日本熱帯医学会雑誌

第30巻　第３号　平成14年９月

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METEOROLOGICAL FACTORS INFLUENCING THE INTENSITY OF MALARIA OUTBREAK IN ZIMBABWE*

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Accepted September, 10, 2002

Abstract: Correlation between meteorological data observed at Gokwe and intensity of malaria outbreak or the number of clinical malaria cases occurring at malaria season in whole Zimbabwe was studied. Meteorological year (Met Year) in this country starts in July at the coldest month and ends in the next June, and malaria peak season lasts from January to May. The correlation of the number of clinical malaria cases at peak season in thousand (Mp) and meteorological factors was calculated from the data in 8 years from Met Year 1990/1991 to 1997/1998.

Among single factors, correlation was highest with a total rainfall (mm) in a year (Rt) followed by that in January (R1), in February (R2) and average temperature in August (Av8), showing the coefficients of 0.873, 0.870, 0.862 and 0.739, respectively. The adjusted R^2 of the above factors were 0.722, 0.717, 0.700 and 0.470, respectively, where Av8 was non significant statistically. In two meteorological factors, the correlations higher than a single factor were a combination of R1+R2 with an adjusted R^2 of 0.792. Malaria at peak season will be increased by more rainfall in January, February and total in a year, and may be high average temperature in August. Formulae of regression lines are as follow, and by these, intensity of malaria outbreak at malaria season will be indicated.

1. Mp = 361.30×Av8 - 6,182.96 (approximation)
2. Mp = 3.12×R1+43.37 (good fit)
3. Mp = 1.82×R1+2.47×R2 - 15.02 (best fit)
4. Mp = 1.463×Rt - 323.21 (good fit for retrograde study)

Key words: Malaria, Intensity of outbreak, Meteorological factors, rainfall, Zimbabwe

INTRODUCTION

Malaria is an important disease in Zimbabwe, even if its intensity of outbreaks (the number of malaria cases in a year) was reported to be hypo- or meso-endemic in nature (Taylor and Mutambu, 1986). A retrograde study on malaria outbreaks in Zimbabwe (Freeman, 1995) suggested that among meteorological factors, only temperature in September influenced the intensity of malaria outbreaks in a year, and this analysis was reported later (Freeman and Bradley, 1996). The study was interesting and important, but no one has followed these findings later. In the above reports, a parameter used for the intensity of malaria was the number of malaria cases among inpatients and outpatients at Central Hospital at Harare, the capital city of Zimbabwe where no malaria occurs at altitude of 1,450 m. The meteorological data were referred to the weather station at the same city. There is a question whether the number of patients in a hospital coming from malarious areas outside of the City, and meteorological survey data at its neighbour actually represented the intensity of malaria outbreaks in this country and climate of malarious areas, respectively. The present authors attempted to make clear the relationship between climate and the intensity of malaria outbreak using more direct parameters.

MATERIALS AND METHODS

For the intensity of outbreaks, monthly incidence of clinical malaria cases reported by rural health centres was used. The meteorological data in the malarious area were collected, and a set of nearly complete data was found at

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*This study was performed as a part of activities of MOHCW-JICA Project on Infectious Disease Control in Zimbabwe supported by Japan International Cooperation Agency.

Gokwe Town and was taken into consideration in this study.

As the season of malaria transmission is a period from January to May in this country, the malaria peak occurs at the beginning of a calendar year. The study of influence of climate to the intensity of outbreak in a calendar year was considered to be inappropriate since meteorological factors after malaria season had no influence to the preceding malaria outbreak.

By chance, referring to the records of meteorological surveys in this country, the Meteorological Year (Met Year) has already been defined in starting in July, the coldest month, and ends in the next June. Since the malaria season comes at the last part of the Met Year, use of this calendar was found to fit our purpose.

The meteorological factors defined in this country and referred to the present study are rainfalls measured by mm, and average, maximum and minimum temperatures at degree Celsius in each month.

Monthly occurrence of all clinical malaria cases detected in the rural health centres in Zimbabwe was collected in 8 years from Met Year 1990/1991 to 1997/1998. Then correlation coefficients (r) were calculated between a total number of clinical malaria in a Met Year and corresponding meteorological factors such as average, maximum and minimum temperatures, and rainfalls in every months.

The coefficients were not so high with any factors of climate. Comparatively higher coefficients are shown in the maximum temperature in June, rainfalls in January and in December where the coefficients (r) were 0.774, 0.766 and -0.748, respectively, at risks between 5% and 1%. Although the coefficients were significant, they were not satisfactorily high, and rainfall in December showed negative correlation. The meaning of preliminary study was not readily understood.

Then, another modification was given to the number of malaria cases under an assumption that any meteorological factors before malaria season might influence the number of malaria cases at the high transmission period form January to May (peak season). The numbers of malaria cases at peak season were calculated by each Met Year, and correlation coefficients between meteorological factors and the intensity of outbreaks were calculated to find out what factors were more influential to malaria outbreaks.

The correlation coefficients of each meteorological factor to the intensity of malaria outbreak, and their significant levels are shown in Table 1. The meteorological factors showing the coefficient (r) higher than 0.7 were the average temperature in August, rainfalls in January and February, and a total rainfall in a Met Year with such high coefficients as 0.739, 0.870, 0.862 and 0.873, respectively, at risks of < 1% except the average temperature in August (Table 1). Rainfalls were generally more influential to the malaria outbreak than the temperature.

The important figures of meteorological data and malaria occurrence at peak season in 8 years are listed up in Table 2, and the regression line analysis was made based on this table.

Table 1 Pearson correlation calculated by multi- regression analysis and their significant levels

Pearson Correlation	Mp	Av8	R1	R2	Rt
Mp	1.000	0.739	0.870	0.862	0.873
Av8		1.000	0.778	0.541	0.758
R1			1.000	0.762	0.884
R2				1.000	0.892
Rt					1.000
Significant Level					
Mp	/	0.180	0.002	0.003	0.002
Av8		/	0.011	0.083	0.015
R1			/	0.014	0.002
R2				/	0.001
Rt					/

Table 2 Observed data of important meteorological factors in 8 years and the number of clinical malaria cases at transmission season used for regression line analyses.

Met Year	Mp Y	Av8 X1	R1 X2	R2 X3	Rt X4
1990/1991	308.16	18.2	165.8	74.2	489.0
1991/1992	172.54	19.3	106.0	14.3	526.7
1992/1993	657.74	18.2	81.3	156.1	639.9
1993/1994	421.78	18.2	187.4	146.4	637.0
1994/1995	363.17	18.3	70.4	40.4	329.6
1995/1996	1,186.58	20.4	333.2	251.7	1,005.0
1996/1997	1,138.05	19.6	354.0	207.6	1,069.0
1997/1998	1,169.84	19.7	328.4	149.7	775.1

Met Year; Meteorological year
Mp; Malaria cases at peak season in thousand
Av8; Average temperature in August
R1; Rainfall in January
R2 Rainfall in February
Rt Total rainfall in a Met Year

RESULTS

Single meteorological factors: The formulae of regression lines obtained by each of single factors are as follows;

1. $Mp = 361.30 \times Av8 - 6182.96$

where Mp is no. of clinical malaria in thousand at peak season, and Av 8 is the average temperature at degree Celsius in August.

2. $Mp = 3.12 \times R1+43.37$

where R1 is rainfall in January in mm.

3. Mp = 4.49 × R2+92.75

where R2 is rainfall in February.

4. Mp = 1.463×Rt - 323.21

where Rt is the total rainfall in a Met Year.

Using the above 4 formulae, estimation of the intensity of malaria outbreaks at peak season in the same Met Year is described with r^2 and adjusted R^2 in Table 3. The figure r^2 tends to fit optimistically how well the estimate fits the observed figure. Adjusted R^2 attempts to correct r^2 to more closely reflect the goodness of fit. The correlation coefficient (r) of each model was considered here as the coefficient in the multiple regression analysis (R) when only one dependent variable is used. The fit of calculated figures to the observed ones of malaria cases is better in the order of a total rainfall in a year, monthly rainfalls in January and February, and average temperature in August, according to adjusted R^2 (Table 3).

Multiple meteorological factors: Multiple regression analyses and ANOVA were performed using the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) to calculate formulae of regression lines using the above mentioned 4 meteorological factors. All the 4 factors were entered first to the model, and factors were removed one by one from the model according to the backward method. All the formulae are presented with multiple regression coefficient (R), adjusted R^2, F-value of the result of ANOVA, and its probability.

The formulae using all 4 meteorological factors are as follows:

a. Mp =-0.45 × Rt+3.39 × R2+1.39 × R1+141.59 × Av8 - 2425.55

(R=0.936, Adjusted R^2=0.712, F=5.33, p=0.10)

Then the total rain was removed first from the model:

b. Mp = 2.62 × R2+1.13 × R1+108.67 × Av8 - 1955.79

(R=0.933, Adjusted R^2＝0.774, F=8.98, p=0.03)

Average temperature of August was removed next from the model.

c. Mp = 2.47 × R2+1.82 × R1 - 15.02

(R=0.923, Adjusted R^2＝0.792, F=14.34, p=0.008)

Finally, R2 was removed and R1remained in the formula

d. Mp = 3.12 × R1+43.37

(R=0.870, Adjusted R^2＝0.717, F=18.71, p=0.005)

Estimation by formulae using more factors is usually considered to be closer to the observed figures, but the adjusted R^2 is largest in formula c, using 2 meteorological factors, R 1 and R2. This was presumably caused by only 8 sets of the corresponding meteorological data and observed malaria cases in 8 Met Years.

Two meteorological factors: The formulae constructed by two meteorological factors, other than formula c, are shown below in the order of larger adjusted R^2.

e. Mp = 3.41 × R2+188.39 × Av8 - 3343.9

(R=0.921, Adjusted R^2＝0.786, F=13.94, p=0.009)

f. Mp = 1.62 × R1+0.80 × Rt - 194.77

(R=0.898, Adjusted R^2＝0.729, F=10.41, p=0.017)

g. Mp = 2.13 × R2+0.85 × Rt - 182.78

(R=0.892, Adjusted R^2＝0.714, F=9.74, p=0.019)

h. Mp = 1.23 × Rt + 88.84 × Av8 - 1852.2

(R=0.881, Adjusted R^2＝0.686, F=8.66, p=0.024)

i. Mp = 2.68 × R1+76.30 × Av8 - 1316.86

(R=0.876, Adjusted R^2＝0.673, F=8.22, p=0.026)

There was no combination of 2 factors to elevate R^2 figure than the above formula c involving R1and R2.

Table 3 Calculated estimation of malaria cases by single factorial formulae and observed figures at each peak season

Formula no.	1 Av8	2 R1	3 R2	4 Rt	Mp observed
1990/1991	392.70	5560.67	425.91	390.73	308.16
1991/1992	790.13	374.09	156.96	445.77	172.54
1992/1993	392.70	297.03	793.64	611.04	657.74
1993/1994	392.70	628.06	750.09	606.81	421.78
1994/1995	428.83	263.02	274.15	158.01	363.17
1995/1996	1,187.56	1,082.95	1,222.88	1,144.09	1,186.58
1996/1997	898.52	1,147.85	1,024.87	1,237.53	1,138.05
1997/1998	934.65	1,067.98	764.90	808.44	1,169.84
r^2	0.546	0.757	0.743	0.847	
adjusted R^2	0.470	0.717	0.700	0.722	

Table 4 Calculated estimation of malaria cases by single- and bi-factorial formulae

Time of estimation	Sep.	Feb.		Feb.	Mar.		Mar.	Mp
Formula no.	1	2	or	i	c	or	e	Observed
1990/1991	392.70	560.67		516.14	470.01		337.82	308.16
1991/1992	790.13	374.09		439.81	213.22		340.79	172.54
1992/1993	392.70	297.03		289.68	518.51		617.10	657.74
1993/1994	392.70	628.06		574.03	687.66		584.02	421.78
1994/1995	428.83	263.02		268.10	212.90		241.40	363.17
1995/1996	1,187.56	1,082.95		1,132.64	1,213.10		1,357.55	1,186.58
1996/1997	898.52	1,147.85		1,127.34	1,142.03		1,056.46	1,138.05
1997/1998	934.65	1,067.98		1,066.36	952.43		877.86	1,169.84
adjusted R^2	0.470	0.717		0.673	0.792		0.786	

Formula 1; involves Av8
2; R1
i; Av8, R1
c; R1, R2
e; Av8, R2

Estimation of intensity of malaria at peak: The above mentioned formulae can be utilised for obtaining approximate estimation of intensity of malaria outbreak in a Met Year (Table4). Useful formulae among all are the formula 1, 2, c and e with statistical significance. In early September, using Av8, the first approximation is available (Formula1, adjusted R^2= 0.470 without statistical significance). Then in early February, an estimation is made by using R1 (Formula 2, adjusted R^2= 0.717). Then in early March, better estimation is available using R1 and R2 (Formula c, adjusted R^2= 0.792). The total rainfall in a Met Year also showed a high correlation (adjusted R^2= 0.722) which can be used for a retrograde study. When the malaria data and meteorological data are accumulated more in the future, the reliability of the above proposed formulae will be examined for their goodness of fit, and also the formulae using more parameters may predict the intensity of malaria outbreak precisely.

DISCUSSION

The reliability of meteorological factors which influenced quantitatively the intensity of malaria outbreaks with highly statistical significance, can be extended to their relationships in the future. It means that the above formulae with highly statistical significance are useful for estimating the intensity of outbreaks in the future. Another issue in this study is a value of clinical malaria cases since proven malaria cases are not available in field health stations. However, at malaria peak season, high ratio of proven malaria is involved in clinical malaria, whereas in the other seasons, proven ones are involved only at around 5%.

In the present study, the rainfall was more influential to the intensity of malaria outbreak, and this finding was different from the results by Freeman and Bradley (1996) who gave an importance only to the temperature in September, although some meaning was given also to the temperature in August with a low significance in this study. Important results in this study are to point out the value of rainfall in January, February and a total of the Met Year by highly statistical significance, and their values will be consistent in the future.

The estimation of malaria occurrence, however, was changed much by the month of calculation in the same year. For example in Met Year 1991/1992 (Table 4), the estimated figure of malaria was comparatively high, but it was corrected to a lower level by less rain in January (Table 2) and further by drought in February (Table 2).

The results obtained herein gave an acceptable understanding of the physical influence of climate to the vector *Anopheles*. A higher temperature in August, possibly in September too, at the early spring in this country may enhance the start of hatching of the vector mosquitoes, and develop larvae to make larger population at the starting point of vector proliferation and increase the outbreak. The rainfalls in January and February also stimulate the growth of vector population. On the contrary, even if the estimate in September is large, the transmission will be suppressed by less rain in January and February. We are grateful, if this short note stimulates the interest of workers who will make better and precise correlations between meteorological factors and intensity of malaria outbreaks, leading to a better forecast.

ACKNOWLEDGMENTS

We appreciate very much to Dr. B. Piotti and Mr. Takamanya of Division of National Health Information System in Ministry of Health and Child Welfare for submitting

monthly occurrence of clinical malaria cases, and to Mrs. Chipindu, Mrs. Kasehe and Mrs. Chiramba of the Meteorological Station at Harare and to Mr. Shambare of WHO, Southern African Inter-country Office for providing meteorological survey data.

