日本熱帯医学会雑誌

第23巻 第3号 平成7年9月

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日本熱帯医学会雑誌
第23巻第３号
1995年９月15日　印刷
1995年９月15日　発行

発行所　日本熱帯医学会
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諫早市長野町1007-２（〒854）
☎0957-22-6000

本雑誌の刊行にあたりその費用の一部を文部省科学研究費補助金
（研究成果公開促進費）によった。

Jpn. J. Trop. Med. Hyg., Vol. 23, No. 3 (1995) pp. 151~157

ORAL TREATMENT OF NEW WORLD CUTANEOUS LEISHMANIASIS WITH ANTI-MALARIAL DRUGS IN ECUADOR: A PRELIMINARY CLINICAL TRIAL

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Received April 24, 1995/Accepted June 14, 1995

Abstract: The current study was designed to evaluate anti-leishmanial activity of mefloquine hydrochloride (Mephaquin®) and artesunate (Plasmotrium®) which are currently being used as malarial drugs. A total of 17 patients (volunteers) with cutaneous leishmaniasis were treated with these drugs in this study. Of these subjects, 16 were treated by the oral administration of a total dosage of 1,500mg (1 Lactab® each for 6 days) mefloquine, 4.2mg/kg/day for 6 days, and if necessary the dosage was repeated with 3 weeks intervals. The majority of cutaneous lesions healed within 2 to 3 weeks after the commencement of mefloquine treatment, showing an average of 3.6 weeks of healing times with 100% cure rate. One slowly healing within 8 weeks after the commencement was observed; this case grew worse because of infection with *Tunga penetrans* at the late healing phase of leishrnaniasis. The remaining one patient with an ulcer lesion was treated by the oral administration of a total dosage of 1,200mg (2 Lactab® each for 3 days) artesunate, i. e., 6.7mg/kg/day for 3 days, and the same dosage was repeated 2 weeks later. The lesion healed within 6 weeks after the commencement of artesunate treatment. In the present study, all the patients received mefloquine or artesunate were treated without admission, performing their normal daily activities. No specified adverse reaction was noticed.

INTRODUCTION

The pentavalent antimonials sodium stibogluconate and meglumine antimonate remain as the first choice of drugs in the clinical treatment of different types of leishmaniasis, such as cutaneous, mucocutaneous and visceral forms (Bryceson, 1980; WHO, 1990). Since the introduction of these antimonials more than 50 years ago, many investigations have been done in order to find more efficient treatment without side effect for the disease. No satisfactory effective new drugs, however, have been developed, though several important advances have been made (Berman, 1988; Croft, 1988). There is, therefore, still a need to search for a new drug that is fully effective and orally applicable for most clinical forms of Old and New World leishrnaniasis.

To date, trials of search for new drulgs and treatment have been performed *in vitro* and *in vivo* using experimental animals and/or volunteer patients with leishmaniasis. In the present study we tried to treat cutaneous leishmaniasis patients with two types of anti-malarial drugs, mefloquine and artesunate, which are clinically being used. The current paper describes the anti-leishmanial activities of these drugs, based on the data obtained from clinical trials at endemic areas of Ecuador.

MATERIALS AND METHODS

In the present study, a total of 17 patients with cutaneous leishmaniasis were examined. They came from different endemic areas, Zhucay and Manta Real (Province of Cañar), Zapotal (Province of Guayas), Muisne (Province of Esmeraldas) and Caluma (Province of Chimborazo). Of these subjects, 16 were treated orqlly with a total dosage of 1,500mg (1 Lactab® each for 6 days) mefloquine (Mephaquin®, Mepha Ltd., Aesch-Basle, Switzerland; each Lactab® contains

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mefloquine hydrochloride corresponding to 250mg mefloquine base), i.e., 4.2mg/kg/day for 6 days, and if necessary the dosage was repeated with 3 weeks intervals after the end of initial treatment. In patients treated with mefloquine, 13 out of 16 were male and 3 were female, aged from 3 to 81 years. The remaining one patient (14 years old male) was treated orally with a total dasage of 1,200mg (2 Lactab® each for 3 days) artesunate (Plasmotrium®, Mepha Ltd., each Lactab® contains artesunate 200mg), i.e., 6.7mg/kg/day for 3 days, and the same dosage was repeated 2 weeks later. All the subjects were informed of the purpose of the study and gave permission for drug administration and repeated physical examinations. The patients received treatment during their daily activities without admission. Almost all the volunteers (patients) lived in mountainous and dense forest areas in which no transportation systems are available (Fig. 1). Their dwellings are located at very remote areas from our laboratory of health centers in each endemic area. All the volunteers were in a very poor economic condition. In such a field situation and an ethical consideration, no placebo treatment was performed in the current trial.

All the cutaneous leishmaniasis patients treated in this study were diagnosed by the demonstration of *Leishmania* amastigotes in smear specimens from the lesions. Treated patients received a follow-up physical examination every 2 or 3 weeks, and they were recorded photographically at the same time. When their dermal lesions were partially still active in the examination, an additional administration of the drug was made as mentioned above. During the treatment, the patients were asked for the presence of any complaint, such as vomiting, nausea, diarrhoea, fever and etc.

The evaluation of the results of treatment was made as described by El-On *et al.* (1986) but partially modified as follows: 1) rapidly effective (grade 1), no parasites detected in cutaneous lesions, followed by total healing within 1 -3 weeks after the commencement of treatment; 2) less rapidly effective (grade 2), the same process (no parasites and total healing) occurring within 4-6 weeks after the commencement; 3) effective (grade 3) , no parasites detected but healing within 7-8 weeks; 4) ineffective (grade 4), parasites still present in lesions and/or no clinical healing after 9 or 10 weeks of the commencement of treatment.

In the present subjects, species of *Leishmania* are not identified precisely, but our previous work indicates that *L.* (*Viannia*) *panamensis* is the most frequently identified organism by zymodeme and serodeme analyses, followed by *L.* (*V.*) *guyanensis* in the surrounding regions (unpublished data).

RESULTS

The number of cutaneous lesions per person ranged from 1 to 4 with different size of lesions, ranging from 3 × 3 mm to 30 × 30 mm in diameter (Table). All the present patients had ulcerative lesions; the majority of these lesions were located on the exposed body surface, such as forearm, foot and face. The duration time of infection varied from one to 12 months (average: 3.6 weeks) at the time of the commencement of mefloquine treatment. Nine (56.3%) of the 16 patients healed within 3 weeks after the commencement of oral treatment of mefloquine, showing grade 1 category (Fig. 2A-C), and other 6 cases took 4-6 weeks (grade 2) for healing. Only one case (patient No. 3) took 8 weeks for complete healing, because of *Tunga penetrans* infection in the cutaneous lesion which had been at the late phase of healing (4 weeks after the commencement of mefloquine treatment). No ineffective case was found in this study using mefloquine; the cure rate showed 100%. In the present trial of treatment with mefloquine, age or sex of the patients, number and size of the lesions and duration time of the infection did not show any influence against the efficacy of drug. However, heavy bacterial infections and other infection such as *Tunga penetrans* were fully influential for the healing time, especially in lesions located on the lower extremities.

In a patient (14 years old male) treated with artesunate (Plasmotrium®), an ulcer (20 × 17mm) on the forearm healed within 6 weeks after the commencement of treatment (Fig.3A-C) and no recurrence was found after 5 months (Fig. 3D). In comparison with mefloquine, however, artesunate showed a slow effectiveness on the healing of the dermal lesion, especially in the early phase (1 week later) of oral treatment.

All the present patients treated with mefloquine or artesunate lived and worked in a rural and humid area with hygienically bad conditions. There is no specified adverse reaction in the patients treated with the drugs, including skin eruptions or pruritus. Therefore, none of the subjects treated were withdrawn from the study because of adverse reactions.

DISCUSSION

For the treatment of most forms of New and Old World leishmaniasis, pentavalent antimonial compounds are still remain as the drugs of choice. These drugs are given intramuscularly or intravenously, and generally

Table. Clinical data on the parasitologically-positive patients with cutaneous leishmaniasis who received Mephaquin® therapy

Patient No.	Age	Sex	No. of lesions	Size of lesions(mm)	Site of lesions	Type of lesions	Duration time of infection	Times (wks) for healing
1	41	♂	1	35×25	forearm	ulcer	2M*	6
2	16	♂	2	20×15 5×5	nose cheek	ulcers	12M	3
3	8	♂	2	30×30 10×5	foot	ulcers	2M	8
4	12	♂	2	5×5 5×5	forearm	ulcers	3M	5
5	19	♂	1	20×10	forearm	ulcer	3M	5
6	81	♂	4	20×10 15×15 10×10 10×10	forearm shoulder	ulcers	2M	4
7	26	♂	3	20×20 10×10 10×10	forearm	ulcers	1M	4
8	17	♂	1	15×10	neck	ulcer	1M	2
9	4	♂	4	10×10 5×5 5×5 4×4	face	ulcers	5M	2
10	22	♂	1	20×10	forearm	ulcer	1M	2
11	3	♂	2	4×4 3×3	face	ulcers	8M	2
12	13	♀	1	15×5	forearm	ulcer	2M	3
13	22	♂	1	4×3	forearm	ulcer	3M	3
14	22	♀	1	30×10	foot	ulcer	8M	3
15	31	♀	1	20×10	front	ulcer	2M	2
16	14	♂	1	34×26	foot	ulcer	3M	6

* M : month.

cause serious adverse reactions, such as renal and liver disfunctions, nausea, headache and arthragia, in addition to pain at the injection site. For the treatment of Old World cutaneous leishmaniasis, El-On *et al.* (1986) reported topical application of paromomycin ointment, obtaining satisfactory cure rate as good as any currently used therapy. Recently, the therapy was also used for New World cutaneous leishmaniasis in Ecuador (Nonaka *et al.*, 1992; Krause *et al.*, 1994) and Belize (Weinrauch *et al.*, 1993), confirming the results reported by El-On *et al.* (1986). However, such a topical treatment using ointment has limitations in its usage even in cutaneous leishmaniasis cases. Application of ointment would be only useful for relatively mild and simple lesions which are caused by *L.* (*L.*) *mexicana* groups. It is however not feasible for cutaneous disease forms caused by *L.* (*V.*) *braziliensis* (Weinrauch *et al.*, 1993) and mucocutaneous or visceral forms caused by other *Leishmania* agents.

To date, various oral anti-leishmanial drugs, such as metronidazole, rifampicin, levamizole, ketokonazole, co-trimoxazole, dapsone and etc., have been used for different disease forms with variable results. In the present study, anti-malarial drugs, mefloquine and artesunate were selected for clinical trials in the continuing search for oral anti-leishmanial drugs. These drugs were found to be effective against malarial parasites in the Old and New World. Mefloquine, a long-acting quinine analogue is a schizonticide and destroys the erythrocytic, asexual forms of the *Plasmodium* parasites in man, and the mean elimination half-life of the drug is calculated as 21.4 days ranging from 15 to 33 days (Desjardins *et al.*, 1979; Schwartz *et al.*, 1980, 1982). According to Schwartz *et al.* (1980, 1982), maximum plasma concentrations are reached 2-12 hours after a single oral dose and plasma concentrations approaching 1 $\mu g/ml$ are present after a dose of 1, 000mg of mefloquine. They also showed that similar

Figure 1 A dwelling site of inhabitants in an endemic area (Manta Real) surrounded by a dense forest where the present patients (volunteers) came from.

Figure 2A–C A cutaneous leishmaniasis lesion located on the lower extremity of a 22 years old female patient (No. 14) . A, An ulcer (34 × 26mm) with typically elevated border and marked induration around the lesion, before mefloquine treatment. B, After 1 week of the commencement of oral administration of mefloquine, the lesion reduced slightly in size and showed a gradual disappearance of the ulcer border and induration. A marked epidermidalisation was observed on the surface of the lesion. C, After 3 weeks of mefloquine treatment, the lesion was completely covered by epidermis without induration.

Figure 3A–D A cutaneous leishmaniasis lesion located on the upper extremity of a 14 years old male patient. A, A lesion (20 × 17 mm) showing a typical ulcer border, before artesunate treatment. B, After 2 weeks of the oral administration of artesunate, the lesion showed a marked granulation and reduced ulcer border. C, The cured lesion completely covered by epidermis without induration, after 6 weeks of artesunate treatment. D, The lesion (arrow) after 5 months of the treatment, showing a typical scar.


3A
3B
CAL 9
9/2/94
3C
3D

maximum concentrations are present in the steady state after administration of 250mg (1 Lactab®) weekly; the concentration in the erythrocytes is almost twice as high.

In this study, using mefloquine, almost all cutaneous lesions healed within 6 weeks after the commencement of treatment showing 100% cure rate, in spite of a relatively low daily dosage (250mg/day) of the drug compared with the dosage used in malarial cases. The precise mode of action of mefloquine against *Leishmania* has not been determined, although it has been shown that more than 98% of the active substance against *Plasmodium* schizonts is bound to plasma proteins (Schwartz *et al.*, 1982). To some extent, the mode of action of mefloquine against *Leishmania* parasites might be similar to that found in malarial cases, affecting amastigote-macrophage interactions.

In oral treatment using artesunate, only one case was experienced in this preliminary trial, suggesting that the drug might remain as a candidate for future study. Artesunate, a preparation for the killing of erythrocytic stage of *Plasmodium* asexual form, reacts with intraparasitic heme in its mechanism of antimalarial action (Meshrick *et al.*, 1991), but its mode of action against *Leishmania* parasites is still unknown precisely. According to Jiang *et al.* (1982), an advantage of artesunate administration is the speed of onset of action and inhibitory effect on the maturation of malarial parasites. On the other hand, in the present leishmaniasis case, it is likely that the drug has a tendency to act slowly as compared with mefloquine, especially at the early phase of treatment.

The current treatment with mefloquine or artesunate was done in the subjects who made their normal daily activities without admission. When a similar treatment using these drugs was performed in well-controlled subjects under admission, more rapidly healing would be found. Furthermore, a rapid healing might occur when antibiotics are used as complemental treatment to eliminate bacterial infections of cutaneous lesions. With regard to oral administration of the present drugs used, more suitable and effective dosage should be examined, in addition to their pricise mechanism(s) of anti-leishmanial action.

In conclusion, the significant efficacy of anti-malarial drugs, mefloquine and artesunate against *Leishmania* following the oral delivery suggests that the novel anti-leishmanial activities of these drugs should be investigated further, and their potential as drugs for various clinical forms of leishmaniasis including visceral forms needs more study.

ACKNOWLEDGEMENTS

We are grateful to Dr. Alexandra Sari Bravo and Ms. Gloria Esthela Losa of Subcentro de Salud de Zhucay, and to Miss Monica Pauta of Subcentro de Salud de Manta Real, Province of Cañar, Ecuador. This investigation was supported by the Ministry of Education, Science and Culture, Japan (Research grant no. 05041115) and Mepha Ltd., Aesch-Basle, Switzerland.

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Jpn. J. Trop. Med. Hyg., Vol. 23, No. 3 (1995) pp. 159~164

DRUG SENSITIVITY OF *VIBRIO CHOLERAE* AND *SHIGELLA* SPECIES IN THE WORLD

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Received July 20, 1995/Accepted August 16, 1995

Abstract: Recently isolated *Vibrio cholerae* and *Shigella* pecies from 6 countries were examined for their drug sensitivities. The sensitivities of *V. cholerae* were characterized by a narrow inhibitory concentration ranges without any resistant strain. However, the sensitivities of *Shigella* species were variable and mostly resistant to tetracycline and ampicillin. Japanese isolates of *Shigella* species were relatively more sensitive to tetracycline and ampicillin than the isolates from the other countries.
Indonesian isolates of *Shigella* species were relatively more resistant than those of the other countries, even to the new antimicrobials such as ofloxacin and cefdinir, and a highly resistant strain against ofloxacin was found.

INTRODUCTION

Cholera and bacillary dysentery (Shigellosis) are the most notorious illness among diarrheal diseases in the world. It has generally been said that *Shigella* species are mostly resistant to traditional antibiotics such as tetracycline and ampicillin (Carlson *et al.*, 1983),while *Vibrio cholerae* are sensitive to them, although emergence of tetracycline resistant *Vibrio cholerae* has occasionally been reported (Glass *et al.*, 1980; Ramamurthy *et al.*, 1992; Towner *et al.*, 1980; Yamamoto *et al.*, 1995). However, the drug sensitivities of the pathogenic organisms are variable from place to place where they are isolated, and from time to time of isolation. Therefore, the drug sensitivities should intermittently be examined for better understanding of the epidemiological feature. This paper described the drug sensitivities of *V. cholerae* and *Shigalla* species recently isolated from a variety of places in the world.

MATERIALS AND METHODS

Bacterial strains: *V. cholerae* were collected from Argentina, Indonesia, Laos, India, Bangladesh and Thailand in total of 159 strains. The isolates from the former 3 countries are *V. cholerae* O1 El Tor and those from the latter 3 countries are *V. cholerae* O139 synonym Bengal. The strains of *Shigella* species were collected from Bolivia, Dominican Republic, Indonesia, Laos, Kenya and Japan in total of 191 strains. All strains were isolated during the period between 1992 and 1995.

Drugs examined: Ampicillin (ABPC), Tetracycline (TC), Erythromycin (EM), and Ofloxacin (OFLX) were examined for all isolates. Additionally, Polymyxin B (PLB) for *V. cholerae* and Cefdinir (CFDN) for *Shigella* were examined.

Sensitivity tests: Minimum inhibitory concentration (MIC) of the drugs were examined by plate dilution technique. Heart infusion agar plates containing the drug at the serial 2-fold concentrations from $0.025\mu g/ml$ to $100\mu g/ml$ were prepared. The organisms to be examined were cultured in heart infusion broth over night and the culture fluids were diluted 1 : 10 with normal saline solution for the inoculum (ca. $10^7/ml$). They were inoculated by using microplanter (Sakuma Co. MITP#00257) and determination of MIC was made after incubation at 37 C for 24 hours.

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RESULTS

V. cholerae: The drug concentration ranges to inhibit the growth of organisms were 3.13 to 12.5μg/ml of ABPC, 0.2 to 0.4 of TC, 0.4 to 12.5 mostly 3.13 to 6.25 of EM, and less than 0.05 of OFLX. Except PLB, there was no resistant *V. cholerae* strain. The sensitivity pattern was characterized by a narrow inhibitory concentration ranges of each drug. There was no variability depending on the place of isolation (Table 1). *V. cholerae* O139 were resistant to PLB like *V. cholerae* O1 El Tor with the MICs more than 25 μg/ml, mostly 100μg/ml.

