 1999

Notes:

＊1. *Simulium* (*Montisimulium*) sp. G is known only from larval specimens collected at Ang Ka, Doi Inthanon National Park [11].

＊2. *Simulium* (*Gomphostilbia*) *burtoni* is newly recorded based on three adult females collected on a human attractant at Tambol Ban Laung, Doi Inthanon National Park, in January and February 2004, by W. Choochote, and a female specimen emerged from the pupa collected at Hauy Mor, Chiang Mai Province, in June 2001, by W. Choochote and H. Takaoka. Identification of *S. burtoni* is tentative because adult female specimens of *S. burtoni* collected from northern Thailand seem to differ from the original description [12] in that they have a shiny fifth tergite and dark subbasal spot on the hind tibiae. However, our reexamination of the type female specimen shows that the fifth tergite of *S. burtoni* is shiny (it was wrongly noted to be dull in the original description). It is also shown that there appears to be no dark subbasal spot on the hind tibiae of the type specimen, as described in the original description. However, it is difficult to conclude that its hind tibiae lack the dark subbasal spot since the type specimen was dried and pinned soon after it had emerged from the pupa, and then preserved in 70% ethanol. It is possible that the dark subbasal spot was as yet undeveloped in the freshly emerged adult or that it faded in alcohol. Additional adult specimens of *S. burtoni* from the type locality of Malaysia are needed to solve this problem.

＊3. *Simulium* (*Simulium*) sp. D, reported from six females collected while biting on human attractant in Doi Inthanon National Park by Takaoka and Suzuki [1], was described as a new species, *S.* (*S.*) *setsukoae*, by Takaoka and Choochote [9].

＊4. *Simulium* (*Simulium*) sp. E, which shows a much paler coloration of the female legs than *S.* (*S.*) *rufibasis* [1], is tentatively included in *S.*(*S.*) *rufibasis*, since apart from the female leg coloration, there is no morophological difference in the female, male and pupal stages between *S.* (*S.*) sp. E and *S.* (*S.*) *rufibasis*.

＊5. *Simulium baimaii*, left unassigned to any subgenus by Takaoka and Kuvangkadilok [4], is treated under the subgenus *Simulium* s. str. for convenience' sake.

KEYS TO THE SUBGENERA OF *SIMULIUM* S. L. IN THAILAND

ADULT FEMALES＊

1. Katepisternum haired……*Gomphostilbia*
   Katepisternum bare……2
2. Claw with a large basal tooth……*Nevermannia*
   Claw simple or with a small subbasal tooth……3
3. Paraproct with a cluster of dark spines……*Daviesellum*
   Paraproct without any dark spine……*Simulium* s. str.

(*The female of *Montisimulium* is not included because that of *S.* (*M.*) sp. G is unknown)

ADULT MALES＊

1. Katepisternum haired……*Gomphostilbia*
   Katepisternum bare……2
2. Coxite longer than style……*Nevermannia*
   Coxite shorter than style……3
3. Coxite much longer than wide……*Daviesellum*
   Coxite as long as, or slightly shorter than, wide……*Simulium* s. str.

(*The male of *Montisimulium* is not included because that of *S.* (*M.*) sp. G is unknown)

PUPAE＊

1. Grapnel-like hooklets present on the last abdominal segment……*Gomphostilbia*
   Grapnel-like hooklets absent on the last abdominal segment……2
2. Cocoon with an anterodorsal projection……*Nevermannia*
   Cocoon without any anterodorsal projection……3
3. Dorsal surface of abdominal segment 2 with stout hooks similar in size to those on abdominal segments 3 and 4……*Daviesellum*
   Dorsal surface of abdominal segment 2 with spines (in place of such distinct hooks) much smaller than hooks on abdominal segments 3 and 4……*Simulium* s. str.

(*The pupa of *Montisimulium* is not included because that of *S.* (*M.*) sp. G is unknown)

MATURE LARVAE

1. Hypostomium very wide, with 13 apical teeth·····*Daviesellum*
   Hypostomium of moderate width, with 9 apical teeth····2
2. Last abdominal segment lacks ventral papillae or with small ones·····*Simulium* s. str.
   Last abdominal segment with large ventral papillae·····3
3. Postgenal cleft vestigeal·····*Montisimulium*
   Postgenal cleft distinctly formed·····4
4. Lateral margin of hypostomium serrated·····*Nevermannia*
   Lateral margin of hypostomium smooth·····*Gomphostilbia*

KEYS TO THE SPECIES OF THE SUBGENUS *DAVIESELLUM*

ADULT FEMALES

1. Genital fork with a distinct projection directed anteriorly·····*courtneyi*
   Genital fork without such a projection·····*pahangense*

ADULT MALES

1. Ventral plate much longer than wide when viewed ventrally·····*courtneyi*
   Ventral plate much shorter than wide when viewed ventrally·····*pahangense*

PUPAE

1. Frons densely covered with tubercles·····*courtneyi*
   Frons mostly bare (though narrow portion near the lower margin tuberculate)·····*pahangense*

MATURE LARVAE

1. Postgenal cleft long, but its apex not reaching the posterior margin of hypostomium·····*courtneyi*
   Postgenal cleft long, its apex reaching the posterior margin of hypostomium·····*pahangense*

KEYS TO THE SPECIES OF THE SUBGENUS *GOMPHOSTILBIA*

ADULT FEMALES*

1. Antenna composed of 10 segments·····2
   Antenna composed of 11 segments·····3
2. Tergite 5 shiny·····*burtoni*
   Tergite 5 not shiny·····*chumpornense*
3. Mid and hind femora almost yellow except apical cap dark·····*dentistylum*
   Mid and hind femora alomst dark except base pale·····4
4. Hind tibia mostly dark·····*siamense*
   Hind tibia whitish yellow or dark yellow on basal 2/5 or more·····5
5. Hind tibia with a dark subbasal spot·····6
   Hind tibia without such a dark subbasal spot·····8
6. Sensory vesicle of medium size, 0.3 times as long as 3rd maxillary palpal segment·····*angulistylum*
   Sensory vesicle enlarged, 0.5 or 0.6 times as long as 3rd maxillary palpal segment·····7
7. Arms of genital fork with a short projection directed anteriorly·····*decuplum*
   Arms of genital fork without such a projection·····*parahiyangum*
8. Hind tibia whitish yellow on basal 2/3; sensory vesicle of moderate size, 0.3 times as long as 3rd maxillary palpal segment·····*asakoae*
   Hind tibia whitish yellow on basal 2/5 or 1/2; sensory vesicle enlarged, 0.57 or 0.68 times as long as 3rd maxillary palpal segment·····9
9. Frons-head ratio 1.0 : 4.8: sensory vesicle enlarged, 0.57 times as long as 3rd maxillary palpal segment·····*inthanonense*
   Frons-head ratio 1.0 : 5.7; sensory vesicle enlarged, 0.68 times as long as 3rd maxillary palpal segment·····*sheilae*

(*The female of *S. gombakense*, which is unknown, is not included)

ADULT MALES

1. Antenna composed of 10 segments·····2
   Antenna composed of 11 segments·····3
2. Ventral plate produced ventrally·····*burtoni*
   Ventral plate nearly flat·····*chumpornense*
3. Hind basitarsus enlarged·····4
   Hind basitarsus slender, much thinner than hind tibia····6
4. Eye with 16 horizontal rows of large facets·····*inthanonense*
   Eye with 13 horizontal rows of large facets·····5
5. Hind tibia mostly medium brown to brownish black with basal 1/3 or a little less somewhat pale·····*sheilae*
   Hind tibia whitish on a little less than basal 1/2 and brownish black on the rest·····*asakoae*
6. Mid and hind femora yellow except apical cap dark·····*dentistylum*
   Mid and hind femora almost dark except base pale·····7
7. Hind tibia almost dark except base pale·····8
   Hind tibia whitish on basal 1/2 or more·····10
8. Abdominal segments 2, 5, 6 and 7 each with a dorso-

lateral pair of shiny whitish-pruinose patches……*siamense*

Abdominal segments 2, 6 and 7 each with a dorsolateral pair of shiny whitish-pruinose patches……9

9. Upper eye with 12 or 13 vertical columns and 15 horizontal rows of large facets……*decuplum*

Upper eye with 17 vertical columns and 15 horizontal rows of large facets……*parahiyangum*

10. Hind tibia whitish on basal 2/3, with a dark subbasal spot……*angulistylum*

Hind tibia whitish yellow on basal 1/2, without such a dark subbasal spot……*gombakense*

PUPAE

1. Gill of much inflated structure with 2 triplet groups of finger-like projections and 8 slender filaments……*gombakense*

Gill with 8 or 10 filaments……2

2. Gill with 10 filaments……*decuplum*

Gill with 8 filaments……3

3. Gill filaments all shorter than pupal body (shorter than 1.6 mm), short-stalked……4

Gill filaments subequal to, or longer than, pupal body (longer than 1.9 mm), moderately-stalked……7

4. Antennal sheath smooth……5

Antennal sheath with tubercles……6

5. Gill filaments arranged in 2 groups, i.e.1dorsal (4 individual and 2 paired filaments) and 1ventral (2 paired filaments)……*chumpornense*

Gill filaments arranged in 3 groups (3+3+2 filaments from dorsal to ventral)……*angulistylum*

6. Antennal sheath with marked ridges each corresponding to flagellar segments 1–9, each ridge covered with several tubercles; gill filaments arranged in 3 groups (3+3+2 filaments from dorsal to ventral)……*parahiyangum*

Antennal sheath with less marked ridges corresponding to flagellar segments 1–9, each ridge covered with a few tubercles; gill filaments arranged in 4 groups (2+1+3+2 filaments from dorsal to ventral)……*dentistylum*

7. Dorsal and middle triplet groups and ventral pair group arising basally at the same level……*siamense*

Dorsal and middle triplet groups sharing a short stalk, which arises, with a stalk of ventral pair group, from short common basal stalk……8

8. Dorsal and middle triplet groups consisting of 1 individual filament and 2 paired filaments with a very long stalk……*burtoni*

Dorsal and middle triplet groups consisting of 1 individual filament and 2 paired filaments with a short or medium-long stalk……9

9. Cocoon with a distinct anterodorsal projection……*inthanonense*

Cocoon with an anterodorsal bulge……10

10. Terminal hooks weakly undulate on outer margin……*sheilae*

Terminal hooks weakly serrate on outer margin……*asakoae*

MATURE LARVAE

1. Thoracic segment 3 and abdominal segments 1–5 each with 1 or 2 pairs of dorsal and/or dorsolateral protuberances……2

Thoracic segment 3 and abdominal segments 1–5 without any dorsal or dorsolateral protuberance……3

2. Postgenal cleft very long, its apex reaching the posterior margin of hypostomium; thorax and abdomen densely covered with dark spines of various sizes……*parahiyangum*

Postgenal cleft long, but its apex not reaching the posterior margin of hypostomium; thorax and abdomen sparsely or moderately covered with minute setae……*chumpornense*

3. Postgenal cleft very long, its apex nearly or completely reaching the posterior margin of hypostomium……4

Postgenal cleft otherwise……5

4. Abdomen markedly constricted between segments 4 and 5……*dentistylum*

Abdomen not constricted between segments 4 and 5……*sheilae*

5. Postgenal cleft short, much shorter than postgenal bridge……*inthanonense*

Postgenal cleft longer than postgenal bridge……6

6. Abdomen almost bare except the last segment; pharate pupal gill of inflated structure……*gombakense*

Abdomen moderately or densely covered with simple or branched dark spinous setae or spinules dorsally on segments 5–8; pharate pupal gill filamentous……7

7. Abdomen moderately covered with simple minute dark setae dorsally on segments 5–8……*asakoae*

Abdomen moderately or densely covered with branched dark spinous setae or spinules dorsally on segments 5–8……8

8. Thoracic cuticle moderately covered with minute dark spinules with 7–11 branches dorsally; pharate pupal gill with 10 filaments……*decuplum*

Thoracic cuticle almost bare dorsally; pharate pupal gill with 8 filaments……9

9. Minute dark spinous setae on abdominal segments 5–8 somewhat flat and stout basally, with short branches apically……*siamense*

Minute dark spinous setae on abdominal segments 5–8 slender and hair-like basally, with long branches……10

10. Minute dark spinous setae on abdominal segments 5–8 with 0–5 branches……*burtoni*
Minute dark spinous setae on abdominal segments 5–8 with 5–12 (mostly 8–10) branches……*angulistylum*

KEYS TO THE SPECIES OF THE SUBGENUS *NEVERMANNIA*

ADULT FEMALES

1. Antenna yellow with at least 1st flagellar segment darkened; hind tibia yellow on basal 1/2, dark on apical 1/2, and with subbasal dark ring……*aureohirtum*
Antenna almost all darkened or mostly so except a few basal segments pale; hind tibia nearly all brown……2

2. Scutum reddish brown in ground color, with 3 dark longitudinal vittae……*feuerborni*
Scutum brownish black in ground color, without any longitudinal vitta……*caudisclerum*

ADULT MALES

1. Antenna yellow or yellowish brown with 1st flagellar segment darkened; hind basitarsus slender, parallel-sided, much narrower than hind tibia……*aureohirtum*
Antenna almost all darkened; hind basitarsus inflated, its greatest width nearly as wide as that of hind tibia……2

2. Scutum whitish pruinose with 3 dark longitudinal vittae at certain angle of light; paramere with 3 or 4 parameral hooks……*feuerborni*
Scutum whitish pruinose without any longitudinal vitta; paramere with a single parameral hook……*caudisclerum*

PUPAE

1. Gill with 4 filaments……*caudisclerum*
Gill with 6 filaments……2

2. All filaments extending forwards close together, and 2 ventral filaments with rather long stalk……*feuerborni*
All filaments diverging widely from the base, and 2 ventral paired filaments with short stalk……*aureohirtum*

MATURE LARVAE

1. Abdomen with distinct reddish-brown markings dorsally……*feuerborni*
Abdomen without any distinct colored marking dorsally……2

2. Abdomen with accessory sclerite ventrolaterally on each side of the last segment; mandibular serrations composed of 1 well-developed and 1 small teeth and with supernumerary serrations……*caudisclerum*
Abdomen without any accessory sclerite; mandibular serrations composed of 2 well-developed teeth and without supernumerary serrations……*aureohirtum*

KEYS TO THE SPECIES OF THE SUBGENUS *SIMULIUM* S. STR.