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A field evaluation study on the effects of residual spray of Bifenthrin and Deltamethrin on *Anopheles minimus* population in Mae Hong Son Province, northern Thailand.

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Accepted September, 10, 2002

Abstract: A field study was conducted to evaluate the effects of indoor residual house spraying of Bifenthrin and Deltamethrin on malaria vector population of *Anopheles minimus* s.l., from April 1999 to April 2001 at rural villages in Mae Hong Son province, northern Thailand. Nine villages in Mae Hong Son province were selected for the present study (three villages for control and three villages each for insecticide spray). The residual spray of Bifenthrin (25 mg/m^2) showed greater adulticiding effects on *An. minimus* s.l. population than Deltamethrin (20 mg/m^2). In Bifenthrin treated villages, a clear decrease in biting density of *An. minimus* s. l. was found in human bait collection as well as animal bait collection after the insecticide spray. In all of the three villages, the average density after insecticide spray was significantly lower than that observed before the spray. The effects of Deltamethrin on *An. minimus* s. l. density was found only in one village out of the three treated villages. A significant decrease in parous rate after insecticide spray was found in all the villages sprayed with Bifenthrin, whereas no significant changes were observed in control villages. The average parous rate in the villages treated with Deltamethrin became significantly higher after the insecticide spray. These results clearly suggested that the residual spray of Bifenthrin (25 mg/m^2) was more effective than Deltamethrin (20 mg/m^2).

Key words: residual spray, Bifenthrin, Deltamethrin, *Anopheles minimus*, Thailand

INTRODUCTION

The malaria control program in Thailand was started in 1950's and has resulted in an impressive reduction in malaria morbidity and mortality. For more than 40 years, DDT has been used in the malaria control program for indoor residual spraying throughout Thailand. However, the use of DDT for malaria control in Thailand has been decreasing from 1980's and nearly stopped recently because of the development of insecticide resistance in vector populations against DDT and the side effects of DDT spraying on the environment through the biological concentration (Malikul, 1988; Curtis, 1994; Chareonviriyahpop *et al.,* 1999).

The screening of insecticide alternative to DDT has become an important subject for malaria control in Thailand, since insecticide spraying is still the most effective control measure to stop the malaria transmission, especially during its epidemic. Several field studies have been carried out recently in Thailand on the effectiveness of insecticide in malaria vector control (Photijitthi *et. al.,* 1999; Prajakwong *et. al.,* 1997ab; Somboon *et. al.,* 1995; Vongprayoon *et. al.,* 1999, Suwonkerd *et. al,* 1997). Bifenthrin is a newly synthesized pyrethroid and the effectiveness has been studied preliminary in the Office of Vector Borne Diseases Control 2 (VBDO2), Chiangmai, Thailand in 1998. Based on the results of the preliminary study, the present study was conducted to compare the effects of indoor residual house spraying of Bifenthrin and Deltamethrin on malaria vector population of *Anopheles minimus* s.l., from April 1999 to April 2001 at rural villages in Mae Hong Son province, northern Thailand.

MATERIALS AND METHODS

Study area: Mae Hong Son province is located at the Thai-Myanmar border in northwestern Thailand, and the incidence of malaria in the province is the highest in the upper northern part of Thailand (VBDO2, 1999). Nine villages in Mae Hong Son province were selected for the present study. The total number of houses, population, percent-

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age of houses having a bed net and average surface area to be sprayed in each village were shown in Table 1. Three villages each were sprayed with insecticide (Bifenthrin or Deltamethrin) and remaining 3 villages were left without insecticide spraying (control villages).

Insecticide application: Insecticide was applied two times in April and August 2000. Bifenthrin 25 mg/m^2 or Deltamethrin 20 mg/m^2 was applied to the inside wall of household by using Hudson X-pert hand compression pumps with flat fan 8002 nozzles. The actual dosage of insecticides applied on the wall was checked by WHO (Head Quarter in Geneva, Switzerland) by processing the filter papers (Whatman No.1) placed on a wall before the insecticide spraying.

Evaluation of the effect of insecticide spray on vector mosquitoes: The following two different methods were used for evaluating the effects of insecticide spray on vector mosquitoes; (1) survey of wild vector populations and (2) surface contact bioassay.

The wild vector population was surveyed monthly in each village by using an indoor and outdoor human bait collection and animal bait collection. Four houses in each village were selected and a pair of collector sat inside and outside of the houses. Landing mosquitoes were collected for 50 min with a 10 min of break interval from 18:00 to 24:00. For the animal bait collection a cow was tethered inside a gauze net (4 by 4 by 2 m), which was similar to the one described by Service (1993). One collector aspirated mosquitoes landing in and out of the net at every 15 min from 18:00 to 24:00. The collected mosquitoes were kept in a plastic cup hourly for the later identification. During the mosquito collection relative humidity and temperature were recorded. Among female *An. minimus* s. l. collected from human bait collection, at least 50 unfed females were dissected for the parity check (Detinova, 1962) and were checked for malaria sporozoite in salivary glands.

The surface contact bioassay test (WHO, 1975) was carried out monthly after the1st insecticide application. One house in the sprayed villages was selected for the test. Five different places on the inside wall was marked and used for the insecticide exposure test. Twenty females of 3-5 day-old *An. minimus* s. l. laboratory strain (Mae Hong Son strain) were aspirated into each cone, and totally 100 females were used for each test. The females were exposed to sprayed surface for 30 min, and the number of dead and knocked down mosquitoes were counted at the end of exposure. The number was counted again after 24 hr. Abbot's correction was applied to calculate mortality rate based on the mortality rate of control group.

Data analysis: The average density of *An. minimus* s. l. was calculated for two periods, before and after the insecticide spraying, and the significance of the difference was tested by the t-test. The difference in parous rate was analyzed by ANOVA or the t-test after made the arcsine transformation. For the statistical analysis of the surface contact bioassay test, the study period was divided into 3 periods of 4 month, and the average mortality was calculated and compared after the arcsine transformation. Variation in temperature and humidity among village was analyzed by ANOVA, and Tukey's HSD test was used for a pair wise comparison of means. All statistical analysis was performed by using Systat statistical software (Wilkinson, 1996).

RESULTS AND DISCUSSION

Coverage of indoor insecticide spraying and climate conditions during the study: The percentage of houses sprayed with insecticide in this study was shown in Table 1. The coverage of insecticide spray reached more than 89% of the total houses, except for one village, Pong Kan Nai where the coverage was 79.1 and 80.6% at1st and2nd insecticide spray, respectively. Houses without resident or refused by house owner were not treated with insecticide.

Significant differences in humidity were observed among the study villages ($F=5.401$, $p<0.001$, Table 1). The average humidity recorded in the villages selected for Bifenthrin spray were significantly lower than other villages, while no significant differences were found for temperature among villages ($F=1.116$, $p=0.353$).

Effects of insecticide application on wild population of* An. minimus *s. l.: A total of 80,662 anopheline of 22 species were collected in the present study. Among them 30,340 and 98 were morphologically identified as A*n. minimus* s.l. and *An. dirus* s.l. respectively, clearly showing that *An. minimus* s.l. was the most important malaria vector in the study villages. A total of 12,600 *An. minimus* s.l. were dissected for the detection of malaria sporozoite in the salivary gland and all of them were negative.

Table 2 compares the average density of *An. minimus* s. l. before and after the insecticide spray. The residual spray of Bifenthrin showed greater effects on *An. minimus* s.l. population than Deltamethrin. The seasonal changes in *An. minimus* s.l density observed before the insecticide spray was similar to that reported in previous papers (Ismail *et al.,* 1974; Ismail *et al.,* 1975; Suwonkerd *et al.,* 1995; Takagi *et al.,* 1995; Suwonkerd *et al.,* 1997). However, in Bifenthrin treated villages a clear decrease in biting density of *An. minimus* s. l. was found in human bait collection as well as animal bait collection after the insecticide spray. In all of the three villages, the average density after the insecticide spray was significantly lower than that before the spray (t-test, $p<0.05$).

Table 1. Demographic parameters, climate conditions and results of insecticide application at each study village in Mae Hong Son, northern Thailand.

	Village								
	Tong Muang	Pakolo	Huey Pong On	Pong Kan Nai	Sao Tao	Chang Chum	Mai Sape	Huey San Nok	Tobsok
Number of houses	49	52	82	61	136	101	92	36	74
Population	141	181	304	326	556	410	426	119	276
Average (±sd) temperature during the study*	25.0^{a}±4.0	24.3^{a}±3.4	23.6^{a}±4.8	23.9^{a}±4.1	25.3^{a}±3.1	23.4^{a}±3.4	23.1^{a}±3.9	24.7^{a}±3.2	25.2^{a}±2.7
Average (±sd) humidity during the study*	68.8^{bc}±8.8	68.8^{c}±8.2	67.6^{c}±7.8	75.8^{ab}±13.3	77.9^{ab}±9.6	79.1^{ab}±9.1	76.2^{ab}±7.3	77.5^{ab}±6.6	80.2^{a}±17.3
% houses having a bed net	93.8	98.0	85.2	100	87.5	93	93.5	86.1	87.8
Average surface to be sprayed (m^2)/house	163.8	206.2	175.6	198.9	280.9	266.9	184.8	247.8	223.8
Insecticide sprayed	Bifenthrin			Deltamethrin			No spray		
% houses sprayed**									
1st application	92.6	96.4	90.2	79.1	91.6	94.9	—	—	—
2nd application	92.3	93.3	89.4	80.6	92.8	92.8	—	—	—

*Averages in the same row followed by the same letter were not significantly different (Tukey's HSD test, p>0.05).
**1st application = 24-27 April 2000; 2nd application = 23-25 August 2000.

Table 2. Comparisons of average density (±sd) of *An. minimus* s. l. observed before and after the insecticide spray in Mae Hong Son, northern Thailand from April 1999 to April 2001.

Village	Indoor Collection			Outdoor Collection			Animal bait Collection		
	Before spray	After spray	p	Before spray	After spray	p	Before spray	After spray	p
Bifenthrin sprayed village									
Tong Muang	43.1 ±34.7	3.5 ±3.5	<0.001	73.7 ±40.4	18.9 ±11.0	<0.001	10.1 ±13.0	0.3 ±0.7	0.004
Pakolo	84.8 ±74.1	11.3 ±9.6	<0.001	183.3 ±150.6	82.4 ±88.6	0.039	37.2 ±54.1	2.9 ±8.1	0.012
Hueypong On	18.5 ±18.6	0.8 ±1.4	<0.001	43.0 ±43.0	11.1 ±11.9	0.006	15.4 ±26.7	0	—
Deltamethrin sprayed village									
Pong Kan Nai	68.7 ±87.9	91.4 ±162.6	0.719	176.6 ±211.7	193.8 ±254.7	0.870	33.3 ±45.4	39.5 ±36.4	0.711
Sao Tao	32.1 ±24.9	5.0 ±3.5	<0.001	67.6 ±44.8	22.6 ±12.4	<0.001	0.3 ±0.9	7.8 ±8.0	0.034
Chang Chum	68.6 ±56.8	47.8 ±67.8	0.458	168.1 ±133.7	113.5 ±114.6	0.296	3.2 ±3.9	29.5 ±35.8	0.076
Control village									
Tob Sok	8.0 ±8.8	14.3 ±6.7	0.069	38.7 ±39.4	33.9 ±18.7	0.689	No data	0	—
Mai Sa Pe	2.0 ±3.7	8.62 ±11.0	0.141	8.8 ±13.6	22.8 ±40.2	0.377	1.2 ±1.6	1.0 ±2.4	0.876
Huey San Nok	10.8 ±16.5	62.4 ±53.8	<0.001	47.7 ±53.3	178.6 ±130.5	0.025	1.7 ±4.6	0	—

*The insecticide was sprayed twice; 1st spray= April 2000, 2nd spray= August 2000.

The effects of Deltamethrin on *An. minimus* s. l. density was found only in one village (Sao Tao) out of three villages. The average biting density on human bait was significantly reduced after the insecticide spray, whereas in animal bait collection it showed significant increase after the insecticide spray (t-test, p<0.05).

No significant increase or decrease was found in biting density of *An. minimus* s. l. in control villages.

The average parous rate of mosquitoes was calculated for each village and compared before and after the insecticide spray in Table 3. A significant decrease in parous rate after insecticide spray was found in all the villages sprayed with Bifenthrin, whereas no significant changes were observed in the control villages. The average parous rate in the

Table 3. Comparisons of average (±sd) parous rate before and after the insecticide spray in Mae Hong Son, northern Thailand from April 1999 to April 2001.

	Before spray	After spray	p
Bifenthrin sprayed village			
Tong Muang	70.8 ±13.5	32.3 ±11.2	<0.001
Pakolo	69.3 ±16.7	32.7 ±16.0	<0.001
Hueypong On	72.6 ±12.7	31.7 ±13.5	<0.001
Deltamethrin sprayed village			
Pong Kan Nai	47.3 ±17.5	78.5 ±13.2	0.005
Sao Tao	50.0 ±18.4	84.0 ±6.3	<0.001
Chang Chum	43.3 ±17.6	73.1 ±19.1	0.020
Control village			
Tob Sok	38.9 ±16.3	46.9 ±18.4	0.383
Mai Sa Pe	37.2 ±22.1	44.9 ±18.5	0.624
Huey San Nok	41.5 ±16.3	49.1 ±19.3	0.392

Table 4. Average mortality (±sd) of adult *An. minimus* s. l. observed by the bioassay test in 3 different month periods after the insecticide spray*.

	Period after 1st spray (month)	Bifenthrin	Deltamethrin	p
1st spray				
	1-4	98.50 ±2.00	77.50 ±15.92	<0.001
2nd spray				
	5-8	97.75 ±2.91	79.75 ±8.94	<0.001
	9-12	76.75 ±12.24	72.75 ±32.01	0.674

*The insecticide was sprayed twice; 1st spray= April 2000, 2nd spray= August 2000.

villages treated with Deltamethrin became significantly higher after the insecticide spray. No significant decrease was observed in parous rate as well as the biting density of *An. minimus* s.l. in a village treated with 25 mg/m^2 of Deltamethrin in a previous study (Prajakwong *et al.,* 1997a).

Results of surface contact bioassay test: The mortality of adults exposed to the Bifenthrin-treated wall was always higher than that found on Deltamethrin-treated wall (Table 4). The difference in mortality between Bifenthrin and Deltamethrin was significant during the 1-4 month and 5-8 month, but not during the 9-12 month (Tukey's HSD test). The average mortality of adults exposed to a Bifenthrin-treated wall during the 1-4 month and the 5-8 month was 98.5 and 97.75, respectively, and the difference was not significant (t-test, p>0.05). However, the mortality decreased significantly to 76.75 % in the 9-12 month. On the Deltamethrin-treated wall the adult mortality was less than 80% and not significantly different between the 3 study periods (F=0.019, p=0.981), and within the first 4 months after spray, the mortality of exposed adults decreased rapidly from 92.0 to 67 %. With a higher dosage of Deltamethrin (25 mg/m^2) than the present study (20 mg/m^2), Prajakwong *et al* . (1997a) observed the mortality rate of exposed adult *An. minimus* s. l. from 100-92 % during the first 3 months. The lower residual effects of Deltamethrin, because of the low dosage in this study, might be one of the reasons for the less effectiveness of Deltamethrin to *An. minimus* s.l. population.