Shigella species: Being diffrent from *V. cholerae* most isolates of *Shigella* species were highly resistant to TC and ABPC. However, the sensitivity pattern is different from the place to place of isolation (Table 2). The isolates of Japan were relatively sensitive to these traditional antibiotics. The Indonesian isolates have a tendency to become resistant even against OFLX, and actually there was one strain with the MIC of OFLX at 100 μg/ml. In these sensitivity pattern, a new oral cephem CFDN has a considerably good anti-*Shigella* activity. Visual impression of these different sensitivities is shown in Figure 1.

MIC_{50} and MIC_{90} account for the variety of drug sensitivity of *Shigella* and the resembling sensitivity of *V. cholerae* (Table 3).

DISCUSSION

Results of the present studies revealed that recently isolated cholera vibrios have almost the same drug sensitivity pattern to *V. cholerae* O1 isolated in the past 3 decades (Iwanaga *et al*., 1982; Iwanaga *et al*., 1979). Although *V. cholerae* O139, a new cholera vibrio, is known to be resistant to co-trimoxazole (Yam *et al*., 1994), there must be no difficulties in the antimicrobial therapy for cholera at present. No resistant *V. cholerae* strain against the traditional antibiotics was found in this study. The sensitivity of *V. cholerae* O139 against PLB was different from the previous report by Sarkar et al. in which they described that the MICs were 15 to 50 μg/ml (Sarkar *et al*., 1993).

On the contrary, *Shigella* species showed a variety of drug sensitivities as being expected. It is interesting that the isolates of Japan are relatively more sensitive to tetracycline and ampicillin than those of the other countries. Japanese isolates were obtained from the epidemics in two separated areas of Okinawa. The cases were indigenous and not imported ones. The consumption rate of tetracycline and ampicillin in Japan supposed to be lower than in the other countries. This may be one of the reasons why Japanese isolates are relatively sensitive to these drugs. The resistant rate of *Shigella* against these traditional antibiotics in the recent may have reached maximum, because this kind of sensitivity pattern has been constantly seen in the past decades (Smollan and Block, 1990; Voogd *et al*., 1992). We can not account for the reason why Indonesian isolates have a tendency to be resistant to the new antimicrobials such as cefdinir and ofloxacin. Ofloxacin, (a new quinolone) and cefdinir (a new oral cephem) are excellent to inhibit the growth of *Shigella*. but ofloxacin is not preferable to use in the pediatric cases and cefdinir is expensive. Erythromycin (macrolide) is basically not effective to inhibit the growth of enterobacteriaceae. Actually, high MICs against *Shigella* are shown in the present study. However, there were many facts in the past that erythromycin was effective for the treatment of shigellosis regardless the drug sensitivities (Saito *et al*., 1964; Ukai *et al*., 1965). Recently, moreover, it is widely recognized that erythromycin is effective to panbronchiolitis infected with *Pseudomonas aerginosa* (Unertl *et al*., 1986). Therefore, it may be beneficial to use erythromycin for diarrheal diseases due to bacterial enteritis. Erythromycin is the first choice of drug for *Campylobacter* diarrhea and is known to be effective to *Vibrio* diarrhea (Burans *et al*., 1989; Kobari *et al*., 1967). If eryhromycin is effective to shigellosis, there is a good possibility that it is also effective to *E.coli* diarrhea. Clinical trials and the study to clarify the mechanism of erythromycin activities are required.

This study was supported by a grant-in aid for scientific research from the Ministry of Education,Science,and Culture of Japan (No. 07922097).

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Table 1 MICs of 5 antimicrobial agents against *V. cholerae* O1 and O139

V.cholerae O1

Argentina	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	40	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	0	0	0	0
0.4	0	40	0	0	0
0.8	0	0	0	0	0
1.6	0	0	0	0	0
3.13	0	0	13	0	1
6.25	0	0	27	0	39
12.5	0	0	0	0	0
25	0	0	0	0	0
50	0	0	0	0	0
100	0	0	0	0	0
100<	40	0	0	0	0
total	40	40	40	40	40

Indonesia	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	76	0
0.05	0	0	0	2	0
0.1	0	0	0	0	0
0.2	0	15	0	0	0
0.4	0	63	0	0	1
0.8	0	0	0	0	1
1.6	0	0	0	0	0
3.13	0	0	8	0	16
6.25	0	0	61	0	59
12.5	0	0	9	0	1
25	2	0	0	0	0
50	10	0	0	0	0
100	27	0	0	0	0
100<	39	0	0	0	0
total	78	78	78	78	78

Laos	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	41	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	4	0	0	0
0.4	0	37	0	0	0
0.8	0	0	0	0	0
1.6	0	0	0	0	0
3.13	0	0	2	0	8
6.25	1	0	39	0	33
12.5	0	0	0	0	0
25	0	0	0	0	0
50	1	0	0	0	0
100	37	0	0	0	0
100<	2	0	0	0	0
total	41	41	41	41	41

V.cholerae O139

India	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	8	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	8	0	0	0
0.4	0	0	0	0	0
0.8	0	0	0	0	1
1.6	0	0	0	0	0
3.13	0	0	8	0	7
6.25	0	0	0	0	0
12.5	0	0	0	0	0
25	1	0	0	0	0
50	0	0	0	0	0
100	7	0	0	0	0
100<	0	0	0	0	0
total	8	8	8	8	8

Bangladesh	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	13	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	12	0	0	0
0.4	0	1	0	0	0
0.8	0	0	0	0	1
1.6	0	0	1	0	4
3.13	0	0	11	0	8
6.25	0	0	1	0	0
12.5	0	0	0	0	0
25	0	0	0	0	0
50	2	0	0	0	0
100	11	0	0	0	0
100<	0	0	0	0	0
total	13	13	13	13	13

Thailand	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	31	0
0.05	0	0	0	0	0
0.1	0	1	0	0	0
0.2	0	29	0	0	1
0.4	0	1	0	0	1
0.8	0	0	0	0	0
1.6	0	0	3	0	7
3.13	0	0	28	0	20
6.25	0	0	0	0	2
12.5	0	0	0	0	0
25	1	0	0	0	0
50	9	0	0	0	0
100	20	0	0	0	0
100<	1	0	0	0	0
total	31	31	31	31	31

Table 2 MICs of 5 antimaicrobial agents against *Shigella* spp.

Shigella spp.

Bolivia	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	2	0
0.05	0	0	0	61	0
0.1	37	0	0	5	0
0.2	24	0	0	0	0
0.4	6	0	0	0	0
0.8	0	12	1	0	0
1.6	0	6	8	0	0
3.13	0	1	7	0	0
6.25	1	2	3	0	4
12.5	0	0	0	0	3
25	0	4	1	0	33
50	0	0	1	0	13
100	0	24	24	0	13
100<	0	19	23	0	2
	68	68	68	68	68

Laos	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	0	0
0.05	0	0	0	10	0
0.1	3	0	0	3	0
0.2	6	0	0	0	0
0.4	4	0	0	0	0
0.8	0	1	1	0	0
1.6	0	0	0	0	0
3.13	0	0	3	0	0
6.25	0	0	3	0	0
12.5	0	0	0	0	0
25	0	0	0	0	5
50	0	6	0	0	4
100	0	5	2	0	3
100<	0	1	4	0	1
	13	13	13	13	13

Dominica	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	0	0
0.05	0	0	0	17	0
0.1	8	0	0	4	0
0.2	9	2	0	0	0
0.4	4	5	0	0	0
0.8	0	1	0	0	0
1.6	0	1	0	0	0
3.13	0	0	3	0	1
6.25	0	0	2	0	8
12.5	0	0	1	0	6
25	0	0	0	0	2
50	0	4	0	0	3
100	0	5	0	0	1
100<	0	3	15	0	0
	21	21	21	21	21

Kenya	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	2	0
0.05	0	0	0	8	0
0.1	6	0	0	0	0
0.2	4	0	0	0	0
0.4	0	0	0	0	0
0.8	0	2	1	0	0
1.6	0	0	1	0	0
3.13	0	0	0	0	0
6.25	0	0	3	0	0
12.5	0	0	0	0	0
25	0	0	0	0	8
50	0	1	0	0	1
100	0	6	2	0	1
100<	0	1	3	0	0
	10	10	10	10	10

Indonesia	CFDN	TC	ABPC	OFLX	EM
0.025≧	2	0	0	0	0
0.05	0	0	0	15	0
0.1	18	0	0	9	0
0.2	18	0	0	17	0
0.4	4	2	0	4	1
0.8	2	3	3	0	0
1.6	1	1	7	0	1
3.13	0	0	5	0	0
6.25	1	0	6	0	2
12.5	0	0	0	0	7
25	0	4	0	0	19
50	0	1	0	0	11
100	0	24	12	1	4
100<	0	11	13	0	1
	46	46	46	46	46

Japan	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	0	0
0.05	0	0	0	20	0
0.1	1	0	0	12	0
0.2	28	0	0	0	0
0.4	3	0	0	1	0
0.8	1	8	0	0	0
1.6	0	25	1	0	0
3.13	0	0	26	0	0
6.25	0	0	6	0	0
12.5	0	0	0	0	0
25	0	0	0	0	1
50	0	0	0	0	8
100	0	0	0	0	24
100<	0	0	0	0	0
	33	33	33	33	33

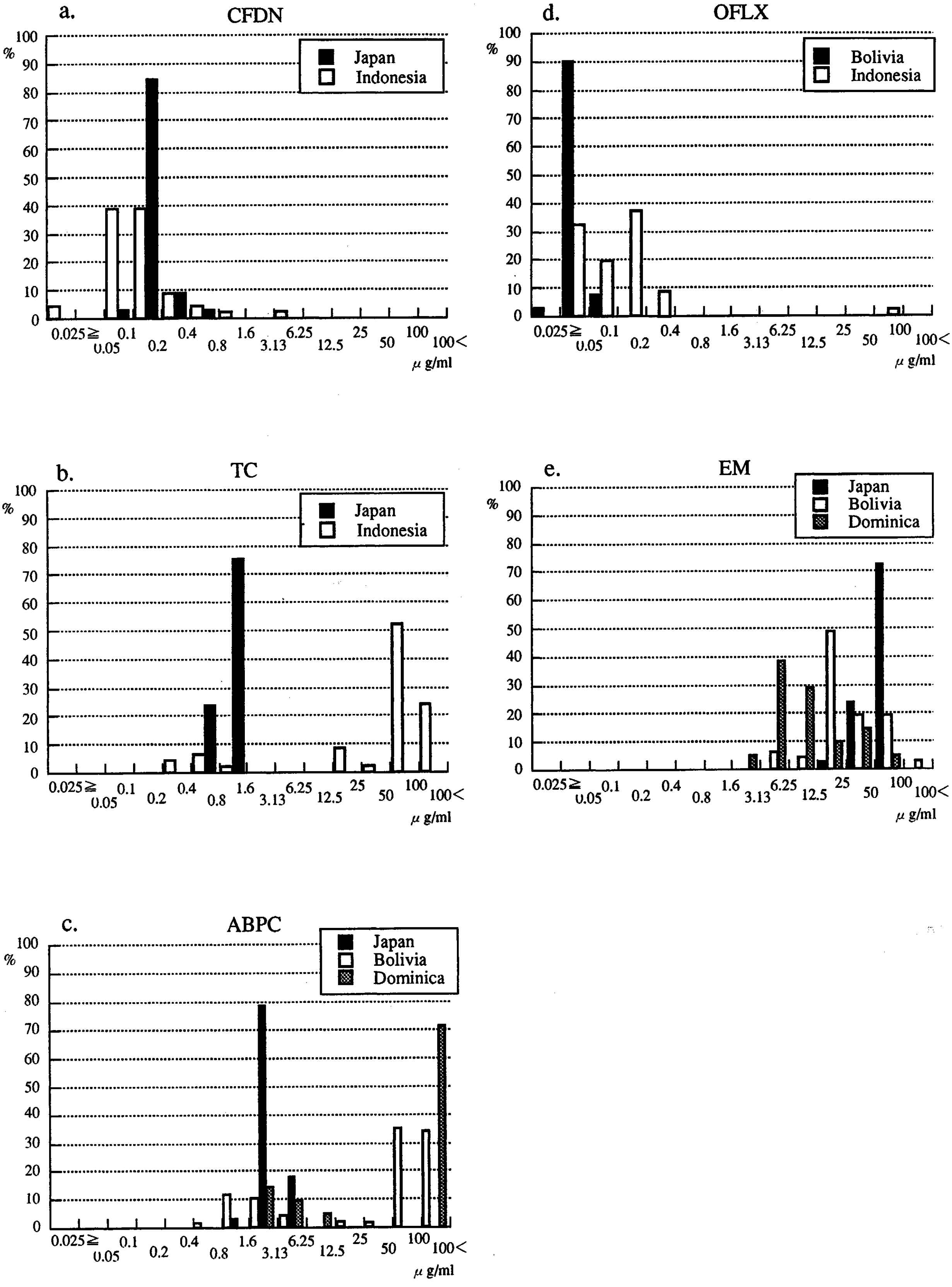

Figure 1 Visual expression of the sensitivities for *Shigella*

Table 3 Variability and Constancy of MIC

Shigella spp.

		Bolivia	Dominica	Indonesia	Laos	Kenya	Japan
CFDN	MIC_{50}	0.1	0.2	0.2	0.2	0.1	0.2
	MIC_{90}	0.2	0.4	0.4	0.4	0.2	0.4
TC	MIC_{50}	100	50	100	50	100	1.6
	MIC_{90}	100<	100<	100<	100	100	1.6
ABPC	MIC_{50}	100	100<	100	6.25	6.25	3.13
	MIC_{90}	100<	100<	100<	100<	100<	6.25
OFLX	MIC_{50}	0.05	0.05	0.1	0.05	0.05	0.05
	MIC_{90}	0.05	0.1	0.4	0.1	0.05	0.1
EM	MIC_{50}	25	12.5	25	50	25	100
	MIC_{90}	100	50	100	100	50	100

V. cholerae O1 & O139

		Argentina	Indonesia	Laos	India	Bangladesh	Thailand
PLB	MIC_{50}	100<	100	100	100	100	100
	MIC_{90}	100<	100	100	100	100	100
TC	MIC_{50}	0.4	0.4	0.4	0.2	0.2	0.2
	MIC_{90}	0.4	0.4	0.4	0.2	0.2	0.2
ABPC	MIC_{50}	6.25	6.25	6.25	3.13	3.13	3.13
	MIC_{90}	6.25	12.5	6.25	3.13	3.13	3.13
OFLX	MIC_{50}	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧
	MIC_{90}	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧
EM	MIC_{50}	6.25	6.25	6.25	3.13	3.13	3.13
	MIC_{90}	6.25	6.25	6.25	3.13	3.13	3.13

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Jpn. J. Trop. Med. Hyg., Vol. 23, No. 3 (1995) pp. 165~167

The frequency of healthy carriers with enteropathogenic Bacteria in Surabaya, Indonesia
—A study in an elementary school—

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Received July 20, 1995/Accepted August 11, 1995

Summary: A total of 953 stool samples from the healthy children of a elementary school located in the suburb of Surabaya City, Indonesia, were examined for *Shigella, Salmonella, Vibrio cholerae, V. parahaemolyticus*, and four kinds of diarrheagenic *Escherichia coli* excluding enteroadherent aggregative *E. coli.* The carrier rate was 6.8% (65 children out of 953). Enteropathiogenic *E. coli* was isolated from 42 children, but the other diarrheagenic *E. coli* were not detected. *Shigella* spp. and *V. cholerae* non-O1 were isolated from 12 and 11 children, respectively.

INTRODUCTION

Although the mortality rate of diarrheal diseases has markedly decreased in the past 10 years, it is still a big health problem in the tropical countries. The use of clean water and the sanitary behavior of the individual are essential in the control of diarrheal diseases in the communities. The sanitary situation of a community can be evaluated by examining the healthy carrier rate in that area. Indeed, secretory diarrhea can now be treated properly at the community level using oral rehydration therapy, but the invasive diarrhea due to *Shigella* species and enteroinvasive *E. coli* are out of hand because of multi-antibiotic resistant organisms. The strategy to eliminate a certain disease from an area is introduced by the precise epidemiological data of the disease. We have been studying the causative agents of diarrheal diseases in Surabaya, Indonesia for the past several years (Iwanaga *et al.*, 1993; Nakasone *et al.*, 1994). Herein, we describe the healthy carrier rate of enteropathogenic bacteria in the pupils of an elementary school located in the suburban area of Surabaya City, Indonesia.

MATERIALS AND METHODS

Stools samples: The stools were collected from a total of 953 children in elementary school in the suburbs of Surabaya City, Indonesia. All stool samples examined were normally formed, and the children were all healthy at least when the stools were collected. The children were between 5 and 15 years old, and the male to female ratio was 505 to 448. Sampling was carried out in 9 days between February 7 and 15, 1995.

Bacteriological examination: All samples were examined for *V. cholerae* O1, non-O1 and *V. parahaemolyticus, Aeromonas, Salmonella* and *Shigella.* We examined 362 samples for diarrheagenic *Escherichia coli* (enteropathogenic-, enterotoxigenic-, enteroinvasive-, and enterohemorrhagic-*E. coli* ; EPEC, ETEC, EIEC, and EHEC, respectively). About 0.1 g of stool was mixed with 1 ml of alkaline peptone water in an Eppendorf tube. After mixing it well by using a vortex machine, large fecal materials in the tube were removed by flashing centrifugation at 15000 rpm for 5 seconds. Fifty micro- liters of the supernatant was placed on a MacConkey agar plate for *E. coli*, and SS agar for *Shigella* and *Salmonella.* The inoculum was spread out

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to a half space of the plate using a glass bar spreader and streaked to the other half space using an inoculum wire loop. The samples in Eppendorf tubes were left at room temperature (Ca. 25°C) overnight, and then 50 μl each of them was inoculated on TCBS agar plate in the same manner as described above. As a rule, 4 colonies each of lactose fermentative and non-fermentative organisms were subcultured on nutrient agar plate (8 strains per plate) to examine for diarrheagenic *E. coli*. The organisms suspected as diarrheagenic *E. coli* were identified by examining their biological properties.