ADULT FEMALES*

1. Claw with a small subbasal tooth……2
Claw without any tooth……6

2. Basal section of radial vein fully haired; fore basitarsus with thick dorsal hair crest……*nigrogilvum*
Basal section of radial vein bare; fore basitarsus with moderate dorsal hair crest……3

3. Mid and hind femora mostly yellowish……*chamlongi*
Mid and hind femora mostly brownish……4

4. Scutum densely covered with yellow hairs……*barnesi*
Scutum moderately covered with brassy hairs……5

5. Antenna brownish black with scape, pedicel and base of 1st flagellar segment yellow; abdominal segment 7 with branched hairs medially on ventral surface……*siripoomense*
Antenna yellow or tawny with 2 apical segments blackish; abdominal segment 7 with simple hairs on ventral surface……*nobile*

6. Scutum with distinct longitudinal vittae……7
Scutum without any vitta……17

7. Fore tibia medium to dark brown, without white area on outer surface; hind tibia dark brown to brownish black with base yellow; inner margin of ovipositor valve with ventrally produced round flap……*chiangmaiense*, *nakhonense* and *quinquestriatum*
Fore tibia with white shiny area on outer surface; hind tibia whitish or yellowish on basal 2/3 or more and dark brown to brownish black on the rest; inner margin of ovipositor valve without such a flap……8

8. Basal section of radial vein fully haired……9
Basal section of radial vein bare……11

9. Hind basitarsus whitish on basal 3/5 and dark brown on the rest; ovipositor valve rounded, covered with short setae, and with elongate internal projection……*grossifilum*
Hind basitarsus whitish on basal 1/2 and dark brown on the rest; ovipositor valve triangular, covered with long stout hairs, and without such an internal projection……10

10. Cibarium with a round short medial projection along posterior margin……*choochotei*

Cibarium with a narrow long medial projection along posterior margin·····*digrammicum*

11. Mid tibia white on basal 2/3 or 3/4 and dark brown or brownish black on the rest; cibarium with a tuberculate medial projection·····12

Mid tibia white on basal 4/5 or 5/6 and light to dark brown on the rest; cibarium with a smooth medial projection·····13

12. Cibarium with tubercles near the base of medial projection·····*suchariti*

Cibarium bare near the base of medial projection·····*maenoi*

13. All femora almost yellow; three spermathecae present·····*triglobus*

At least mid and hind femora mostly dark; one spermatheca present·····14

14. Hind basitarsus whitish yellow on a little more than basal 1/2 and dark brown on the rest·····*fenestratum*

Hind basitarsus whitish yellow on basal 3/5 and dark brown on the rest·····15

15. Sternite 8 with 10−16 long hairs on each side·····*malayense*

Sternite 8 with 23−34 long hairs on each side·····16

16. Mid femur blackish brown with basal 1/5 or 1/4 yellow·····*chaliowae*

Mid femur almost entirely dark brown·····*chainarongi*

17. Mid tarsal segments 2 and 3 entirely yellow·····*nodosum*

Mid tarsal segments 2 and 3 light brown to brownish black·····18

18. Mid femur entirely yellow·····*rudnicki*

Mid femur almost dark brown·····19

19. Abdominal segment 7 with a pair of clustered hairs ventrally·····20

Abdominal segment 7 without such a pair of clustered hairs ventrally·····22

20. A pair of clustered hairs on abdominal segment 7 short, subequal in length to those on the surrounding area·····*setsukoae*

A pair of clustered hairs on abdominal segment 7 much longer than those on the surrounding area·····21

21. Mid tibia almost light brown with base somewhat yellow·····*weji*

Mid tibia white on basal 1/2 or more and medium brown on the rest·····*rufibasis*

22. Hind basitarsus white on a little more than basal 1/2 and brownish black on the rest; scutum covered with yellowish hairs as well as dark ones·····*yongi*

Hind basitarsus white on basal 3/5 or more and brownish black on the rest; scutum covered with dark brown hairs only·····23

23. Sensory vesicle enlarged, 0.7 times as long as the 3rd maxillary palpal segment·····*tani*

Sensory vesicle medium-sized, 0.3 times as long as the 3rd maxillary palpal segment·····*brevipar*

(*The females of *S. baimaii* and *S. thailandicum,* which are unknown, are not included)

ADULT MALES*

1. Basal portion of radial vein fully haired·····2

Basal portion of radial vein bare·····4

2. Abdomen with a pair of shiny whitish-grey pruinose spots dorsally or dorsolaterally on segments 2, 5, 6 and 7; width of style nearly the same from base to apical tip when viewed ventrolaterally·····*choochotei*

Abdomen with a pair of shiny white pruinose spots dorsally or dorsolaterally on segments 2, 6 and 7; width of style becoming much narrower apically·····3

3. Fore basitarsus with thick dorsal hair crest; hind basitarsus nearly parallel-sided; ventral plate nearly quadrate, parallel-sided when viewed ventrally·····*nigrogilvum*

Fore basitarsus with moderate dorsal hair crest; hind basitarsus spindle-shaped; ventral plate gradually narrowed posteriorly when viewed ventrally·····*digrammicum*

4. Scutum broadly silvery pruinose with transverse, inverted-V-shaped, black band·····5

Scutum otherwise·····6

5. Ventral plate with a narrow body parallel-sided when viewed ventrally·····*nobile*

Ventral plate with a wide body broadened medially when viewed ventrally·····*nodosum*

6. Mid femur and tibia almost yellow·····*rudnicki*

Mid femur and tibia otherwise·····7

7. Mid tibia mostly dark (including posterior surface)·····8

Mid tibia whitish or yellowish (at least on posterior surface) on basal 1/2 or more and dark brown on the rest·····16

8. Fore tibia medium brown, with white shiny area on outer surface·····9

Fore tibia medium to dark brown, without white area on outer surface·····15

9. Hind basitarsus white or whitish yellow on basal 1/2 or less and dark brown to brownish black on the rest·····10

Hind basitarsus white or whitish yellow on more than basal 1/2 and light to dark brown on the rest·····13

10. Hind basitarsus somewhat enlarged, about 5.0 times as long as wide and much narrower than hind tibia·····*chamlongi*

Hind basitarsus much enlarged, 3.2−3.5 times as long as wide, and subequal to, or a little wider than, the greatest width of hind tibia·····11

11. Upper eye with 20 vertical columns of large facets····· ·····*setsukoae*
Upper eye with 15–17 vertical columns of large facets ·····12
12. Hind basitarsus whitish yellow on basal 1/3 and brownish black on the rest·····*rufibasis*
Hind basitarsus whitish yellow on basal 1/2 or a little less and brownish black on the rest·····*tani*
13. Hind basitarsus, narrow (similar to that of female), parallel-sided, white with apical 1/6 light brown····· ·····*siripoomense*
Hind basitarsus enlarged, wedge-shaped, whitish yellow on basal 3/5 or a little less and dark brown on the rest·····14
14. Abdominal segments 2, 5, 6 and 7 each with a pair of silvery iridescent spots dorsolaterally·····*weji*
Abdominal segments 2, 6 and 7 each with a pair of silvery iridescent spots dorsolaterally·····*brevipar*
15. Hind basitarsus whitish yellow on basal 1/2 or a little more or less and brownish black on the rest····· ·····*quinquestriatum*
Hind basitarsus whitish yellow on basal 3/5 and brownish black on apical 2/5····· ·····*chiangmaiense, nakhonense* and *thailandicum*
16. Hind basitarsus whitish on basal 1/2 or a little less and brownish black on the rest; body of ventral plate wider than long·····17
Hind basitarsus entirely light to dark brown, or so with basal 2/5 whitish yellow or dark yellow; body of ventral plate longer than wide·····18
17. Body of ventral plate about 1.8 times as wide as long, and much shorter than arms·····*yongi*
Body of ventral plate about 2.3 times as wide as long, and much longer than arms·····*grossifilum*
18. Hind basitarsus entirely light to dark brown·····19
Hind basitarsus brownish black with basal 2/5 whitish yellow or dark yellow·····20
19. Ventral plate subquadrate when viewed ventrally, and with distinct teeth on its posterior surface····*chaliowae*
Ventral plate gradually narrowed posteriorly when viewed ventrally, and without any tooth on its posterior surface·····*maenoi*
20. Upper eye with 20 vertical columns and 20 horizontal rows of large facets·····*chainarongi*
Upper eye with 15–17 vertical columns and 16 or 17 horizontal rows of large facets·····21
21. Upper eye with 15 vertical columns and 16 horizontal rows of large facets; ventral plate with 2 vertical rows of teeth nearly parallel-sided on its posterior surface ·····*malayense*
Upper eye with 17 vertical columns and 17 horizontal rows of large facets; ventral plate with teeth irregularly situated on its posterior surface·····*fenestratum*

(*The males of *S. baimaii*, *S. barnesi* and *S. suchariti*, which are unknown, are not included)

PUPAE

1. Gill of inflated form·····2
Gill filamentous·····4
2. Gill with 2 filaments arising from long inflated trunk ·····*baimaii*
Gill with 3 or 6 inflated tubes with rounded apex·····3
3. Gill with 3 inflated tubes·····*nodosum*
Gill with 6 inflated tubes with minute spines····· ·····*grossifilum*
4. Gill with 6 filaments·····5
Gill with 8 or 10 filaments·····19
5. Cocoon simple wall-pocket-shaped·····6
Cocoon shoe-shaped or boot-shaped·····16
6. Cocoon with an anterolateral window on each side·····7
Cocoon without lateral window·····10
7. Gill filaments subequal in length and thickness to one another·····8
Gill filaments decreased in thickness from dorsal to ventral·····9
8. Gill filaments short-stalked; dorsal spine-combs present on abdominal segments 7–9·····*siripoomense*
Gill filaments almost sessile; dorsal spine-combs present only on abdominal segment 8·····*rudnicki*
9. Cocoon with a small anterolateral window on each side; frons moderately covered with very large tubercles; terminal hooks present·····*maenoi*
Cocoon with a moderate anterolateral window on each side; frons densely covered with small and medium-sized tubercles; terminal hooks absent·····*suchariti*
10. Integuments of head and thorax bare (except posterior 1/2 of thorax with minute tubercles)·····11
Integuments of head and thorax moderately or densely covered with tubercles·····12
11. Cocoon very thin, transparent, and its anterior margin often not well defined; inner filament of the ventralmost pair narrowed basally; dorsal spine-combs present on abdominal segment 8; terminal hooks absent··· ·····*yongi*
Cocoon thickly woven, not transparent, and its anterior margin well defined; inner filament of the ventralmost pair not narrowed basally; dorsal spine-combs present on abdominal segments 7–9; terminal hooks present·····*chamlongi*
12. Terminal hooks present·····*tani*
Terminal hooks absent·····13
13. Gill filaments with short-stalked; dorsalmost filament

basally directed upward or forward, then curved forward or downward; abdominal segment 7 with spine-combs dorsally·····14
Gill filaments almost sessile; dorsalmost filament basally directed forward or downward; abdominal segment 7 without spine-combs dorsally·····15

14. Two filaments of the dorsal pair subequal in thickness to each other·····*rufibasis*
Dorsalmost filament of the dorsal pair much thicker than the counter filament·····*setsukoae*

15. Thoracic integument with pit-like organ at base of gill ·····*brevipar*
Thoracic integument without such organ at base of gill ·····*weji*

16. Cocoon loosely woven, with many small open spaces in webs·····*choochotei*
Cocoon tightly woven·····17

17. Cocoon with an anterolateral flap and a small anterolateral window on each side; terminal hooks present ·····*digrammicum*
Cocoon without such flap and window; terminal hooks absent·····18

18. Abdominal segment 8 with spine-combs dorsally····· ·····*nobile*
Abdominal segment 8 without any spine-comb dorsally·····*nigrogilvum*

19. Gill with 8 filaments·····20
Gill with 10 filaments·····25

20. Cocoon wall-pocket-shaped·····21
Cocoon shoe-shaped·····22

21. Cocoon with an anterolateral window on each side····· ·····*fenestratum*
Cocoon without any window·····*malayense*

22. Cocoon roughly woven anteriorly, leaving some large open spaces in webs of the anterior collar and many small open spaces near anterior margin·····23
Cocoon without open spaces in the webs·····24

23. Gill filaments subequal in thickness to one another····· ·····*triglobus*
Two ventralmost paired filaments much thinner than the others·····*chiangmaiense*

24. Basal portion of the dorsalmost filament about 1.4 times as thick as the ventralmost one·····*chaliowae*
Basal portion of the dorsalmost filament about twice as thick as the ventralmost one·····*chainarongi*

25. Gill filaments arranged in 2+3+3+2 filaments from dorsal to ventral; all filaments subequal in thickness to one another·····*quinquestriatum*
Gill filaments arranged in 2+2+2+2+2 filaments; 4 or 5 dorsal filaments slightly to markedly thicker than the others·····26

26. Head integument with round tubercles·····*nakhonense*
Head integument with angular tubercles····*thailandicum*

MATURE LARVAE*

1. Last abdominal segment with a distinct accessary sclerite ventrally·····*nigrogilvum*
Last abdominal segment without any accessary sclerite ·····2

2. Abdominal segments 1–5 (or up to 8) each with 1 or more pairs of protuberances dorsally or dorsolateraly ·····3
Abdominal segments lacking protuberances·····9

3. Abdominal segments 1–8 each with 1–6 pairs of protuberances dorsally and dorsolatelly·····*siripoomense*
Abdominal segments 1–5 (or up to 8) each with a pair of protuberances dorsally or dorsolaterally·····4

4. Postgenal cleft very long, its apex reaching the posterior border of hypostomium·····5
Postgenal cleft very long, but its apex not reaching the posterior border of hypostomium·····6

5. Pharate pupal gill with 6 filaments·····*nobile*
Pharate pupal gill with 3 filaments·····*nodosum*

6. Pharate pupal gill with 8 filaments·····7
Pharate pupal gill with 10 filaments·····8

7. Abdominal segments 1–6 each with a pair of protuberances dorsally·····*chainarongi*
Abdominal segments 1–8 each with a pair of protuberances dorsally·····*chiangmaiense*

8. Pharate pupal gill with 10 filaments arranged in 2+3+3 +2 filaments·····*quinquestriatum*
Pharate pupal gill with 10 filaments arranged in 2+2+2 +2+2 filaments·····*nakhonense*

9. Postgenal cleft moderately widened medially, its greatest width much larger than the width at base·····10
Postgenal cleft not widened or slightly so medially, its greatest width subequal to, or slightly larger than, the width at base·····11

10. Pharate pupal gill with 6 filaments·····*chamlongi*
Pharate pupal gill with 8 filaments····· ·····*fensestratum* and *malayense*

11. Abdomen with its greatest width on segment 8·····12
Abdomen with its greatest width on segment 6 or 7····· ·····14

12. Cephalic apotome mostly pale yellow·····*yongi*
Cephalic apotome mostly light to medium brown·····13

13. Posterior circlet with 144–160 rows of hooklets····· ·····*rudnicki*
Posterior circlet with ca. 210 rows of hooklets····· ·····*choochotei*

14. Body longer than 7.0 mm·····*sucharitii*
Body shorter than 7.0 mm·····15

15. Postgenal cleft widely rounded apically·····16
    Postgenal cleft nearly pointed apically·····19
16. Pharate pupal gill with 2 filaments·····*baimaii*
    Pharate pupal gill with 6 or 8 filaments·····17
17. Pharate pupal gill with 8 filaments·····*triglobus*
    Pharate pupal gill with 6 filaments·····18
18. Body length 5.9–6.4 mm; elongate spot on each side of postgenal cleft positive; each lobe of rectal organ with 8–12 finger-like secondary lobules·····*maenoi*
    Body length 5.0–5.5 mm; elongate spot on each side of postgenal cleft negative; each lobe of rectal organ with 14–18 finger-like secondary lobules·····*digrammicum*
19. Postgenal cleft of medium size, nearly as long as wide; pharate pupal gill with 6 inflated tubular filaments·····*grossifilum*
    Postgenal cleft long, much longer than wide; pharate pupal gill with 6 thread-like filaments·····20
20. Cephalic apotome yellow, with dark area medially just in front of posterior margin·····21
    Cephalic apotome pale yellow to pale brown, without dark area medially just in front of posterior margin·····22
21. Body color reddish brown; dorsal pair of pharate pupal gill filaments subequal in thickness to each other·····*rufibasis*
    Body color dark grey to greyish black; one of dorsal pair of pharate pupal gill filaments much thicker than the counter filament·····*setsukoae*
22. Each lobe of rectal organ with 14–16 finger-like secondary lobules; posterior circlet with ca. 86 rows of hooklets with up to 17 hooklets per row·····*weji*
    Each lobe of rectal organ with 7–12 finger-like secondary lobules; posterior circlet with 70–74 rows of hooklets with up to 12 or 14 hooklets per row·····23
23. Body color reddish brown·····*tani*
    Body color dark grey·····*brevipar*

(*The mature larvae of *S. barnesi*, *S. chaliowae* and *S. thailandicum*, which are unknown, are not included)

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THE USE OF TRAVEL VACCINES BY JAPANESE EXPATRIATES IN DEVELOPING COUNTRIES

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Abstract: From 1998 to 2001, using questionnaires, we surveyed the use of travel vaccines among Japanese expatriates in developing countries. The percentage of those using more than one type of travel vaccine before departure increased significantly (45.6% in 1998 to 53.4% in 2001 ($p<0.001$)). In regions such as tropical Africa and South Asia, vaccination rates were high. But the increase was most noticeable in East Asia, the Middle East, and Latin America. Vaccinations against hepatitis A, hepatitis B, and tetanus were high throughout the developing countries. Vaccinations against yellow fever and Japanese encephalitis were high in endemic regions. Vaccination rates were slightly higher for typhoid fever in South Asia and tropical Africa than that in other areas. Vaccination rates for cholera, however, showed yearly declines. These trends seem to reflect a growing awareness among expatriates of the benefits of travel vaccines. Even so, nearly half of those living the countries have not received sufficient vaccination, indicating a need for further education.