These results clearly suggested that the residual spray of Bifenthrin (25 mg/m^2) was more effective than Deltamethrin (20 mg/m^2). However, the average density of *An. minimus* s.l. achieved in Bifenthrin-treated villages was nearly the same level as that observed in the control villages (Table 2). Since indigenous malaria cases, in which the infection was acquired inside the village of residence, were reported every year from the control villages, a higher dosage or more frequent spray of the insecticide is required to control malaria from in these villages.

Acknowledgement

We thank FMC Corporation for financial support and all field staff of Office of Vector Borne Diseases Control, Region 2, Chiangmai, Thailand for their continuous help for the field study.

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Sudomotor modifications by acclimatization of stay in temperate Japan of Malaysian native tropical subjects

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Accepted September, 14, 2002

Abstract: Tropical subjects regulate core temperature with less amount of sweat against heat compared to temperate subjects through long-term heat-acclimatization. The purpose of the study is to determine whether acclimatization in tropical subjects decay during a stay in temperate area. Local sweating response activated by acetylcholine (ACh) applied iontophoretically among Malaysians (n=26) with varying duration of stay in Japan and Japanese residents subjects (J-R n=30). Based on their length of stay, Malaysian subjects were divided into three groups, group M-S (n=9) with a duration of stay of1to 12 months, M-M (n=7) with 13 to 36 months and M-L (n=10) with 37 to 72 months. ACh, the primary transmitter for sudomotor innervation, was iontophoretically administered on the forearm. Sweating response elicited directly (DIR) and indirectly via axon reflex (AXR) were evaluated by quantitative sudomotor axon reflex test. The onset-time of AXR was shortened with the longer duration of stay among Malaysian subjects (P <0.01). DIR, sweat volume directly (ACh muscarinic receptors) induced by ACh for 6-11 min, was 0.68±0.35 mg/cm^2, 1.02±0.64 mg/cm^2, 1.45±0.71 mg/cm^2 and 2.39±0.47 mg/cm^2 in M-S, M-M, M-L and J-R, respectively. These were statistically different (P <0.01). From these results, suppressed neuroglandular response to ACh was confirmed in Malaysians. It is suggested that long-term heat-acclimatization acquired in tropical subjects may decay after immigration to temperate area.

Key words: long-term heat-acclimatization, deacclimatization, sweating response to ACh, Malaysians, Japanese.

INTRODUCTION

Sweating is a mechanism of heat dissipation for humans when exposed to a hot environment. It is known that sweating response to heat is influenced by climatic condition. Heat-tolerance is achieved by the lowering of threshold for sweating and enhanced sweating in short-term acclimation (Nadel *et al.,* 1974, Ogawa and Sugenoya, 1993). In an area where there is a distinct seasonal fluctuation of ambient temperature like Japan, various physiological responses may change season by season. During summer, the sweat rate is higher, with a shorter latent period for sweat onset and lower salt concentration in sweat than in winter (Kuno, 1956; Yoshimura, 1960; Hori *et al.,* 1976; Sugenoya *et al.,* 1995). It is generally accepted that tropical natives having acclimatized to heat for a long time begin to sweat more slowly than temperate natives and the salt concentration in sweat in the former is much lower than in the latter (Kuno, 1956; Yoshimura, 1960; Hori *et al.,* 1976; Ohwatari *et al.,* 1983; Sasaki and Tsuzuki, 1984; Lee *et al.,* 1999).

On the contrary, tropical inhabitants show heat-tolerance with suppressed sweating (Kuno, 1956; Ogawa and Sugenoya, 1993; Matsumoto *et al.,* 1993; Lee *et al.,* 1997; Matsumoto *et al.,* 1997). Adaptation to temporary exposure to heat and acclimatization to tropical climate by permanent residents were distinguishable from each other (Kuno, 1956). The thermotolerance with suppressed sweating and enhanced dry heat loss is more predominant than that with enhanced sweating which is seen in short-term heat acclimatization from the view points of body fluid maintenance and osmoregulation (Lee *et al.,* 1997; Matsumoto *et al.,* 1997).

The natives in torrid zone have the capacity to sweat but have acquired an ability to avoid excessive sweating by acclimatization (Kuno, 1956). For settlers of less than 3 years, the sweat reflex is similar to that of newcomers. It

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has been suggested that more than 6 years of residence in the tropics is necessary to acquire the same capacity as the natives (Kuno, 1956).

An upward shift of threshold core temperature for sweating and decrease in acetylcholine (ACh)-sensitivity of the sweat glands were reported in Thai subjects and Africans. It has been suggested that sudomotor mechanisms is down-regulated both centrally and peripherally in tropical natives (Lee *et al.,* 1997; Matsumoto *et al.,* 1997). In this study to clarify the deacclimatization process of heat-acclimatized tropical subjects through residence in temperate zone, local sweating response activated by ACh applied iontophoretically was compared between Malaysians who stay in Japan for a certain period of time and Japanese permanently staying in Japan under a thermoneutral condition.

MATERIALS AND METHODS

Subjects

Experimental subjects were 56 young sedentary adults male, 26 Malaysians and 30 Japanese between 19-34 years of age with similar physical activity levels. There were significant differences in weight and height (P <0.01). Based on the length of stay in Japan, Malaysian subjects (n=26) were divided into 3 groups, M-S (n=9) with a duration of stay of 2 to 12 months, M-M (n=7) with 13 to 36 months and M-L (n=10) with 37 to 72 months. Japanese subjects (J-R, n=30) were students and staff of Nagasaki University. Physical characteristics of the subjects were as shown in Table1. Malaysia (3 °80' N, 101 °42' E) is located in a tropical zone with dry and wet-seasons with minimal seasonal variations. Annual mean ambient temperature is 26.7℃ and relative humidity of 81%. Nagasaki (32 °44' N, 129 °52' E) is located in a temperate zone with hot summers and cold winters; 16.7℃ and 72% relative humidity. The subjects gave informed consent after having been acquainted with the potential risks associated with experimental procedures. We paid great attention to the subjects in accordance with Helsinki Declaration of 1975.

Measurements and procedures

The experiment was carried out in a controlled climatic chamber at 24.0±0.5℃ with relative humidity of 40 ±3% and less than 1 m/sec air velocity at 2-5 pm. Prior to the test, subjects were dressed lightly and were rested in the climatic chamber for 60 min. Quantitative sudomotor axon reflex test, QSART (Low *et al.,* 1983; Kihara *et al.,* 1993; Lee *et al,* 1997; Chemali *et al.,* 2001) was performed to quantitatively evaluate glandular ACh-sensitivity. The QSART capsule consists of three concentric compartments. ACh iontophoretically applied stimulates the underlying sweat glands in the outer compartment directly while the glands of the skin in the central compartment of the capsule are activated indirectly via axon reflex. Both direct (DIR) and axon reflex-mediated (AXR) sweat responses were measured. Two sets of QSART capsules were attached on the volar aspect of the forearm with rubber bands, one at the mid portion between the wrist and elbow joint and another at 10 cm proximal to the former. The outer compartment of the former capsule was filled with 10% ACh (Ovisot, Daiichi Pharmaceutical Co., Ltd., Japan) solution. A direct current of 2 mA was applied for5min between an electrode on the ACh cell (anode) of the capsule and a flexible plate-electrode (HV-BIGPAD, Omron, Kyoto) (cathode) attached on the forearm skin just proximal to the wrist joint. The central compartment of the ACh capsule served as the site for sudomotor axon reflex, AXR(1), measurement during the 5 min of iontophoresis. Immediately after the cessation of the current loading, sweat capsules were detached, the skin covered with ACh capsule was wiped up and then the two capsules positions were exchanged. This procedure took less than 20 sec. The data was acquired for another5 min to permit the simultaneous observation of DIR and AXR(2) sweating (Lee *et al.,* 1997).

Sweat onset-time, latent period for sweating after current loading, and sweat volume for 5 min, area under the sweating curve, 0-5 min for AXR(1) and 6-11 min for AXR (2) and DIR were used for analysis. Sweat rates were measured by the capacitance hygrometer-ventilated capsule method (Matsumoto *et al.,* 1993; Lee *et al.,* 1999). In brief, nitrogen gas flowed into each compartment with a constant flow rate of 0.3 L/min, and the change of relative humidity of effluent gas was detected by a hygrometer (H211, Technol Seven, Yokohama). Sweating rates were recorded with PC (PC 9801, NEC, Japan) every 5 sec (Lee *et al,* 1997).

Statistical analysis

All data are expressed as means±SD. Statistical significance was determined by ANOVA with Tukey's post hoe test for comparison of mean values among M-S, M-M, M-L and J-R at P < 0.05 level.

RESULTS

The physical characteristics of the 4 groups of subjects are tabulated in Table1. Japanese native subjects were taller and heavier than M-S, M-M and M-L (P <0.01). Typical recording of a single subject is shown in Fig.1. When ACh was applied iontophoretically, AXR sweating occurred after a latent period (onset-time of axon reflex sweat) and reached a plateau phase within a few minutes. At the end of iontophoresis, DIR(2) sweating became sustained, while

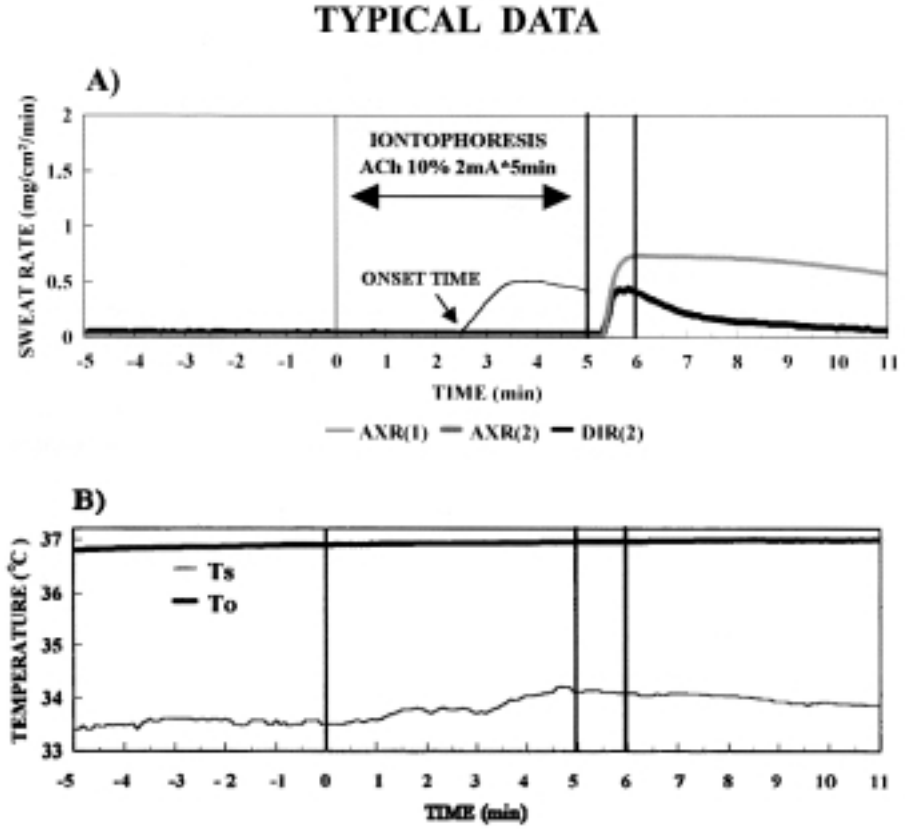

Fig 1. Typical recording of the AXR(1), AXR(2) and DIR sweating (upper panel) and of oral and skin temperatures (lower panel) in a Malaysian native subjects (M-M). Iontophoresis of 10% ACh was performed with 2 mA of direct current for 5 min. Just after the cessation of current loading, the sweat capsules were exchanged each other, then recordings were continued for another 5 min. Sweat onset-time was 2.33 min. Sweat volume was 0.981 mg/cm^2, 0.662 mg/cm^2 and 3.463 mg/cm^2 on AXR(1), AXR(2) and DIR, respectively. Ts: Skin temperature just beside ACh capsule, To: Oral temperature

Table 1. Physical characteristics of the subjects.

	N	Age (years)	Height (cm)	Weight (kg)
J-R	30	25.3±3.9	171.4±4.1	66.90±4.58
M-S	9	24.8±2.4	162.3±3.7*	57.81±3.27*
M-M	7	26.1±3.1	163.2±4.5*	59.20±4.41*
M-L	10	28.4±5.3	165.2±3.9*	60.25±4.83*

Values are means±SD. *Significant difference at P <0.01 compared with J-R.
J-R: Japanese native residents
M-S: Malaysian native subject of staying in Japan for 2- 12 m
M-M: Malaysian native subject of staying in Japan for 13- 35 m
M-L: Malaysian native subject of staying in Japan for 36 - 72 m

AXR(2) sweating declined to the baseline during the observation.

Sweat onset-time of axon reflex (ACh nicotinic receptors) induced by ACh iontophresis, was 2.25±0.43 min, 1.65±0.55 min, 1.45±0.64 min and 1.23±0.38 min in M-S, M-M, M-L and J-R, respectively (Fig. 2). These were statistically different (P <0.01) each other.

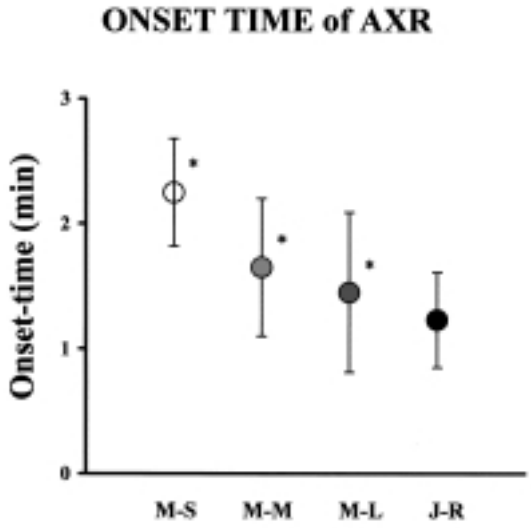

Fig 2. Comparison of the sweat onset-time among Malaysian subjects with different durations of stay in Japan and J-R. Values are means±SD.
Sweat onset-time of axon reflex (ACh nicotinic receptors) induced by ACh iontophresis, was statistically different in compared with J-R (P <0.01).