Serotyping: All *E. coli* strains, *Salmonella, Shigella*, and *V. cholerae* were serogrouped by a slide agglutination test using commercial antisera (Denkaseiken).

Examinations for virulence factors: EPEC was identified by glass slide agglutination test using commercially purchased anti-EPEC sera. Heat labile *E. coli* enterotoxin (LT) was examined by the Biken-method (Honda *et al*., 1981), and heat stable toxin (ST) by the immunoenzyme assay (IEA) using a detection kit, COLIST (Denkaseiken). EIEC and EHEC were screened by sero-agglutination, and then examined by polymerase chain reaction (PCR) according to the methods of Lampel *et al.* (1990) and Kobayashi *et al.* (1990) for the primer sets and conditions of reaction, respectively.

RESULTS

Diarrheagenic *E. coli* was isolated from 42 out of 362 children (11.6%), and all of them were EPEC (Table 1). There were 31 isolates with the serotypes of EIEC or with EIEC biochemical behavior. However, these isolates were all negative for invasive plasmid as examined by PCR. There were three isolates with serotype O157, but the H-antigen of the two isolates was not H7 and one isolate was non-motile. The genes encoding VT-I or VT-II were not detected from the strains with O157 and O26 by PCR. There were many serotypes in the EPEC isolates, and among them, antigen O128 was dominant (Table 2). *Shigella* was isolated from 12 out of 953 children (1.3%) including 7 isolates of *S. boydii*, 3 of *S. flexneri*, 2 of *S. dysenteriae* (not type 1), and none of *S. sonnei* (Table 3). The serotypes of 11 isolates of *V. cholerae* did not belong to O1 or O139. *Salmonella, V. parahaemolyticus*, and *Aeromonas* were not detected.

Table 1 Isolation frequency of enteropathogens

Enteropathogens	No.of positive	(%)	No. examined
EPEC	42	11.6	362
ETEC	0	0	
EHIC	0	0	
EIEC	0	0	
Shigella spp.	12	1.3	953
Salmonella	0	0	
V. cholerae O1	0	0	
V. cholerae non-O1	11	1.2	
V. parahaemolyticus	0	0	
Aeromonas spp.	0	0	

Tabel 2 Serovar distribution of EPEC, 42 strains

EPEC		No. of strain
Class I	O26	1
	O55	2
	O86	3
	O111	1
	O119	6
	O126	2
	O127	3
	O128	11
	O142	1
Class II	O18	5
	O44	4
	O114	3

Tabel 3 Distribution of *Shigella* spp.

Shigella	No.of strain
S. dysenteriae (not type 1)	2
S. flexinneri	3
S. boydii	7
S. sonnei	0

DISCUSSION

In this study, the carrier rate of EPEC was high (11.6%), and no ETEC were isolated. Recently, Albert *et al.* (1995) reported that the carrier rate of EAF positive EPEC (Class I EPEC) in Bangladesh was 5.3% (33/602) and (Class II EPEC) was isolated in 7.3 %. Although the definition of EPEC is still in controversy, *E. coli* bearing the EAF plasmid is considered to be definite EPEC, and called "Class I EPEC" (Levine, 1987). Reviewing the data in the present study in accordance with that classification, Class I specific serotypes were found in 30 out of 362 (8.3%) isolates examined and Class II specific serotypes in 12 (3.3%). The isolation frequency of ETEC from human beings with or without diarrhea is usually less frequent than that of

EPEC (Albert *et al*., 1995; Chatkaeomorakot *et al*., 1987; Echeverria *et al*., 1987; Georges *et al*., 1984; Gomes *et al*., 1989, 1991 ; Toledo *et al*., 1983), and in the present study, no ETEC were isolated. Nevertheless, the detection of ETEC from Japanese travellers returning from the developing countries is far more frequent than that of EPEC (Kudoh *et al*., 1993; Yoshida *et al*., 1992). To explain this discrepancy, continuous monitoring for the detection of ETEC and EPEC is required.

The *Shigella* positive rate of 1.3 % was as expected from our previous experiences. How many of these carriers develop illness is a problem to be clarified in future. *Salmonella* tends to stay in the intestine for a long time, but there were no carriers in the examined population. The distribution density of *Salmonella* must be very low in this area. On the contrary, although *V. cholerae* tends to stay in the intestine for a short period, it was detected from 11 children out of 953 (1.2%). This suggests that the people in this area frequently ingest the organisms, probably because of water contamination. However, the carriers of enterobacteiaceae such as *E. coli, Shigella* and *Salmonella*, suggest the presence of fecal-oral infection. The development of a strategy to prevent diarrheal diseases in this area is awaited.

ACKNOWLEDGMENTS

We thank Kazuhiro Kobayashi, Osaka Prefectural Institute of Public Health, for kindly providing EHEC strains, and Musyadi and Moh. Choesen Masjkoer for their kind assistance.

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Jpn. J. Trop. Med. Hyg., Vol. 23, No. 3 (1995) pp. 169～176

INTESTINAL PARASITIC INFECTIONS IN SOME RURAL AND URBAN AREAS OF THE DOMINICAN REPUBLIC

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Received June 23, 1995/Accepted August 14, 1995

Abstract: A parasitological examination was conducted on fecal samples collected from 1,547 inhabitants residing in 10 rural and two urban localities including a slum in the Dominican Republic. Seven helminth and seven protozoan species were observed. In the helminthic infections, *Trichuris trichiura* was commonest with overall prevalence of 48.4%, being followed by *Ascaris lumbricoides* (prevalence 25.5%) and hookworms (12.9%), while the prevalence of other helminthic infections was quite low, less than 3% for every species. All of the filariform larvae of hookworms were identified as *Necator americanus*. The prevalence of major geohelminth infections markedly differed by locality presumably due to the climatic and socioeconomical differences. The prevalence of *A. lumbricoides* infection showed a peak in the groups of 5-9 or 10-14 years of age in the highly endemic localities, while that of *T. trichiura* and hookworm formed a plateau through the age groups of 5-9, 10-14 and 15-24 years. *Entamoeba coli* was the most prevalent protozoan being detected in 21.1% of the samples, followed by *Giardia intestinalis* (5.4%). The prevalence of protozoan infections was relatively stable among localities, suggesting that contaminated drinking water or foods were the sources of infection.

Key words: Intestinal parasite infection, Dominican Republic, urban slum, rural area, epidemiology.

INTRODUCTION

The Dominican Republic is located in the Caribbean region occupying east two-thirds (ca. 48,442km^2) of Hispaniola Island. The total population is approximately 6 millions of which about 2.4 millions reside in the capital, Santo Domingo. The climate is of tropical oceanic type with monthly mean minumum temperature ranging from 18 to 25°C and mean maximum temperature 28 to 34°C in the areas along the coast. Rainy season lasts from May to September in most regions except northwestern and southwestern regions where rainy season is absent. Annual precipitation is over 2,000mm in northeastern region and 1,000 to 1,400mm in southeastern coastal and west-central regions, and less than 1,000mm in northwestern and southwestern regions.

This country has been known as an endemic area of schistosomiasis and Bancroftian filariasis on which many reports have been made (cf. Vargas *et al*., 1981, 1987, 1990; Vincent *et al*., 1987. Hillyer *et al*., 1986). On the other hand, there have been only sporadic researches on intestinal parasitic infections in limited localities (Mackie *et al*., 1951; Collins and Edwards, 1981; Vargas *et al*., 1987), and any nationwide record has not been available. During the medical cooperation project on gastroenterology between the Dominican Republic and Japan aided by the Japan International Cooperation Agency (JICA), we had an opportunity to make a parasitological examination on feces from the inhabitants in some rural and urban areas. The results are presented in this paper with an epidemiological discussion.

MATERIALS AND METHODS

Twelve localities, two urban and 10 rural, were chosen for survey (Fig. 1). Fecal samples were collected from inhabitants who voluntarily agreed to have

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the parasitological examination. A questionnaire on socioeconomical and sanitary conditions was also made at the delivery of container for fecal collection. Examination of feces was made with both Kato-Katz smear and formalin-ether concentration techniques. Harada-Mori culture was also applied for samples from El Valle and La Ciēnaga. Agar-plate culture of feces for *Strongyloides stercoralis* detection (Arakaki *et al.*, 1988) was done only for 126 samples from La Ciēnaga. All of the inhabitants who were proved to have helminthic and *Giardia intestinalis* infections were treated free of charge.

Statistical analysis of prevalence data between groups was performed by Chi-square test with Yates' correction for continuity (significance level : $p<0.05$).

Outline of The Surveyed Localities

La Ciēnaga is a typical urban slum of Santo Domingo, and is located along the west side of the River Ozama. This area is on the flood plain of the river, and the soil is often moist. Most of the inhabitants are poor immigrants from rural areas and residing in temporary huts made of zinc plates, corrugated cardboards, polyvynyl sheets and wood pieces. In rainy season, floods sometimes sweep away such huts along with inhabitants to the sea. Tap water supply is available only in limited houses, and majority of the inhabitants obtain water from common taps. Most lavatories are of quite simple pit latrine type. Many small streams with filthy water run through the area. The water of the River Ozama is contaminated with sewage from Santo Domingo city, but some poor people of La Ciēnaga utilize it for washing and bathing.

Villa Duarte is located along the east side of the River Ozama, and is on a cliff with 10m height from the river surface. This area is provided with drain system and the soil is usually dried. The inhabitants of this area are composed of middle or somewhat lower classes. Tap water supply is available and most houses have flush toilets or pit latrines. Most of the middle-class inhabitants buy bottled sterilized water for drinking.

Samanā, Las Cuchillas and El Valle are located in the northeastern region of the country (Fig. 1). In Samanā, a small coastal village was investigated. This village is encircled by coconuts plantations, and people engage in agriculture and fishery. The inhabitants of Las Cuchillas and El Valle are mostly farmers cultivating coconuts, oil palm, banana and coffee or cattle breeders. In Samanā and El Valle, water supply is available, but taps are settled outside of the houses. In Las Cuchillas, well water is used for drinking, and rain water is stored in tank for washing and bathing use. In these three areas, pit latrines are predominantly used, but some inhabitants prefer to defecate in nearby bushes. Annual precipitation is more than 2,000mm, and the soil is usually wet.

Dajabōn is located at the northwestern border with Haiti. The altitude is less than 50m from sea level, and its climate is hot and dry. Annual precipitation is less than 800mm and rainy season is absent. People engage in rice paddy cultivation and cattle-breeding, and are relatively wealthy.

Constanza and Jarabacoa are farming areas located in the west-central of the country and with altitude of 1,200m and 500m from sea level, respectively. Monthly mean minimum and maximum temperatures in Constanza are 9-13°C and 23-26°C, respectively. Annual precipitation in Constanza is about 1,000mm. Former Japanese settlements, now inhabited by a few Japanese immigrants and many Dominicans, were chosen for survey. Water from mountain streams is supplied through pipes. The houses of Japanese immigrants have flush toilets or latrines of permanent structure, and the excreta from these lavatories penetrate into bottom soil. Most of the Dominican inhabitants use simple pit latrines.

Juancho, Oviedo, Pedernales and La Cueva are located along coast of southwestern part of the country. Annual precipitation is less than 1,000mm without rainy season, making the climate hot and dry, and the villages are often surrounded by cactus fields. Most people examined were fishermen, cattle-breeders and their family members, and were very poor generally. Tap water supply is not available, and water wagons supply river water once a week. The supplied water is stored in open drum cans in front of the houses. Simple pit latrines are used, but people often defecate within nearby bushes. The data of Juancho and Oviedo were combined together because of small sample size and similar natural and socioeconomical conditions between the localities. With the same reason, the data of Pedernales and La Cueva were joined together.

Results

A total of 1,547 inhabitants (752 males and 795 females) were examined, and seven helminth and seven protozoan species were detected from them. Table 1 shows the parasite prevalence by locality and sex. The most prevalent helminth was *Trichuris trichiura* in all localitles except Dajabōn, where prevalence of soil-transmitted nematode infections was quite low and

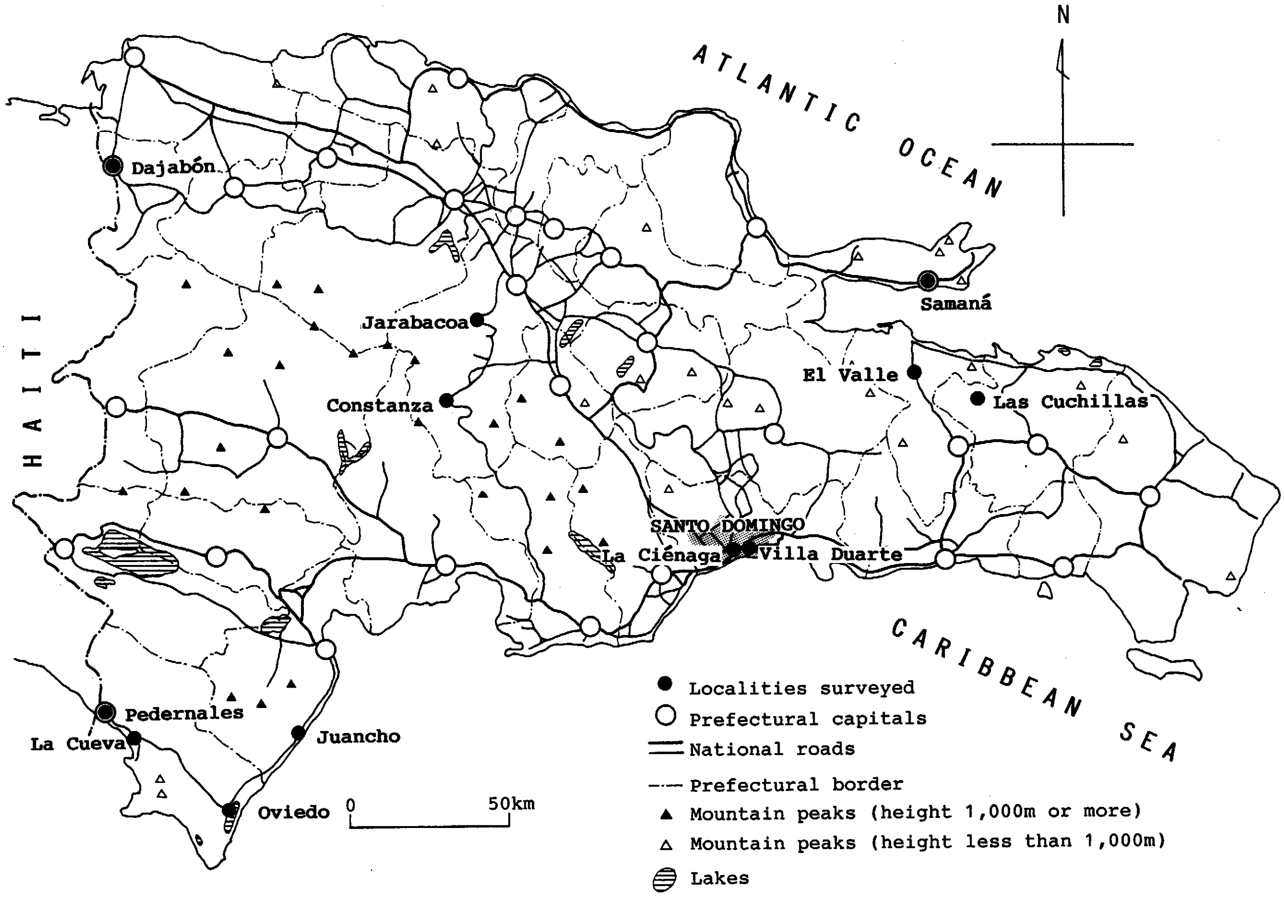

Figure 1 A map of the Dominican Republic showing localities where parasitological examination was made.

Hymenolepis nana was the most prevalent helminth (6.8%) (Table 1). The prevalence of *Trichuris* infection was especially high in El Valle (78.7%) and La Ciénaga (67.1%), and it exceeded 50% in Samaná, Juancho/Oviedo and Pedernales/La Cueva (Table 1). The prevalence of *Trichuris* infection in Villa Duarte, Las Cuchillas, Constanza and Jarabacoa showed no significant difference statistically from each other.

Ascaris lumbricoides was the next prevalent helminth except in the northeastern localities, i.e.Samaná, Las Cuchillas and El Valle, where the prevalence of hookworm infection was much higher (Table 1). Among the localities, the prevalence of *Ascaris* infection was highest in La Ciénaga, but not significantly different from that in El Valle and Juancho/Oviedo. Villa Duarte, Constanza, Jarabacoa and Pedernales/La Cueva showed moderate prevalence of *Ascaris* infection, being not different from each other significantly. *Ascaris* prevalence was quite low in Samaná and Las Cuchillas, where *Trichuris* prevalence was more than eight times as high as *Ascaris* prevalence. In Dajabón, only one child harbored *Ascaris*.

The prevalence of hookworm infection was much higher in the northeastern localities than in other localities (Table 1). It was highest in El Valle where more than 50% of the inhabitants were positive. In La Ciénaga, hookworm prevalene was low (6.3%), and it was much lower (3% or less) in Villa Duarte, Dajabón, Constanza, Jarabacoa, Juancho/Oviedo and Pedernales/La Cueva. All of the filariform larvae of hookworms obtained by Harada-Mori culture were identified as *Necator americanus*. *Strongyloides stercoralis* infection was less common, but was detected also in Jarabacoa and Juancho/Oviedo, where hookworm infection was not proved (Table 1). *Hymenolepis nana* was detected in eight out of the 12 localities surveyed, while *Hymenolepis diminuta* was proved in only one sample (Table 1).

Contrasting to helminthic infections, the prevalence of protozoan infections was relatively stable. The overall protozoan prevalence was ranging from 10 to 30% among the localities except La Ciénaga and El Valle where protozoan infections were rather common (Table 1). The most prevalent protozoan was

Entamoeba coli that was detected in every locality. The prevalence of *E. coli* infection was highest in La Ciênaga and lowest in Villa Duarte (Table 1). However, its prevalence in La Ciênaga was not significantly higher than that in Samanâ, Las Cuchillas, Jarabacoa, Juancho/Oviedo. Also, no significant difference was observed statistically in *E. coli* prevalence among Samanâ, Las Cuchillas, El Valle, Dajabôn, Constanza, Jarabacoa, Juancho/Oviedo and Pedernales/La Cueva. The prevalence of *Giardia intestinalis* infection was highest in La Ciênaga (15.7%), being followed by Las Cuchillas and El Valle (Table 1).