INTRODUCTION

Global business has resulted in an increase in the number of Japanese living abroad long-term. According to statistics from the Ministry of Foreign Affairs, the number of Japanese expatriates in 2002 was 590,000 people, approximately three times as many as in 1980. Of these, about 200,000 people live in developing countries, and are at risk of various local infectious diseases [1].

In order to lower the risk of infectious diseases overseas, travel vaccine can be administered. However, vaccination rates tend to be low among Japanese expatriates. According to research by Basnyat from 1997 to 1998 in Katmandu, 95% of Japanese travelers were not vaccinated against hepatitis A or typhoid fever [2]. Among non-Japanese people, 90% had been vaccinated against these diseases. Therefore, we developed a questionnaire-based investigation to further clarify the situation.

METHODS

Each year, we perform a health consultation service for Japanese expatriates residing in urban areas of developing countries. The subjects of this survey were Japanese expa-

Table 1. Number of Japanese who responded to the questionnaire each year

Area	Countries	1998	1999	2000	2001
Total		3061	2895	2982	2755
East Asia	China	591	490	466	382
South East Asia	Indonesia, Malaysia, Myanmar Philippines, Thailand, Vietnam	917	823	958	878
South Asia	Bangladesh, India, Nepal, Pakistan Sri Lanka	496	513	521	442
The Middle East	Bahrain, Egypt, Iran, Morocco, Oman Qatar, Saudi Arabia, Turkey, UAE	539	549	530	587
Tropical Africa	Ethiopia, Ghana, Ivory Coast, Kenya Nigeria, Tanzania	196	170	146	180
Latin America	Colombia, Costa Rica, Ecuador Guatemala, Mexico. Puerto Rico Panama, Venezuela	322	350	361	286

Japan Labor Health and Welfare Organization, Japan Overseas Health Administration Center

triates who responded to the consultation from 1998 to 2001. The research was limited to those 16 years of age or older. Many of the subjects were employees sent from Japanese companies, and their families.

From 1998 to 2001, during the consultation, questionnaires were given to the subjects. On the questionnaire, the travel vaccines were listed, and the subjects were requested to note the vaccines they had received before departure. Table 1 shows the number of responses corresponding to the year they were received.

The list contained the following 10 vaccines: hepatitis A, hepatitis B, tetanus, rabies, yellow fever, Japanese encephalitis, cholera, typhoid fever, and meningococcal meningitis.

We used the chi-square method to analyze the data.

RESULTS

(1) Total vaccination rate

Table 2 shows the percentage of respondents who received one or more types of travel vaccine before departure. The vaccination rate elevated from 45.6% in 1998 to 53.4% by 2001. The value in 2001 is significantly higher than that in 1998 ($p<0.001$).

On a regional basis, in 1998, the vaccination rates in tropical Africa and South Asia were high and did not vary much during the period of this survey. On the other hand, in East Asia and the Middle East, the vaccination rate in 1998 was only about 30%. The subsequent years showed dramatic increases, and by 2001 both regions showed rates higher than 45%. In Latin America, the rate also rose from 44.7% in 1998 to 56.6% by 2001.

(2) Individual vaccines' vaccination rate

The vaccination rates of individual vaccines excluding cholera and meningococcal meningitis showed an increase from 1998 to 2001 (Table 3). In 2001, the rate for hepatitis A was the highest at 40.3%, hepatitis B and tetanus were in the 30% range, followed by rabies at 18.2%. The rate for cholera decreased, coming to 2.1% in 2001, and that for meningococcal meningitis did not change during the period.

All regions showed increases in rates for hepatitis A, hepatitis B, tetanus, and rabies (Table 4). In 2001, the rate for hepatitis A in tropical Africa was highest at 53.9% and lowest in the Middle East at 30.0%. The rate for hepatitis B was highest in tropical Africa at 50.6%, lowest in the Middle East at 22.7%. The rate for tetanus was highest in South Asia at 54.8% and lowest in East Asia at 22.5%. Rabies was highest in South Asia at 37.3% and lowest in East Asia at 3.9%.

For yellow fever, vaccination rates in endemic regions such as tropical Africa and Latin America were high. In tropical Africa, rates increased only slightly: from 79.1% in 1998 to 81.1% in 2001. In Latin America, rates increased more dramatically: from 15.2% in 1998 to 25.9% in 2001.

Vaccination rates were high for Japanese encephalitis

Table 2. Japanese who received one or more types of travel vaccine (1998 to 2001)

	1998 (%)	1999 (%)	2000 (%)	2001 (%)	P value (1998 vs 2001)
Total	45.6	48.3	49.2	53.4	<0.001
By area					
East Asia	31.6	34.5	35.2	46.6	<0.001
South East Asia	45.6	43.6	43.2	47.8	not significant
South Asia	64.9	67.1	66.0	65.6	not significant
The Middle East	28.9	37.0	40.8	45.0	<0.001
Tropical Africa	86.7	90.0	91.8	86.7	not significant
Latin America	44.7	48.6	54.0	56.6	<0.05

Table 3. Japanese who received travel vaccines (1998 to 2001)

	1998 (%)	1999 (%)	2000 (%)	2001 (%)	P value (1998 vs 2001)
Hepatitis A	31.0	35.0	36.3	40.3	<0.001
Hepatitis B	24.9	28.3	29.3	34.4	<0.001
Tetanus	29.8	34.1	33.9	38.0	<0.001
Rabies	11.9	16.5	16.5	18.2	<0.001
Yellow fever	7.7	7.9	8.4	9.5	<0.05
Japanese encephalitis	5.4	8.9	9.1	10.5	<0.001
Cholera	4.6	3.4	2.7	2.1	<0.001
Typhoid fever	0.9	1.5	2.4	3.6	<0.001
Meningococcal meningitis	2.0	1.2	1.1	1.9	not significant

Table 4. Comparison of vaccine type received by area in 1998 and 2001

		East Asia	South East Asia	South Asia	The Middle East	Tropical Africa	Latin America
Hepatitis A	1998	21.5	32.0	47.8	19.7	46.9	30.4
	2001	40.1***	37.2*	52.9	30.0***	53.9	42.7***
Hepatitis B	1998	17.1	29.3	37.1	12.2	40.8	18.9
	2001	32.5***	32.7	43.4*	22.7***	50.6	26.2*
Tetanus	1998	12.9	26.2	52.0	21.2	57.7	34.5
	2001	22.5***	33.1*	54.8	34.2***	54.4	44.8**
Rabies	1998	2.7	8.4	29.6	4.5	34.2	9.9
	2001	3.9	15.9***	37.3*	10.9***	35.6	18.9**
Yellow fever	1998	0.0	0.3	1.8	3.7	79.1	15.2
	2001	0.0	0.2	3.2	4.4	81.1	25.9**
Japanese encephalitis	1998	4.4	7.5	11.9	0.9	1.0	0.9
	2001	8.9*	14.2***	26.5***	1.0	1.1	1.4
Cholera	1998	2.9	5.6	5.6	1.3	14.8	2.5
	2001	1.0	2.5***	3.2	1.0	4.4***	1.7
Typhoid fever	1998	0.2	0.3	2.8	0.0	4.6	0.3
	2001	0.8	1.0	10.9***	0.9*	16.7***	1.0
Meningococcal meningitis	1998	0.2	0.1	0.6	1.3	25.0	0.3
	2001	0.0	0.0	0.7	2.2	20.6	0.0

P value of 1998 vs 2001: *<0.05 **<0.01 ***<0.001

in endemic regions such as East Asia, Southeast Asia, and South Asia. In these three areas, the rates showed significant increases from 1998 to 2001.

Vaccination rates for typhoid fever in 1998 were low in all areas, although 2001 saw slight increases to 16.7% in tropical Africa and 10.9% in South Asia. Vaccination rates for meningococcal meningitis were very low everywhere except for tropical Africa. Even in that region, there was no major change from 1998 to 2001.

In all regions, the vaccination rates for cholera decreased during the periods of this survey.

DISCUSSION

Infectious diseases are a major health issue for overseas tourists and expatriates in developing countries. For example, Steffen estimated that the infection rate for hepatitis A is 0.2% for people residing in a developing country for a month [1]. In an investigation by Ohara using members of Japan Overseas Cooperation Volunteers as subjects, the disease rate for1year reached 3.9% in 1979 [3]. In a subsequent investigation using the same subjects in 1980, the rate for hepatitis B was 1.09% per year. There have been no cases of rabies among Japanese expatriates since 1970. However, according to an investigation by Takayama, from 1990 to 1996, the number of subjects who visited a metropolitan hospital for post-exposure vaccination of rabies rose to 93 cases [4]. According to a report by the National Institute of Infectious Diseases, 44 cases of typhoid fever and 25 cases of cholera were reported in 2000. There have been no reports of yellow fever among Japanese travelers, but there have been cases among European and American travelers in endemic regions [5].

Travel vaccines are regarded as an effective means of preventing these diseases [6]. For travelers to developing countries, vaccination for hepatitis A, hepatitis B, tetanus, rabies, and typhoid fever are strongly recommended. Also, vaccinations are recommended for yellow fever in tropical Africa and South America and for Japanese encephalitis in Asia.

Since we know that the vaccination rate for Japanese travelers is extremely low [2], we conducted this survey on Japanese expatriates living in developing countries from 1998 to 2001. The results show that the percentage of people receiving one or more types of travel vaccine before departure increased from 45.6% in 1998 to 53.4% in 2001. The vaccination rates in tropical Africa and South Asia were high, but did not increase much from 1998 to 2001. On the other hand, the vaccination rates in East Asia and the Middle East, which had been low in 1998, increased dramatically, reaching over 45% by 2001.

Rates for individual vaccinations of hepatitis A, hepatitis B, and tetanus were high in all regions, while rates for yellow fever and Japanese encephalitis were high only in endemic regions.

During the period of this survey, vaccination rates of all vaccines excluding cholera and meningococcal meningitis increased. The rates of hepatitis A, hepatitis B, and tetanus dramatically increased in East Asia, the Middle East, and Latin America. The increase in rabies vaccination rate

was remarkable in Southeast Asia, the Middle East, and Latin America. Rates for yellow fever vaccinations peaked at 79.1% in tropical Africa in 1998, with almost no change after that. This is perhaps due to the fact that some of the tropical African countries demanded that travelers submit a yellow fever vaccination certificate [6]. Presently there is some doubt about the effectiveness of the cholera vaccine used in Japan, so it is possible that this caused the vaccination rates to decrease in all regions [7].

Vaccination rates for typhoid fever and meningococcal meningitis tended to be low on the whole. This was because these vaccines were not available in Japan [8]. But in 2001, the vaccination rate for typhoid fever in South Asia (10.9%) and tropical Africa (16.7%), and the rate for meningococcal meningitis in tropical Africa (20.6%) were higher than those in another areas. We assume that Japanese people living in highly endemic regions were able to find a way to receive vaccinations. For vaccines not marketed in Japan, it is still possible to receive a vaccination if a doctor privately imports the vaccines. We need to make this fact better known to Japanese medical personnel.

Looking at the overall picture, the increasing rates indicate a growing awareness among Japanese expatriates and medical personnel of the benefits of vaccination. However, nearly half of Japanese people residing in developing countries are not vaccinated. For short-term visitors, the vaccination rate is probably even lower. In a recent investigation by Kikuchi, which surveyed a Japanese tourist group visiting tropical Africa, the vaccination rate for yellow fever was high at 80% [9]. However, the rates of other vaccines such as hepatitis A and rabies were below 5%. An investigation on German tourists receiving hepatitis vaccine before going to developing countries showed a very high rate of 59% [10].

In summary, it is clear that despite some improvement, vaccination rates are still alarmingly low among Japanese travelers. The solution to this problem is better education and knowledge transfer, both to the travelers who should receive these vaccines, and to the medical personnel who consult with travelers and administer vaccinations.

ACKNOWLEDGMENT

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DETECTION RATES OF ROTAVIRUS ANTIGEN FROM DIARRHEAL PATIENTS IN LAO PEOPLE'S DEMOCRATIC REPUBLIC

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Abstracts: The detection rate of rotaviruses from diarrheal stools in Lao People's Democratic Republic (Lao PDR) was studied in the period from 1994 to 2003. Rotavirus antigen was detected using latex agglutination kit. The average detection rate was 2.4%, or 18 of 738 cases examined in total. Rotavirus was not detected from 175 cases examined in 1995, 1998, 2000 and 2003, but 8 of 85 cases (9.4%) examined in 1997 were positive for rotavirus. The detection rate was 6.0% in the age group younger than 2 years and 0.6% in the age group older than 2 years. These detection rates were markedly lower than those in neighboring countries such as Vietnam and Thailand.

Rotaviruses are a major cause of diarrhea in infants and young children. The infections are also common in adult. Rotavirus infections are frequently seen throughout the year in tropical areas, but they are predominant during the winter season in the temperate zone. On the basis of reported data, the detection rate of rotavirus from pediatric diarrhea is about 30% on average〔1-11〕. A high frequency of rotavirus infection has been reported in Vietnam and Thailand, countries neighboring Lao PDR. Some reports show that the detection rate of rotavirus in pediatric diarrhea was 50 to 60% in Vietnam and 30 to 40% in Thailand〔3,7,8〕. Although available from many other countries, no data on the detection frequency of rotavirus from diarrheal stools has been reported from Lao PDR. In the present paper, therefore, we present the detection rate of rotavirus from the stools of diarrheal patients in Lao PDR over the past decade.

Stool samples submitted to our Laboratory (Center for Laboratory and Epidemiology, Ministry of Health, Lao PDR) for analysis of enteropathogens from a number of hospitals and clinics in Vientiane were examined. The samples were collected from diarrheal patients in the period from 1994 to 2003. All samples were taken in plastic containers, and a total of 738 stool samples were examined. Rotavirus was detected using a commercially available latex agglutination kit (MICROGEN, Bioproducts LTD., England UK). Watery stools were directly treated with an equal volume of extraction buffer included in the kit. Muddy stools were first diluted double with normal saline solution and 100 μl of the diluent was treated with 100 μl of extraction buffer. The treated samples were centrifuged at 10,000 rpm for 5 min. A drop of the sensitized latex solution was placed at 3 places on the special sheet (black background) for the agglutination test. A drop of positive and negative control solution was added to the first and second latex, respectively, and a drop of stool supernatant was added to the third latex. Each sample was mixed well using a toothpick and then gently tilted several times. Agglutination was visually observed.

The detection rate of rotavirus from diarrheal stools was generally very low. Only 18 of 738 cases examined over the past 10 years were positive for rotavirus (2.4%). No positive case was found among the 175 cases examined in 1995, 1998, 2000 and 2003. The highest positive rate was observed in the 1997 samples, but this was still lower than 10% (Table 1). Rotavirus was mainly detected in the younger age group, a finding consistent with other reports. In the age group younger than 2 years, 15 of 250 cases examined (6.0%) were positive, but in age group older than 2 years, the positive rate was 0.6%, or 3 of 488, which was one-tenth that of the former group (Table 2). In spite of this fact, high infection rates such as 30 to 60% of pediatric diarrhea were reported in Vietnam and Thailand〔3,7,8〕 countries neighboring Lao PDR. Although the reason for the low infection rate in Lao PDR is unclear, population density, which is markedly lower in Lao PDR than Vietnam and Thailand, may influence the infection rate. If person to person infection is the main infection route of rotavirus,

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Table 1. Detection rate by year

Year	No. examined	No. positive (%)
1994	84	1 (1.1)
1995	67	0
1996	113	2 (1.8)
1997	85	8 (9.4)
1998	24	0
1999	32	1 (3.1)
2000	21	0
2001	84	1 (1.1)
2002	165	5 (3.0)
2003	63	0
Total	738	18 (2.4)

Table 2. Distribution by age group

Age group (year)	No. examined	No. positive (%)
1>	143	9 (6.2)
1-2	107	6 (5.6)
2-5	59	0
5-15	42	1 (2.3)
15<	387	2 (0.5)
Total	738	18(2.4)

population density may play an important role in the spreading of the disease. According to a report in 1994 〔4〕, rotavirus infection rate among children was 0.96% (16 of 1,668) in New Caledonia where the population density is lower than in Lao PDR. The detection method used in the present study may also have contributed to the low detection rate. A comparative study on the ability of commercially available ELISA kits and RPLA kits to detect rotavirus in stools revealed that the sensitivity was about 88% in ELISA and 70% in RPLA 〔12〕. The studies in Thailand and Vietnam used ELISA while the present study in Lao PDR used RPLA to detect rotavirus. Most samples in the present study were collected in the dry season, and there seems to be some seasonal variation on the detection rate. Rotavirus is antigenicaly classified into 5 groups, A to E. These groups have independent RNA. Type A rotavirus is the most prevalent in humans throughout the world, but infections with type B and C have also been reported 〔9,10〕. Therefore, non-type A rotavirus should also be considered when discussing the infections in Lao PDR.