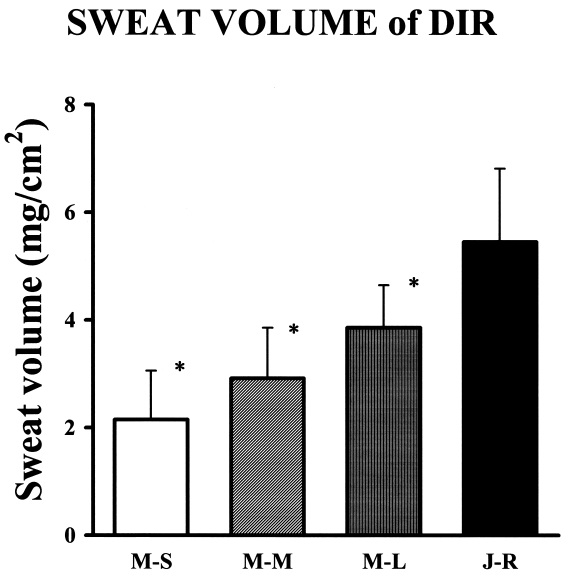

Fig 3. Comparison of DIR, directly activated sweat volume for 6-11 min among Malaysians with different durations of stay in Japan and J-R. Values are means±SD. These were statistically different in compared with J-R (P < 0.01).

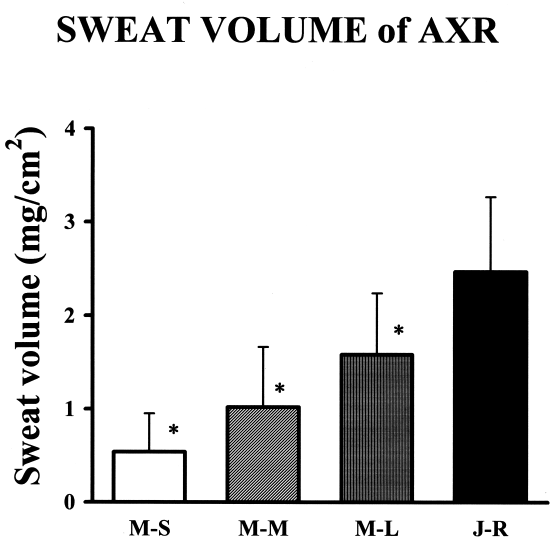

Fig 4. Comparison of AXR, axon reflex-mediated sweat volume for 0-5 min, among Malaysians with different durations of stay in Japan and J-R. Values are means±SD. These were statistically different in compared with J-R (P <0.01).

DIR, sweat volume directly (ACh muscarinic receptors) induced by ACh for 6-11 min, was 2.15±0.91 mg/cm^2, 2.92±0.94 mg/cm^2, 3.86±0.79 mg/cm^2 and 5.45±1.37 mg /cm^2 in M-S, M-M, M-L and J-R, respectively (Fig. 3). The differences were statistically significant (P <0.01).

AXR, sweat volume indirectly induced via axon reflex (ACh nicotinic receptors) by ACh for 0-5 min, was 0.54± 0.41 mg/cm^2, 1.02±0.64 mg/cm^2, 1.58±0.66 mg/cm^2 and 2.47±0.80 mg/cm^2 in M-S, M-M, M-L and J-R, respectively (Fig. 4). These were statistically different (P <0.01) each other.

There was a positive correlation between DIR and the duration of stay in Japan among Malaysian subjects, r= 0.588, P <0.001 (Figure not shown).

DISCUSSION

In this study, to clarify whether heat-acclimatized tropical subjects loose their nature of acclimatization through residence in a temperate area, we examined the ACh-sensitivity of the sweat glands by QSART in the tropical Malaysians staying in temperate Japan.

Suppression of sweating response to ACh applied iontophoretically has been shown in Thai and tropical Africans (Lee *et al.,* 1997; Matsumoto *et al.,* 1997). In this study we showed suppressed sweating response to ACh in Malaysians.

Expression levels of nicotinic ACh receptors and muscarinic ACh receptors may be genetically determined and/or regulated by environmental factors such as physiological stress and infection (Sato *et al.,* 1999).

AXR (sweating of ACh nicotinic receptors) and DIR (sweating of ACh muscarinic receptors) in both M-S, M-M and M-L were significantly smaller than those in J-R. Sweat onset-time in Malaysians was longer than in Japanese with the significant differences (P <0.01).

Heat-tolerance is achieved by the lowering of threshold for sweating and enhanced sweating in short-term acclimation (Nadel *et al.,* 1974; Ogawa and Sugenoya, 1993). During acclimation through daily exercise in a hot environment, these thermoregulatory changes are about three-quarters developed by the end of the first week of exposure and is generally thought be complete after 10-14 days (Pandolf and Young, 1999). Heat acclimation is transient and gradually disappears if not maintained by repeated heat exposure. It is believed that heat acclimation might be retained for 2 weeks after the last heat exposure but then be rapidly lost during the next 2 weeks (Lind, 1964).

According to our knowledge, this is the first report, except our preliminary report (Saat *et al,* 1999), showing deacclimatization process of heat-acclimatized tropical subjects through residence in a temperate area. DIR (sweating of ACh muscarinic receptors) sweating of Malaysian subjects showed a positive correlation with duration of stay in Japan (P <0.01). It might indicate that long-term heat-acclimatization acquired in tropical subject who had born and raised in a tropical area decays after immigration to a

temperate area. It is suggested that long-term heat-acclimatization is, at least some part, a phenotypic phenomenon.

ACKNOWLEDGEMENT

The authors would like to express sincere thanks to Miss Junko Kawashima and Miss Junko Hayashima for excellent secretarial assistance.

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AN ENDEMIC HUMAN INFECTION WITH *HETEROPHYES NOCENS* ONJI *ET* NISHIO 1916 AT MIKKABI-CHO, SHIZUOKA, JAPAN

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Accepted August, 11, 2002

Abstract

Human *Heterophyes nocens* infection was confirmed in 2 areas of Mikkabi-cho, Shizuoka Prefecture where high prevalence of *Metagonimus yokogawai* infection had been reported. The total prevalence showed 9.6% by stool examination. Among the patients, the older age's males predominated, and the infection within the same family was often observed. Adult worms were collected from stool samples of the patients after treatment with praziquantel. The worms were identified as *H. nocens* with morphological characteristics. The average size of eggs (27.8 x 16.1 μm) appeared in stools of the patients was slightly larger than that reported earlier, and rather equivalent to eggs of *M. yokogawai.* Questionnaire study revealed a correlation of a habit of eating raw fish, mullet in particular, and the trematode infection in Hamana Lake.

INTRODUCTION

Heterophyid trematodes have occurred in various areas in the world. Among them *Metagonimus yokogawai* and *Heterophyes nocens* are known to be distributed mainly in Korea and Japan (Ito, 1964; Saito, 1999; Chai and Lee, 1990; Chai *et al.,* 1994; 1997; 1998). While human cases with *M. yokogawai* are common, those with *H. nocens* are rather fewer and most cases in Japan have been reported from western areas except Chiba Prefecture (Yokogawa *et al.,* 1965). This distribution pattern may be related to the habit of eating raw fish that is the second intermediate host of *H. nocens* (Ito, 1964).

In Shizuoka Prefecture, *M. yokogawai* is widely distributed and the metacercariae are found in the sweetfish, *Plecoglossus altivelis* (Ito *et al.,* 1967a, b; Mochizuki and Ito, 1975). Areas surrounding Hamana Lake in western part of the prefecture were also reported to be endemic for *M. yokogawai* (Ito *et al.,* 1991). In 1997, a stool examination at a junior high school in Mikkabi-cho revealed 10 egg positive children with a rate of 1.5% for the heterophyid fluke. These children lived various places in the area, and it was suggested that the infection has been endemic throughout the area. In Mikkabi-cho, however, there are no rivers where the sweetfish can grow and the endemicity of *Metagonimus* infection could not be explained. This suggests that the infection was caused by other fish species in Hamana Lake.

The present study aimed to clarify prevalence of the infection in Mikkabi-cho and to determine the causative parasite species to the infection among the residents in the area.

MATERIALS AND METHODS

1) Study site

Mikkabi-cho is located in the north end of Hamana Lake in Shizuoka Prefecture and its population was 16,300 in 1997. Two districts were selected for the survey: Nueshiro and Daifukuji, located at lake side and at mountain side, respectively, with the population of 600 each.

2) Stool examination

Containers for stool collection were distributed to all residents in both districts and collected after a few days. Stool samples were examined for trematode eggs with the Kato's cellophane thick smear technique. Each sample was

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examined 3 times under a microscope.

3) Treatment

Subjected people with egg positive were informed the result and asked to admit local clinics and to have praziquantel (Biltricide®) at a dose of 20mg/kg.

4) Adult recovery

Whole stool at the first defecation after treatment was collected. The stool samples were dissolved with tap water and washed several times to remove soluble contents and fine particles. Then sediments were sieved with a wire mesh to remove large particles, and remaining sediment was examined for adult worms under a dissecting microscope. When the worm was found, it was fixed with 70% alcohol and stained with borax carmine.

5) Measurement of eggs

Eggs were collected from positive stool samples with the centrifugation technique and the size was determined under a light microscope.

6) Questionnaire

A questionnaire was sent to 140 families, who cooperated to the stool examination, to ask on eating habit of fishes, fish species, way of cooking and frequency of eating fishes in Hamana Lake. Results were analyzed using χ^2 test with the contingency table.

RESULTS

A total of 457 stool samples was collected from the two districts, Nueshiro and Daifukuji. Results of stool examination are shown in Table 1. Eggs detected were small and ovoid in shape, and the operculum was seen at one end, showing characteristics of heterophyid trematode eggs (Fig. 1). Prevalence rates of the fluke infection were 7.5% (10/134) in Nueshiro and 10.5% (34/323) in Daifukuji, respectively, and with a total prevalence of 9.6% (44/457). The rates seemed higher in male than in female in both areas, but the differences were not significant. Among 44 positive persons, 15 persons belonged to 7 families and the number of positive family was 36 out of 140 families subjected (25.7%). Persons at age of 30's or older composed of 90.9% (40/44) of the patients (Fig. 2). Out of 4 remaining

Table 1
Result of stool examination in 2 areas in Mikkabi-cho

District	No. of people examined			positive for heterophyid eggs (%)		
	M	F	Total	M	F	Total
Daifukuji	153	170	323	20 (13.1)	14 (8.2)	34 (10.5)
Nueshiro	61	73	134	6 (9.8)	4 (5.5)	10 (7.5)
Total	214	243	457	26 (12.1)	18 (7.4)	44 (9.6)

M: male, F: female

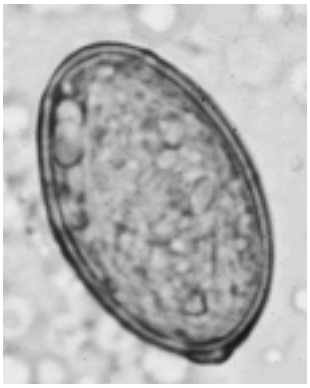

Figure 1 An egg found in stool of a patient in Mikkabi-cho.

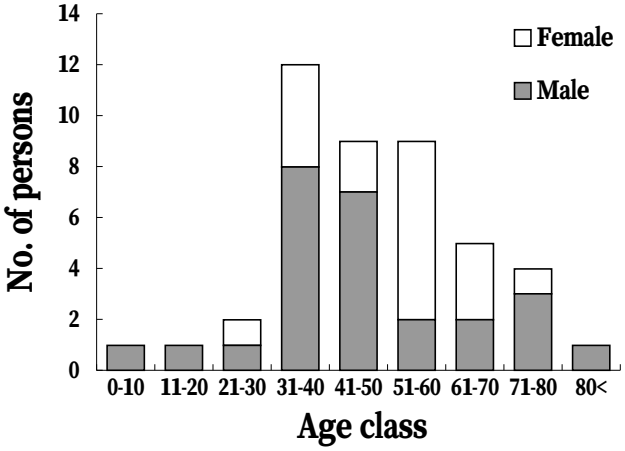

Figure 2 Age distribution of egg positive persons in Mikkabi-cho.

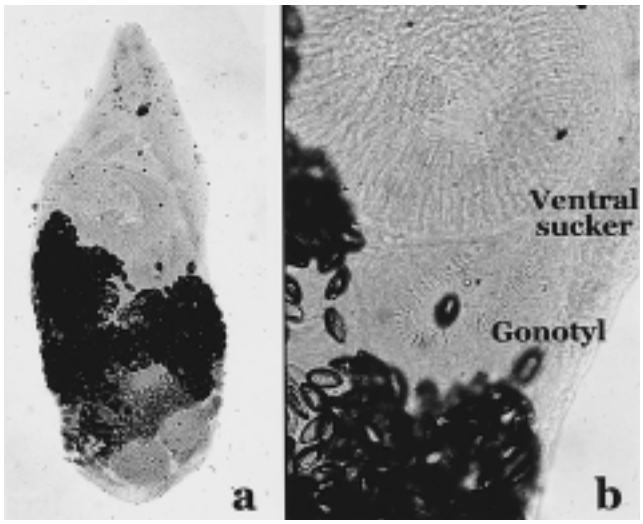

Figure 3 a, Adult worm of *Heterophyes nocens* found from human stool after treatment with praziquantel.
b, Enlarged view of ventral sucker (upper) and gonotyl (lower). Chitinous rodlets are seen on the gonotyl.

Table 2
Measurement of adult worms shown in range among 5 worms (μm).

Body length	975 - 1365
Body width	335 - 546
Oral sucker	47-55 x 51-86
Pharynx	50-70 x 31-70
Esophagus	62 - 132
Acetabulum	156-273 x 226-351
Gonotyl	94-140 x 128-171
Ovary	64 - 132
Testis	94-148 x 78-105
No. of chitinous rodlets	49 - 58

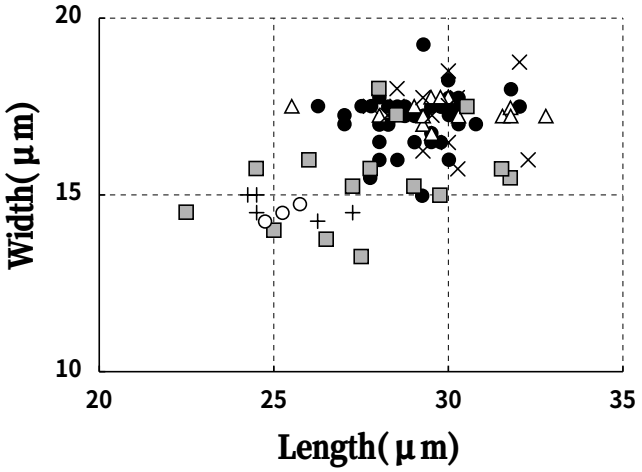

Figure 4 Size distribution of eggs from 6 patients. Each symbol represents each patient.

younger positive persons, 2 had another positive person in the same family.

A total of 30 stool samples was collected from the patients after treatment, and adult worms were found from one patient in each area; 2 worms in Nueshiro and 8 worms in Daifukuji (Fig. 3). Although the worms were loosened and elongated, all worms had ventral sucker (acetabulum) and gonotyl indicating that they belong to genus *Heterophyes* (Fig. 3b). The worms were identified as *H. nocens* Onji *et* Nishio, 1916 from this finding and results of measurement shown in Table 2.

The size of 98 eggs from 6 patients is shown in Fig. 4. The size of eggs varied from 22.5 to 32.8 μm in length and 13.3 to 19.3 μm in width with an average of 27.8 x 16.1 μm.