Multiple infections were demonstrated in 44.8% of the inhabitants examined in total (22.9% with 2 parasite species, 11.8% with 3, 3.6% with 4, 1.0% with 5 and 0.3% with 6). The frequency of multiple infections showed a clear correlation with the prevalence of paraistic infections in the localties. In El Valle and La Ciênaga where overall prevalence was about 90% (Table 1), multiple infection was observed in 69.8% and 64.4% of the inhabitants, respectively. In the localities with overall parasite prevalence ranging from 65 to 75%, multiple infection was found in 30 to 45% of the inhabitants. In the localities with parasite prevalence of 45 to 50%, multiple Infection was found in 17 to 26%. Triple or more infections were found in 43.2, 31.8 and 15.0% among the inhabitants in El Valle, La Ciênaga and Samanâ, respectively, while such infection was less than 10% in other localities. In Dajabōn where the parasite prevalence was lowest (32.5%), double infection was demonstrated only in 1.7% of the inahbitants and triple or more infection was not observed.

The prevalence of *Trichuris*, hookworm and *H. nana* infections was significantly higher in males than in females in total ($p<0.005$), while *Ascaris* prevalence was not different significantly between sexes. The protozoan prevalence showed no clear difference between sexes except that *E. coli* infection was significantly higher in females (Table 1). This was due to the much higher prevalence of *E. coli* infection in females (36.8%) than in males (28.6%) among adults (aged 15 or more) in La Ciênaga, while it was same between sexes among young inhabitants (aged 0 to 14 years).

The age-related prevalence of three major soil-transmitted helminthic infections by locality was shown in Figs. 2-4. The data of the west-central two localities and the southwestern four localities were combined together. The data of Villa Duarte and Las Cuchillas were excluded becuase of small sample size. In La Ciénaga and the southwestern localities, the prevalence of *Ascaris* infection was already more than 30% in the youngest age class (0-4 years) (Fig. 2). In all localities

Table 1 Prevalence of intestinal parasitic infections in the Dominican Republic, 1993-1995.

Locality	Urban Areas: Santo Domingo (Capital)		Rural Areas: Northeastern Region			Northwestern Region	West-central Region		Southwestern Region		Sex		Total
	La Ciênaga (slum)	Villa Duarte	Samanâ	Las Cuchillas	El Valle	Dajabôn	Constanza	Jarabacoa	Juancho/ Oviedo	Pedernales/ La Cueva	Males	Females	
No. examined	395	70	180	85	169	237	147	78	87	99	752	795	1574
Helminths													
Ascaris lumbricoides	46.3%	25.7	7.2	4.7	37.9	0.4	22.4	17.9	37.9	32.3	25.8	25.3	25.5
Trichuris trichiura	67.1	31.4	58.3	42.4	78.7	4.2	29.9	33.3	56.3	58.6	52.5	44.4	48.4
Hookworm	6.3		30.0	22.4	54.4	1.7	1.4			3.0	15.6	10.3	12.9
Strongyloides stercoralis	2.5		9.4	4.7	4.1	0.4		3.8	2.3	1.0	3.3	2.5	2.9
Enterobius vermicularis	0.5		0.6		0.6	0.4	0.7				0.8		0.4
Hymenolepis nana	0.8				0.6	6.8	1.4	1.3	3.4	1.0	1.6	1.9	1.7
Hymenolepis diminuta									1.1		0.1		0.1
Subtotal	75.2	47.1	68.9	54.1	86.4	13.5	40.1	39.7	66.6	63.6	62.0	53.2	57.5
Protozoans													
Entamoeba histolytica	4.1	1.4			3.0		0.7				1.7	1.3	1.5
Entamoeba hartmanni	2.8	1.4	3.3	4.7	1.2				3.4	2.0	2.1	1.6	1.9
Entamoeba coli	28.4	7.1	23.9	20.0	18.9	20.3	13.6	17.9	20.7	17.2	18.4	23.6	21.1
Endolimax nana	13.9			3.5	13.0		0.7				5.3	5.2	5.2
Iodamoeba buetschlii	9.1				3.0						2.4	2.9	2.7
Giardia intestinalis	15.7	2.9	0.6	7.1	6.5		0.7			1.0	5.1	5.8	5.4
Chilomastix mesnili	1.3	1.4			1.2						0.7	0.4	0.5
Subtotal	54.7	11.4	26.1	28.2	36.7	20.3	15.0	17.9	24.1	19.2	28.6	33.5	31.1
Grandtotal	88.9	50.0	73.9	67.1	91.1	32.5	47.6	48.7	73.6	68.7	70.2	65.3	67.7

except Samaná and Dajabón, it showed a rapid increase with age attaining to more than 40% in 5-9 years age class. In La Ciénaga, *Ascaris* prevalence further elevated to form a peak of about 70% in 10-14 years age class, while it decreased in the elder groups in El Valle and west-central and southwestern localities (Fig. 2). In Samaná and Dajabón, no clear peak was observed in the age-related prevalence of *Ascaris* infection (Fig. 2).

In *Trichuris* infection, the prevalence was already 50% or more in the youngest age class in the localities except Dajabón and Constanza/Jarabacoa, and formed a plateau through the next three or four age classes, although it showed a decrease in 10-14 years age class in Samaná. *Trichuris* prevalence gradually decreased thereafter but still remained at high level in elder groups in these localities (Fig. 3). In Constanza/Jarabacoa, the prevalence in the youngest age class was 22%, but increased rapidly with age attaining a peak of 61.1% in 10-14 years age class, then decreased rapidly again (Fig. 3). In Dajabón, the prevalence was generally low throughout all age classes, forming a low peak in 10-14 years age class (Fig. 3).

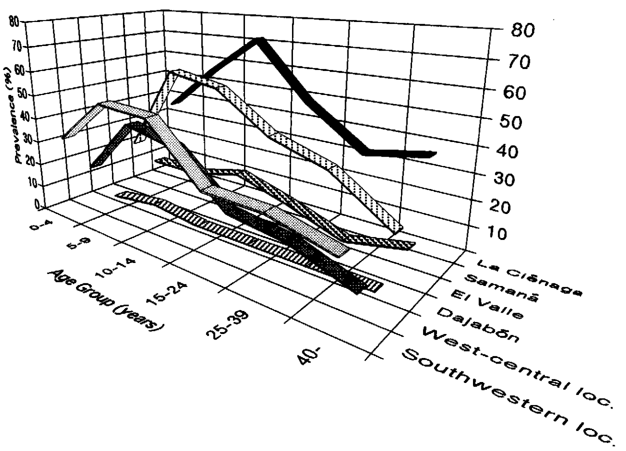

Figure 2 Age-related prevalence of *Ascaris lumbricoides* infection by locality in the Dominican Republic. West-central loc.: Constanza and Jarabacoa; Southwestern loc. : Juancho, Oviedo, Pedernales and La Cueva.

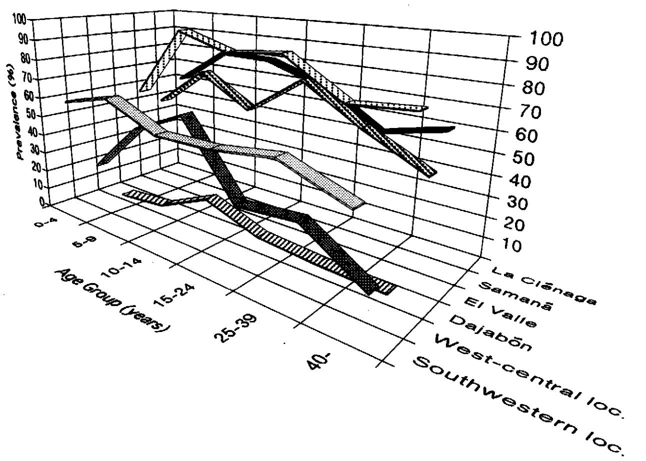

Figure 3 Age-related prevalence of *Trichuris trichiura* infection by locality in the Dominican Republic. West-central loc.: Constanza and Jarabacoa; Southwestern loc. : Juancho, Oviedo, Pedernales and La Cueva.

In El Valle and Samaná, the prevalence of hookworm infection was about 20% and 15%, respectively, in the 0-4 years age class, rapidly increased to form a plateau of more than 60% and 30%, respectively, in the next three age classes, then decreased gradually but again increased in the eldest group (Fig. 4). In La Ciénaga, the hookworm prevalence in the first age class was only 1.4%, then increased to form a low peak (16%) in 15-24 years age class, then gradually decreased (Fig. 4). In the west-central, northwestern and southwestern localities, the prevalence of hookworm infection was very low throughout all age classes (Fig. 4).

DISCUSSION

The helminths detected in the present study are of simple life cycle except *H. diminuta* that requires an intermediate arthropod. Hato Mayor Prefecture where El Valle belongs has been known as one of the endemic areas of schistosomiasis mansoni, and control measures have been conducted from 1970 (Hillyer *et al.*, 1986; Vargas *et al.*, 1987,1990). Hillyer et al. (1986) proved

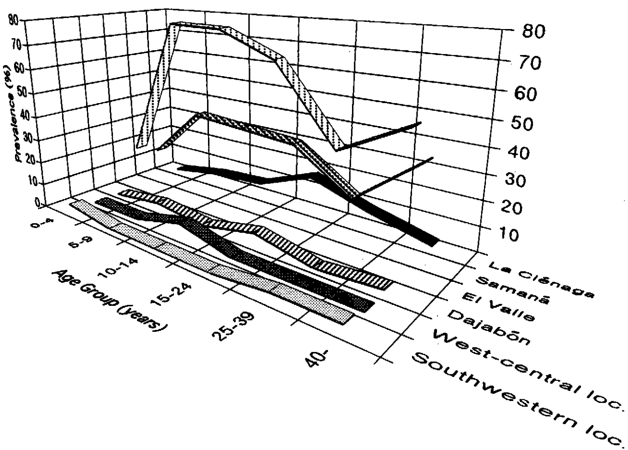

Figure 4 Age-related prevalence of hookworm infection by locality in the Dominican Republic. West-central loc.: Constanza and Jarabacoa; Southwestern loc.: Juancho, Oviedo, Pedernales and La Cueva.

positive rate of 5.0% by radioimmunoassay in 1983 and Vargas *et al.* (1990) detected schistosome eggs in 7.8% of inhabitants during the period from 1982 to 1987 in El Valle. However, no schistosome egg was observed in the present material, suggesting that the rate of infection has been greatly reduced nowadays. Besides these species, *Taenia* sp. has been recorded from Dominican inhabitants, but its prevalence was very low (less than 0.5%; Mackie *et al.*, 1951; Vargas *et al.*, 1987). The absence of habit of eating raw meat or raw fish among the Dominicans may be the cause of the low prevalence of food-borne cestode and trematode infections.

The present results showed marked differences in the prevalence of soil-transmitted helminthic infections among localities (Table 1). One of the major causes of these discrepancies among the localities seems to be the climatic difference. The northeastern region of the Dominican Republic has large amount of rainfall, over 2,000mm/year, keeping the environment moist. Such condition is especially suitable for larval development and transmission of hookworms (cf. Schad and Warren, 1990). The high hookworm prevalence in the northeastern region may be caused by this abundant precipitation and the low sanitation level. Meanwhile, the amount of annual precipitation in the northwestern and southwestern regions is less than 1,000mm. Such dry condition would inhibit development and survival of hookworm larvae, as was reflected by the low hookworm prevalence in these areas.

It has been well known that *Ascaris* eggs are more resistant to desiccation than are those of *Trichuris* (cf. Beaver *et al.*, 1984). It was therefore unexpected that the *Ascaris* prevalence in Samaná and Las Cuchillas was found to be quite low, while the prevalence of *Trichuris* infection was rather high (Table 1). At the present time, no reasonable explanation on the cause of this phenomenon is possible. However, it is suspected that some natural or artificial factors might be involved. Such low *Ascaris* and high *Trichuris* prevalence has been also recorded on some other Caribbean islands (Bundy,1986), suggesting the presence of common cause(s) of this unbalanced prevalence of these orally-transmitted geohelminth infections.

La Ciénaga is on the flood plain of the River Ozama and the soil is usually moist. Moreover, inhabitants are often barefooted and latrines are of quite inadequate structure. Nevertheless, the hookworm prevalence was less than 10% although *Ascaris* and *Trichuris* infections were considerably prevalent (Table 1). Similar status has been also reported in some urban slums of developing countries (Bundy *et al.*, 1988; Ferreira *et al.*,1994). The cause of such unexpectedly low prevalence of hookworm infection may be the pollution of environment with substances that may antagonistic to development of hookworm larvae. In urban slums, the environment is often contaminated with sewage containing chemical substances including synthetic detergent and decomposed organic matter that would kill hatched larvae of hookworms. Meanwhile, *Ascaris* and *Trichuris* eggs are more resistant in having thick shells, and their infections may be enhanced by the crowded condition of the urban slums.

Villa Duarte, Constanza and Jarabacoa shared almost same parasitological status characterized by relatively low *Trichuris* and *Ascaris* infections (about 30% or less) and quite low hookworm prevalence (less than 5%) (Table 1) . In these areas, inhabitants are generally wealthier than those in other localities except Dajabón, human population is less crowded, and/or the latrines and drainage system are of adequate structure. These conditions may reduce the opportunity of geohelminth transmission. In Dajabón, where the living condition resembles those in Constanza and Jarabacoa, the prevalence of the orally-transmitted geohelminth infections was significantly lower than these localities (Table 1). Possibly, much drier climate of Dajabón might also attribute to such low prevalence.

The parasitological status of the southwestern localities, i.e. Juancho/Oviedo and Pedernales/La Cueva, also resembled each other in having high prevalence of *Trichuris* infection (more than 50%), moderate and low prevalence of *Ascaris* and hookworm infections, respectively (Table 1). The low hookworm prevalence seems to be due to the dry climate as in Dajabón. Meanwhile, the inadequate structure of the pit latrines and shortage of water may make contamination of the living environment with *Trichuris and Ascaris* eggs that are more resistant against desiccation than hookworm eggs and larvae.

Unlike the soil-transmitted helminthic infections, the prevalence of protozoan infections was relatively stable among the localities as shown above (Table 1). This suggests that transmission of the intestinal protozoans is not greatly influenced by the difference in climate by locality. Infection of intestinal protozoans is acquired by ingesting drinking water or foods contaminated with fecal material (cf. WHO, 1987). In the surveyed localities, people often utilize unboiled water for drinking and foods seem to be polluted with protozoan cysts through dirty hands or flies. In La Ciénaga, the people are residing in quite unhygienic condition, and thus transmission of protozoan cysts may occur frequently as was reflected by the higher prevalence of protozoan infections. Meanwhile, in Villa Duarte that is on the opposite bank of the River Ozama, the inhabitants are wealthier and live in much better condition than in La Ciẽnaga, and the prevalence of protozoan infections was significantly lower. This fact may clearly indicate that the paraiste prevalence is greatly influenced by the economical condition.

About 45 years ago, Mackie *et al.* (1951) examined 1,139 sugar cane plantation workers in two southeastern localities. The most prevalent helminth was hookworm (overall prevalence 59.2%) being followed by *Trichuris* (58.9%) and *Ascaris* (20.1%), and the most common protozoan was *E. coli* (78.1%), being followed by *Endolimax nana* (47.7%), *Entamoeba histolytica* (34.2%) and *Iodamoeba buetschlii* (26.3%). In 1977, Collins and Edwards (1981) examined 137 fecal samples in the southcentral region and found that *Trichuris* infection was commonest (prevalence up to 35.8%) being followed by *Ascaris* (13.1%) and hookworm (8.8%) infections. They observed only two protozoans, *E. coli* (24.8%) and *G. intestinalis* (0.7%). In 1980s, Vargas *et al.* (1987) examined 1,025 inhabitants in Higuey, the capital city of eastern-most prefecture, and found that *Trichuris* infection was most prevalent (59%), being followed by hookworms (20%), *Ascaris* (17%) and *Schistosoma. mansoni* (11.8%). Other helminthic infections were less than 1% in total. The commnonest protozoan was *E. coli* (33%), being followed by *G. intestinalis* (5%) and *E. histolytica* (0.1%).

Because these studies were done in the localities different from those in the present survey and the employed techniques were also different, it is inappropriate to compare their data directly with the present results. However, it is suggested that the status of intestinal parasitic infections, especially helminthic ones, have remained almost unchanged during the recent five decades in rural areas. Moreover, recent urbanization has produced numerous slum dwellers, who are continuously exposed to parasite infections as shown in the present data of La Ciẽnaga. The intestinal helminthic infections have been often considered as a too large problem to be challanged by public health service because they occur most commonly in the poorest communities of the developing countries (cf. Savioli *et al.*, 1992). However, recent advances of theoretical and practical studies have provided realistic and cost-effective options of control by chemotherapy (cf. Bundy *et al.*, 1992; Guyatt *et al.*, 1995; Medley *et al.*, 1993). Some appropriate measures of intestinal parasite control should be taken in order to improve the public health status in the Dominican Republic.

ACKNOWLEDGMENTS

Sincere thanks are rendered to Lic. Dorcas Paniagua, Centro de Gastroenterologia, Hospital "Dr. Luis E. Aybar", for her kind collaboration in the field and laboratory works. This research was supported by the Japan International Cooperation Agency.

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SEASONAL DENSITY AND MALARIA VECTOR COMPETENCE OF *ANOPHELES MINIMUS* AND OTHER ANOPHELINES AT A SHALLOW VALLEY IN NORTH THAILAND

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Received May 30, 1995/Accepted June 28, 1995

Abstract: Biting density of anopheline mosquitoes was monitored in both the rainy and dry seasons at 6 outdoor stations in two villages of north Thailand by 3 human and 1 water buffalo bait. A total of 19 *Anopheles* was collected. The most predominant species were *An. vagus* and *An. annularis.* They were followed by *An. hyrcanus* group, *An. minimus, An. aconitus, An. nivipes* and *An. kochi.* The rest species were not abundant. The fauna of *Anopheles* changed seasonally. *An. minimus, An. aconitus* and *An. barbirostris* were abundant in the dry season, while *An. maculatus* sensu lato, *An. kochi, An. tesselatus, An. vagus, An. annularis* and *An. hyrcanus* group were the rainy season *Anopheles.* The 'crude feeding index' was relatively high in *An. maculatus* and *An. minimus.* They were followed by *An. tesselatus* and *An. splendidus,* while that of *An. vagus* and *An. kochi* was less than 1/10 of *An. minimus. An. minimus* was regarded as the most important vector. The biting density of *An. minimus* was higher at the peripheral area of villages with scarce dwellings and rich vegetation. At stations with dense dwellings and adjoining to an open field, the density of *An. minimus* was low, and the malaria risk was probably higher.