The present paper briefly reported the distribution of rotavirus in Lao PDR summarizing the accumulated laboratory data. Since the epidemiological information from this country has been very limited, the data will contribute to discussions on the worldwide epidemiology of rotavirus infection.

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第44回日本熱帯医学会　第18回日本国際保健医療学会　合同大会

シンポジウム３　「熱帯感染症の臨床：途上国の実態と輸入感染症の動向」報告

日時：2003年10月11日（土）15：15～17：00
会場：北九州国際会議場　国際会議室

わが国における輸入感染症としての熱帯病
増田　剛太，大石　和徳

タイ国北部地域における HIV 陽性患者に合併した市中呼吸器感染症の臨床的検討
麻生　憲史

ラオス村落住民におけるタイ肝吸虫の頻度と超音波肝胆道所見
新里　敬，Bouakham Vanachone，Chantavilay，Rattanavong，仲宗根啓樹，宮城　啓，小林　潤

わが国におけるマラリア患者の動向
中村　哲也

デング熱の臨床と診断
吉田邦仁子

旅行者下痢症－横浜市立市民病院の症例を中心に－
足立　拓也

輸入感染症患者に対する臨床現場での初期対応
岩﨑惠美子

わが国における輸入感染症としての熱帯病

増田　剛太，　　大石　和徳
東京都立北療育医療センター，長崎大学熱帯医学研究所感染症予防治療分野

◆日本の国際化と海外旅行者数の増加

第二次世界大戦敗戦後の混乱期にはわが国にも様々な伝染性疾患（赤痢や腸チフス・パラチフスなどの腸管疾患。結核，ジフテリア，痘瘡，マラリア，日本脳炎など）が流行した。しかし，1955年の国連加盟，さらに，1964年の東京オリンピックを契機として，日本におけるその発生数は著しく減少し，今日に至っている。伝染性疾患減少の要因としては上下水道の整備や病原体媒介動物の制御などを初めとする衛生環境改善，各種ワクチンや抗菌薬による感染症予防・治療手段の進歩，伝染病予防法などによる防疫業務の遂行などが挙げられる。これに加え，日本が四面を海に囲まれた島国であり，従来は，これらの疾患がわが国へ簡単に侵入できなかったことが国内の感染症激減に大きな意味を持った。しかし，日本国内からこれらの伝染性疾患（その多くは今日では熱帯地方に常在する）が姿を消したことは，その後のわが国に「国内は清潔で安全であり，熱帯病や伝染性疾患は存在しない」という認識が定着する結果を招いた。

◆国際化の進行

その後も日本は経済大国化への道を直進し，1966年度には国民総生産（GNP）が世界第３位となり，昨今見られる日本人の海外旅行ブームの基礎を築いた。さらに，1970年に始まったジャンボジェット機航空網の整備は大量の旅行客を短時間内に遠隔地へ運搬することを可能にした。1980年以降にも日本人海外旅行者数は増加を続け，1990年に年間1000万人の大台を突破してからもなお増加し，2002年には1650余万人となった（図１）。その大部分は短期間旅行者であり，彼らは短期旅行後に帰国（再入国）する。他方，外国人入国者（来航者）数も増加しており，2002年には570余万人を数えた（図１）。すなわち，この１年間だけでも2220余万人のわが国への入国者（リピーターを含む延べ数）が存在したわけである。これらのうち熱帯地方（発展途上国）への旅行者が占める割合は必ずしも明かではないが，数百万人規模になると考えられ，これらの人々は来航地で流行している疾患を輸入感染症として日本国内に持ち込む可能性がある。島国であるわが国がジャンボジェット機の登場により，機能的にはこれら地域と陸続きになってしまったとも解釈できる。

WHO によれば発展途上国（熱帯地方）への旅行で最も高頻度に遭遇する感染症は旅行者下痢症であるが，さらにマラリア，腸チフス，ウイルス性出血熱や各種寄生虫性病などの今日のわが国に流行していない伝染性疾患が常在する地域がある。

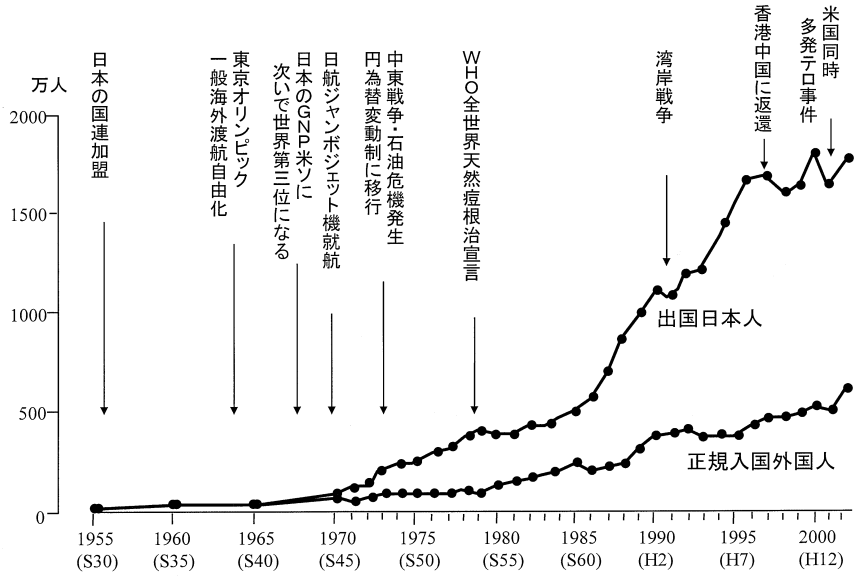

図1.日本人海外旅行者数・外国人日本入国者数の経年変化

表１．渡航地・来航地別にみた2000年の流行感染症と出国日本人数・正規入国外国人数

渡航地／来航地	出国日本人数*1) 17,818,590人	正規入国外国人数 5,272,095人	感　染　症*2)
アジア（東アジア，東南アジア，中南アジア，西アジア）	8,481,472人	3,222,982人	マラリア，腎症候性出血熱，デング熱，日本脳炎，A型肝炎，B型肝炎，E型肝炎，メリオイドーシス，レプトスピラ症，ペスト，ブルセラ症，髄膜炎菌性髄膜炎，ツツガムシ病，コレラ，ポリオ，細菌性赤痢，その他細菌性下痢症，腸チフス，パラチフス，ランブル鞭毛虫症，赤痢アメーバ感染症，クリミア・コンゴ出血熱，リーシュマニア症(皮膚，内臓)，オンコセルカ症，AIDS，狂犬病，各種寄生虫症
北アメリカ	5,519,652人	890,771人	サルモネラなどによる細菌性下痢症，ライム病，ロッキー山脈紅斑熱，ハンタウイルス肺症候群，デング熱，マラリア，リーシュマニア症(皮膚，皮膚・粘膜)，腸チフス，パラチフス，ブルセラ症，赤痢アメーバ感染症，細菌性赤痢，AIDS，狂犬病，西ナイル熱
ヨーロッパ	2,374,845人	814,912人	サルモネラ症，カンピロバクター腸炎，ジフテリア，AIDS，ライム病，A型肝炎，狂犬病
オセアニア	1,267,492人	184,974人	デング熱，マラリア，腸チフス，A型肝炎，細菌性下痢症，フィラリア症，AIDS
アフリカ	106,470人	20,643人	マラリア（特にサハラ以南），フィラリア症，回帰熱，リーシュマニア症（皮膚，内臓），トリパノソーマ症（睡眠病），ペスト，ラッサ熱，エボラ出血熱，クリミア・コンゴ出血熱，マールブルグ出血熱，リフトバレー出血熱，西ナイル熱，黄熱，メジナ条虫症，髄膜炎菌性髄膜炎，ブルセラ症，住血吸虫症（ビルハルツ），細菌性赤痢，コレラ，その他感染性下痢症，赤痢アメーバ感染症，ランブル鞭毛虫症，A型肝炎，E型肝炎，腸チフス，狂犬病，蠕虫症，AIDS，狂犬病
南アメリカ	68,420人	135,770人	マラリア，アメリカトリパノソーマ症（シャガス病），リーシュマニア症（皮膚，皮膚・粘膜），ペスト，黄熱，デング熱，腸チフス，パラチフス，A型肝炎，B型肝炎，赤痢アメーバ感染症，コレラ，住血吸虫症（ビルハルツ），髄膜炎菌性髄膜炎，AIDS，狂犬病
その他・無国籍	239人	2,043人	――

＊1）入国日本人数17,655,946人　＊2）下線は発熱を主症状とする疾患

◆渡航地・来航地別にみた流行感染症と出国日本人数・正規入国外国人数

2000年の出国日本人数・正規入国外国人数を大陸別（WHOによる）に分け，各大陸で流行している特異的な感染症と対比した資料を示す（表１）。日本人出帰国記録は2001年７月以降分が廃止された。従って，日本人旅行者の旅行地別集計はここに示した2000年が最後の成績になる。日本人の渡航地で最も多いのはアジアで840余万人，来航外国人数も320余万人であり，合計1160余万人がアジア地域から日本に入国したことになる。アジアはきわめて範囲が広く，地域により事情がかなり異なるが，さまざまな熱帯病や伝染性疾患の侵淫地が散在する。アフリカや南アメリカなどのマラリアや各種ウイルス性出血熱などの急性感染症がとくに広く流行している地域への旅行者・来航者は現時点ではそれほど多くないことが分かるが，今後，その動向を知る上でも日本人出帰国記録は不可欠ではないだろうか。

以上にわが国を中心にみて人的交流が盛んになり，それに伴い，来航先で流行している感染症，とくに熱帯病が輸入感染症としてわが国に持ち込まれる可能性が増加していることを示した。今日のわが国ではこれらの疾患に関する認識がきわめて低いために，臨床の場で見逃され，誤診される可能性が高いことが大きな問題点として指摘できる。今後は，国際化により，多くの熱帯病・伝染性疾患が日本国内に侵入していること，とくにマラリアやチフス性疾患など急変する可能性がある疾患，住血吸虫など慢性に経過する疾患など現在の日本人に余り知られていない疾患に関する国内向けの啓蒙活動，さらに熱帯病の克服に向けた研究事業による国際貢献が重要な課題になる。

タイ国北部地域における HIV 陽性患者に合併した市中呼吸器感染症の臨床的検討

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はじめに

2002年末現在の世界の HIV 感染者および AIDS 患者数は4200万人，アジア・太平洋地域にはこのうち720万人が存在すると推計されている。そのうちアジアの中で推定 HIV 感染率が１％を超えているのはカンボジア，ミャンマーおよびタイの３カ国のみである。このうちタイでは政府の政策が功を奏して感染者が減少に転じているものの，年間の新規 AIDS 発生は２万人程度認められ依然重要な問題である。

このような背景のもと，我々はタイ国北部地域における HIV 陽性患者に合併した市中呼吸器感染症の病態を明らかにする目的で，1996年12月より2002年１月の間にチェンマイのナコンピン病院において入院治療を行った HIV 感染者に合併した市中呼吸器感染症191症例192エピソードについて起炎病原体と治療法の実態調査および治療効果判定を行なった。とりわけ，*Streptococcus pneumoniae* と *Rhodococcus equi* は HIV 陽性患者に合併した市中呼吸器感染症の主要な起炎菌であり，以下の解析を行なった。

Ⅰ．*Streptococcus pneumoniae*

１．緒言

肺炎球菌は肺炎のみならず中耳炎，副鼻腔炎，菌血症，髄膜炎の起炎菌として知られている。元来，ペニシリンに高感受性を示していたが，近年ペニシリン耐性肺炎球菌が世界的に広まっており，その耐性はペニシリンのみにとどまらず，多剤耐性の傾向を認める。タイ国においては1992年から1994年にかけての急性呼吸器感染症の小児の鼻咽腔から分離された肺炎球菌の37.2%がペニシリン耐性菌であったとの報告があるものの，成人におけるペニシリン耐性肺炎球菌の現状は明らかではない。

２．対象と方法

チェンマイ大学医学部附属病院で，1999年９月から2000年６月の間に，93症例の種々の検体より分離された肺炎球菌93株について最小発育阻止濃度（以下 MIC）および血清型を測定し，年齢別の耐性化の状況等について検討した。また，ペニシリン高度耐性菌（PRSP）についてはパルスフィールド電気泳動法（PFGE）による解析を行い，国際流行株との比較を行った。

３．結果

93株中29株（31.2%）はペニシリン感受性株（PSSP），24株（25.8%）はペニシリン低感受性株（PISP），そして40株（43.0%）は PRSP であった。その他の薬剤についてはセフェム，クロラムフェニコール，テトラサイクリン，マクロライド，アミノグリコシドなどに対し多剤耐性傾向を示したが，カルバペネム，グリコペプチドには感受性を有しており，その薬剤感受性成績はペニシリンの薬剤耐性化がやや高度であることを除けば日本とほぼ同様の傾向であった。年齢別では，５歳以下において PSSP が３株（21.4%）と他の年齢層（30%～60%）に比べて少なく，耐性菌の検出率が高い傾向にあった。血清型の分布は PSSP では多様性を示したが，PRSP では６B，19F，23F の３つの血清型で全体の90%を占めていた。Figure.１に示すように，この３つの血清型を示す PRSP における PFGE の解析では血清型19F を示す16株中，国際流行株台湾19F のサブタイプが２株しか認められず，これとは異なる PFGE 型別を示す群が12株と半数以上を占めた。血清型６B では，11株全てが同じ型別か，そのサブタイプであった。血清型23F を示す９株中，７株が国際流行株スペイン23F と同じ型別かサブタイプで，２株がそれとは異なる PFGE 型別を示した。[1)]

４．考案

タイ国北部地域においてもペニシリン耐性肺炎球菌は本邦同様高頻度に認められ，一部の薬剤を除いて多剤耐性傾向が確認された。タイは近年 AIDS 症例の発生数が減少傾向に転じたものの，いまだ世界の中では AIDS 患者が非常に多い地域なので，今後，今回の MIC 成績を基にした治療戦略の見直しに加えて，AIDS 症例などのハイリスク患者においては肺炎球菌ワクチンの検討が望まれる。

Ⅱ．*Rhodococcus equi*

１．緒言

Rhodococcus equi は元来，家畜，特に子馬に呼吸器感染症を引き起こす原因菌として知られていた。1967年に初めて人への感染が報告されて以来，日和見感染症の原因菌として認識されてきた。その後1980年代の AIDS の流行により，人においての呼吸器感染症の報告が増加し，既にタイ国北部地域ではまれな疾患ではなくなっている。*R. equi* の治療には erythromycin と rifampin（RFP）の併用が推奨されているが，既に RFP 耐性株は少数ではあるが報告さ