Questionnaire for fish eating habit of residents was returned from 72 families (51.4%). The positive families were more (61.1%) than that of negative families (48.1%). Approximately 70% of the families had a habit of eating fishes caught in Hamana Lake. Among the fishes, sweetfish, mullet (*Mugil cephalus*) and gobby (*Acanthogobius flavimanus*) were preferably eaten by a half of the families. Mullet was eaten raw by 49% of the family, similarly, 23% for gobby and 26% for Shirauo (*Salangichthys microdon*). Seventy three percent of positive families particularly had the habit of eating raw mullet and it was significantly higher than that of negative families ($P<0.05$). This tendency was higher in Daifukuji, but not signicant in Nueshiro. There was no significant relation between infection and eating raw fish for gobby and Shirauo with the fluke infection.

DISCUSSION

A relatively high level of human infection with heterophyid trematode has been known in areas around Hamana Lake and the infection was thought as that with *M. yokogawai* (Ito *et al.,* 1991). However, the present result showed that the infection in Mikkabi-cho was caused by *H. nocens.* *M. yokogawai* is mainly found in the sweetfish in Miyakoda River flowing into Hamana Lake at northeast end of the lake, but there are no rivers where the sweetfish grows in Mikkabi-cho. These evidences suggest that the human infection with *H. nocens* is caused by catch and eating the fishes in the lake.

The adult worms collected from the patients were all relaxed and partly degenerated probably due to the treatment with praziquantel, therefore they may have caused a difference in the sizes. Even so, the measurements were among the ranges reported earlier for *H. nocens* (Suzuki *et al.,* 1982; Chai *et al.,* 1984; 1985; 1994; Sohn *et al.,* 1989). The number of chitinous rodlets on the gonotyl can be a key to distinguish species of genus *Heterophyes.* Imported human cases with *H. heterophyes* have been reported in Korea (Chai *et al.,* 1986; Chai and Lee, 1990) and it seems important to distinguish the present worms from *H. heterophyes.* The adult worms in the present study harboured the rodlets in a range from 49 to 58 as shown in Table 2. This range is apparently fewer than that of 68 to 85 for *H. heterophyes* (Chai *et al.,* 1986).

The size of eggs was slightly larger than that of earlier reports for *H. nocens* in Korea (Lee *et al.,* 1984; Chai and Lee, 1990), and rather equivalent to that of *M. yokogawai* appeared in those reports. The difference could be attributed to that the eggs measured in the present study were collected from human stool and the others were in adult uteri of *H. nocens.* The average size in individual patients varied in a wide rage. This suggests that the egg size may not be consistent among different geographical locations.

Questionnaire study well revealed the relationship between the infection and habit of eating raw fish caught in Hamana Lake. And it was strongly indicated that the significant correlation of prevalence with *H. nocens* was due to

eating raw mullet, the second intermediate host of *H. nocens.* Predominance of males and older persons among the patients suggests a possible association with a general habit of alcohol intake among such persons. Although gobby is also known to harbour *H. nocens* and eaten raw by residents in the study area, no significant correlation was detected. Examination for metacercariae from the fishes including gobby will further clarify the role of these fishes in the life cycle of *H. nocens* in the area.

Recently, metagonimiasis has been increasingly found on stool examination at human dry dock in Japan (Ishizu *et al.,* 1997). These infections may have been overlooked because of having no particular symptoms. This suggests that the infection has not necessarily increased and only opportunity to be detected has increased. In such cases the infection is usually diagnosed as metagonimiasis. However, it may also include infection with *H. nocens* or other heterophyid trematodes. In fact, the relatively high prevalence rates of heterophyid infections in the areas around Hamana Lake were once thought as metagonimiasis (Ito *et al.,* 1991). This could be the case also in other areas in Japan where people have the habit of eating raw mullet or gobby. In Korea, for example, endemic foci of *H. nocens* infection have been reported in southern coastal areas where *M. yokogawai* is common among residents (Chai *et al.,* 1994; 1997; 1998). Confirmation of species with adult worms should be required.

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PREVALENCE OF TOXOPLASMOSIS IN CHILDREN IN NORTHEASTERN BRAZIL

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Accepted September, 10, 2002

Abstract: We conducted a survey of the prevalence of *Toxoplasma gondii* infection in children Iiving in Jaboatão dos Guararapes, Pernambuco, Brazil from 1998 to 1999. This city is situated adjacent to Recife, capital of the state of Pernambuco. We determined the prevalence of specific anti-*T. gondii* IgG antibodies in 196 individuals from 2 to 16 years of age. Individuals who were IgG antibody negative also underwent specific anti-*T. gondii* IgM testing to detect recent infection with the parasite. Seroprevalence was 79.1%, and high titers of IgG antibody were observed in 49% of positive subjects. The prevalens of antibodies to *T. gondii* increased with age, with a multiple correlation coefficient (R) of 0.709; however, there was no significant difference in antibody status by sex. Out of 47 children who did not have specific anti-*T. gondii* IgG antibody, 4 were determined by specific anti-*T. gondii* IgM antibody analysis to have been recently infected. Stool was examined for soil-transmitted helminth eggs to assess the frequency of soil contact in the entire study population. Stool examination in the population revealed that soil-transmitted helminthiases was highly endemic, with an infection rate of 85.2% in this area. These results suggest that the primary mode of *T. gondii* transmission to humans is through contact with soil.

Key words: Toxoplasmosis, Soil-transmitted helminthiases, Northeastern Brazil, IgG, IgM, ELISA

INTRODUCTION

Toxoplasma gondii infection in man is widespread throughout the world. Its transmission to humans takes place by the ingestion of food or water contaminated with oocysts from cat feces, the ingestion of tissue cysts in raw or inadequately cooked meat or in uncooked food that has come in contact with contaminated meat, or transplacentally (Dubey and Beattie, 1988; Desmonts and Couvreur, 1974). Generally, the symptoms are mild, causing malaise, lethargy and rash, many times going unnoticed. However, immunosuppressed patients, e.g., AIDS patients or patients undergoing immunosuppressive treatment during organ transplantation, who were previously infected with *T. gondii* may develop fatal disseminated toxoplamosis or toxoplasmic encephalitis by reactivation of the quiescent parasite (Bertoli et al., 1995; Luft and Remington, 1992). In congenital *T. gondii* infection, relapsing chorioretinitis develops when cysts rupture and bradyzoites are trasfoemed into tachyzoites (Remington et al., 1995).

Infection in man and animals varies in different geographical areas within a country. Little is known about the causes of these variations. Enviroumental conditions, cultual habits, and animal fauna are some of the factors that may determine the degree of natural spread of *T. gondii*. Infection is more prevalent in hot and humid areas than in dry and cold climates (Gibson and Coleman, 1958).

A few epidemiological surveys of the seroprevalence of toxoplasmosis in northeastern Brazil have been reported (Hamada et al., 1990; Cerqueira et al., 1998; Gondim et al., 1999). However, the prevalence of the infection differs greatly among studies, probably due to the reasons described above. As to source, raw or inadequately cooked pork was considered to be an important source of *Toxoplasma* infection in the USA (Dubey, 1991). There was a suspicion of a correlation between the prevalence of toxoplasmosis and that of soil-transmitted helminthiases in Brazil (Hamada et al., 1990).

In the present study, serodiagnosis of toxoplamosis and stool examination were performed on young inhabitants in Jaboatão dos Guararapes, Pernambuco, Brazil, in order to elucidate the period and route of infection in individuals.

MATERIALS AND METHOD.

Study areas.

Jaboatão dos Guarerapes is located adjacent to Recife, capital of the state of Pernambuco, northeastern Brazil.

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This is a typical suburb with approximately 580,000 inhabitants and, with a few surrounding cities, forms the so-called Metropolitan Recife. Most of the city lacks waterworks, sewerage systems and periodic trash collection, and the population is of low socioeconomic standing (Figure 1). Stool and serum samples were collected from 196 individuals (from 2 to 16 years of age) in a rural area of the city. Informed consent was obtained from all subjects (and from parents of minors) according to guidelines from institutional review boards at the School of Medicine, Chiba University, and Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco.

Figure 1 Map of study area, Jaboatão dos Guararapes and Recife, PE, Brazil.

Stool examination.

Stool examination for helminth eggs was performed by the concentration technique of Ritchie (1948).

Serological examination.

The IgG ELISA kit (TOXOPLASMA IgG-EIA™, DENKA SEIKEN Co., LTD., Tokyo, Japan) and the IgM ELISA kit (TOXOPLASMA IgM-EIA™, DENKA SEIKEN Co., LTD., Tokyo, Japan) were used in this study. For each set of tests, kits with the same lot number were used before their expiration date. Instructions provided by the manufacturer were strictly followed. In the IgG assay, antibody titer was indicated by EIVALUE as described in the manual. A re-assay was performed with a 2-fold diluted serum sample if the titer was higher than 1600.

Statistical analysis.

Qualitative variables were analyzed using the chi-square test. P value less than 0.05 were considered significant. Correlation between antibody status and age was determined using regression analysis.

RESULTS

One hundred and ninety six blood samples were analyzed for specific IgG anti-*T. gondii* antibodieds by ELISA. Of the blood samples, 155 (79.1%) were positive and 3 (1.5%) were doubtful (Table 1). Prevalence did not differ significantly according to gender, however, age was associated with increased antibody presence (R=0.709, p= 0.003089: Figure 2). It is noteworthy that even before the age of 5 years, 71.8% (28/39) of children had acquired immunity against *T. gondii*. This indicates that the transmission of *T. gondii* takes place at an early age in this area. Interestingly, a high frequency of high antibody levels was

Table 1 Prevalence of specific anti-*T. gondii* IgG antibody by age and sex in Jaboatão dos Guararapes

	No. Examined			No. Positive (%)					
Age	M	F	Total	M		F		Total	
2	4	6	10	3	(75)	4	(67)	7	(70)
3	7	4	11	4	(57)	3	(75)	7	(64)
4	7	11	18	4	(57)	10	(91)	14	(78)
5	7	5	12	5	(71)	4	(80)	9	(75)
6	9	9	18	6	(67)	7	(78)	13	(72)
7	8	3	11	8	(100)	2	(67)	10	(91)
8	8	13	21	5	(63)	10	(77)	15	(71)
9	7	8	15	3	(43)	6	(75)	9	(60)
10	12	10	22	10	(83)	7	(70)	17	(77)
11	12	8	20	12	(100)	8	(100)	20	(100)
12	7	5	12	7	(100)	2	(40)	9	(75)
13	4	4	8	4	(100)	4	(100)	8	(100)
14	6	4	10	6	(100)	3	(75)	9	(90)
15	3	2	5	3	(100)	2	(100)	5	(100)
16	2	1	3	2	(100)	1	(100)	3	(100)
Total	103	93	196	82	(79.6)	73	(78.5)	155	(79.1)

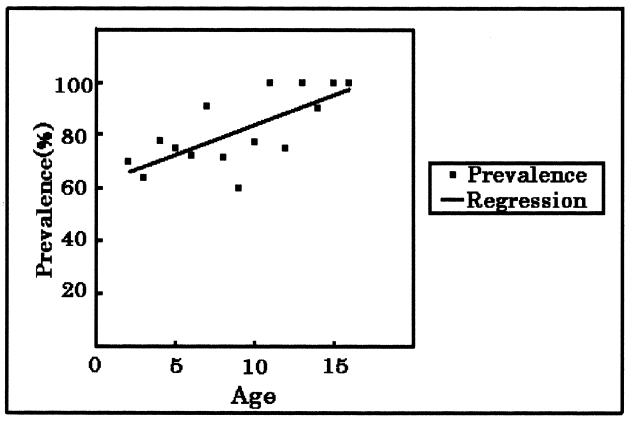

Figure 2 Correlation between age and prevalence of specific anti-Toxoplasma IgG antibody. In comparison of two regression parameters, correlation was observed between age and prevalence. R=0.7088, P=0.003089

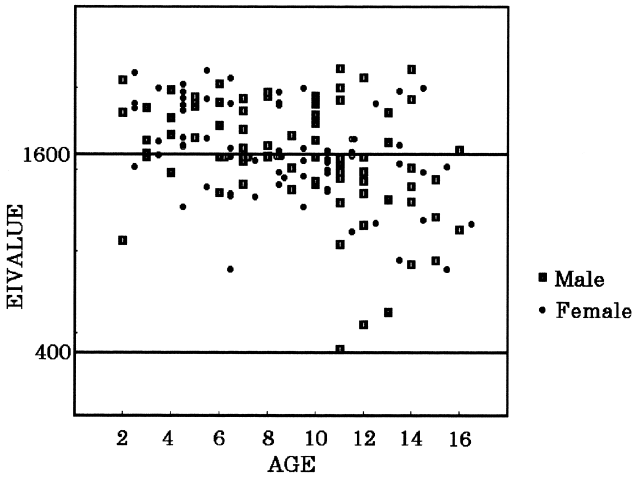

Figure 3 Distribution of IgG antibody titer by age and sex. EIVALU was determined accoeding to the manufacturer's instructions. Out of 82 male and 73 female positive individuals, 40 (48.8%) and 36 (49.3%), respectively, had a markedly high antibody titer.

Table 2 Specific anti-*T. gondii* IgM antibody by age and sex*

	No. IgG Negative (IgG Doubtful)			No. IgM Positive (IgM Doubtful)		
Age	M	F	Total	M	F	Total
2	1	2	3			0
3	3	1	4	(1)		0 (1)
4	3	1	4	(1)	(1)	0 (2)
5	2	1	3	1	(1)	1 (1)
6	3	2	5			0
7	0	1	1			0
8	3	3	6		1 (1)	1 (1)
9	4	2	6			0
10	2	3	5	(1)	1	1 (1)
11	1 (1)	0	1 (1)			0
12	1 (1)	3	4 (1)		1	1
13	1 (1)	0	1 (1)			0
14	0	1	1			0
15	0	0	0			0
16	0	0	0			0
Total	24 (3)	20	44 (3)	1 (3)	3 (3)	4 (6)

*Specific anti-*T. gondii* IgM antibody was determined in anti-*T. gondii* IgG antibody negativa or doubtful children.

observed. Figure 3 shows the quantitative expression of antibody in positive individuals as determined according instructions in the manual provided by the manufacturer. Of the 155 seropositive individuals, 76 (49%) had antibody titers higher than 1600.

Specific IgM anti-*T. gondii* antibody analysis was performed in the 47 test subjects (including 3 doubtful) who lacked anti-*T. gondii* IgG antibody production by IgG analysis. Four were determined to be positive and 6 doubtful (Table 2). All of those positive for specific anti-*T. gondii* IgM antibody were among those who were IgG antibody negative. The fact that specific anti-*T. gondii* IgM antibody was still detected in a 12-year-old child suggested that particular transmission sources still existed in this area.

Stool examination for helminth eggs was performed by the concentration technique. Table 3 shows results of stool examinations for *Ascaris*, *Trichuris* and Hookworm, which are categorized as soil-transmitted helminths (STH). Out of 196 stools, 167 (85.2%) were positive for one or more STH, 112 (57.1%) for *Ascaris*, 122 (62.2%) for *Trichuris* and 60 (30.6%) for Hookworm. Additionally, no correlation was observed between the positive rate and age or sex.