Key words: anopheline fauna, vector competence, shallow valley, north Thailand

INTRODUCTION

In Thailand a clear peak of malaria cases appears in dry season, especially in November and December. The prevalence of potential malaria vectors affects this seasonality (Ismail *et al.,* 1978; Harbach *et al.,* 1987; Rosenberg *et al.,* 1990; Gingrich *et al.,* 1990). Five *Anopheles* were incriminated as the malaria vector in Thailand (Ismail & Phinichpongse, 1980; Malikul, 1988). Among them, *Anopheles minimus* has been considered as the primary vector at the mountain foot, forested foothills and forest fringes because the species is abundant at these areas, and preferably breeds in slow running streams located there (Ismail *et al.,* 1978; Harbach *et al.,* 1987; Ratanatham *et al.,* 1988).

These areas also were one of the most delicate and unstable areas because man has actively touched the natural environment by deforestation, human settlement, agricultural activities, and so on, and drastic environmental changes have occurred in many places. These changes must affect the bionomics of anopheline mosquitoes as suggested by Ismail *et al.* (1978) and Rao (1984). Therefore, it is quite necessary to re-examine the bionomics of anophlines occupying these areas. We conducted an investigation of anopheline fauna both in dry and rainy seasons from 1988 to 1990 in two villages located in a shallow valley on the forest fringe, aiming to clarify the seasonal density, the local distribution of major potential vector species, and the risk of bite to human, all of which were important parameters in the

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This study was partially supported by a grant under The Monbusho International Scientific Research Program (#63044112), and by The Monbusho Overseas Research Fund.

malaria epidemiology. Results obtained from the investigation were reported in this paper. In discussion, we also evaluated the vector competence of these species from the ecological point of view, and suggested an epidemiological scope of malaria in the mountain foot area.

MATERIALS AND METHODS

Two villages studied were in a shallow valley and surrounded by forested mountains, ca. 450 m above the sea level, 98° 52′ E and 19° 11′ N, 50 km north from Chiangmai city. Several clumps of dwellings were scattered within 4 km along an unpaved road. Reported number of population was 1335 individuals during the study period. Taking into the number and body size, human, water buffaloes and cows were main blood sources for malaria vectors in the study villages although dogs, cats, poultry and several swine were also reared. There were two slow running streams, which were a main breeding place of *An. minimus*. The flat part of land was used as terraced rice fields or dwellings. The lower part of slopes behind the flat area was mainly occupied by rhychee orchards, while the upper part was covered with the secondary forest.

A weekly population census for adult and larval mosquitoes was made from December, 1988 to February, 1989 in the dry season, from June to November, 1989 in the rainy season, and May to June, 1990 in the intermediate season in these villages. Adult collection was made at 2 stations selected among 6 stations in one night by a set of human and animal bait collections. In the human bait collection, 3 collectors exposed their legs to mosquito bite, sat outdoors, and caught all mosquitoes landed to them by an aspirator with a 50 minute collection followed by a 10 minute break from 18:00 to 24:00. In the animal bait collection, one water buffalo was tethered in a net ($4 \times 4 \times 2$ m), which was similar to one described by Service (1993), and one collector caught mosquitoes landing in and out of the net using an aspirator at every 15 minutes from 18:00 to 24:00. The mutual distance between stations was 300-500 m.

The crude feeding index (Service, 1993) on human was calculated using the following 4 parameters; the registered number of human population in the study area [1335], the number of water buffaloes plus cows counted by questionnaire [165], and the average numbers of anopheline mosquitoes caught by a human bait and by a water buffalo bait per half night. The risk of bite to human also was estimated by multiplying the crude feeding index by overall adult density.

Larval collection was made in 2 slow running streams targeting *An. minimus*. Four blocks each covering 300 m of the streams were selected near the adult collection stations. Twenty samples each composed by 30 dippings were collected at each regular

Table 1 Environmental conditions of 6 stations where an anopheline mosquito collection was made

Station	General topography	Vegetation	No. of houses	No. of water buffaloes & cows	Distance from a stream
1	An end of village B Adjoining to a rice field Distribution of dwellings; moderately dense Sparse canopy	Rice field	73	52	50 m (stream B)
2	A pass between Station 1 and 3 Forest located behind plantations	Sugar cane plantation Secondary forest	0	0	500 m (stream B)
3	Distribution of dwellings; Sparse, in a forest A bottle neck of a valley	Secondary forest	20	16	1000 m (stream A)
4	Adjoining to a wide rice field Widest part of a shallow valley Distribution of dwellings; moderately dense Sparse canopy	Rice field	115	55	300 m (stream A)
5	Adjoining to a wide rice field A center of village A Distribution of dwellings; dense Sparse canopy	Rice field	121	33	250 m (stream A)
6	An end of village A Distribution of dwellings; Sparse, in orchards Adjoining to a rice field Canopy composed mainly by orchards	Rice field Rhychee orchard	8	9	200 m (stream A)

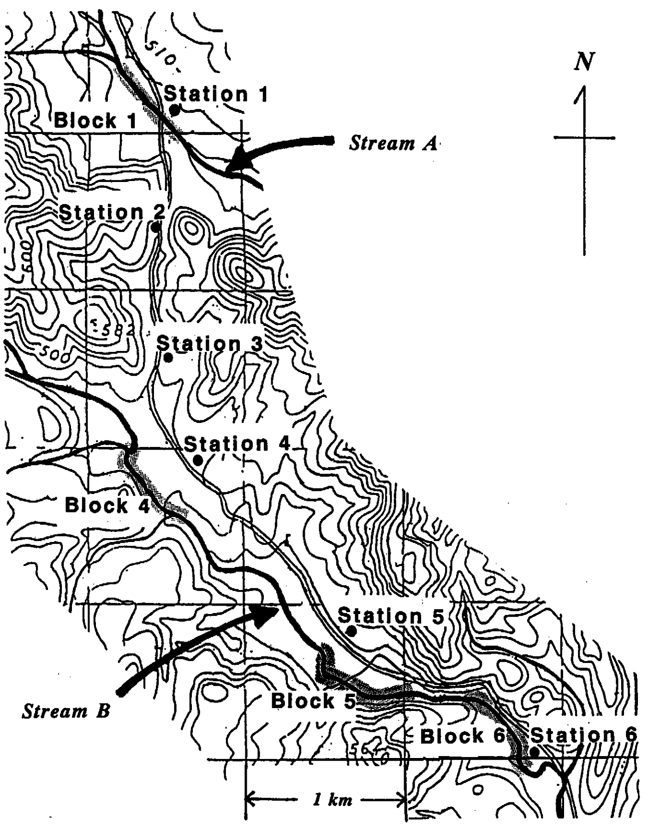

Figure 1 Map showing 6 stations for adult collection and 4 blocks for larval collection.

census. A round dipper sized 13.0 cm in diameter and 7.5 cm in depth was used.

Locations of 6 adult collection stations (1-6) and 4 larval collection blocks (1, 4-6) were illustrated in Fig. 1, and the environmental conditions of the stations were summarized in Table 1.

RESULTS

The anopheline fauna and the seasonal density of adults

During the study period, a total of 19 species of *Anopheles* was collected outdoors by human and water buffalo bait collections. They were *An. hyrcanus* group, *An. barbirostris* Van der Wulp in the subgenus *Anopheles*, and *An. minimus* Theobald, *An. aconitus* Donitz, *An. maculatus* sensu lato, *An. annularis* Van den Wulp, *An. nivipes* Theobald, *An. varuna* Iyengar, *An. kochi* Grassi, *An. tesselatud* Theobald, *An. vagus* Donitz, *An. splendidus* Koidzumi, *An. jamesii* Theobald, *An. stephensi* Liston, *An. philippinensis* Ludlow, *An. jeyporiensis* James, *An. pallidus* Theobald, *An. culicifacies* Giles and *An. dirus* Peyton & Harrison in the subgenus *Cellia.*

The most predominant species were *An. vagus* and *An. annularis.* They were followed by *An. hyrcanus* group, *An. minimus, An. aconitus, An. nivipes* and *An. kochi.* The rest species were not abundant. The average biting density (number of females per one bait per half night) of 12 dominant species, which were collected more than 200 females during the study period, were shown in Table 2.

In the dry season, *An. minimus, An. aconitus, An. annularis* and *An. hyrcanus* group were predominant, and the density of *An. minimus* caught by a human bait was outstanding. They were followed by *An. nivipes* and *An. barbirostris.* But the density of this second

Table 2 Average adult density (no./1 bait/half night) of 12 major anopheline mosquitoes collected by 3 human (H) and 1 water buffalo (B) baits in 2 mountain-foot villages in north Thailand

Season	No. of half nights	Bait	Average density												
			min	aco	niv	mac	koc	ann	var	tes	vag	hyr	bar	spl	Total
	53	B	48.9	54.8	29.4	3.3	8.5	48.1	3.6	2.3	7.4	43.6	27.7	3.5	281.1
Dry	47	H	3.1	1.8	0.4	0.2	0.1	0.7	0.1	0.1	0.0	0.5	0.3	0.1	7.4
		B+H	52.1	56.5	29.7	3.4	8.6	48.8	3.7	2.3	7.4	44.1	28.1	3.6	288.5
	53	B	8.3	2.7	26.6	15.4	40.7	133.8	0.0	12.5	138.6	73.2	5.7	6.4	464.8
Rainy	48	H	2.1	0.3	0.7	1.6	0.3	2.9	0.0	1.1	0.5	1.1	0.0	0.5	11.2
		B+H	10.3	3.1	27.3	17.0	41.0	136.7	0.0	13.6	139.1	74.4	5.7	6.9	476.0
	24	B	7.7	0.7	1.1	1.6	0.1	0.5	0.0	0.0	3.5	0.9	0.8	0.1	17.1
Intermediate	24	H	0.6	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9
		B+H	8.3	0.7	1.1	1.8	0.1	0.6	0.0	0.0	3.5	0.9	0.9	0.1	18.1
	130	B	24.7	23.6	23.0	7.9	20.1	74.3	1.5	6.0	60.2	47.8	13.8	4.1	307.3
Overall	119	H	2.2	0.8	0.4	0.8	0.2	1.4	0.0	0.5	0.2	0.7	0.1	0.3	7.6
		B+H	26.9	24.4	23.5	8.7	20.2	75.7	1.5	6.5	60.4	48.5	13.9	4.3	314.9

min, *An. minimus*; aco, *An. aconitus*; niv, *An. nivipes*; mac, *An. maculatus* sensu lato; koc, *An. kochi*; ann, *An. annularis*; var, *An. varuna*; tes, *An. tesselatus*; vag, *An. vagus*; hyr, *An. hyrcanus* group; bar, *An. barbirostris*; spl, *An. splendidus*.

abundant group was about a half of that of *An. minimus.* Other anophelines did not over 10 individuals per half night. The total anopheline density by human and water baffalo baits was 288.5 individuals.

In the rainy season, predominant species changed. *An. vagus* was strikingly abundant, though it was hardly collected by a human bait. *An. annularis* also was collected abundantly by a water buffalo bait. *An. maculatus, An. kochi, An. tesselatus* and *An. hyrcanus* group also were more abundant than in the dry season. On the other hand, the biting densities of *An. minimus* and *An. aconitus* in this season were evidently lower than those in the dry season. However, the density of *An. minimus* in human bait collection was the second highest. Total biting density by human and water baffalo baits (476.0) was much higher in the rainy season than that in the dry season.

In the intermediate season, May and June, all species were not abundant, and the anopheline mosquito fauna was poor. *An. minimus* and *An. maculatus* were the only 2 species collected by a human bait.

The difference of the adult density among stations

The frequency distributions of each species among stations were calculated using the average densities (Fig. 2). The predominant species could be classified into 4 groups depending on the frequency distribution. In the first group including *An. kochi, An. maculatus* and possibly *An. hyrcanus* group, a large proportion of samples were obtained at station No. 2 (Fig. 2a), while in the second group of *An. minimus, An. aconitus, An. varuna* and possibly *An. splendidus,* the distribution was characterized by high frequency at station No. 3 and 6, and by low frequency at No. 4 (Fig. 2b). In the third group including *An. annularis, An. nivipes* and *An. vagus,* the frequency at station No. 4 was the highest, and those at No. 2, 3 and 6 were low (Fig. 2c). *An. tesselatus* were clasified as the fourth group which was characterized by the high frequency at station No. 1 and 6, and low at station 4 and 5 (Fig. 2d).

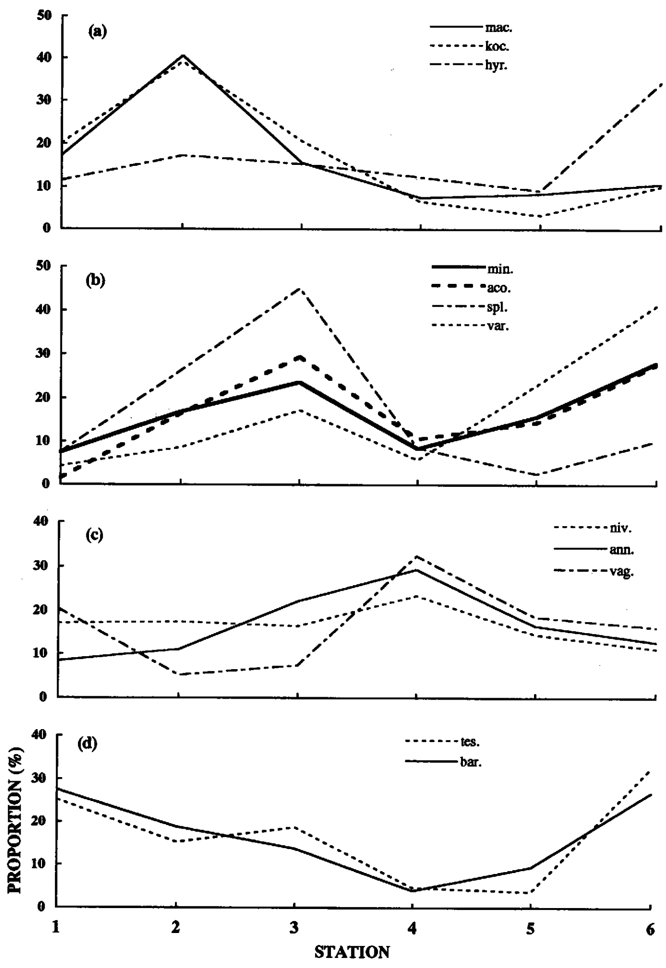

Figure 2 Proportion (%) of major anophelines (adult) at 6 stations.

Table 3 Average larval density (no./30 dips) of anophelines at 4 blocks of 2 slow running streams and average adult density (no./1 bait/half night) of *An. minimus* at the nearest stations to the blocks

Season	Parameter	Block and station						
		1	2	3	4	5	6	Average
Dry	Larval density	3.3	—	—	4.4	4.5	3.1	3.8
	% of *An. minimus* larvae	90.3	—	—	72.9	96.5	94.4	86.7
	% of young larvae	39.5	—	—	39.0	42.9	45.8	41.6
	Adult density of *An. minimus*	15.4	52.1	81.1	12.8	58.9	92.2	52.1
Rainy	Larval density	0.9	—	—	1.0	0.8	0.3	0.7
	% of *An. minimus* larvae	86.7	—	—	73.4	60.8	38.5	67.5
	% of young larvae	59.6	—	—	56.8	50.7	70.0	58.8
	Adult density of *An. minimus*	9.5	7.9	10.3	12.7	6.3	11.9	10.3

The larval population census in slow running streams

Results of the larval collection were summarized in Table 3. In the two slow running streams examined, the predominant species was always *An. minimus*. The proportion of this species among all anophelines collected in the streams was more than 90% in the dry season except at block 4. It decreased in the rainy season especially at blocks 5 and 6 but more than 2/3 of samples was still occupied by *An. minimus* on the average. Other collected species were *An. aconitus* and *An. hyrcanus* group. The former was more in the dry season, while the latter was more in the rainy season. The proportion of young larvae was higher in the rainy season. The average larval density of *An. minimus* among 4 blocks (only 3rd and 4th instar larvae were taken into account) was nearly similar to each other ranging only 3.1-4.5 per 30 dips in the dry season. It was reduced to less than 1.0 in the rainy season, and its reduction rate was larger from block 1 toward block 6. The frequency distribution of larval population of the species among blocks did not coincided to that of adult population among stations.

The host preference and the risk of bites to human

The human, baffalo and cow were considered as the main blood sources in the study villages. The host preference of females was roughly evaluated by the crude feeding index (Service, 1993) on human calculated by 4 parameters described in MATERIALS AND METHODS, and was shown in Table 4. Because the indices were rather small, all species encountered in this study were found to be zoophilic. Among 12 species, *An. minimus* and *An. maculatus* showed relatively strong preference to human. *An. tesselatus* and *An. splendidus* followed the former 2 species. *An. kochi* and *An. vagus* were strongly zoophilic because the indices of these species were less than 0.1 times of those in *An. minimus* and *An. maculatus*.

Multiplying the crude feeding index by the overall adult density shown in Table 2 enabled to estimate more realistic risk of bite to human by a certain malaria vector. The values calculated for each species were also shown in Table 4. *An. minimus* showed the largest value. This species was the most important malaria vector in the study area. The value of *An. annularis* was approximately 60% of *An. minimus*. Because of great abundance, this species should be dangerous in the rainy season. Both *An. aconitus* and *An. maculatus* showed moderate values. The values of other species were apparently smaller than these species.