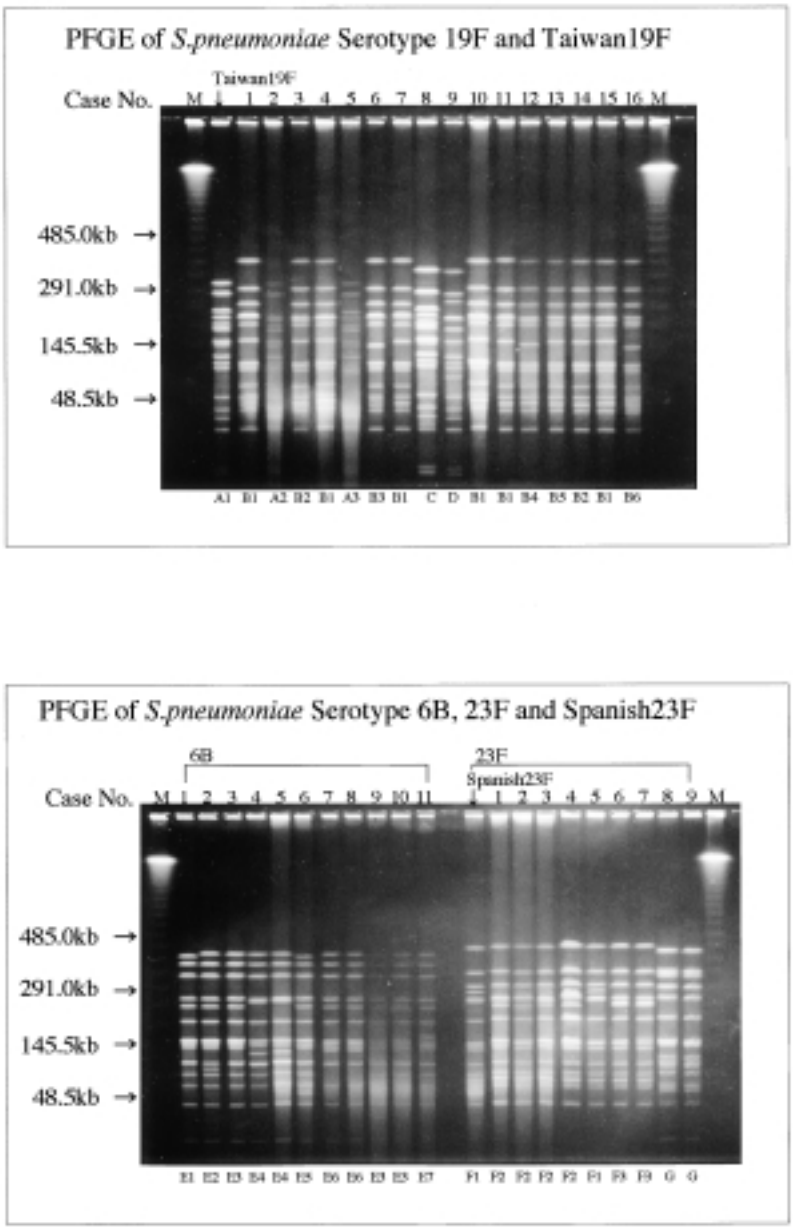

Figure. 1

れている。近年 Fines らは結核菌などと同様に子馬から分離された *R. equi* において RFP 耐性に関与する *rpoB* 遺伝子のアミノ酸変異を報告している。しかし，人由来の耐性株がどのような遺伝子変異を持つのかは解っていない。

２．対象と方法

タイ国北部地域において1993年より2001年の間に分離された *R. equi*30株に対し RFP に対する MIC を測定した。その結果，26株は MIC １μg/ml 以下の感受性株であったが，１株は MIC 8μg/ml を示す軽度耐性株で，残りの３株は MIC 64μg/ml 以上を示す高度耐性株であった。これらの軽度耐性株と高度耐性株は1998年から2001年の間に分離されたものであり，その耐性株４株と，同時期に分離された RFP 感受性株４株の合計８株を対象として *rpoB* 遺伝子のアミノ酸変異とパルスフィールド電気泳動法による分子疫学的検討を行った。PFGE は菌株を BHI ブロスで35℃一夜静置培養したものを遠心し，沈渣を PettIV で懸濁。懸濁液を２％Incert Agarose を用いてブロックとした。ブロック内の *R. equi* を Lysostaphin で溶菌後，Proteinase K で蛋白質を消化した。制限酵素 PshBI で切断後，CHEF Mapper を用いて電気泳動を行った。泳動時間は20時間18分とした。また，PCR は RFP 耐性に関与する *rpoB* 遺伝子の増幅を

行い，この PCR 産物をオートシークエンサーを用いて塩基配列を決定し，アミノ酸配列に翻訳した。

３．結果

Table. 1 に示すように２株の高度耐性株は PFGE で A pattern, DNA sequencing にて531位のセリンがトリプトファンへの変異を示し，１株の高度耐性株は PFGE で B pattern, DNA sequencing にて526位のヒスチジンがチロシンへの変異を示し，軽度耐性株は PFGE で C pattern，DNA sequencing にて509位のセリンがプロリンへの変異を認めた。一方，４株の感受性株は PFGE で耐性株とは違った型別を示し，*rpoB* 遺伝子のアミノ酸変異は認めなかった。[2)]

４．考案

今回，初めての人由来 RFP 耐性 *R. equi* の検討で，526位のヒスチジンがチロシンへ変異し高度耐性を獲得していることを明らかにした。変異したアミノ酸は異なるものの，この部位の変異は家畜由来の *R. equi* にても確認されており，やはり RFP へ高度耐性を示していた。また，531位のセリンがトリプトファンへ変異し高度耐性を獲得したが，これも変異したアミノ酸は異なるものの，531位のセリンの変異は家畜由来の *R. equi* にても確認されている。ただし，この場合は軽度耐性を示していた。一方，結核菌においてもこの部位の変異は認められており，その際は高度耐性に関わっていた。そして今回，軽度耐性株において509位のセリンがプロリンへ置換されていたが，この位置の変異は今まで *R. equi* においての報告はない。以上，我々の結果は，タイ国北部地域の AIDS 患者において数タイプの RFP 耐性 *R. equi* が広まりつつあることを示した。

Table. 1 RFP MICs, RpoB amino acid substitutions, and PFGE patterns of *Rhodococcus equi* detected from AIDS patients

Strain No.	RFP MIC (μg/ml)	Amino acid substitution (*E. coli* numbering)	PFGE patterns
1	>128	Ser 531 Trp	A1
2	>128	His 526 Tyr	B
3	64	Ser 531 Trp	A2
4	8	Ser 509 Pro	C
5	0.5	None	D
6	0.5	None	D
7	0.5	None	E1
8	0.5	None	E2

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ラオス村落住民におけるタイ肝吸虫の頻度と超音波肝胆道所見

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緒　言

タイ肝吸虫（*Opisthorchis viverrini*）は生魚の摂食により感染し，ヒトの胆管や胆嚢に寄生する。タイ東北部では住民の約60～70%にタイ肝吸虫の感染が認められ，胆管癌との関連も指摘されている（Kullavanijaya et al., 1999）。

メコン川を挟んでタイ東北部と国境が接し，生魚の摂食という同じ食習慣を持つラオス人民共和国においても，タイ肝吸虫症は公衆衛生上の主要課題の一つである。小林らによるラオス国カムワン県における15歳以下の住民の糞便調査では，感染率は37.5%と報告された（Kobayashi et al., 1995）。

我々は1996～1997年にかけて，ラオス国カムワン県の３つの村で住民の糞便調査を行った。その調査結果（Kobayashi et al., 2000）の概要を紹介するとともに，*O. viverrini* 陽性者と陰性者における腹部超音波検査所見の比較検討を行ったので報告する。

対象と方法

１．糞便中のタイ肝吸虫虫卵調査

ラオス国カムワン県で，日本－ラオス国際技術協力公衆衛生プロジェクトの寄生虫コントロールプログラムにおける調査対象地域となっていた３つの村（Sisomsouen, Phavang, Thakhek Neua）の住民を対象とした。Sisomsouen と Phavang は県庁所在地の Thakhek 市から約55㎞北に位置する村で，両村とも村の周囲には田園しかない。両村の違いは，Sisomsouen の約20%の家にトイレが設置されたが，Phavang には１個もトイレはなかったことである。一方，Thakhek Neua は Thakhek 市の中にあり，経済的にも衛生的にも比較的恵まれた環境にあった。いずれの村民も，生魚をよく摂食する習慣があった。

３つの村民から671検体（Simomsouen 189，Phavang 258，Thakhek Neua 224）の糞便を回収した（回収率44～73%）。

２．方法

回収された便検体は２日以内に Kato-Katz 厚層塗抹法で検鏡された。感染強度は33mg の便中に含まれる総虫卵数で表した。

虫卵陽性者で無症状の Thakhek Neua 住民のうち，同意の得られた84名（男性47名，女性37名，７～66歳：中央値37歳）に対し腹部超音波検査を施行し，肝胆道系所見の有無を調べた。また，カムワン県病院を何らかの消化器症状または腹部痛で受診し，腹部超音波検査の依頼があった患者のうち同意の得られた53名（男性11名，女性42名，３～77歳：中央値36歳）に対して，糞便検査を施行し，タイ肝吸虫の虫卵の有無を調べ，腹部超音波検査の肝胆道系所見と合わせて検討した。腹部超音波検査は，肝胆道系疾患を

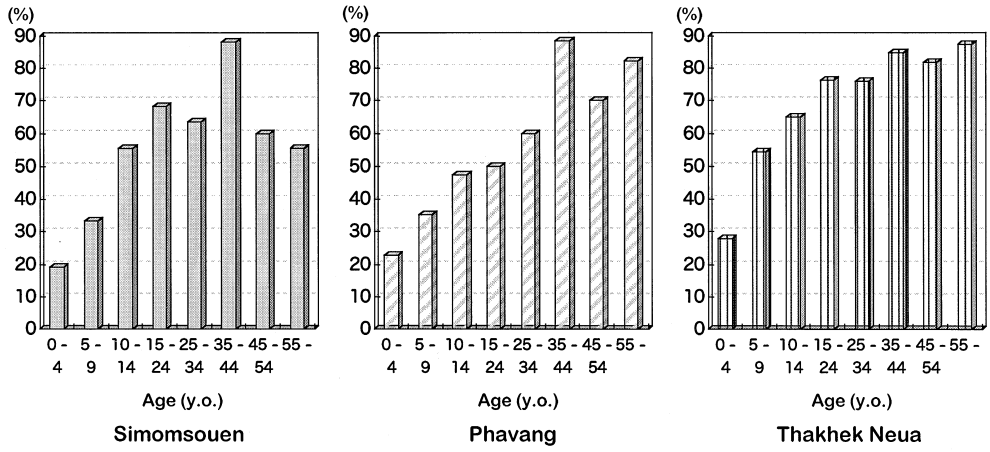

Figure 1　Prevalence of *Opisthorchis infection* in 3 villages.

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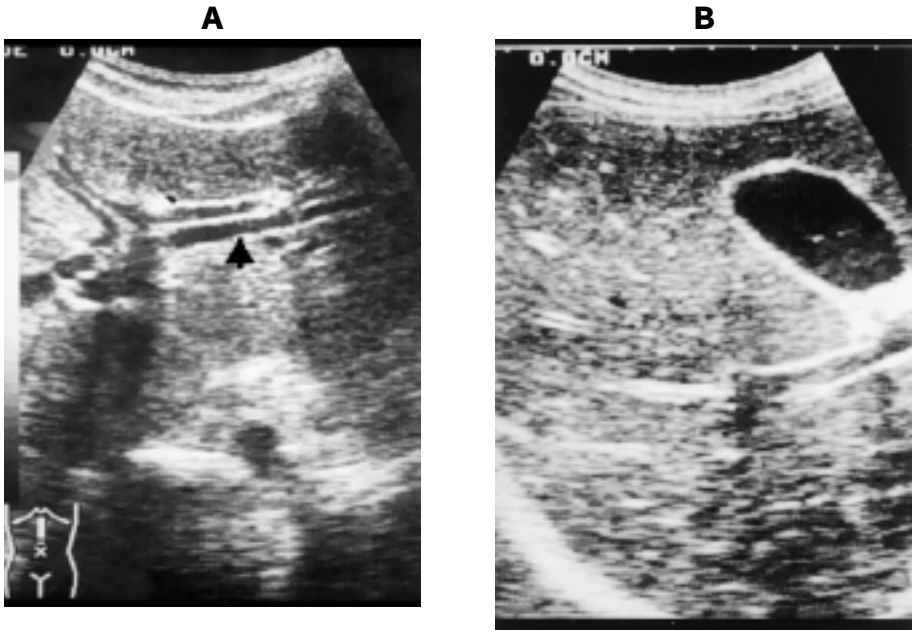

Figure 2　Ultrasonographic hepatobiliary findings in case of *Opisthorchis infection*: 2-A and 2-B showed dilatation of intrahepatic bile duct (arrow) and intrahepatic enhanced echoes, respectively.

専門とする日本人医師と Khammouane 県病院の腹部超音波検査担当医師により施行された。

検定

データの解析は統計解析ソフト（SPSS）を用いて行った。P値が0.05以下を有意差ありとした。

結　果

1．タイ肝吸虫保有率

O. viverrini の陽性率は671名中375名（55.9%）で，うち成人の虫卵陽性者は294名中215名（73.1%）と高率であった。村ごとの陽性率は Simomsouen で52.9%，Phavang で55.0%，Thakhek Neua で60.7%と，３群間での有意差は認められなかったが，年代別に比較すると，３村とも年齢の増加とともに陽性率が上昇し，15歳以上では感染率は50%を超えていた。特に Thakhek Nuea では10歳未満の陽性率が他の２村と比較して高率であった（Figure１）。

20歳以上の成人294名中215名（73.1%）が虫卵陽性で，３つの村の住民で糞便中のタイ肝吸虫虫卵数（EPG：便１ｇ当たりの虫卵数）を比較した結果を Table１に示した。成人の虫卵陽性率は Thakhek Neua が多く，また，糞便中の虫卵数も他の２村の住民に比べ多かった。

Table 1　Intensity distribution of liver fluke egg counts (EPG, eggs per gram of feces) of the examied cases over the age of 20 in 3 villages.

EPG group	Sisomsouen	Phavang	Thakhek Neua
0	33(35.1)	31(24.8)	15(20.0)
1-1,000	49(52.1)	54(43.2)	28(37.3)
1,000-6,000	12(12.8)	37(29.6)	26(34.7)
>6,000	0(0)	3(2.4)	6(8.0)

The number in parentheses indicates the proportion of the examined cases.

２．超音波検査結果

腹部超音波検査の肝胆道系所見の結果を虫卵陽性者群と陰性者群，および有症状群（病院受診患者）と無症状群（住民）とに分けて，Table２に示した。虫卵陽性者群では，虫卵陰性者群に比べ正常所見であった人は少なく，逆に胆管の拡張所見が認められた人が有意に多かった。有症状群と無症状群の比較では，有症状群で正常所見者が少なく，胆管拡張所見の認められた人が多かった。

虫卵陽性者における典型的な腹部超音波所見を Figure２に示した。虫卵陰性者群と比較し，肝内胆管の拡張と胆石・胆泥が有意に認められた。また，肝実質の粗造と点状エコー像も認められたが，糞便中の虫卵数の多い人で所見が強く出る傾向にあった。

便中の虫卵数と腹部超音波所見との関連を調べたが，虫卵数が多い人（5,000個/ｇ以上）に胆管拡張や肝実質の変化が多く認められる傾向にあったが，明確な関連は認められなかった。また，有症状群と無症状群間での虫卵数と腹部超音波所見の関連も認められなかった。

腹部超音波検査で診断された疾患は，胆管細胞癌は１例（0.73%）のみ（タイ肝吸虫虫卵陽性）で，脂肪肝18例，慢性肝炎２例，肝硬変１例，肝細胞癌４例，胆嚢結石９例であった。肝胆道系以外の疾患として，腎結石４例，膀胱結石１例，膀胱癌１例，卵巣嚢腫３例，子宮筋腫１例が認められた。

Table 2 Prevalence of ultrasonographic hepatobiliary findings of the group who provided a stool sample and those who were examined by ultrasound.