Table 3 Results of stool examination for soil-transmitted helmithes by age and sex

Age	No. Examined			STH*			*Ascaris*			*Trichuris*			Hookworm		
	M	F	Total	M	F	Total(%)	M	F	Total	M	F	Total	M	F	Total
2	4	6	10	4	3	7 (78)	3	1	4	1	2	3	1	1	2
3	7	4	11	6	3	9 (82)	3	3	6	4	3	7	2	1	3
4	7	11	18	5	10	15 (83)	3	7	10	4	8	12	2	3	5
5	7	5	12	6	5	11 (92)	4	2	6	5	4	9	2	2	4
6	9	9	18	8	8	16 (89)	7	3	10	4	7	11	2	2	4
7	8	3	11	8	3	11(100)	5	2	7	5	2	7	3	1	4
8	8	13	21	8	10	18 (86)	5	6	11	4	8	12	3	3	6
9	7	8	15	6	8	14 (93)	4	5	9	4	5	9	2	2	4
10	12	10	22	10	9	19 (86)	8	5	13	7	6	13	3	3	6
11	12	8	20	8	7	15 (75)	5	6	11	8	7	15	5	2	7
12	7	5	12	5	4	9 (75)	4	3	7	3	5	8	2	3	5
13	4	4	8	3	4	7 (88)	3	2	5	2	3	5	2	0	2
14	6	4	10	5	3	8 (80)	4	1	5	4	3	7	3	1	4
15	3	2	5	3	2	5(100)	2	3	5	1	1	2	2	1	3
16	2	1	3	2	1	3(100)	2	1	3	1	1	2	1	0	1
Total	103	93	196	87	80	167	62	50	112	57	65	122	35	25	60
(%)				(84.5)	(86.0)	(85.2)	(60.2)	(53.8)	(57.1)	(55.4)	(69.9)	(62.2)	(34.0)	(26.9)	(30.6)

*STH: Soil-transmitted helminth. Child with one or more species of eggs, was considered SHT positive.

DISCUSSION

The study reported here estimated the *T. gondii* seroprevalense to be 79.1% in 1998-1999 in a population of children between the ages of 2 - 16 years in Jaboatão dos Guararapes in Metropolitan Recife, PE, Brazil. This compares with an estimated 16.7% of children in Santo Inácio and 50% of children in Iraquqra, the state of Bahia, northeastern Brazil, in 1995 (Cerqueira et al., 1998). The state of Bahia borders on the state of Pernambuco on the north, and the two towns are approximately 900 Km from Jaboatão. On the other hand, a serological survey of slum-living children (younger than 18 years old) in Rio de Janeiro, Brazil, in 1999 estimated the *T. gondii* seroprevalense to be 70% (Bahia-Orliveira et al., 2001).

These results indicate that the status of seroprevalense is affected by multiple factors. There are several potential sources of *T. gondii* infection for humans. Ingestion of the cyst, which can be found in raw or inadequatey cooked meat or in uncooked food in contact with contaminated meat, is the most common cause of the infection in many countries and regions (Walton et al., 1966, DAFSIS, 1995). Oocyst transmission, which can take place by ingestion contamnated uncooked vegetables or water or by handling infected cat feces or contaminated soil, is also a principal cause of the infection (Kodym et al., 2001; Benenson et al., 1982; Weigel et al., 1999). Therefore, the human acquisition of *T. gondii* is associated with behavior and preferences of people as well as conditions in which they live. In northeastern Brazil, pork is consumed less than beef or chicken. Recently, Dubey and cowoekers (2002) isolated *T. gondii* at the high rate of 27% from chickens collected in rural areas surrounding São Paulo, Brazil. However, people in northeast Brazil are not in the habit of eating uncooked fowl. Congenital toxoplasmosis plays a very small role in prevalence in the test population because around 75% women of childbearing age in Pernambuco already have immunity against this parasite (Author's unpublished deta).

The parasitological survey of 3 selected intestinal helminth infections in Jaboatão can be considered to be an accurate indicator of sanitary conditions. There are few reports on the prevalence of intestinal parasite infections in northeastern Brazil. In comparison with our result of 85.2% egg-positivity, the incidence of soil-transmitted helminthiases was 89% in school children in São Lorenço, a neighboring city of Jaboatão, in 1981 (Yokogawa et al., 1984) and 68% in school children in Varzea, Recife, in 1988 (Hamada et al., 1990). The high prevalence rate of STH infections suggests that children have frequent soil contact in this district and that environmental hygiene had not improved for more than 20 years.

No correlation was observed between the prevalence of STH infections and age, although the prevalence of anti-*T. gondii* IgG antibody correlated with increases in age. However, the prevalence of STH infections under the age of 7 correlated with increases of age within the age group of 1 to 7 years (γ=0.901). The results of stool examination indicates the state of current infection, but gives no information on the history of infection in an individual (Needham et al., 1992). On the other hand, specific IgG production continues perpetually in toxoplasmosis, unless one becomes immunodeficient. It would be of interest to learn how toddlers have ingested this pathogen.

Most anti-*T. gondii* IgM antibody studies have demonstrated that IgM is a reliable marker and can persist for 3 months to one year after acute infection (Del Bono et al., 1989). The prevalence of specific anti-*T. gondii* IgM antibody in children without specific anti-*T. gondii* IgG antibody was remarkably low in our survey. However, it is of interest that anti-*T. gondii* IgM positivity was distributed over a wide range of ages (2-12 yers old). This phenomenon suggests that the source of the infection still exists and the protozoa is transmitted continuously, rather than through a sudden and temporary outbreak via contaminated pipe water in this area (Burnett et al., 1998; Isaac-Renton et al., 1998).

Another interesting observation was the high specific IgG antibody levels. High IgG antibody levels are typically observed in patients with acute phase (Flegr et al., 2000) or with reactivated infection. However, when we performed venipuncture we did not encounter any individuals among our test population who had a fever or complained of painful lymph nodes and were suspected of having acute toxoplasmosis.

It is presently impossible to determine if a person was initially infected by ingection of *T. gondii* oocysts or through consuption of infected meat containing tissue cysts. Lind et al., (1997) reported that the anti-*T. gondii* IgG antibody level in pigs experimentally infected by oocysts was higher than in those infected by tissue cysts. Detecting antibody to *T. gondii* in more than 70% of children indicates numerous sources of exposure to infection. Furthermore, the presence of antibody to *T. gondii* associated with age and the demonstration of recent infection by anti-*T. gondii* IgM antibody analysis suggest the presence of factors that facilitate continuous infection in the population. Children 2-16 years of age were chosen for the study since travel is limited among this age group. The high prevalence of STH infection in the population proves that these children have frequent soil contact. Therefore, the study is consistent with the theory of transmission by oocysts in this area. Unfortunately, we could not carry out a survey on the number

of cats in the area. However, we encountered many free-roaming cats with kittens. In tropical or neotropical districts, domestic cats deliver more than twice per year. However, considering that oocysts of *T. gondii* are shed by cats for only a short period of their life, especially in kittenhood (Dubey et al., 1995), controlling the kitten population by various means would not be difficult.

ACKNOWLEDGMENT

The authors thank all staff members of Sector Imunologia, Laboratório de Imunopatologia Keizo Asami, UFPE for providing all facilities for fieldwork. This study was partially supported by grants from the Oyama Foundation.

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DESCRIPTION OF A NEW SPECIES OF *SIMULIUM* (*SIMULIUM*) FROM HOKKAIDO, JAPAN (DIPTERA: SIMULIIDAE)

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Accepted September, 10, 2002

Abstract: A new black-fly species, *Simulium nemuroense* sp. nov., is described from pharate female, pharate male, pupal and larval specimens collected from Hokkaido, Japan. This new species is assigned to the *slossonae* species-group within the subgenus *Simulium* (*Simulium*), by having the following characters: in both sexes of adults, no hairs on the basal portion of radial vein; in the female, the claw with a large basal tooth and the genital fork with two projections on each arm, one directed anteriorly and the other posteromedially; in the male, the ventral plate lamellate with a median wide projection, the style longer than the coxite and with a prominent basal protuberance; and in the larva, the postgenal cleft very deep and the presence of the ventral papillae. *S. nemuroense* sp. nov. is easily distinguished from all the three known species by several characters including the male genitalia with a wide ventral plate and a hairy basal protuberance of the style, and the cocoon with an anterodorsal projection. This is the first record of the *slossonae* species-group in Japan.

Key words: Simuliidae, *Simulium*, black-fly, Diptera, Japan, new species

Recently, one of us (KS) found a new black-fly species while collecting simuliid pupae and larvae for faunal and ecological studies in Hokkaido, Japan. This species is assigned to the *slossonae* species-group within the subgenus *Simulium* (*Simulium*), recently created by Currie (1997), which includes only three known species found in Holarctic Region (Crosskey, 1999), all having been previously placed in the subgenus *Simulium* (*Parabyssodon*) by Crosskey and Howard (1997).

This new species is here described from the pharate females and pharate males, pupae and mature larvae. It should be noted that some adult body parts, such as head, thorax and legs, were not fully developed in coloration, making their descriptions incomplete. The morphological features and terms used herein follow mostly those of Crosskey (1969), and partially those of Takaoka (1983).

Simulium (*Simulium*) *nemuroense* sp. nov.

DESCRIPTION. **Female.** Body length 3.5 mm. ***Head.*** Narrower than thorax. Frons dark, shiny, with several dark hairs in vertical row along each lateral margin; frontal ratio 1.4:1.0:1.4. Frons-head ratio 1.0:4.2. Clypeus dark, shiny, moderately covered with dark hairs laterally but widely bare on median longitudinal area. Fronto-ocular area (Fig. 1) well developed, triangular. Proboscis 0.64× as long as clypeus. Antenna composed of 2+9 segments. Maxillary palp (Fig. 2) consisting of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:2.4; 3rd segment not enlarged, thinner than 4th segment, with sensory vesicle of variable shapes and sizes: sensory vesicle ellipsoidal, 1.7× as long as wide and ca. 0.3× as long as 3rd segment in the holotype female (Fig. 3), while much elongated, 2.9× as long as wide and ca. 0.4× as long as 3rd segment in the paratype female (Fig. 4). Maxillary lacinia with 10 or 11 inner and 13 or 14 outer teeth. Mandible with ca. 26 inner and 8 outer teeth. Cibarium (Fig. 5) smooth on posterior margin, with cornuae directed upwards. ***Thorax.*** Scutum shiny, with slightly darker portion along each lateral margin and on prescutellar area, densely covered with recumbent scale-like hairs. Scutellum with many hairs. Postscutellum bare. Pleural membrane bare. Katepisternum longer than deep and bare. ***Legs.*** Foreleg: at least apical caps of femur and tibia, and whole of tarsus darkened; basitarsus much dilated, ca. 4.6× as long as its greatest width. Midleg: at least coxa, apical caps of femur and tibia, apical 1/2 of basitarsus, and whole of remaining tarsal segments dark. Hind leg: at least coxa, apical caps of femur, tibia and basitarsus, and most of remaining tarsal segments dark; basitarsus (Fig. 6) parallel-sided; calcipala well developed, nearly as long as wide; pedisulcus well developed. Claws (Fig. 7) each with large basal tooth 0.5× as long as claw. All femora and mid