DISCUSSION

Anopheles fauna in the study villages was mainly composed of 19 species, and the species diversity and density were higher in the rainy season. Five *Anopheles* were incriminated as the malaria vector in Thailand (Ismail & Phinichpongse, 1980; Malikul, 1988). Among these 5 species, 4 species except *An. sundaicus* were collected in our study. *An. dirus* is the primary vector in deep forests and mountains with serious malaria endemicity (Ismail *et al.*, 1974, 1975; Gingrich *et al.*, 1990; Rosenberg *et al.*, 1990). Although this species is highly anthropophilic, only one adult female was caught by human bait in our study area located in a shallow valley of forest fringes and surrounded by low mountains. The scarcity of *An. dirus* in forest fringes was also confirmed by Ismail *et al.* (1978). Therefore, the important malaria vectors in this area should be the rest of 3 species, *An. minimus, An. aconitus* and *An. maculatus* sensu lato.

The calculation of the crude feeding index and the risk of bite to human suggested that *An. minimus* was the most important malaria vector in the study area because of both its relatively high density and high feeding index to the human bait. The highest crude feeding index in *An. maculatus* was noteworthy. Although the crude feeding index was not large, *An.*

Table 4 Crude feeding index on human* and the risk of bite to human† in major anopheline mosquitoes

Parameter	min	aco	niv	mac	koc	ann	var	tes	vag	hyr	bar	spl
Crude f. ix.	0.0110	0.0043	0.0023	0.0119	0.0010	0.0024	0.0040	0.0094	0.0004	0.0017	0.0013	0.0078
Ratio to *min*	1.0000	0.3953	0.2105	1.0786	0.0925	0.2186	0.3623	0.8570	0.0398	0.1587	0.1211	0.7116
Risk of bite	0.2963	0.1061	0.0543	0.1028	0.0206	0.1821	0.0061	0.0611	0.0264	0.0846	0.0185	0.0339
Ratio to *min*	1.0000	0.3580	0.1833	0.3470	0.0695	0.6146	0.0205	0.2062	0.0891	0.2856	0.0626	0.1143

min, *An. minimus*; aco, *An. aconitus*; niv, *An. nivipes*; mac, *An. maculatus* sensu lato; koc, *An. kochi*; ann, *An. annularis*; var, *An. varuna*; tes, *An. tesselatus*; vag, *An. vagus*; hyr, *An. hyrcanus* group; bar, *An. barbirostris*; spl, *An. splendidus*.
*Density in human bait × water buffalo plus cow populations/density in buffalo bait × human population
†Crude feeding index × overall density

annularis was probably more important than *An. aconitus* in the study area because the species was rather abundant throughout the year. It should be noted that *An. annularis* was regarded as an important malaria vector in some localities in Sri Lanka (Ramasamy *et al.*, 1992) and India (Rao, 1984).

Four types of frequency distribution of *Anopheles* were observed in the study area. *An. minimus* was abundant at a peripheral part of villages with scarce dwellings and rich vegetation. In contrast with this, this species was not abundant at a part with dense dwellings. These facts suggested that malaria risk transmitted by *An. minimus* might be larger in scarcely populated area such as periphery of villages and between villages. It was possibly due to more attacks by the species to limited blood sources, and concentrated distribution to the abundant resting places.

The topographic and vegetational characters of the study area, such as locating in a shallow valley with slow running streams and adjoining a forest fringe, are widely observed in inland of southeast Asia. Drastic environmental changes also have been occurred in this part of Asia by deforestation, human settlement, agricultural activities, and so on (e.g. Walsh *et al.*, 1993). These changes must affects the bionomics of anopheline mosquitoes, especially *An. minimus* as suggested by Ismail *et al.* (1978). A continuous monitoring of both the environment and the vectors by a standardized method is still necessary to yield more harmonious vector control strategy.

ACKNOWLEDGMENTS

We thank all field staff of Malaria Center Region 2 for their continuous help for the field survey.

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SEASONAL AND SPATIAL DISTRIBUTION OF 3 MALARIA VECTORS AT THREE MOUNTAINOUS VILLAGES IN NORTH THAILAND

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Received June 28, 1995/Accepted August 29, 1995

Abstract: Malaria vector occurrence in mountainous villages was monitored for 2 years in north Thailand. Both larval and adult densities of *Anopheles miniumus* peaked in May or June and in November or December. Stable conditions in streams during these periods possibly favored the breeding of *An. miniumus*. Higher lalval density also was encountered in streams where ca. 3/4 of the water surface was sunlit, portions with rapid currents and slow currents alternate, and the water was clear. *An. maculatus* sensu lato peaked in June or July, while *An. dirus* scarcely changed seasonally. The densities of the three malaria vectors were higher in more mountainous villages.

Key words: malaria vectors, occurrence, mountainous villages, north Thailand

INTRODUCTION

In Thailand, the mortality due to malaria has reduced from 351 per 100,000 population in 1947 to 3.9 in 1980, and to 2.1 in 1991 by an intensive nation wide control program (Malaria Division, 1991). The vector control, one of principal measures in the program, mainly depended on the residual spraying of DDT. This measure could reduce the frequency of man-vector contact (Ismail *et.al.,* 1975). Although the residual spraying of DDT was highly effective and practical in areas with easy access, its applicability was limited in remote areas because of high per-capita costs (Schlissmann, 1983). Another problem in the DDT application is change in vector behavior (Ayurakit-Kosol and Griffith, 1963). *Anopheles minimus* avoided DDT-sprayed wall surface, and malaria transmission continued by outdoor biting (Ismail *et.al.*,1975). Under these situations, larval control should be considered, because *An. minimus* prefers to breed only at a limited part of slow running streams (Muirhead Thomson, 1940).

The vector occurrence was monitored for two years in three villages with different slow running streams to collect baseline data for more effective and economic malaria vector control in rural areas. Results obtained in the monitoring were reported in this paper with special attention to the environmental differences among larval habitats.

MATERIALS AND METHODS

This study was carried out from January 1987 to January 1989 in three villages locating in mountainous areas with two natural slow-running streams in Lumphun and Lampang provinces. These were Ban Mae Bon Tai (village A) and Ban Mae Bon Noau (village B), Banpoung canton, King Toung Hua Chang district, and Ban Na-Lam (village C), Sreamsrai canton, Sreamngram district. Distances between neighboring two villages were ca. 3 km (Fig. 1).

A monthly larval survey was conducted at 20 fixed observation points, each in a section of 100 m of a stream passing through Village A and B, and at 15 points in another stream along Village C. Larval collection was made by two collectors using a plate of 9 inches in diameter. Samples were kept separately by observation

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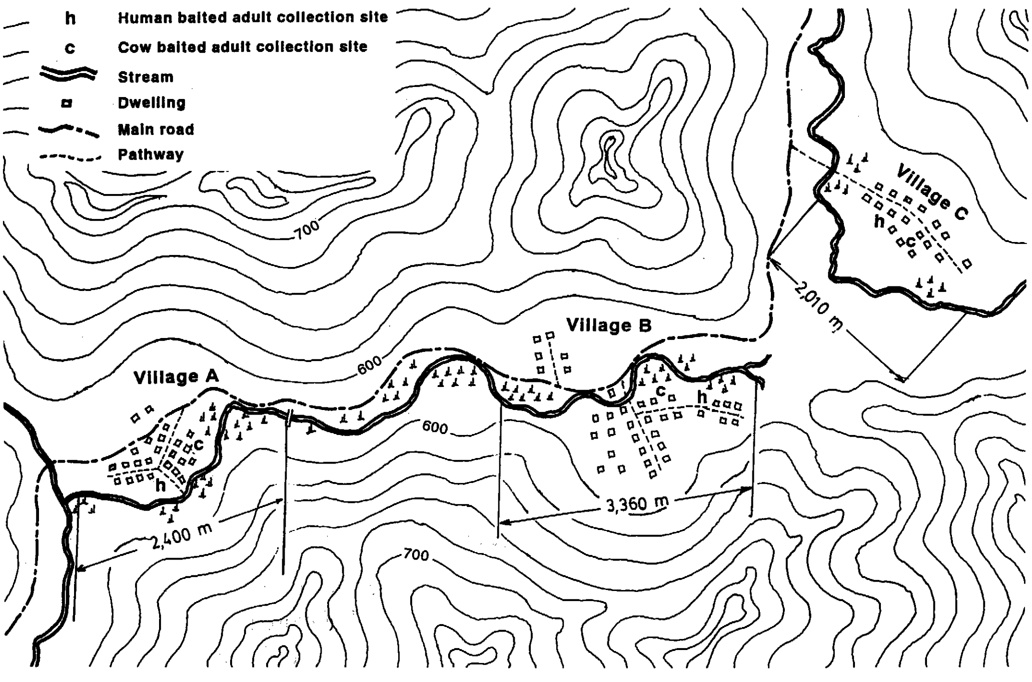

Figure 1 Sketch map of the study area.

points in small vials with 4% formalin solution, and identified to species under a microscope in the laboratory.

In every survey, presence or absence of water at the fixed points was recorded, and the percent of water coverage (100 x no. of points with water / no. of points examined) was calculated. The monthly precipitation record was collected from the nearest meteorological office.

The light condition, velocity of water current and water clearness at each fixed point were recorded by direct observation. The light condition was classified into 3 classes (+, ± and −). One m^2 of water surface was examined at each point, and if the water surface was fully sunlit, the point was classified to '+'. If half-sunlit and half-shady, the point was classified to '±', and '−' was for the fully shady point. The velocity of water current was recorded following Muirhead Thomson (1940). A table tennis ball was released at upper edge of a certain point, and the velocity was measured by observing the ball drifting downstream. When the ball moved ≥ 2.0, 2.0-0.3 and 0.3-0.0 feet / second, the point was classified to '+' (fast), '±' (slow) and '−' (stagnant), respectively. The water clearness also was classified into 3 classes. If a part of the bottom of a stream was clearly seen up to 30 cm in depth, the clearness of the point was classified to '+', and, if not seen and smelled, the point was classified to '−'. A point with the bottom not seen and without smell was classified to '±'. All observations were made between 10:00 and 14:00.

Human and cow baited adult collection was made monthly for two nights between 18:00 - 24:00 at a fixed station in each village. Two human and one cow were used as baits.

Human baits sat outdoor, and collected landing mosquitoes by an aspirator. Fifty minutes collection and 10 minutes break were repeated hourly. A cow bait was tethered inside a gauze cage shown by Service (1993), and one collector collected mosquitoes resting on walls of the cage by an aspirator every 15 minutes.

Results And Discussion

The seasonal prevalence of *An. minimus* larvae at each village is shown in Fig. 2. The average larval density fluctuated similarly in 3 villages with two peaks in a year. The first peak was observed in May or June in the early rainy season, and the second peak in November or December at the onset of the dry season. The water coverage of the streams, being affected by the seasonal pattern of precipitation, increased just before the first peak of the larval occurrence, and decreased just after the second peak of it as shown in Fig. 3. These facts suggested that the amount and pattern of precipitation decisively influenced the seasonal occurrence of *An. minimus*. Too much rain in the middle of the rainy season possibly lowered the larval density by flooding or fast currents of water, while too little rain late in the dry season also lowered it by reducing the available larval habitat. Stable water conditions in slow-running streams provided by moderate amounts of rainfall is a requisite to the occurrence of abundant *An. minimus*.

The average of *An. minimus* larvae /100 dips was 1.29 (SD 2.13), 2.22 (SD 2.81) and 3.71 (SD 8.79) in Village A, B and C, respectively (Table 1). As to the light condition, a stream along Village A was the most sunlit. It was followed by another stream along Village C. The water current was slower in a stream along Village A than that along Village B locating upstream. In another stream along Village C, points with and without fast water currents were subequal in number. The stream water was the clearest at a section of a stream along Village C, followed by a stream along Village B and A. Therefore, it was suggested that the larval density was high in such a stream as in Village C, where ca. 3/4 of the water surface was sunlit, portions with fast and slow water currents appeared alternative-

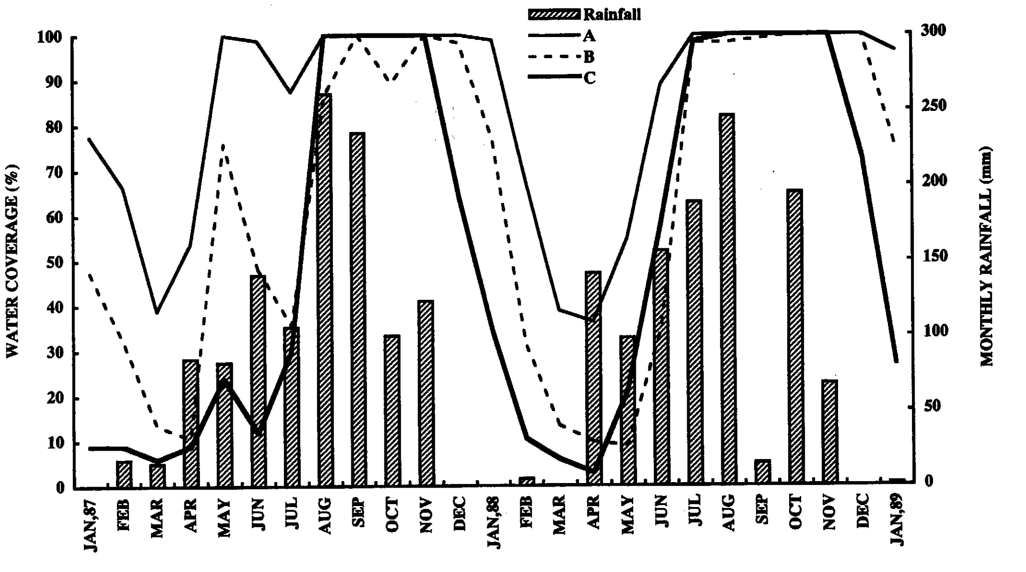

Figure 2 Seasonal density of 4th instar larvae of *An. minimus* in slow-running streams along 3 study villages.

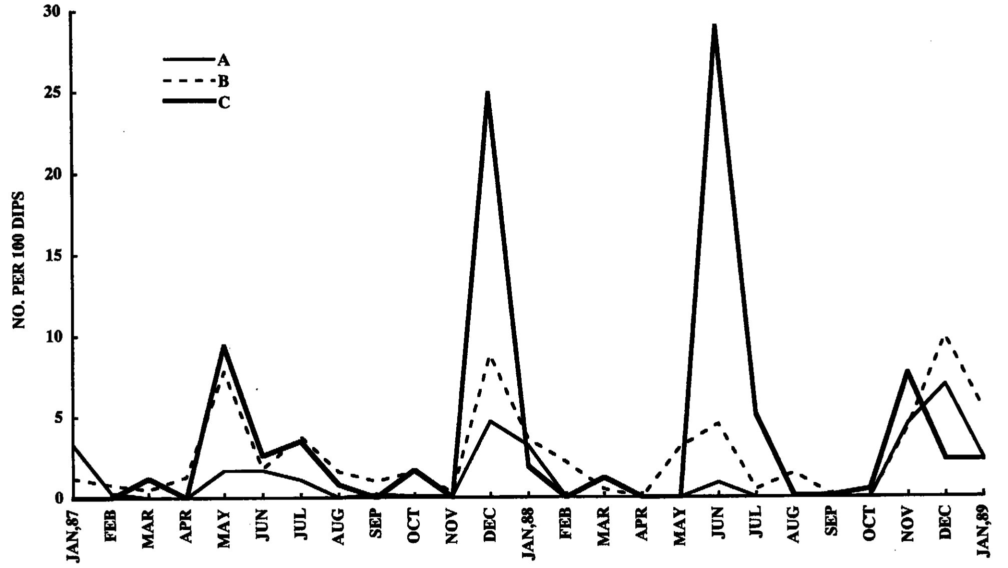

Figure 3 Monthly amount of rainfall at the nearest meteorological office (histogram), and the water positive rate at fixed sampling points (lines) in study streams.

Table 1 Average density of 4th instar *An. minimus* larvae (number per 100 dips), and average proportions (%) of 3 classes of light condition, water current and clearness at 20 larval sampling points in 3 villages during 25 months.

Village	Larval density	Class	Sunlight	Water current	Clearness
		+	95.2	3.5	11.9
A	1.29	±	4.3	29.5	15.3
		−	0.5	64.9	72.6
		+	1.5	8.9	59.2
B	2.22	±	97.3	63.9	23.8
		−	1.2	24.5	17.0
		+	48.9	45.9	99.5
C	3.71	±	50.6	5.1	0.5
		−	0.4	46.5	0.0

Table 2 Average number of females per half night and the proportion (%) of 3 major malaria vectors (parenthesize) collected by 2 human plus 1 cow baits during 25 months.

Village	*An. minimus*	*An. dirus*	*An. maculatus*	Total *Anopheles*
A	5.7(14.5)	1.0(2.6)	6.1(15.5)	39.5(100.0)
B	24.2(13.7)	2.3(1.3)	48.0(27.2)	176.3(100.0)
C	20.2(4.6)	2.0(0.5)	64.2(14.5)	443.0(100.0)

ly, and the water was fairly clear.

Three potential malaria vectors, *An. minimus*, *An. dirus* and *An. maculatus* sensu lato, were collected in 3 villages by humans and cow-baited adult collections. The average number of females per half night and the proportion of these species during the study period is summarized in Table 2. All of these species were more abundant at Village B and C, both of which were more mountainous than Village A. However, the proportion of these vector species to the total *Anopheles* was different among villages. The proportion of *An. minimus* and *An. dirus* rather decreased from Village A to C in this order,

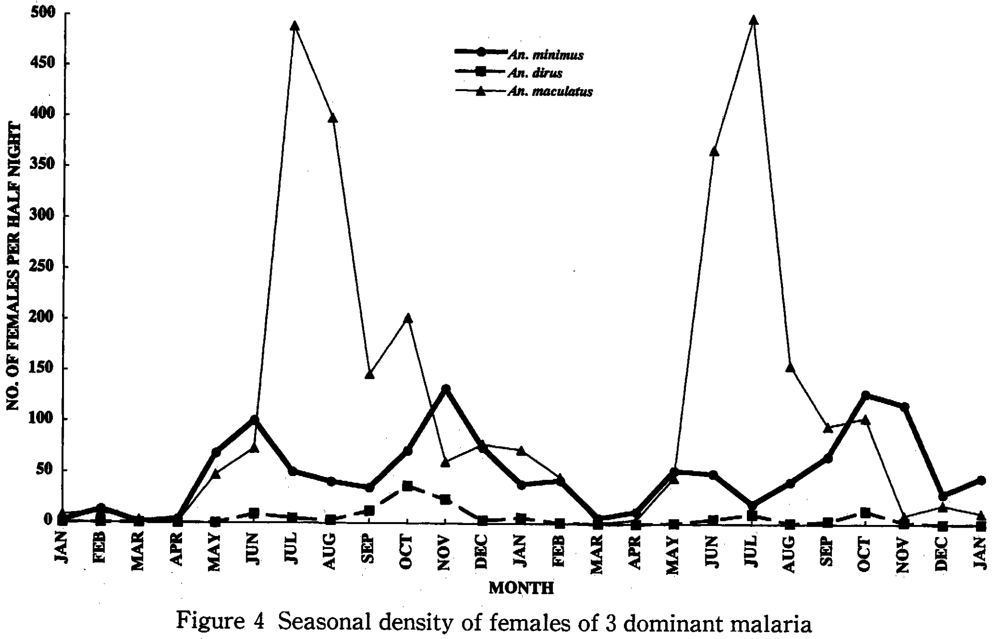

Figure 4 Seasonal density of females of 3 dominant malaria vectors collected by human and cow baited half-night collection.

while that of *An. maculatus* sensu lato was highest at Village B.