Examined group	Ultrasonographic findings	Egg positive (91 cases)		Egg negative (46 cases)
		Number of cases	Mean egg count (per1gram feces)	Number of cases
Asymptomatic residents (n=53)	Normal	20 (54.1)*	1721	11 (68.8)
	Intrahepatic enhanced echoes	9 (24.3)	1986	4 (25.0)
	Gall-bladder abnormality	1 (2.7)	333	0 (0)
	Gall-bladder sludge and stones	3 (8.1)	1526	2 (12.5)
	Dilatation of intrahepatic bile ducts	12 (32.4)†	1315	0 (0)
Symptomatic patients (n=84)	Normal	17 (31.5)†‡	3456	18 (60.0)
	Intrahepatic enhanced echoes	18 (33.3)	3698	8 (26.7)
	Gall-bladder abnormality	1 (1.9)	3666	1 (3.3)
	Gall-bladder sludge and stones	6 (11.1)†	1030	0 (0)
	Dilatation of intrahepatic bile ducts	28 (51.9)†‡	2808	7 (23.3)

The number in parentheses indicates the proportion of the findings that was detected by ultrasound examination.
*$p<0.005$ and †$p<0.001$, compared to egg negative group; ‡$p<0.001$, compared to asymptomatic group

考　察

本研究の結果により，隣国のタイの住民同様に，ラオス人民共和国の住民ではタイ肝吸虫の感染率が高いことが示された。タイの村落における同様の調査結果では，子供の感染率が10%未満で年齢とともに次第に微増していくのに対し（Brockelman et al., 1987: Kurathong et al., 1987: Haswell-Elkins et al., 1991），今回のラオスでの調査では，子供の感染率が20%以上と高く，その感染率は年齢とともに急増し，15歳以上で50%を超える結果となった。Kobayashi らが以前に行った別の村の糞便調査でも子供の感染率は同様の結果であったことから（Kobayashi et al., 1996），ラオスの乳幼児期から生魚を摂食するという習慣がタイ肝吸虫の感染率に大きく関わっているものと考えられた。３つの村のうち，都市部の村である Thakhek Neua での陽性率が高かったのは興味深いが，これは他の村落では平地ラオ族と中地ラオ族の混合によってなっているが，この村落は大部分が平地ラオ族のみで形成されていることが影響しているかもしれない。ラオスは多くの民族から形成されているが，大きく平地，中地，高地ラオ族に分けられる。魚の生食は平地ラオ族に多いといわれており，ラオス保健省の調査や Rim らによれば，高地ラオ族や中地ラオ族の多い県では感染率は少ない（Rim et al., 2003）。

ラオスの住民は乳幼児期からタイ肝吸虫の感染に曝されていることから，肝胆道系疾患のリスクが大きいと考えられる。タイ肝吸虫症は疫学的にも実験的にも胆管癌との強い関連性が指摘されている（Watanapa and Watanapa, 2002: Kullabanijaya et al., 1999）。今回の調査では，超音波検査を施行した137例中１例（0.73%）で胆管細胞癌が見つかった。この頻度はタイ東北部における同様な調査結果とほぼ近いが（Haswell-Elkins et al., 1994），今回の検討における超音波検査施行症例数は比較的少なく，今後症例数を増やしての検討が望まれる。

タイ肝吸虫症の腹部超音波所見に関しては，肝左葉の大きさの増大，胆嚢壁の不明瞭化，胆嚢内胆泥，胆石，肝実質の粗造と点状エコー像，肝内胆管拡張などの所見が，特に重感染者で顕著に認められると言われている（Mairiang et al., 1992）。今回の我々の検討でも，肝実質の粗造と点状エコー像に加え，虫卵陽性者では特に肝内胆管の拡張と胆石・胆泥が多く認められ，糞便中の虫卵数が多い症例ほど超音波検査所見も強い傾向にあった。

以上の結果より，タイ肝吸虫はラオス住民の間に乳幼児期より高頻度に感染しており，腹部超音波検査結果と合わせて考えると，タイ肝吸虫症は比較的中等度から重篤な肝胆道疾患との関連性があることが示唆された。タイ肝吸虫症のコントロールには，生魚の摂食を避けるという公衆衛生の教育と啓蒙が重要であるとともに，ラオスにおいても胆管細胞癌などの重篤な肝胆道系疾患の早期診断と治療に関する対策が今後重要となってくると思われる。

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PREVALENCE AND ULTRASONOGRAPHIC HEPATOBILIARY FINDINGS OF LIVER FLUKE, *OPISTHORCHIS VIVERRINI*, IN LAO VILLAGES

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Abstract: Infection with the liver fluke, *Opisthorchis viverrini*, remains a major public health problem in Lao P.D. R. Cholangiocarcinoma caused by the liver fluke is the most common form of liver cancer in Northeast Thailand where an about 60 to 70% of the population are infected with the fluke. The parasite is also common to the lowlands of Lao among people with close ethnic ties to northeast Thai, but the prevalence and incidence of the infection in Lao are still not uncertain. The objective of our report is to investigate the prevalence of chronic infection with *O. viverrini* and to identify chlanginocarcinoma within a population-based survey of the infection in Lao. Stool samples from 670 residents (0-87 y.o) from 3 villages in Khammouane Province were examined for intensity of liver fluke infection. People from varying egg count categories were selected for ultrasound examination to identify hepatobliary disease. Patients coming to provincial hospital with abdominal symptoms were examined in the same way as the residents were done. The rate of the parasite was 56% among the residents and reached up to 73% in age group greater than 20 years old. The parasite was found even in 20% of age group below 5 years. Only one subclinical case of cholangiocarcinoma were diagnosed among 137 people, based on ultrasonographic evidence. Dilatation of intrahepatic bile ducts and Gall-bladder sludge and stones were observed higher within the moderate and heavy liver fluke-infected group. This survey suggests prevalence of the liver fluke is higher than previously thought, and that the intensity of the parasite might be related with the formation of hepatobiliary lesion. although it could not elucidate the relation with cholangiocarcinoma.

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わが国におけるマラリア患者の動向

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1．はじめに

わが国におけるマラリア患者のほとんどは海外で感染したいわゆる輸入症例である。例外として，航空機により国内に持ち込まれた感染蚊から感染したと思われる「空港マラリア」がまれに報告されている。マラリア患者数の動向は，感染症法（旧伝染病予防法）に基づく届出と，各施設の報告またはアンケートによる全国集計から推測するとができる。後者に関する過去の主な報告を表１にあげた（１-８）。本稿では，表１のうち主に木村らの報告と東京大学医科学研究所附属病院での集計結果をあわせ，わが国におけるマラリア患者の動向を解析する。

表１　わが国のマラリア患者動向の報告

発表年	発表者(文献)	調査対象	調査時期	調査期間	症例数	症例／年
1992	大友弘士(1)	全国集計	最近20年間	20年	1678例	84人
1993	大友弘士(2)	全国集計	1988-1992	５年	423例	85人
1994	増田剛太(3)	東京都立駒込病院	1975-1992	18年	109	６人
1996	大西健二(4)	東京都立墨東病院	1987-1995	９年	34	４人
2001	大友弘士(5)	全国集計	1999-2000	２年	226	113人
2001	増田剛太(6)	東京都立駒込病院	1975-2000	25年	274	11人
2002	三浦聡之(7)	東京大学医科学研究所附属病院	1992-2001	10年	170	17人
2003	木村幹男(8)	全国集計	1990-2000	11年	1253	114人

2．総患者数

感染症法（旧伝染病予防法）に基づく届出状況によれば，我が国におけるマラリア患者数は1998年までは年間60人前後であったが，その後140人前後に増加している（図１参照）（９）。一方，大友らによる全国調査では，法規に基づく届出数よりも多くの患者数が報告され，年間120名程度である（８）。したがって，最近の法規に基づく届出数の増加傾向は感染症法の施行に伴う届出頻度の増加の影響が大きいと推測される。東京大学医科学研究所附属病院の集計では，各年度ごとにばらつきはあるがおおむね10～20名のマラリア患者が受診している（図１）（８）。これらの結果を総合すると，わが国におけるマラリア患者数は年間120～140人でほぼ一定しているのではないかと思われる。

3．推定感染地域および原虫種

推定感染地域は，木村らの全国集計（1990-2000年）によればアフリカとアジアがそれぞれ約40%で，残りがオセアニア，中米となっている。東京大学医科学研究所附属病院での過去10年間（1992-2001）の集計では，アフリカが約60%，アジアが約30%と全国集計に比べアフリカが多い傾向にあった。原虫種は，アフリカでの感染の90%以上が熱帯熱マラリアで，それ以外の地域では２／３以上が三日熱マラリアであった。マラリア患者全体に閉める熱帯熱マラリアの頻度は，経時的にやや増加している傾向にあった。

4．治療と予防

東京大学医科学研究所附属病院では，原則として熱帯熱マラリアに対してはメフロキンで，非熱帯熱マラリアに対してはクロロキンで治療を行っている。したがって，使用頻度は原虫種頻度に比例し，約50%がメフロキン，約40%がクロロキンを投与されている。予防薬としてメフロキンが2001年に認可されて以来，東京大学医科学研究所附属病院では，年間約30人にマラリア予防用のメフロキンを処方している。

5．死亡率

わが国の1990年から2000年までのマラリア患者1253名中，死亡例が16名あり死亡率は1.3%であった。これは，欧米での死亡率の報告(英国：7801名中51名，0.65%，ドイツ：3747名中135名，3.60%)に比べほぼ同等の数字である(10)。

6．わが国におけるマラリア治療および予防の問題点

わが国で抗マラリア薬として認可されている薬剤は，メフロキン（治療と予防）とファンシダール（治療のみ）のみである（経口のキニーネも認可薬であるが，ほとんど用いられることはない)。その他，ドキシサイクリンも使用可能であるが，わが国ではマラリアに対して適応が認められていない。このような貧弱なマラリア診療体制を補うた

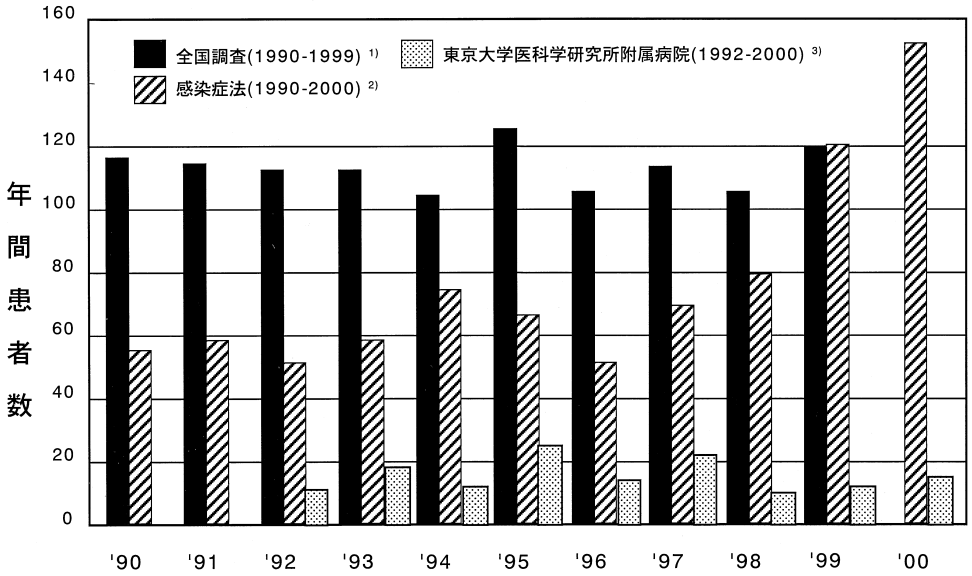

図１　わが国における年間マラリア患者数
木村らによる全国集計（1990-1999，文献８）：■，感染症法（旧伝染病予防法）に基づく届出数（1990-2000，文献９）：▨，東京大学医科学研究所附属病院の集計（1992-2000，文献７）：▩を示す。

めに，創薬等ヒューマンサイエンス財団総合研究事業「熱帯病に対するオーファンドラッグの開発研究」班が抗マラリア薬を輸入，保管している。2003年12月の段階で，静注用キニーネ，リン酸プリマキン，硫酸クロロキン，アーテスネート, アトバコン／プログアニル合剤, アーテメーター／ルメファントリン合剤を準備し，東京大学医科学研究所附属病院が中央保管施設としてこれらの薬剤を備蓄し，使用頻度の高い薬剤および緊急性のある薬剤を全国22の保管施設に配布している。保管薬剤の詳細と保管施設に関しては，研究班ホームページ（http://www.ims.u-tokyo.ac.jp/didai/orphan/index.html）をご参照いただきたい。

認可薬および研究班保管薬により，マラリア治療に関しては一応の体制が整っているといえるが，研究班の保管薬剤の輸入および薬剤の患者への供給体制については解決すべき問題が残されている。また，研究班保管薬は予防のための供給は行っておらず，わが国で使用できるマラリア予防薬はメフロキン１剤という状況である。WHO は，渡航する国ごとにメフロキン，クロロキン，プログアニル，ドキシサイクリンのいずれかをマラリア予防に推奨している。わが国においてこのうちの１剤しか処方できないという現実は，年間1000万人以上のヒトが海外に出かけ，自衛隊の海外派遣が行われている現状を考えると憂慮すべき問題と思われる。行政の対応を期待したいところである。

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デング熱の臨床と診断

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はじめに

デング熱（Dengue fever; DF）は再興感染症の一つであり，全世界では年間約１億人がデング熱を発症し，50万人がデング出血熱（dengue haemorrhagic fever; DHF）を発症すると推定される。熱帯・亜熱帯地域への渡航者増加に伴い，近年我が国でも増加傾向にある輸入感染症であり，その臨床像および各種検査を通じた早期診断が重要な鍵となる。

本稿では，デング熱及びデング出血熱に関し概説するとともに，我々が過去３年間に経験した，デング熱８症例の臨床的検討を行う。

病原体及び感染経路

デングウイルスは，フラビウイルス科に属し，４つの血清型が存在する。同型に対しては終生免疫を獲得するが，他の血清型に対する交叉免疫は数ヶ月で消失し，二次感染時にデング出血熱になる確立が高くなるといわれている。感染媒体は主にネッタイシマカであり，時にヒトスジシマカも媒介する。流行地域は，ネッタイシマカの生息地である，北緯および南緯35度の範囲に含まれる，熱帯・亜熱帯地域の100カ国以上にも及ぶ。発症１日前から発症後５日頃の，ウイルス血症期の感染者を吸血した蚊が感染し，８～12日の潜伏期の後，吸血を繰り返すことでヒトにウイルスを感染させる。同一蚊による頻回の吸血のため家族や同居者などが次々と数日の間に発症することを認めることがある。

臨床像及び診断

その臨床像から，非特異的症状のデング熱・出血傾向を伴うデング出血熱・ショック症状を伴い重症化するデングショック症候群の３つに大別される。WHO による，デング出血熱の重篤度による分類を示す（Table１）。

Table 1: Grading severity of Dengue haemorrhagic fever.