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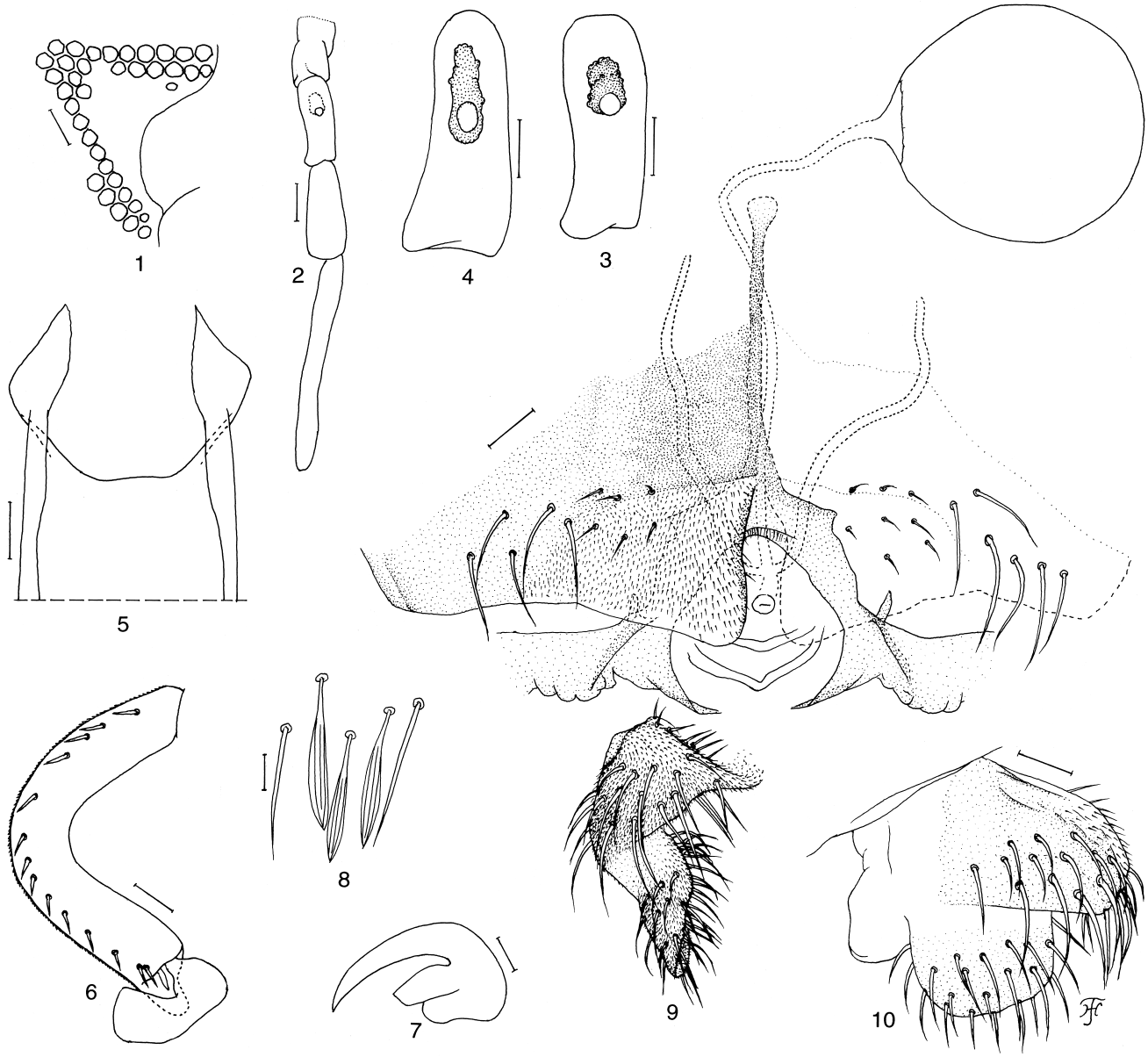
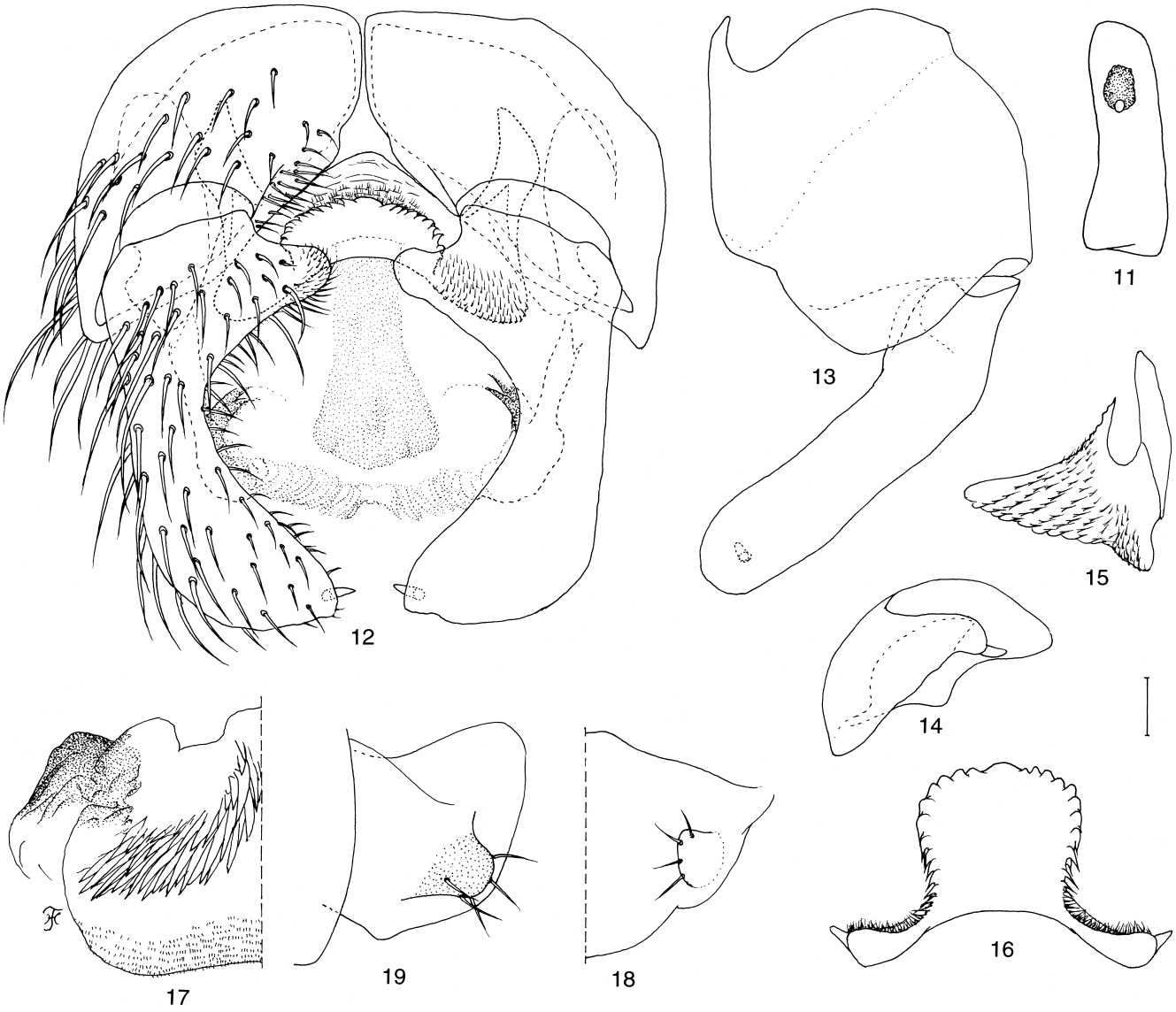
and hind tibiae covered with broad scale-like hairs as well as simple hairs (Fig. 8). ***Wing.*** Costa with 2 parallel rows of short spines as well as hairs. Subcosta bare. Basal portion of radius bare. Basal cell absent. ***Abdomen.*** Basal scale light brown, with fringe of pale long hairs. Dorsal surface of segment 2 pale, with pair of large shiny spots on anterior 1/2, medium brown on posterior 1/2; that of remaining segments medium brown, sparsely covered with short hairs; terga of segments 6-8 shiny when illuminated; ventral surface of segment 7 with large sternal plate medially. ***Genitalia*** (Figs. 9 & 10). Sternum 8 wide, bare medially but furnished with 5 or 6 long hairs as well as a few short setae on each side. Anterior gonapophysis triangular, thin, membraneous except inner margin narrowly sclerotized, densely covered with microsetae, interspersed with 3-5 short setae. Genital fork of inverted Y-form, with slender, well-sclerotized stem and wide arms; each arm with large lobe-like projection directed posteromedially and



Figures 1-10. Female characters of *Simulium nemuroense* sp. nov. 1, fronto-ocular area; 2, maxillary palp; 3 & 4, 3rd segments of maxillary palp with sensory vesicle; 5, cibarium; 6, hind basitarsus and 2nd tarsal segment; 7, claw; 8, simple and scale-like hairs on outer surface of hind tibia; 9, genitalia *in situ* (ventral view), showing 8th sternum, anterior gonapophyses, genital fork, spermatheca with main and accessory ducts, and right paraproct and cercus; 10, right paraproct and cercus (lateral view). Scale bars 0.01 mm for figs. 7 & 8; 0.03 mm for figs. 1, 3-5, 9 & 10; 0.05 mm for figs. 2 & 6.

small slender projection directed forward. Paraproct of usual form, somewhat protruding ventrally. Cercus in lateral view rounded posteriorly, ca. 0.5× as long as wide. S-permatheca globular, strongly sclerotized except small area around juncture to duct, and duct itself unsclerotized, with distinct reticulate surface pattern, and with numerous internal setae; main spermathecal duct narrow, while both accessory ducts slightly wider than main duct and with tapered apex.

Male. Body length 3.5 mm. ***Head.*** Slightly wider than thorax and holoptic. Upper eye consisting of large facets in 27 vertical columns and 25 horizontal rows. Clypeus dark, shiny, widely bare medially, but moderately covered with hairs along lateral margins. Antenna composed of 2+9 segments, dark brown except base of 1st flagellar segment pale; 1st flagellar segment somewhat elongate, ca. 1.4× as long as 2nd flagellar segment. Maxillary palp composed of 5 segments, brown, proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.5; 3rd segment (Fig. 11) of moderate size; sensory vesicle small, ellipsoidal, ca. 0.2× as long as 3rd segment. ***Thorax.*** Scutum dark brown, with silvery



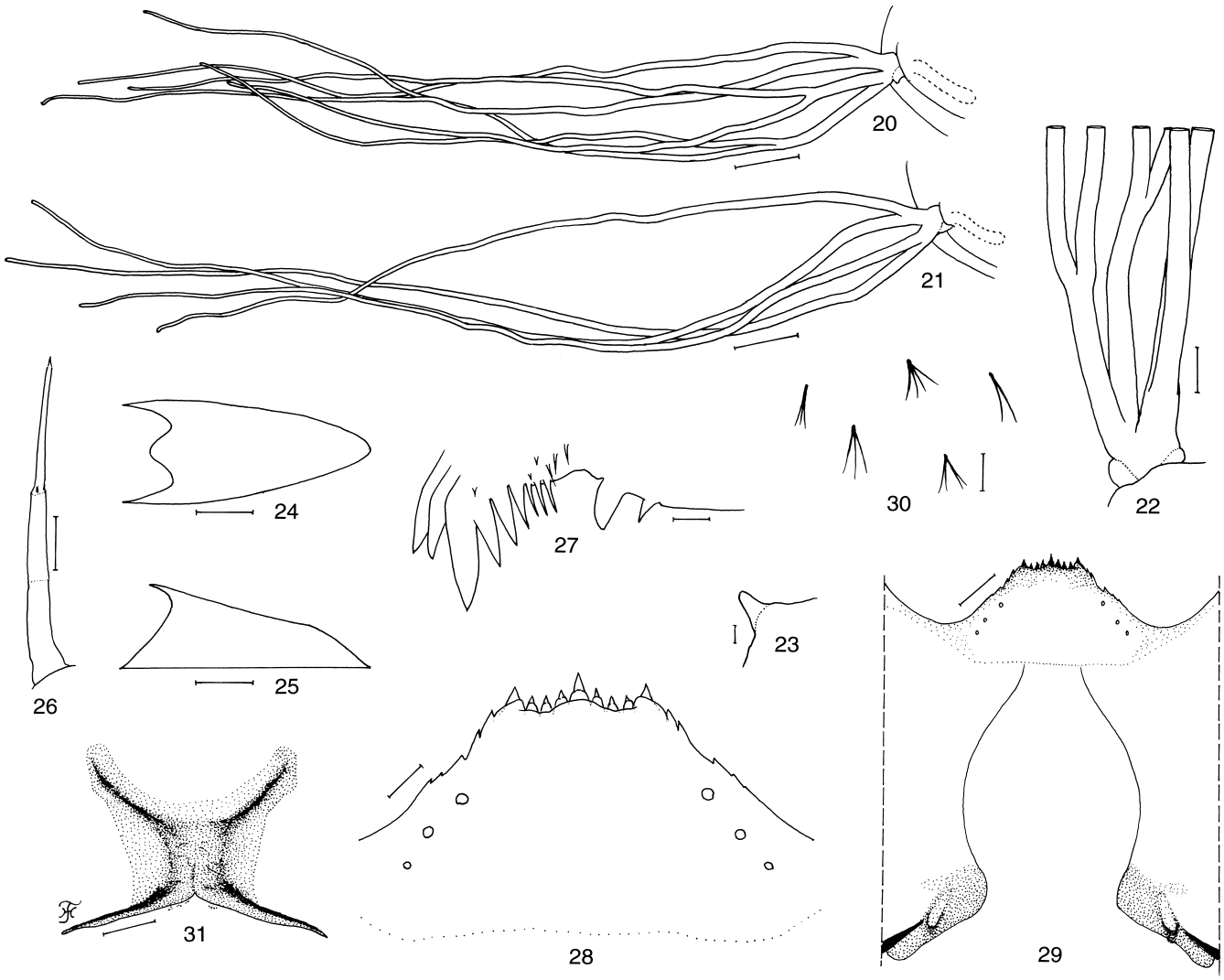
Figures 11-19. Male characters of *Simulium nemuroense* sp. nov. 11, 3rd segment of maxillary palp with sensory vesicle; 12, genitalia *in situ* (ventral view), showing coxites, styles, ventral plate, parameres and median sclerite; 13, right coxite and style (lateral view); 14, right style (end view); 15 & 16, ventral plates (15, lateral view; 16, end view); 17, right paramere and aedeagal membrane (end view); 18 & 19, posterior tips of abdomen showing cercus (18, end view; 19, lateral view). Scale bar 0.03 mm for all figures.

shiny marking consisting of pair of narrow crescent-shaped spots each directed from anterolateral corner toward posteromedially on each shoulder, narrow band along each lateral margin, and large spot on prescutellar area, all connected to one another, densely covered with recumbent fine hairs, and with several longer hairs on prescutellar area. Scutellum dark, with many hairs. Postscutellum dark and bare. Pleural membrane bare. Katepisternum medium brown, longer than deep, and bare. ***Legs.*** Foreleg: coxa yellow except inside surface light brown; trochanter medium brown; femur dark yellow with apical cap medium brown; tibia pale except apical cap and inner surface of basal 1/4 or 1/3 medium brown; outer surface largely white shiny; tarsus medium brown; basitarsus slender, moderately dilated, ca. 4.8× as long as its greatest width. Midleg: coxa and trochanter medium brown; femur dark yellow or light brown with apical cap medium brown; tibia light brown with base yellow and apical cap medium brown; tarsus light to medium brown. Hind leg: coxa medium brown; trochanter dark yellow; femur dark yellow or light brown, with apical cap medium brown; tibia light to medium brown with base dark yellow and apical cap dark brown; basitarsus dark yellow with apical 1/4 medium brown, other tarsal segments light brown; basitarsus slender, parallel-sided, much narrower than hind tibia and femur, as in female. Calcipala well developed, nearly as long as wide; pedisulcus well developed. All femora and mid and hind tibiae covered with broad scale-like hairs as well as simple hairs, as in female. ***Wing.*** As in female. ***Abdomen.*** Basal scale light brown, with fringe of dark long hairs. Dorsal surface of segment 2 pale on anterior 1/2 with pair of shiny spots, medium brown on posterior 1/2; that of other segments medium to dark brown, with dark simple hairs; segments 5-7 each with paired shiny dorsolateral spots. ***Genitalia*** (Figs. 12-19). Coxite large, cylindrical. Style elongate, ca. 1.2× as long as coxite, flattened dorsoventrally, narrowed from base to middle, then widened apically, with apical spine; style with prominent hairy basal protuberance with rounded apex directed medially. Ventral plate in ventral view lamellate, much shorter than wide, with its lateral and posterior margins widely depressed, and anterior margin convex, moderately covered with setae on ventral surface except most of anterior portion bare, with stout arms somewhat diverged, and prominent plate-like median projection along posterior margin; anterior surface of this median projection moderately and elaborately covered with spinous setae but posterior surface with no setae; lateral margins of this median projection appearing toothed when viewed from end. Paramere of normal form, with numerous hooks. Median sclerite simple, plate-like, slightly widened toward apex. Aedeagal membrane densely covered with spinous microsetae. Ventral surface of 10th abdominal segment bare. Cercus small, rounded in lateral view, and with 4 or 5 simple hairs.