The seasonal change in adult density of 3 malaria vectors is illustrated in Fig. 4. *An. minimus* showed a clear bi-modal pattern with peaks in May-June and October - November. This seasonal pattern corresponded to that of the larval population of the species described in the above. In contrast with *An. minimus*, *An. maculatus* sensu lato was abundant during the rainy season. The seasonal pattern of occurrence in our study areas agreed with the observations made in Wang Thon district, Pitsanuloke province and in Muaklek district, Saraburi province (Ismail *et al*., 1974, 1975, 1978) for *An. minimus* and in Pakchong district, Nakhon Ratchasima province and in Mae Tang district, Chiangmai province (Upatham *et al*, 1988; Takagi *et al*., 1995) for *An. maculatus*. Different seasonal prevalence from ours also was reported in *An. minimus* (Ratanatham *et al*., 1988; Leemingsawat, 1989; Gingrich *et al*., 1990; Takagi *et al*., 1995) in different provinces of Thailand, even if the seasonal precipitation pattern was nearly similar each other.

Our study lightened favorite conditions in the larval habitat on breeding of *An. minimus*. However, above-mentioned various seasonal prevalence of the species still requests a more analytical study focusing to the relationship among the rainfall pattern, the larval habitat quality and the malaria vectors occurrence. The study will provide to predict the seasonal abundance and to evaluate the feasibility of larval control of the species.

Owing to its low density, the seasonal prevalence of *An. dirus* in the study area was indefinite.

ACKNOWLEDGEMENT

We are indebted to Dr. Udom Chitprarop, the former director of Malaria Center 2, for his encouragement during the study. We thank all collectors for their excellent work throughout the study period and Prof. Yoshito Wada for critical reading the manuscript. We also thank Dr. Yoshio Tsuda for valuable comments.

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BLACK FLIES (DIPTERA: SIMULIIDAE) FROM HONG KONG: TAXONOMIC NOTES WITH DESCRIPTIONS OF TWO NEW SPECIES

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Received July 28, 1995/Accepted August 24, 1995

Abstract: Light-trapped adult specimens of Hong Kong Simuliidae, together with a few pupae and larvae collected from streams were examined. Eight species were recognized, consisting of two new species and six newly recorded ones. All the eight species belong to the genus *Simulium* Latreille s. l. and are further placed in three subgenera: *Nevermannia* Enderlein (1 sp.), *Gomphostilbia* Enderlein (2 spp.) and *Simulium* s. str. (5 spp.). Descriptions of the two new species are given.

No black-fly species has been reported so far from Hong Kong. In this paper we record six known species and describe two new species, chiefly based on adult specimens collected using light traps by one of us (D. Dudgeon). Collecting methods and a description of the collecting site (Tai Po Kau Forest Stream) were given by Dudgeon (1988). Specimens obtained by other investigators and held in Bishop Museum, Hawaii, U.S.A., have been examined also.

The morphological features and terms used follow those of Crosskey (1969). The systematic placement is done at subgeneric and species-group levels according to Takaoka and Davies (1995a).

Type specimens of two new species will be deposited at Bishop Museum, Hawaii, U.S.A.

Family Simuliidae Newman
Subfamily Simuliinae Newman
Tribe Simuliini Newmann
Genus *Simulium* Latreille s. l.

Subgenus *Gomphostilbia* Enderlein

1. ***Simulium* (*Gomphostilbia*) *dudgeoni* Takaoka & Davies, sp. nov.** Figs. 1-15

DESCRIPTION. **Male.** Body length ca. 3.0 mm. ***Head.*** Slightly wider than thorax. Upper eye consisting of 13 or 14 vertical columns and 15 or 16 horizontal rows of large facets. Clypeus brownish black, whitish pruinose, covered densely with whitish yellow pubescence interspersed with several dark hairs. Antenna composed of 2+9 segments, brown with scape, pedicel and base of 1st flagellar segment yellow; 1st flagellomere elongate, ca. 2.0 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0 : 1.1 : 2.8 ; sensory vesicle (Fig. 1) small, 0.15 × as long as 3rd segment, with an opening near distal end. Thorax. Scutum brown, with trace of 3 fine longitudinal vittae, densely covered with bright golden yellow recumbent pubescence; scutum entirely whitish pruinose on anterodorsal surface when illuminated dorsally and viewed from front; also largely white pruinose on prescutellar area in certain angle of light. Scutellum brown, with bright golden yellow pubescence and several dark long marginal hairs. Postscutellum brown, whitish pruinose, bare. Pleural membrane bare. Katepisternum longer than deep, with dark hairs and pale ones. ***Legs.*** Foreleg: coxa yellow; trochanter dark yellow to brown; tibia brown with outer median surface largely somewhat pale; tarsus brown to dark brown; basitarsus slender, ca. 8.0 × as long as its greatest width. Midleg: coxa dark brown; trochanter and femur brown; tibia brown with base yellow; tarsus dark brown with basal 1/3 to 1/2 somewhat pale. Hind leg (Fig. 5): coxa brown; trochanter yellow to dark yellow; femur brown

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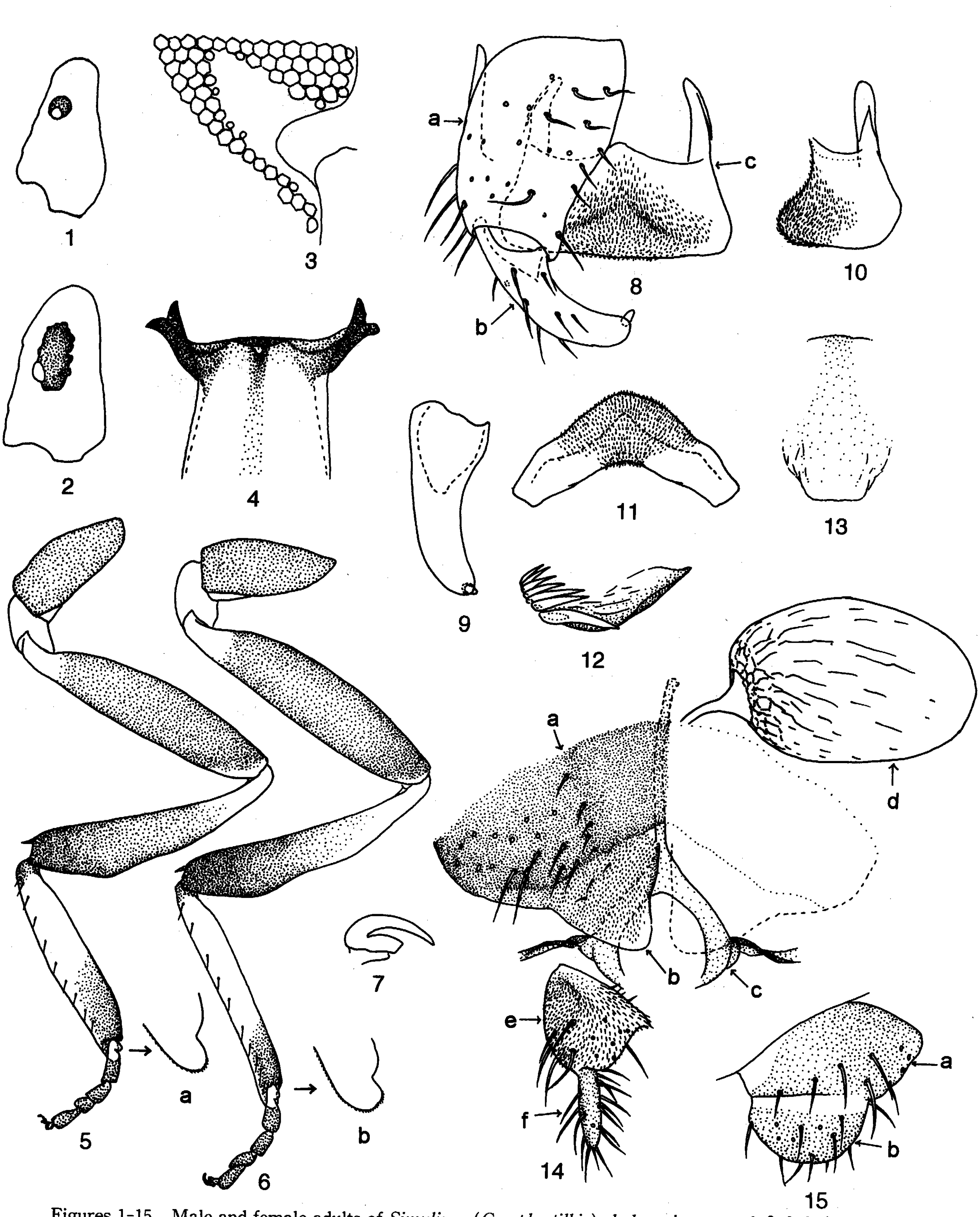
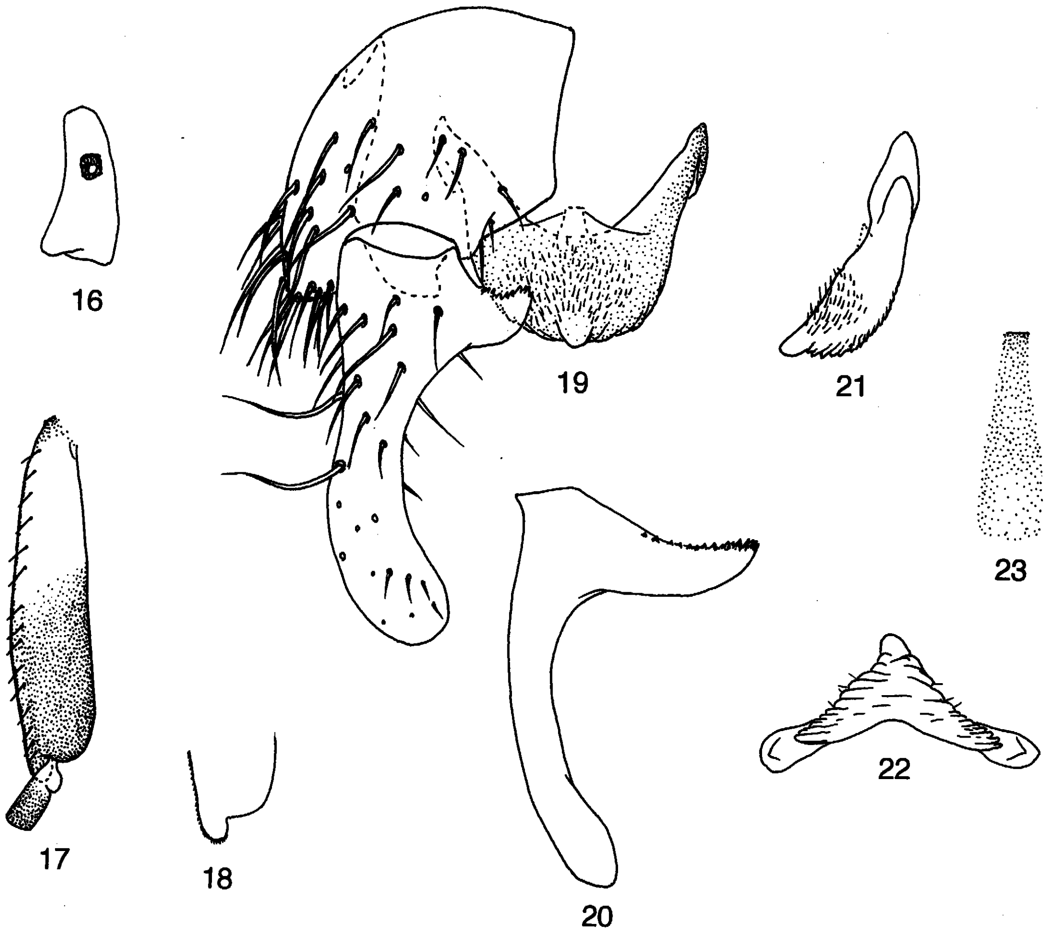


Figures 1-15 Male and female adults of *Simulium* (*Gomphostilbia*) *dudgeoni* sp. nov. 1 & 2, 3rd maxillary palpal segment (1, ♂; 2, ♀); 3, ♀ fronto-ocular area; 4, ♀ cibarium; 5 & 6, hind legs with calcipala (a, b) (5,♂; 6, ♀); 7, ♀ claw; 8, ♂ gentalia in situ (ventral view; a, coxite; b, style; c, ventral plate); 9, style (ventrolateral view); 10 & 11, ventral plate (10, lateral view; 11, end view); 12, paramere; 13, median sclerite; 14, ♀ genitalia in situ (ventral view; a, 8th sternite; b, anterior gonapophysis; c, gental fork; d, spermatheca; e, paraproct; f, cercus); 15, paraproct (a) and cercus (b) (lateral view).

with base yellow; tibia brown with basal 1/5 to 1/4 yellow, gradually darkened toward distal tip; tarsus brown with basal 1/2 and a little more of basitarsus and basal 2/5 of 2nd segment whitish yellow; basitarsus moderately inflated, spindle-shaped, tapering at both ends, ca. 4.8 × as long as wide, ca. 0.75 × as wide as hind tibia which is subequal in greatest width to femur. Calcipala (Fig. 5a) well developed, slightly shorter than wide. Pedisulcus well developed. ***Wing***. Length 2.0 mm. Costa with spinules and hairs. Subcosta bare. Basal portion of radius fully haired. Hair tuft on stem vein brown. ***Abdomen***. Basal scale brown, with fringe of dull brown hairs. Dorsal surface of abdomen brownish black, covered with dark hairs; segments 2, 5-7 each with a dorsolateral pair of shiny, whitish pruinose patches; those on segment 2 widely connected in middle. ***Genitalia*** (Figs. 8-13). Coxite nearly rectangular in ventral view. Style ca. 0.8 × length of coxite, somewhat wider at base in posterolateral view, gradually tapered toward apical tip, gently curved inward, with apical spine. Ventral plate transverse, ca. 0.5 × as long as wide, distinctly widened posteriorly, with nearly straight posterior margin in ventral view; ventral plate widely produced ventrally along posterior margin as seen in lateral view; ventral plate moderately covered with microsetae on ventral and posterior surface; basal arms somewhat converging; Paramere with 1 distinct parameral hook and several medium ones. Median sclerite thin, plate-like, weakly sclerotized.

Female. Body length ca. 2.5 mm. ***Head***. slightly narrower than thorax. Frons dark brown, whitish grey pruinose, densely covered with whitish yellow recumbent pubescence interspersed with a few dark hairs; frontal ratio 1.7 : 1.0 : 3.1. Frons-head ratio 1.0 : 5.7. Fronto-ocular area (Fig. 3) well developed. Clypeus dark brown, whitish grey pruinose, densely covered with whitish yellow pubescence interspersed with several dark hairs. Antenna composed of 2+9 segments, brownish black except scape, pedicel and base of 1st flagellar segment yellowish; lst flagellomere somewhat elongate, ca. 1.6 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0 : 1.0 : 2.7 ; 3rd segment not so enlarged, sensory vesicle (Fig. 2) oblong, ca. 0.4 × as long as 3rd segment, with a medium opening near distal end. Maxillary lacinia with 13 or 14 inner and 14 outer teeth. Mandible with ca. 28 small inner teeth and 8 outer ones. Cibarium (Fig. 4) medially forming low, rectangular, moderately sclerotized plate folded forward from posterior margin; cibarium medially with heavily sclerotized longitudinal dark band with dorsal end forked. ***Thorax***. Scutum brown in ground color, shiny, whitish grey pruinose, with trace of 3 faint, fine longitudinal vittae. Scutellum brown with whitish yellow pubescence and long erect dark hairs along posterior margin. Postscutellum brown, whitish grey pruinose, bare. Pleural membrane bare. Katepisternum dark brown, with numerous hairs, longer than deep. ***Legs***. Foreleg: coxa white; trochanter yellow; femur brown; tibia yellowish brown on basal 3/4, brown on distal 1/4, with outer surface largely pale on basal 3/4; tarsus dark brown; basitarsus slender, ca. 7.7 × as long as its greatest width. Midleg: coxa brown; trochanter yellow; femur brown; tibia yellowish white on basal 1/3, brown on rest, posterior surface of basal 2/3 largely white shining when illuminated; tarsus brown with basal 1/2 or a little less of basitarsus yellowish white. Hind leg (Fig. 6): coxa brown; trochanter yellow; femur brown; tibia yellowish white on basal 1/3 or 1/2, brown on apical 1/2, though its border not well defined, posterior surface of basal 2/3 largely white shining when illuminated; tarsus brown with basal 2/3 of basitarsus and basal 1/2 of 2nd segment white; basitarsus slender, parallel-sided, ca. 6.8 × as long as wide. Calcipala (Fig. 6b) well developed, slightly shorter than wide. Pedisulcus well developed. Hind femur and tibia ca. 1.9 × and 1.6 × as wide as hind basitarsus, respectively. Claws (Fig. 7) each with a large basal tooth 0.51 × as long as claw. ***Wing***. Length 2.0 mm. Costa with spinules as well as hairs. Subcosta fully haired. Hair tuft of stem vein fell, then coloration unknown. Basal portion of radius fully haired. ***Abdomen***. Basal scale dark brown with fringe of pale yellow hairs. Dorsal surface of abdomen brownish black; tergite of 2nd segment whitish pruinose, tergites of segments 6-8 shiny. ***Genitalia*** (Figs. 14 & 15). Sternite 8 bare medially, with ca. 18 dark macrosetae on each side. Anterior gonapophysis thin, membraneous, nearly triangular in shape, rounded posterointernally, covered densely with microsetae and a few short setae near anterior border; inner margin well sclerotized. Genital fork of usual inverted-Y form, with arms folded medially, bearing inner margins slightly converging posteriorly. Paraproct moderately produced ventrally, 0.5 × as long as wide. Cercus ca. 0.5 × as long as wide, with posterior border rounded when viewed laterally. Spermatheca ellipsoidal, well sclerotized except a small area near tubal juncture; internal setae absent.