Grade１	発熱・非特異的症状， Tourniquet test 陽性・打撲後の皮下出血
Grade２	Grade１に加え，自発的出血の存在
Grade３	頻脈・脈拍微弱・脈圧低下（20㎜Hg 以下） 皮膚冷湿感・不穏等の循環障害
Grade４	ショック状態，血圧・脈圧測定不可

※Grade３・４がデングショック症候群（Dengue Shock Syndrome, DSS）に相当する。

デングウイルスに感染した場合，不顕性感染に終わる割合が高く，症状を示す場合は，多くがデング熱と呼ばれる一過性熱性疾患を呈する。２～15日の潜伏期間の後，突然の発熱，頭痛特に眼窩痛，筋肉痛，関節痛を呈する。解熱時に一致して，胸部・体幹から始まる斑状丘疹が出現し，四肢・顔面へ広がるが，この発疹は数日で自然消褪する。これらの症状は１週間程度で消失し，通常後遺症なく回復する。血液所見では白血球減少，相対的リンパ球増加，血小板減少，肝機能障害などを認める。

デング熱とデング出血熱を病態生理学的に区別するものは，血管透過性の亢進による血漿漏出である。通常のデング熱とほぼ同様に発症するが，第３～５病日に血漿漏出と出血傾向を主症状とする重篤な病態を示す。血液検査においても，血小板数低下（＜10.0×10^4／μl），ヘマトクリット値20%以上の上昇，血液凝固時間延長等を認める。血漿漏出が進行すると，循環血液量の不足からデングショック症候群を呈する。出血傾向としては，Tourniquet Test 陽性，出血班，鼻血，消化管出血等が半分以下の症例で認められ，重篤な例では DIC に陥ることもある。

以上のような渡航歴・臨床所見に加え，確定診断には，血清 ELISA 法によるデングウイルス特異的 IgM 抗体・IgG 抗体の検出，HI 抗体価等が用いられ，その他ウイルス分離や，PCR 法によるウイルス遺伝子の検出も非常に有用である。初感染・二次感染の鑑別に関しては，HI 抗体価の推移及び IgM・IgG 比に注目する必要がある。

デングウイルスに対する特異的治療はなく，補液・疼痛コントロールなどの対症療法が中心となる。一般にデング出血熱の患者は症状が回復し始めると後遺症を残さず迅速に回復する。患者の隔離は不要であるが，感受性のある媒介蚊が生息している可能性があれば患者から吸血しないよう蚊の進入を阻止する必要がある。

当院におけるデング熱症例

次に，当院にて過去３年間で経験した，デングウイルス感染症８症例の臨床的検討を行った。Table１に，渡航歴・臨床及び血液検査所見を，Table２に血清学的診断及びデング出血熱重篤度 Grading を提示した。対象は2000年７月～2003年３月に当科を受診し，デング熱の診断に至った８

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Table 2: 8 cases diagnosed Dengue between July 2000 and May 2003.

症例	年齢	性別	発症日	感染推定国	発症～受診の期間	皮疹部位	出血傾向および血漿漏出症状	最小の血小板数（10^4/ul）	基準値よりのHt上昇度（%）	IgM Ab	IgG Ab	血清PCR	DHF Grading
1	28	女	2000．7．14	ドミニカ	3日	上下肢	－	4.8	－	＋	－	施行せず	－
2	29	男	2001．1．10	インドネシア	3日	－	－	5.7	－	－	－	Type 2	－
3	26	男	2002．5．8	タイ	5日	全身	－	2.0	3.0	＋	＋	Type 1	－
4	30	女	2002．6．8	カンボジア	1日	上下肢	Tourniquet test 陽性	6.8	22.0	－	－	Type 4	Grade 1
5	25	女	2002．6．14	ホンジュラス	28日	－	胸水・腹水	2.0	25.5	－	＋	－	Grade 2
6	25	男	2002．8．4	タイ	17日	上下肢	眼底出血	6.8	18.0	＋	－	Type 1	Grade 2 の可能性
7	22	女	2002．8．5	ラオス	7日	下肢	眼底出血・鼻出血	(22.7)※	(－)※	＋	＋	施行せず	Grade 2 の可能性
8	21	女	2003．2．25	フィジー	5日	上肢	Tourniquet test 陽性	5.1	21.0	＋	＋	施行せず	Grade 1

症例で，男性３例，女性５例，平均年齢は25.7歳であった。主訴は７例が39℃以上の発熱，１例が視野障害であった。感染推定国は，東南アジアが５例，中米が２例，オセアニアが１例であり，すべて過去にデング出血熱の報告のある地域に合致していた。夏季・冬季の長期休暇に渡航し，現地の雨季に合致したケースが多く，発症後受診までの期間は１～28日で，平均11日であった。うち６例が入院加療を必要とした。臨床症状は，平均６－７日間持続した39℃以上の発熱を全症例に認め，筋肉痛４例，頭痛２例を認めた。皮疹は６例に四肢を中心に認め，出現時期は，発症後平均７日目であった。血液検査では，６例に白血球減少を,7例に血小板減少を，５例にマトクリット値の上昇を認めた。症例７は，発熱後３日目に鼻出血を認め，現地ラオスの病院にて血液検査・点滴加療を受け，その後視野異常が出現するも放置，帰国後当科受診，眼底出血を指摘されたケースであった。当科受診時は data 改善を認めているものの，経過中に血小板減少を認めていたものと推察された（※）。有熱期を過ぎ発症後平均10日より，全例に軽度から中等度の肝機能障害を認め，うち２例に２－３横指の肝腫大を認めた。

デング出血熱としての，出血傾向・血漿漏出傾向に関しては，Tourniquet Test 陽性２例，眼底出血２例，血管透過性亢進による血漿漏出症状として，胸・腹水の存在を１例に認めた。これら総合して考えると，WHO デング出血熱病態分類では，２例が Grade１，１例が Grade２に相当した。また，受診迄に時間を要した症例６・７では，当院での検査結果では基準に達しなかったものの，経過中に Grade２に相当していた可能性が示唆された。デングショック症候群に相当した症例はなかった。いずれの症例も当院においては輸液などの保存的加療にて症状軽快したが，症例５の25歳女性は現地ホンジュラスの病院で血小板輸血を10単位×２回受け，その後当院に搬送されたという経緯があった。有症状期間は平均15日間であった。

確定診断は，ELISA 法によるデングウイルス特異的 IgM 抗体・IgG 抗体の検出を，さらに可能な症例では PCR 法によるデングウイルス遺伝子の検出も行った（協力：国立感染症研究所ウイルス第１部）。PCR 陽性例は４例であり，血清型は１型２例，２型１例，４型１例であった。症例２及び４は共に発症初期であり，IgM 抗体陰性・PCR 陽性で，早期診断において PCR 法が有用であった。PCR 陰性であった症例５は，前述の如く治療後受診であり，発症後17日目の血清を用いたため，ウイルス分離に至らなかったと考えられた。

おわりに

デング熱は約３年周期で流行することが知られているが，最近の outbreak は2001年９月ハワイ・マウイ島，2002年ブラジル南東部・中米（ホンジュラス・エルサルバトル）・東南アジア（マレーシア・タイ・ベトナム），2003年２月オーストラリア（ケアンズ）などで認められている。今回当科において経験した８症例は，これら流行地域に合致していた。中でも，ネッタイシマカが絶滅したとされるマウイ島での outbreak は，大量発生したヒトスジシマカによって媒介されたことが確認された。ヒトスジシマカは，本邦でも東北地域以南に生息し，1942年から1945年にかけて長崎・大阪で認められた大流行は，本種が媒介したとされている。今後，新たな国内二次感染の可能性の示唆され，警鐘を鳴らすものである。

我々医療従事者は，帰国後不明熱の鑑別疾患として，デング熱の重要性を再確認し，その動向に充分注意を払う必要があると考えられる。

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Dengue and Dengue haemorrhagic fever.

KUNIKO YOSHIDA

Abstract: Dengue fever is an important health problem for travelers to all endemic countries. Dengue virus which has four major serotypes is transmitted by the mosquito, *Aedes aegypti.* New infection with a particular serotype causes antibody-dependent enhancement. Dengue fever causes negligible mortality, but re-infection to the other serotype leads to the risk of developing the significantly more dangerous dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). We reviewed 8 cases who were consulted to our clinic after returning from tropical and subtropical countries and diagnosed Dengue between July 2000 and May 2003. 3 cases were male and 5 were female whose mean age was 25.7 years old. 5 cases from Southeast Asia, 2 from Central America and1from Oceania. To differentiate dengue cases, clinical evaluation and laboratory diagnosis were used. 7 cases (87%) complained high fever at the first examination. The most common presenting features of Dengue were thrombocytopenia (87%) and leukopenia (75%) in laboratory. Clinically, high fever (87%), rash (72%), myalgia (50%), headache (25%), pain in orbit (13%), visual impairment (13%) were inspected. Those who complained visual impairment were pointed as hemorrhage in the eyeground. The dengue cases were classified as dengue fever (3 cases, 37.5%), DHF (5 cases, 62.5%), and no DSS. With serological confirmation, primary antibody response was observed in 6 cases (75%), Dengue virus isolation were done for 5 cases, 4 (80%) were positive. Dengue serotypes were 2 cases were typel, 1 was type 2 and 1 was type 4. The prevalence of dengue fever appears to be increasing in travelers, which calls special attention for health care professionals. The clinical presenting features provide important guides to establishing the diagnosis.

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旅行者下痢症　－横浜市立市民病院の症例を中心に－

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はじめに

海外渡航者が健康上の問題から医療機関を受診する際，圧倒的に多い症状は下痢である。ことに発展途上国からの帰国者に多く，いわゆる熱帯地域を１か月間旅行すると，３～８割の旅行者が一過性にせよ下痢を経験するといわれる(2)。

本稿では経口による感染経路の共通性から，下痢が症状である旅行者下痢症に，症状としては発熱性疾患である腸チフス・パラチフスを加え，輸入腸管感染症としても述べることとする。

方　　法

旅行者下痢症と腸チフス・パラチフスの臨床的特徴を検討する目的で，2001年１月１日から2003年６月30日までの２年６か月の期間に当院を受診した計307症例を，レトロスペクティブに分析した。渡航中あるいは帰国後に下痢をきたした，いわゆる旅行者下痢症が298例，腸チフス・パラチフスが９例である。男性166例，女性141例，受診時の平均年齢は32.5歳であった。

Table 1. Countries and areas where enteric infections were acquired during international travel:Yokohama Municipal Citizen's Hospital, between 1 January 2001 and 30 June 2003

Country and area	No. of cases	(%)
Asia		
Indonesia	62	19.6
Thailand	49	15.5
India	43	13.6
Vietnam	19	6.0
Cambodia	19	6.0
China	15	4.7
Malaysia	8	2.5
Philippines	8	2.5
Nepal	8	2.5
Korea	5	1.6
Hong Kong	4	1.3
Taiwan	4	1.3
Other countries	17	5.4
Subtotal	261	82.3
Africa	34	10.7
Latin America	10	3.2
Australia/Pacifics	6	1.9
Other areas	6	1.9
Total *	317	100.0

＊Total patients 307, including multiple-country visitors 10

結果および考察

下痢を発症したか，発症する直前に滞在していた国・地域を，推定感染国としてTable１に示す。インドネシア（19.6%），タイ（15.5%），インド（13.6%）の３か国で

Table 2. Causative agents of enteric infections among international travelers:Yokohama Municipal Citizen's Hospital, between 1 January 2001 and 30 June 2003

Infectious agent	No. of strains	(%)
Bacteria		
Shigella species*	30	20.1
Diarrhogenic *E. coli* †	26	17.4
Campylobacter jejuni	20	13.4
Campylobacter coli	2	1.3
Nontyphoidal *Salmonella* ‡	17	11.4
Salmonella Typhi	4	2.7
Salmonella Paratyphi A	5	3.4
Plesiomonas parashigelloides	9	6.0
Vibrio parahaemolyticus	4	2.7
Aeromonas hydrophila	2	1.3
Subtotal	119	79.9
Parasites		
Giardia lamblia	14	9.4
Cryptosporidium	2	1.3
Metagonimus yokogawai	3	2.0
Others §	10	6.7
Virus		
SRSV	1	0.7
Total	149	100.0

＊*S.sonnei* 23, *S.flexneri* 6, *Shigella* UT 1
† ETEC (O6) 9, etc
‡ Serotypes O76, O84, O94, O3 & 103
§ *Trichuris trichura* ova 2,
Taenia saginata 2,
Strongyloides stercoralis 1,
Ancylostoma ova 1,
Heterophyes 1,
Endolimax nana 1,
Iodamoeba buetschlii 1,
Pentatrichomonas hominis 1

133 cases were positive for causative infective agents out of 307 patients, and 149 strains were isolated, including 15 cases of multiple infections

ほぼ半数を占め，アジア地域が全体の８割を占めた。出張や赴任目的の渡航が多いはずの中国，韓国，台湾は相対的には下位であり，仕事目的の海外渡航者と比較して，観光目的の渡航者が旅行者下痢症の多くを占めていることがうかがわれた。こうした渡航者の問診では，観光旅行中の生水や生の食品の飲食，屋台での喫食，河川での水泳などの行動歴が少なからず聴取され，こうした旅行中の気軽な行動が感染リスクを高めている一因となっていることが推定された。

307症例のうち133例から病原微生物が証明された（陽性率43%）。病原微生物149株の内訳を Table２に示す。多いものから，赤痢菌（20.1%），下痢原性大腸菌（17.4%），カンピロバクター（14.7%），非チフス性サルモネラ（11.4%）であり，細菌性腸炎が４分の３を占め，チフス性疾患が6.1%を占めた。細菌以外では，ジアルジア症が全体の9.4%とかなりの割合を占めた。少数ながら寄生虫疾患も散見された。

２類感染症である細菌性赤痢については，最近では *Shigella sonnei* や *S. flexneri* などによる軽症例がほとんどで，*S. dysenteriae* 感染により発熱・腹痛・頻回の膿粘血便をきたし，重症となる例は今回の調査では認めなかった。自覚症状は軽微であるが，検疫所の検便で *S. sonnei* などが検出されて受診に至る例もしばしば経験した。治療はニューキノロン常用量を５日間経口投与する。

下痢原性大腸菌の中では，突然の水様下痢をきたし，コレラと臨床症状が似ている毒素原性大腸菌（Enterotoxigenic *Escherichia coli*: ETEC）感染症が比較的多くみられた。経口ニューキノロンが有効であるが，自然経過でも治癒し，予後は良好である。腸管出血性大腸菌（Enterohemorrhagic *E. coli*: EHEC）感染症は腸管出血や腎障害をはじめ，急速に全身状態が悪化するため，最も注意が必要なものである。しかしながら，今回の２年半の観察期間ではみられなかった。

次いで，カンピロバクター，サルモネラと，国内でもしばしば経験する腸炎起炎菌が続くが，これらの臨床的特徴は国内発生例と同様と考えてよい。いずれも一時的な禁食と輸液などの対症療法のみでも自然治癒する，予後良好な疾患であるが，カンピロバクターでは最近のキノロン耐性株の増加が問題となっている。

２類感染症である腸チフス・パラチフスは，汚染された水や食物を経口摂取することで感染が成立する点は，他の感染性腸炎と同様であるが，腸管内のマクロファージに貪食されたのち血流に入り，細胞内で増殖する，本態は菌血症と考えてよい発熱性疾患である。第一選択薬は細胞内移行性の優れているニューキノロンで，常用量の３分の４を２週間経口投与する。いわゆるオールドキノロンであるナリジクス酸（Nalidixic acid: NA）に耐性を有するものはニューキノロンの最低発育阻止濃度（Minimal inhibitory concentration: MIC）も上昇しているため，ニューキノロン低感受性株と呼ばれ，ニューキノロンによる治療が奏効しにくくかつ再発しやすい。インド亜大陸や東南アジアでこうした低感受性株が急速に増加していることは深刻な問題である。同地域から帰国した腸チフス・パラチフス患者は NA 耐性の有無を調べ，NA 耐性であればニューキノロンと第三世代セフェムを併用することで，より高い治療効果が期待できる(1)。

コレラは旅行者下痢症のなかでも大量の下痢により急速な脱水をきたす，臨床上きわめて重要なものであるが，その頻度は低く，今回の症例のなかには認めなかった。最近は古典的なアジア型より病原性の低いエルトール型が多い。

原虫のなかで最多のジアルジア症も，臨床症状から細菌性腸炎と区別するのは困難である。便鏡検で栄養型または嚢子を証明することで診断する。治療はメトロニダゾール経口が有効であるが，難治性のものや再発する場合は，再治療やチニダゾールへの薬剤変更が必要になることもある。男性同性愛者の性行為感染症の一面もあるため，難治例では注意深い問診と，パートナーの検便，陽性例では両者の治療が必要である。