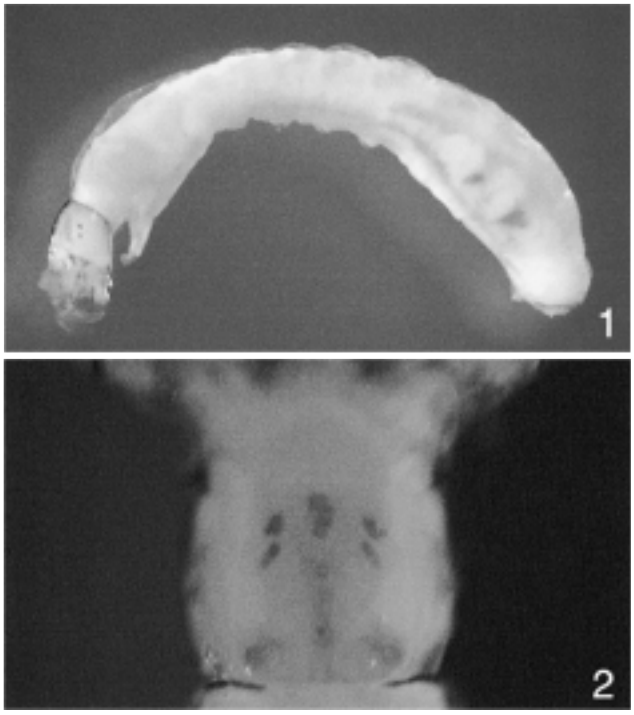
Pupa. Body length 3.2-3.6 mm. ***Head.*** Integument yellow, moderately covered with small round tubercles; frons with 3 simple (or rarely bifid) medium-long trichomes on each side, of which 2 trichomes situated close together but the dorsalmost trichome separated at some distance from the others, while face with 1 simple medium-long trichome on each side. ***Thorax.*** Integument yellow, moderately covered with small round tubercles, with 3 simple long trichomes mediodorsally, 2 simple long trichomes mediolaterally, 1 simple medium-long trichome posterolaterally, and 3 simple medium-long trichomes ventrolaterally, on each side. Gill either with 6 long slender filaments arranged in 3 pairs (Fig. 20), as in 24 of 32 pupae and pupal exuviae examined, or with 4 long slender filaments arranged in 2 pairs (Fig. 21), as in remaining 8 pupae; in both cases, gill divided basally into 2 (dorsal pair and ventrolateral one) basally, then each divided again into 2 filaments (dorsal and ventral filaments) from very short stalk (Figs. 20 & 21); in case of 6 filaments (Fig. 22), each filament of ventrolateral pair divided further into 2 filaments from moderate to very long secondary stalk, i.e., out of 24 pupae, 14 with all 4 secondary stalks (2 each on left and right side) of moderate length, 8 with all long or very long secondary stalks (bifurcation occurring near apical tip of filaments in 3 pupae), and 1 with 3 long secondary stalks and 1 moderate one, and 1 with 2 long secondary stalks and 2 moderate ones; ventral filament of dorsal pair 3.5-4.1 mm long, subequal to or a little longer than pupal body, usually slightly longer and thicker than others (1.2-1.4× as long as shortest filament, which is 2.5-3.6 mm long); all filaments pale, generally directed forward; their cuticular surface with distinct sharp annular ridges and furrows (though ridges becoming indistinct near apex), and densely covered with minute tubercles. ***Abdomen.*** Terga 1 and 2 yellowish, bare; tergum 1 with 1 simple slender medium-long seta on each side; tergum 2 with 1 simple slender medium-long seta and 5 short spinous setae on each side; terga 3 and 4 each with 4 hooks and a few spinous setae on each side; tergum 5 bare; terga 6-9 each with transverse row of spine-combs directed backward on each side; tergum 9 with pair of distinct conical terminal hooks (Fig. 23). Sternum 4 with 1 bifid hook (subequal in size to those on sterna 5-7), 1 simple hooklet and a few slender setae on each side; sternum 5 with pair of bifid hooks and a few slender setae on each side; sterna 6 and 7 each with 1 bifid hook submedially and 1 simple hook laterally and a few slender setae on each side; each side of last segment with 2 simple slender setae but no grapnel-shaped

hooklets. ***Cocoon*** (Figs. 24 & 25). Simple, wall-pocket-shaped, moderately woven without open spaces or windows in web, with anterodorsal projection of short or moderate lengths; individual threads generally visible; 3.8-5.0 mm long × 1.7-2.2 mm wide.

Mature larva. Body length 5.0-6.0 mm. Body creamy white, somewhat light brownish on dorsal surface of abdominal segments 5-8 in some larvae (there is a possibility that color markings on the larval body were probably faded out since the larvae had been long preserved in ethanol solution); in dorsal view, thoracic segments somewhat swollen like a barrel, abdominal segments 1-4 somewhat narrower than thorax, with moderate intersegmental constrictions, and segments 5-9 swollen also like a barrel, with widest portion on segment 6; in lateral view, larval body markedly arched dorsally (Photo. 1). Cephalic apotome (Photo. 2) pale, with distinct positive head spots; eye brow faintly positive; 2 large spots posterior to eye-spot region distinctly positive; isolated spots below eye-spot region indistinct. Cervical sclerites composed of 2 small rod-like pieces, not fused to occiput, widely separated medially from each other. Antenna (Fig. 26) consisting of 3 segments and apical sensillum, slightly shorter than stem of labral fan;



Figures 20-27. Pupal and larval characters of *Simulium nemuroense* sp. nov. 20-25, pupa; 26-31, larva. 20, gill with 6 filaments (lateral view); 21, gill with 4 filaments (lateral view); 22, basal portion of left gill with 6 filaments (dorsal view); 23, terminal hook (lateral view); 24 & 25, cocoons (24, dorsal view; 25, lateral view); 26, left antenna (dorsal view); 27, apex of mandible; 28, hypostomium; 29, head capsule (ventral view) showing hypostomium and postgenal cleft. Scale bars 0.01 mm for figs. 23, 27 & 30; 0.02 mm for fig. 28; 0.05 mm for figs. 26, 29 & 31; 0.1 mm for fig. 22; 0.3 mm for figs. 20 & 21; 1.0 mm for figs. 24 & 25.



Photos. 1 and 2. Larva of *Simulium nemuroense* sp. nov. 1, whole body of mature larva (lateral view); 2, head showing positive head spots on clypeus (dorsal view).

proportional lengths of 1st, 2nd and 3rd segments 1.0:1.0: 1.3. Labral fan with ca. 30 main rays. Mandible (Fig. 27) with 2 usual mandibular serrations; comb-teeth composed of 3 teeth, decreasing in length from 1st to 3rd; supernumerary serrations absent. Hypostomium (Fig. 28) with anterior row of 9 teeth; median and corner teeth well developed; 3 intermediate teeth on each side also sharply pointed; lateral serrations moderately developed; 3 hypostomal bristles lying subparallel to lateral margin on each side. Postgenal cleft (Fig. 29) deep, reaching posterior margin of hypostomium. Thoracic and abdominal cuticle sparsely covered with dark minute spines with 0-4 branches (Fig. 30) dorsally and dorsolaterally; last segment also covered with colorless short setae on each side of anal sclerite; cluster of minute spines around anus absent. Rectal papilla of 3 lobes each with 6 or 7 finger-like secondary lobules. Anal sclerite (Fig. 31) of usual X-form, with posterior arms nearly as long as anterior ones; basal portion of arms widely sclerotized. Accessory sclerite absent. Ventral papillae (Photo. 1) moderately developed, conical and placed ventrally, then, well discernible even when the larva is viewed laterally. Posterior circlet with 56-60 rows of up to 13 hooklets per row.

TYPE SPECIMENS. Holotype female, dissected out of a pupa, collected from the Nishimarubetsu River, just below the Barasan Bridge, Bekkai Town, Notsuke County, Nemuro District, eastern Hokkaido, Japan, 23. VII. 1998, by K. Saito and A. Kanayama. Paratypes, 1 pharate female, 4 pharate males, 13 pupae, 19 pupal exuviae, 14 mature larvae, same data as holotype. Holotype and most of paratype specimens will be deposited at the Natural History Museum, London, UK.

BIOLOGICAL NOTES. The immature stages of *S. nemuroense* sp. nov. occur in a small, somewhat muddy stream (2-3 m wide, ca. 0.5 m deep, and exposed to the sun), very slowly flowing in the marshy area (ca. 6 m in altitude) near the coast of the Nemuro Bay. They were found on the surface of grass leaves trailing in the water. This species was collected together with *S. yonagoense* Okamoto, 1958.

DISTRIBUTION. Limited to the type locality in Nemuro, Hokkaido, Japan.

ETYMOLOGY. The species *nemuroense* is named after the district name, Nemuro, where this species was collected.

REMARKS. This new species is assigned to the *slossonae* species-group within the subgenus *Simulium* (*Simulium*), by having the following characters: in both sexes of adults, no hairs on the basal portion of radial vein; in the female, the claw with a large basal tooth (Fig. 7) and the genital fork with two projections on each arm, one directed anteriorly and the other posteromedially (Fig. 9); in the male, the ventral plate lamellate with a wide median projection and the style with a prominent basal protuberance (Fig. 12); and in the larva, the postgenal cleft very deep (Fig. 29) and the presence of the ventral papillae (Photo. 1). This is the first record of the *slossonae* species-group in Japan.

The *slossonae* species-group is a small group, consisting of three known species found in the Holarctic Region [i.e., *S. rugglesi* Nicholson & Mickel, 1950, from USA and Canada, *S. slossonae* Dyar & Shannon, 1927, from USA, and *S. transiens* Rubtsov, 1940, from Alaska, Canada, China, Finland, Mongolia and Russia (Crosskey, 1999; Crosskey and Howard, 1997)].

S. rugglesi is similar to the new species in the female genitalia but differs by the following characters: in the female, the fifth maxillary palpal segment very short, nearly as long as fourth segment; in the male, the ventral plate nearly quadrate; in the pupa, the gill with eight filaments per side, and the cocoon simple, with no anterodorsal projection (Davies *et al*., 1962; Stone, 1964; Adler and Currie, pers. commun.).

S. slossonae resembles this new species in the female

genitalia but is different by having the male ventral plate nearly quadrate, the pupal gill composed of six filaments with three long stalks, the cocoon simple, and the larval postgenal cleft deep but not reaching the hypostomium (Dyar and Shannon, 1927; Stone and Snoddy, 1969; Adler and Currie, pers. commun.).

S. transiens appears to have much closer similarities to this new species by having the broad male ventral plate and the deep larval postgenal cleft reaching the hypostomium, but is easily distinguished by the following characters: the female genital fork with narrow arms, the male style with spinous basal protuberance; the pupal gill with four filaments per side, pupal head and thoracic integuments with sharp conical tubercles, and the cocoon simple (Rubtsov, 1989; Adler and Currie, pers. commun.).

ACKNOWLEDGEMENTS

We thank Dr. P. Adler, Clemson University, USA, Dr. D. Currie, Royal Ontario Museum, Canada, and Dr. D. Craig, Alberta University, Canada, for providing valuable information and references on the *slossonae* species-group. Thanks are due to Dr. R. W. Crosskey, The Natural History Museum, UK, for his kind suggestion on this study. Our appreciation goes to Dr. A. Kanayama, who kindly helped in the colleciton of black flies in Hokkaido, and to Dr. Y. Otsuka for his kind help in taking the photographs of the larval characters.

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Short Communication
Seroprevalence of hepatitis D virus infection among HBsAg carriers in northern Thailand

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Accepted September, 10, 2002

Key words: HDV, Northern Thailand, HBsAg carrier

INTRODUCTION

The hepatitis delta virus (HDV) is a small, single stranded RNA hepatotropic virus that depends on hepatitis B virus (HBV) for its survival and replication (Polish *et al*., 1993; Purcell, 1994; Karayiannis, 1998). The hepatitis B virus (HBV) provides the envelope for the HDV, which consists of hepatitis B surface antigen (HBsAg). Because of this relationship, HDV infection occurs only in persons with hepatitis B, either as a co-infection or as a superinfection of a carrier of HBsAg (Rizzetto *et al*., 1980).

The mode of transmission of HDV infection appears to have two patterns: (i) endemic, associated with non-parenteral spread in Italy and (ii) sporadic, associated with parenteral transmission in almost all other areas of the world (Rizzetto *et al*., 1984). In regions where HDV infection is not endemic, the disease is mostly confined to groups at high risk of acquiring HBV infection and high-risk HBV carriers (Polish *et al*., 1993).

Human HBsAg carriers express delta antigen in the liver but do not circulate detectable delta antigen in the blood. Most patients develop antibody to HDV (anti-delta). Detection of anti-delta virus antibodies can indicate an ongoing or a past infection with HDV. The serological detection of this antibody by a sensitive enzyme immunoassay (EIA) provides a tool for recognizing HDV infection and for studying its epidemiology.

Because this viral infection can cause fulminant as well as chronic liver disease, spread of HDV into areas where HBV infection is endemic has serious clinical implications. Prevention depends on the widespread use of hepatitis B vaccine (Polish *et al*., 1993). But those who already have chronic HBV infection continue to be at risk of being infected with HDV (Purcell, 1994). Our study has indicated that northern Thailand belongs to an intermediate prevalence region of HBV infection with 8.7% (Jutavijittum *et al*., 1999) and it needs to be aware of the possibility of superinfection or co-infection of HBV with HDV. Hence, we plan to determine the prevalence of HDV infection among HBsAg carriers in northern Thailand.

MATERIALS AND METHODS

Serum samples from voluntary blood donors in Chiang Mai, Chiang Rai, Lampang, and Lamphun provinces in northern Thailand were screened for blood-transmitted pathogens at the 10th Regional Blood Center office in Chiang Mai. None of the blood donors complained the subjective symptoms of liver dysfunction. Testing for HBsAg was performed using a commercial ELISA kit, Enzygnost® HBsAg 5.0 (Dade-Behring, Marburg Germany). From 1998 to 2000, samples that were HBsAg-positive were collected and stored at −20℃. A total amount of 395 HBsAg-positive (287 males and 108 females) serum samples were obtained, 110 from Chiang Mai, 97 from Chiang Rai, 88 from Lampang, and 100 from Lamphun. The range of donor's age was from 17 to 52 years. The samples were tested for the presence of antibody to hepatitis delta antigen(anti-HD) using ETI-AB-DELTAK-2®, an ELISA kit from Dia-Sorin, Saluggia (Vercelli), Italy.

RESULTS

No anti-HDV was detected among 395 voluntary

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blood donors in 4provinces of northern Thailand.

DISCUSSION

The incidence of HDV varies around the world. Of patients with chronic liver disease in Africa, 73% are positive for HBsAg and 75% of these are also positive for anti-HDV antibodies (Cenac *et al*., 1995). Even in non-endemic areas, HDV antibodies are found in～20% of patients with chronic hepatitis B and acute hepatitis superimposed on chronic hepatitis B infection (Jacobson *et al*., 1985). In contrast, asymptomatic carriers of HBV are only rarely positive for anti-HDV antibodies (Jacobson *et al*., 1985; Louisirirotchanakul *et al*., 1988). In Asia, despite a rich reservoir of HBV carriers, the prevalence of HDV infection is considered to be low, found in～9% (Hao *et al*., 1992; Arakawa *et al*., 2000) although there are areas with high prevalence such as Fiji, Samoa and some areas of China (Vranckx *et al*., 1988).

HDV markers were more frequent in chronic liver disease with 18% than in asymptomatic HBV carriers with 2% (Jacobson *et al*., 1985). In Taiwan, the anti-HDV prevalence among HBsAg carriers was significantly high in STD patients (9.6%), prostitutes (33.1%), and drug abusers (68.1%) than in blood donors from the general population (2.2%) (Chen *et al*., 1992). In Thailand, HDV infection was generally found to be uncommon among cases of HBsAg-positive individuals, 0/27 of asymptomatic HBsAg carriers (Chainuvati *et al*., 1987). About 10% of patients with chronic liver disease and cirrhosis have anti-HDV antibodies, in contrast to～60% of intravenous drug users, and no anti-HDV demonstrated from 46 asymptomatic HBsAg carriers (Louisirirotchanakul *et al*., 1988). We demonstrated that all 395 voluntary blood donors in 4provinces of northern Thailand were negative for anti-HDV. This concurs with previous epidemiological surveys which indicate that in Thailand where HBV infection is endemic, delta infection is rare among asymptomatic HBsAg carriers.

ACKNOWLEDGEMENTS

This study was supported by a grant for International Health Cooperation Research (12C-4) from the Ministry of Health and Welfare, Japan

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ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

VOL.30 No.3 SEPTEMBER 2002

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JSTM FOUNDED 1959

Jpn. J. Trop. Med. Hyg.

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