一般には，旅行者下痢症をきたす疾患は重症化するものは少なく，当院の症例も治療により，または自然経過で全例が治癒した。重篤な合併症はみられなかった。

汚染された水や食物を介しての経口感染であるので，生水・生の食品・屋台で売られている食品を避けることと，石鹸での食前手洗い励行が予防として勧められる(3)。

旅行者下痢症の治療は，経口補液も含めて第一に輸液であり，ほとんどの場合はこれのみで十分である。発熱または血便を伴う場合は，便検体を採取後に経口ニューキノロンを３日間投与する。妊婦や小児の場合はニューキノロンが原則禁忌であるため，輸液を中心とした対症療法を行い，検便の結果に基づいて特異的抗菌治療の必要性を検討する。原虫・寄生虫疾患の可能性もあるため，細菌培養だけでなく便鏡検もあわせて行う。

従来ニューキノロン感受性であった起炎菌のうちいくつかの属種で耐性株が増加しているため，起炎菌同定・薬剤感受性をふまえた，特異的な治療薬の選択が望ましい。

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Traveler's diarrhea

TAKUYA ADACHI

Abstract: Diarrhea is by far the most common health problem encountered with travel to developing countries.
298 cases of traveler's diarrhea and 9 cases of typhoid and paratyphoid fever were summarized as intestinal infection. All patients were affected with either diarrhea or enteric fever during or shortly after their trip overseas and visited the Department of Infectious Diseases at Yokohama Municipal Citizen's Hospital from January 2001 to June 2003.
The most common countries of disease acquisition were Indonesia (20%), Thailand (16%), and India (14%). 82% of all cases were acquired in Asian countries.
From 133 among 307 patients (43%), 149 strains of causative agents were identified. The leading causes were *Shigella* (20%), diarrhogenic *Escherichia coli* (17%), *Campylobacter* (15%), nontyphoidal *Salmonella* (11%), and *Salmonella* Typhi and Paratyphi A (6%). 80% of causative agents were bacteria. Protozoa and parasites were less likely causes, accounting for 19% of infection, including 9% of giardiasis.
The most important factor in treating diarrhea is the replacement of lost fluids. Antibiotics are usually not required with acute watery diarrhea, which are usually self-limited with supportive treatment. If blood occurs in the stool, or if there is fever, empiric antibiotic therapy should be considered. A short course of fluoroquinolone can be considered in such cases.
There have been increasing cases of enteric fever with decreased susceptibility to fluoroquinolones. Resistance to nalidixic acid on disk diffusion susceptibility testing and clinical fluoroquinolone treatment failure are the key findings of decreased susceptibility strains. Intravenous administration of a third generation cephalosporin in addition to fluoroquinolones is useful to treat such resistant strains.
There is a tendency that the longer the traveler stays abroad the greater the risk is to acquire enteric fever or intestinal parasite infection.
Attention to food and beverage preparation can decrease the likelihood of developing traveler's diarrhea. Bacteriological stool test is incredible for appropriate diagnosis and treatment.

Department of Infectious Diseases, Yokohama Municipal Citizen's Hospital

輸入感染症患者に対する臨床現場での初期対応

岩﨑惠美子
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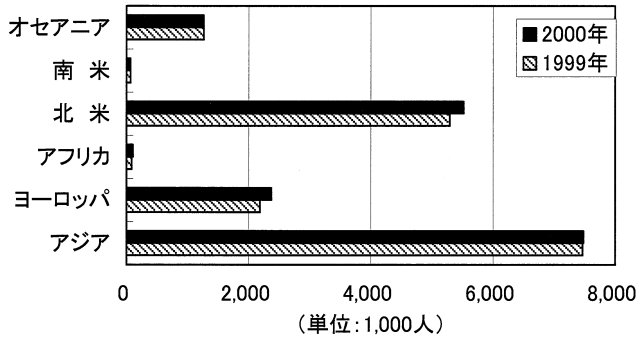
1．はじめに

アジアの一地域で始まったSARS（重症呼吸器症候群）の流行は，現代社会の中での感染症の脅威，すなわち，感染症が短時間で地球規模の流行に発展する可能性があることを私達に実感させてくれた。実際，海外では，動物との接点から発生する重篤で感染力の強い感染症が多く，交通機関の発達した現代では，これらが国際交流によって頻繁になった人の移動とともに，容易に国境を越えるようになってきたのである。このような状況の中で，感染症から国民の健康を守るためには，感染者を早期に発見し，良質で，適切な医療を提供することで患者を救うことと同時に，そこからの二次感染を防ぐことが必要になってくる。すなわち，医療機関での感染患者への初期対応が，患者の早期発見や適切な医療の内容につながることから，その役割は非常に大切になってくる。従って，現在の日本の医療関係者には，海外の感染症の流行情報や，その診断，治療などに必要な知識が求められることとなる。

2．日本の海外渡航者と渡航先での感染症事情

交通機関の発達は，人や物の国際交流を盛んにした。日本では，年間1,600万人の日本人が海外に渡航し，さらに年間500万人の外国籍の人が来日している。日本人旅行者の特徴は，その年齢分布でも顕著なように20歳代の女性が最も多く，ほとんどが一週間以内の短期旅行であり，その旅行先として三分の一がアジアを選んでいることである。

図１：地域別渡航者数の推移



また，日本に来日する人の多くもアジアの人々であり，日本はアジアと密接な関係にある。

このように，多くの人々が往来しているアジアは，また，多くの感染症が日常的に発生し，注意を要する地域でもある。特にアジア地域は腸管系感染症の多発地帯の一つであり，渡航先での食事などが原因で，罹患して帰国する人は後を絶たない。また，この地域の環境衛生などは，決して良好ではなく，蚊やダニ，ネズミなどの媒介動物や，病原体を体内で増やしている増幅動物（リザーバー）が普通に生息しており，多くの感染症の流行を起こしている。その代表として，マラリア，デング熱，レプトスピラ症などが挙げられ，これらは動物が関係していることから，季節によって流行に差がみられる。

このようなアジアの環境の中では，これらの感染症に罹患して帰国する人もみられるのは当然であろう。

また，近年では，このような古典的な感染症のみならず，国際的に感染拡大する可能性の高い，強い感染力を持つ感染症の発生もみられている。まさに，今回流行したSARSはその典型であろう。アジアでは人と動物の接点が近いために，動物由来の強い感染力を持つ重篤な感染症に人が罹患する機会は多く，その上，アジアの高い人口密度や頻繁な人の移動などによって，世界的な流行を引き起こす可能性は高くなっている。

3．輸入感染症対策の中での検疫所の役割

日本では海外から入ってくる感染症を監視しているのが検疫所である。交通機関の発達とともに，国境を越えての人の往来はほとんどが航空機によるものとなり，人の動きはますます高速かつ広範囲になってきた。その結果，人とともに動く感染症の動きも，より複雑でスピーディーになってきた。

また，従来，国内で注意すべき輸入感染症と考えられてきた感染症は，腸管系感染症ではコレラ，細菌性赤痢，腸チフスなど，そして近年，日本の多くの若者が免疫を持たないことで感染するA型肝炎，さらに，蚊によって媒介されるデング熱，マラリアなどであり，検疫所でもこれらの感染症に関しては水際での検査体制の充実を図り，患者の発見に力を注いできた。しかし，地球上での感染症の動向を考えると，これらだけでは済まなくなってきており，ますます水際である検疫所の感染症の監視は難しくなってきている。

その原因の一つには，特に感染力の強い重篤な感染症では，その潜伏期が長いことがあげられる。日本人の海外旅行の形態をみると，多くが短期旅行であることなど，感染症の症状が現れないうちに空港検疫所を通過し入国するため，空港検疫所での患者の発見が難しくなっている。また，検疫のための手続きや検査などの煩わしさから，入国者に

対する検疫の際に虚偽の申告をする場合が多く，これらも入国時の監視を困難とする原因の一つとなっている。

これらの背景を考慮し，検疫所では水際での対応だけでなく，海外から入ってくる感染症に対応する医療機関の支援，入国者の健康管理や感染拡大防止を図るための施策も展開している。

⑴感染予防

感染症対策の基本は予防にある。特に海外で流行する感染症は，国内で患者発生のみられる感染症とは異なるものが多いために，それに対する知識を日本の医療関係者は十分に有していない。それらを考慮し，検疫所では，医療関係者が診察する際の助けとなるよう，医療関係者に対しても海外で流行している感染症の流行情報を HP などを通じて提供している。

また，市民に対しては，予防接種の実施により，海外の旅行先での感染を防ぐことの可能であることなど，感染症の予防啓発を HP やパンフレットなどを通じて行っている。予防接種に関しては，検疫所で「トラベルメイト」という予防接種記録の手帳を配布し，その手帳の利用を旅行者に呼びかけている。しかし，現在までに，この手帳の普及は十分ではない。

また，予防接種によって防ぐことの出来る感染症から国民を守るために，海外へ渡航する前に予防接種を勧めると同時に，検疫所でも海外旅行のための予防接種を実施している。いくつかの検疫所では海外旅行外来を地域の医療施設と共同で開設し，そこで予防接種を実施するとともに予防教育の場としても活用している。

実際，検疫所は交通の不便な空港ないしは港などにあることが多いため，そこでの予防接種は利用されにくい。その点からも，市中の医療機関などでの海外旅行外来の開設は，国民にとって極めて有効である。

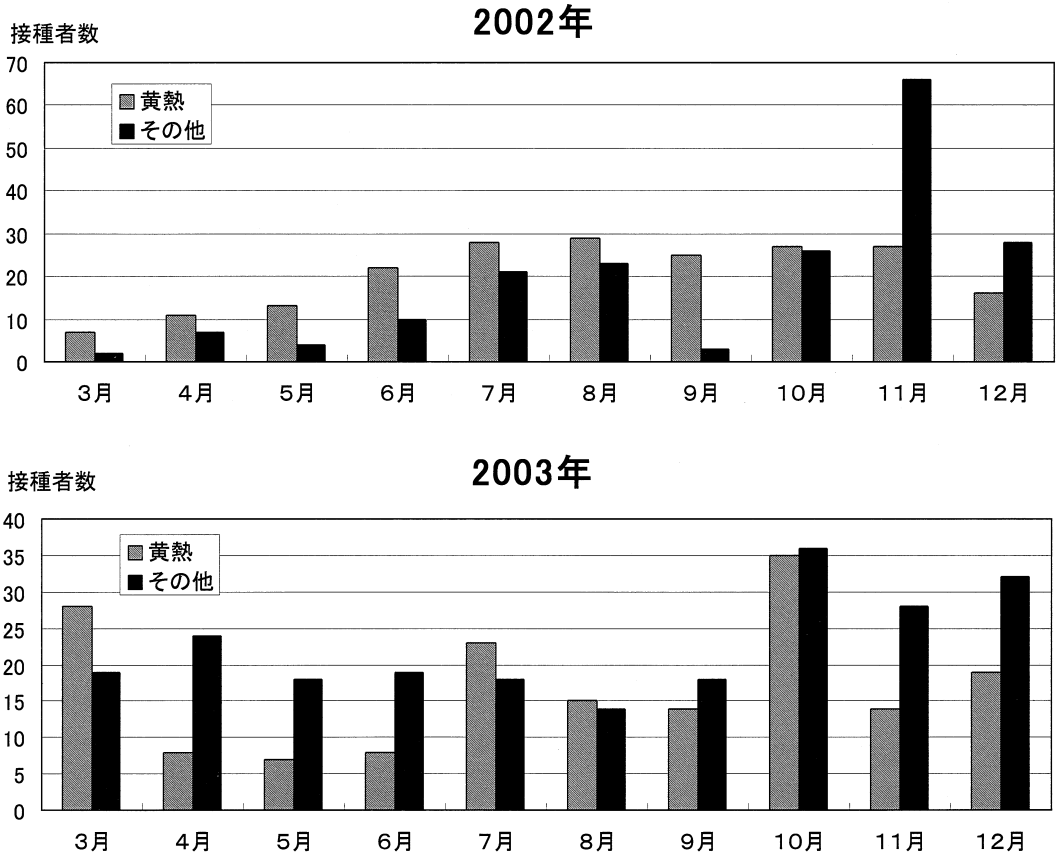
⑵海外旅行外来

先進国では，海外へ旅行する人，特に途上国への旅行を目指す人の多くは，海外旅行外来を訪れ，そこで渡航先に応じた予防接種やその相談，感染予防の知識，予防薬などの処方を受けている。また不幸にも感染して帰国してきた場合にも，この海外旅行外来を相談，診察，治療のために利用している。

しかし，日本ではこのような外来を開設している施設は極めて少なく，それが海外旅行前の予防接種の普及を阻んでいる。

そこで，海外旅行外来の重要性から，検疫所でも外来開

図２：海外旅行外来での予防接種



※その他：破傷風，A 型肝炎，B 型肝炎，狂犬病，ポリオ

設を考え，いくつかの検疫所でスタートし始めたところである。

仙台検疫所では海外旅行外来を国立仙台病院，国立療養所盛岡病院で開設し，予防接種，相談業務を行っている。その外来の開設は，実際に寄せられるメールやFAXなどでの相談や電話相談も増やしており，特にSARSのような国際的に感染拡大し，多くの人々にとって関心のある感染症が流行しているときには，相談窓口として大いに役立っていることが統計からも判る。

実際，感染患者の早期発見が感染拡大にとっては非常に意味があり，旅行者からの相談によって，症状が重症化する前に適切な医療機関に誘導することや，医療関係者への適切な情報の提供がそれに役立つことはいうまでもない。

このような医療機関と市民の対応窓口としても，海外旅行外来の役割は大きいと考える。

⑶入国後の健康異変に対する対応

感染症に罹患した患者をできるだけ迅速に医療機関へ誘導し，適切な医療を提供することが感染拡大を防止するためには必要不可欠である。特に海外からの感染症の多くは重篤で感染力が強いことを考えると，発症してから医療を受けるまでの時間の短縮が通常の感染症以上に大切になる。検疫所では従来から，電話などでの相談を通じ，医療機関への誘導を行ってきたが，一般への知名度は低く，利用は少ないのが現状である。しかし，現在のような感染症の動向を考えると，より早い対応や有効な感染症対策が必要であり，そのためには検疫所による積極的な関与が求められてきている。今回，11月に行われた法律の一部見直しで，SARSに関しては流行地域から入国した人の10日以内に発生した健康異変を検疫所に届け出ることが義務付けられ，少しでも患者発見までの時間の短縮を図り，感染拡大や流行阻止の一端を担うために積極的に検疫所が関わるような体制に整備された。

４．輸入感染症などに対する医療機関の対応

輸入感染症の特殊性を考えると，感染症の原因となる病原体を知ることが非常に大切であり，その上で適切な医療が行われることが必要となる。実際，日本の医療機関では，腸管系感染症に関しても，一般外来でその病原体の検査も実施せずに，いきなり抗生物質投与が始められる場合が多いことがアンケート調査でも明らかとなった。

東北地域の臨床内科医会のメンバーにアンケート調査を実施したが，675名から回答が得られ（回答率は50%），その中で下痢症の患者に治療を始める前に検便などの病原体の検査をする医師が７%にも満たないことが判明した。この結果が全てとは思わないが，日本の医療関係者の感染症に対する知識や治療法などに大いに問題があることを示唆していると思われる。

従来，エボラ出血熱などの重篤な感染症の流行現場では，医療関係者による感染症対策の不備が深刻な問題を生んできた。今回のSARSでも同様に多くの国で医療従事者の感染が流行を大きくしており，感染症患者の治療に際しては，診断，治療に関する充分な知識も必要であるが，それと同時に，日頃からの医療関係者の標準予防策の徹底が求められ，病院での感染対策の充実が必要になっている。

５．おわりに

SARSの流行が私たち医療関係者に残してくれたものは，感染症の怖さだけでなく，もっと大切なものを数多く残してくれたと思われる。

特に，感染症に関する情報，治療法などの知識もさることながら，このような感染症に罹患した患者に日常の診療で遭遇する可能性が高いことを教え，そして，それらに自らが感染しないように，また，自らが原因となって感染を拡大させないように努力しなさいと諭してくれたような気がする。

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