TYPE SPECIMENS. Holotype ♂, slide mounted, HONG KONG: New Territories, Tai Po Kau Forest Stream, light trap, by D. Dudgeon, 2. VI. 1983. Allotype ♀, slide mounted, same data as holotype. Paratypes: 2



Figures 16–23 Male adult of *Simulium* (*Simulium*) *taipokauense* sp. nov. 16, 3rd maxillary palpal segment; 17, hind basitarsus & 2nd tarsomere; 18, calcipala; 19, genitalia in situ (ventral view); 20, style (medial view); 21 & 22, ventral plate (21, lateral view; 22, end view); 23, median sclerite.

♂, same data as holotype; 1 ♂, same data as holotype except 14–15.II.1984; 1 ♂, same data as holotype except 12–13.IV.1984.

DISTRIBUTION. Hong Kong.

REMARKS. The species name *dudgeoni* is given after Dr. D. Dudgeon, The University of Hong Kong, who collected this species.

This species is assigned to the *ceylonicum*-group of the subgenus *Gomphostilbia* by the inflated male hind basitarsus (Fig. 5). The spindle-shaped male hind basitarsus separates this species from most known species of this species-group, of which basitarsus is wedge-shaped. S. (*G.*) *ceylonicum* from Sri Lanka has an inflated, spindle-shaped male hind basitarsus but it is much wider (Length : Width = 3.4 : 1.0) (Davies and Györkös, 1987). The female of S. (*G.*) *ceylonicum* differs from the present species by the coloration of the hind tibia which is pale on the basal 3/5 and by the wider fore basitarsus (L : W = 5.7 : 1.0) (Davies and Györkös, 1987). This new species is also similar to *S.* (*G.*) sp. collected from Taiwan by having the spindle-shaped male hind basitarsus, though somewhat wider (L : W = 4.2 : 1.0) (Takaoka, unpublished data). The male of *S.* (*G.*) sp. differs by the coloration of the hind tibia with the basal 1/2 whitish.

2. *Simulium* (*Gomphostilbia*) *asakoae* Takaoka & Davies, 1995

Simulium (*Gomphostilbia*) *asakoae* Takaoka & Davies, 1995b : 53–58.

The male and female adult specimens collected from Hong Kong show almost the same characters of *S.* (*G.*) *asakoae* recently described from West Malaysia (Takaoka and Davies, 1995b). This species belongs to the *ceylonicum*-group by having the inflated, wedge-shaped, male hind basitarsus, which is remarkably enlarged in this species: that is, 3.3 × as long as its greatest width, subequal to or slightly wider than the hind tibia and 1.4 × width of the hind femur. The hind basitarsus and tibia of the male are white on the basal 1/2 or a little less, and brownish on the rest. The hair tuft on the base of the radial vein of both sexes is yellow. The male is also distinctive by the small number of enlarged facets (i.e., 10 vertical columns and 12 or 13 horizontal rows on each side).

SPECIMENS EXAMINED. 1 ♂, HONG KONG: New Territories, Sai Kung, light trap, by W. J. Voss & Hui Wai Ming, 15. X. 1964; 1 ♀, 1 ♂, 12. II. 1965; 1 ♀, 24. II. 1965; 1 ♀, 8.III.1965; 1 ♂, 17.III.1965; 1 ♂, 2. IV. 1965; 1 ♂, 17. XI. 1964; 2 ♀, 25. XI. 1964; 3 ♂, 9. XII. 1964; 1 ♀, 10. XII. 1964; 1 ♂, 18. XII. 1964; 1 ♀, 4.I.1965; 1 ♀, 11. I. 1965; 1 ♂, 15-16. I. 1965; 1 ♂, 22. I. 1965; 1 ♂, 23. I. 1965; 2 ♂, 25. I. 1965; 1 ♂, 26. I. 1965; 1 ♂, 29. I. 1965; 1 ♀, 10 ♂, 30. I. 1965; 1 ♀, 10. V. 1965 (all pinned, Bishop Museum). 1 ♂, Tai Po Kau, Malaise trap, by Lee Kit Ming & Hui Wai Ming, 1. VI. 1964; 1 ♀, 1 ♂, by W. J. Voss & Hui Wai Ming, 6. VI. 1964; 1 ♀, 11. VI. 1964; 7 ♀, 15. VI. 1964; 1 ♀, 9 ♂, 16. VI. 1964; 1 ♀, by W. J. Voss, 20. IV. 1964; 1 ♂, 22. VI. 1964; 1 ♀, by W. J. Voss & Hui Wai Ming, 22-24. VI. 1964; 3 ♀, 1 ♂, by W. J .Voss, 26. VI. 1964; 1 ♀, by W. J. Voss & Hui Wai Ming, 27. VI. 1964; 1 ♀, 2 ♂, 30. VI. 1964; 1 ♀, 6 ♂, by Lee Kit Ming & Hui Wai Ming, 2-6.VII. 1964 ; 6 ♀, 19 ♂, 3-4. VII. 1964; 1 ♂, 6-8.VII. 1964; 2 ♀, 17. VII. 1964; 1 ♂, W. J. Voss & Hui Wai Ming, 4. IV. 1965; 1 ♀, 17. V. 1965; 1 ♀, 3. VI. 1965; 1 ♀, 14. VI. 1965; 1 ♀, 15. VI. 1965; 4 ♀, 17. VI. 1965; 4 ♀, 28. VI. 1965; 1 ♀, 2. VII. 1965; 1 ♀, 5. VII. 1965; 1 ♀, 12. VII. 1965; 1 ♂, 15-17. VII. 1965 ; 1 ♀, 26. VII. 1965; 1 ♀, 1 ♂, 27.VII.1965; 1 ♀, 29. VII. 1965; 1 ♀, 13. VIII. 1965; 1 ♀, hand net, 19. VIII. 1965; 1 ♀, 4 ♂, 20. VIII. 1965 ; 1 ♀, hand net, 24. VIII. 1965; 3 ♀, 2 ♂, 2. IX. 1965; 1 ♀, 3.IX. 1965; 2 ♀, 1 ♂, 6. IX. 1965; 1 ♂, 9. IX. 1965 ; 1 ♀, 1 ♂, 15. IX. 1965 (all pinned, Bishop Museum). 1 ♀, pinned, New Territories, Castle Peak, by W. J. Voss, 29.VII.1964 (Bishop Museum). 1 ♂, Tai Po Kau Forest Stream, 10. V. 1975, by D. Dudgeon; 5 ♂, light trap, 2. VI. 1983 ; 1 ♀, 1 ♂ , 17-18.I.1984; 26 ♂, 14-15. II. 1984; 1 ♂, 7-8. III. 1984; 2 ♂, 28-29. III. 1984; 6 ♂, 12-13. IV. 1984 (all in alcohol). 1 ♀, pinned, Lantau Island, Trappist Monastary near Silver Mine Bay, by W. J. Voss, 22. VII. 1964 (Bishop Museum).

DISTRIBUTION. Hong Kong (new record), West Malaysia.

subgenus *Nevermannia* Enderlein

3. *Simulium* (*Nevermannia*) *aureohirturm* Brunetti, 1911

Simulium aureohirtum Brunetti, 1911: 283-88 (male); Edwards, 1934: 134-37 (female, pupa and larva).

Simulium (*Nevermannia*) *aureohirtum*: Ogata, 1956: 61-62; Ogata, 1966: 129; Crosskey, 1988: 459; Takaoka and Roberts, 1988: 194-95; Crosskey and Lowry, 1990: 204; Takaoka and Davies, 1995b: 85-86.

Simulium (*Eusimulium*) *aureohirtum*: Puri, 1933: 1-7; Ogata and Sasa, 1954: 325; Ogata, Sasa and Suzuki, 1956: 73; Crosskey, 1973: 423; Takaoka, 1976: 170-71; Takaoka, 1979: 382-84; Datta, 1983: 225; Takaoka and Suzuki, 1984: 11-12; An, 1989: 182.

Eusimulium aureohirtum: Orii, Uemoto and Onishi, 1969: 1-13. *Simulium* (*Eusimulium*) *tuaranense* Smart and Clifford, 1969:40-43. Syn. by Crosskey, 1973.

Simulium (*Eusimulium*) *philippinense* Delfinado, 1962: 47-62. Syn. by Takaoka, 1983.

The morphological characters of female and male adult specimens from Hong Kong agree with the redescription of this species given by Takaoka (1979) and Takaoka and Roberts (1988) except that the scutellum of the present male specimens bears golden yellow long hairs instead of brown hairs. This species is a member of the *ruficorne*-group defined by Crosskey (1969), and is widely distributed in the Oriental Region and also parts of the Palaearctic Region. This is the first record of this species from Hong Kong.

SPECIMENS EXAMINED. 2 ♂, HONG KONG: New Territories, Sai Kung, light trap, by W. J. Voss & Hui Wai Ming, 8. X. 1964; 2 ♀, 2 ♂, 9. X. 1964; 1 ♀, 3. XI. 1964; 1 ♀, 17. XI. 1964; 1 ♀, 1 XII. 1964; 1 ♀, 2. XII. 1964; 2 ♀, 2. XII. 1964; 1 ♂, 9. XII. 1964; 2 ♀, 1 ♂, 18. XII. 1964; 1 ♂, 20-21. I. 1965; 1 ♀, 25. I. 1965; 1 ♂, 26. I. 1965; 1 ♂, 29. I. 1965; 1 ♀, 6 ♂, 30. I. 1965; 1 ♀, 12. II. 1965; 1 ♂, 15. II. 1965 (all pinned, Bishop Museum). 2 ♂, Tai Po Kau, Malaise trap, by W.J.Voss & Hui Wai Ming, 16.VI.1964; 1 ♂, 27. VI. 1964; 1 ♀, 30-31.VI.1964; 1 ♂, 20. VIII.1965; 1 ♂, 23. VIII. 1965; 1 ♀, by Lee Kit Ming & Hui Wai Ming, 1. IX. 1965 (all pinned, Bishop Museum). 2 ♀, Lantau Island, Trappist Monastary near Silver Mine Bay, by W. J. Voss, 22. VII. 1964 (all pinned, Bishop Museum). 2 ♀, Tai Po Kau Forest Stream, light trap, by D. Dudgeon, 2. VI. 1983; 1 immature larva, 18. XI. 1976 (all in alcohol); 1 immaure larva (in alcohol), Sam A Chung Stream, by D. Dudgeon, 18. XII. 1979.

DISTRIBUTION. China, Hong Kong (new record), India, Japan, Java, Philippines, Sabah, Sri Lanka, Sulawesi, Sumatra, Taiwan, Thailand, West Malaysia.

Subgenus *Simulium* Latreille s. str.

4. *Simulium* (*Simulium*) *taipokauense* Takaoka, Davies & Dudgeon sp. nov. Figs. 16-23

DESCRIPTION. **Male.** Body length 2.8-3.5 mm. ***Head.*** As wide as or slightly wider than thorax. Upper eye consisting of 19 horizontal rows and 19-21 vertical columns of large facets. Clypeus dark brown, whitish grey pruinose, iridescent when illuminated, covered with long dark hairs along both lateral margins. Antenna composed of 2+9 segments, dark brown to brownish black with scape, pedicel and base of 1st flagellar segment pale; 1st flagellomere elongate, ca. 1.8 × as long as 2nd flagellomere. Maxillary palp composed of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0 : 1.1 : 2.8; 3rd segment of normal size, with small, round sensory vesicle (Fig. 16), 0.18 × length of 3rd segment. ***Thorax.*** Scutum dark brown, with white pruinose and iridescent pattern differing with angles of light: when illuminated posterodorsally and viewed dorsally scutum shows an anterior pair of narrow rectangular white iridescent bands separated medially by dark brown area (width a little narrower than 1/3 of that of scutum) , and a wide white iridescent posterior spot occupying ca. 2/5 of scutum; when illuminated dorsally and viewed anterodorsally anterior bands fade and are replaced by subanterior pair of white iridescent bands of similar size; when illuminated in certain angle of light these anterior and subanterior bands on each side are fused forming a large iridescent spot on each shoulder, which extends from anterior corner to wing base along lateral margin in a wide band which is widely connected to a posterior spot; scutum uniformly covered with pale pubescence, interspersed with long dark erect hairs on prescutellar area. Scutellum dark brown, whitish pruinose, with several dark erect hairs and pale pubescence. Postscutellum dark brown, whitish pruinose, iridescent under certain lights, bare. Pleural membrane and katepisternum bare. ***Legs.*** Foreleg: coxa white; trochanter and femur dark yellow; tibia brown with an outer surface largely pale and white iridescent in light; tarsus brown; basitarsus moderately dilated, 5.6-6.3 × as long as wide. Midleg: coxa dark brown; trochanter, femur and tibia yellow, tibia with a large white sheen on posterior surface; tarsus brown except basal 4/5 of basitarsus and base of 2nd tarsomere yellowish white. Hind leg: coxa brown; trochanter white; femur dark yellow or yellowish brown with base white and distal cap brown; tibia dark brown with base white; tarsus dark brown with a little less than basal 1/2 of basitarsus and of 2nd tarsomere white; basitarsus (Fig. 17) wedge-shaped (in some specimens spindle-shaped), expanded, 3.7-4.2 × as long as its greatest width, and its width ratio to that of hind tibia and femur 1.00:1.04-1.12:0.96-1.08 (n=4) ; calcipala (Fig. 18) small, slightly shorter than wide, not reaching pedisulcus; pedisulcus distinct at basal 1/3 of 2nd tarsomere. ***Wing.*** Length 2.3-2.4 mm; costa with spinules and hairs; subcosta bare; basal portion of vein R entirely bare; hair tuft at base of R dark brown. ***Abdomen.*** Basal scale dark brown with long dark hairs. Dorsal surface of abdomen dark brown, with dark hairs; segments 2, 5-7 each with a pair of silvery iridescent areas dorsolaterally, those on segment 2 connected broadly in middle. ***Genitalia*** (Figs. 19-23). Coxite in ventral view nearly quadrate; style elongate, 1. 45 × as long as coxite, spatulate ventrodorsally, without terminal spine; style with prominent, black basal protuberance pointed dorsally, its anterior margin furnished with several small spines. Ventral plate in ventral view broad, saddle-shaped, with rounded posterior margin, setose on ventral surface, with thick basal arms diverged; ventral plate with a small, nipple-shaped, bare median process; ventral plate weakly dentate along posterolateral margins. Parameres wide, each with several distinct hooks. Median sclerite club-shaped, slightly widened toward apical tip.

Femals, Pupa and **Larva.** Unknown.

TYPE SPECIMENS. Holotype ♂, slide mounted, HONG KONG: Tai Po Kau Forest Stream, light trap, 14-15.II.1984, by D. Dudgeon. Paratypes: 14 ♂, same data as holotype; 1 ♂, same data as holotype except 17-18. I. 1984; 1 ♂, same data as holotype except 12-13. Ⅳ. 1984.

DISTRIBUTION. Hong Kong.

REMARKS. The style of male genitalia of this new species bears an elongate basal protuberance (Fig. 20) , a character commonly seen in all species of the *multistriatum*-group and in some species of the *griseifrons*-group of the subgenus *Simulium* s. str., defined by Takaoka and Davies (1995a). The pale midleg of this species resembles that of *S.* (*S.*) *ufengense* reported from Taiwan (Takaoka, 1979) and *S.* (*S.*) *fuzhouense* from Fujian, China (Zhang and Wang, 1991), both, of the *griseifrons*-group. However, the basal protuberance of the style is much longer than those of these two known species, and the ventral plate is saddle-shaped, with a nipple-shaped median process (Fig. 19) in this new species but is flat and quadrate in shape, without any median process in the latter two species. This speices differs from all the other species of the *griseifrons*-group and all the species of the *multistriatum*-group by the shape of the ventral plate. This species is tentatively assigned in the *griseifrons*-group.

5. ***Simulium (Simulium) sakishimaense* Takaoka, 1977**

Simulium (Simulium) sakishimaense Takaoka, 1977: 197-201; Takaoka, 1979: 398; Takaoka and Suzuki, 1984: 39-40; An, 1989: 188.

The female, male and pupal specimens collected from Hong Kong almost agree with the original description given by Takaoka (1977). The upper eye of males from Hong Kong shows somewhat more facets: i.e., 19 horizontal rows and 18 vertical columns (15 horizontal rows in the type specimen). This species belongs to the *multistriatum*-group redefined by Takaoka and Davies (1995a). This is the first record of this species from Hong Kong.

SPECIMENS EXAMINED. 2 ♂, HONG KONG: New Territories Sai Kung, light trap, by W. J. Voss & Hui Wai Ming, 26. I. 1965; 1 ♂, 27. I. 1965; 1 ♀, 30. I. 1965; 1 ♂, 8.II.1965; 1 ♂, 24.II.1965 (all pinned, Bishop Museum). 1 ♂, Tai Po Kau, hand net, by Lee Kit Ming & Hui Wai Ming, 26. VI. 1965; 1 ♂, Malaise trap, 27. VII. 1965; 1 ♀, Malaise trap, 29. VII. 1965; 1 ♀, light trap, 14. VIII. 1965; 2 ♀, light trap, 2. IX. 1965; 1 ♂, hand net, 6. IX. 1965 (all pinned, Bishop Museum). 3 ♀, 13 ♂ Tai Po Kau Forest Stream, light trap, by D. Dudgeon, 2. VI. 1983; 1 ♂, 20. VII. 1983; 1 ♀, 1 ♂, 17-18. XI. 1983; 2 ♂, 17-18. I. 1984; 2 ♀, 15 ♂, 14-15. II. 1984; 1 ♀ reared from pupa, 2 pupae, 2 pupal exuviae, 10. V. 1975 (all in alcohol). 2 pupae (in alcohol), Sam A Chung Stream, by Dudgeon, 18. XII. 1979.

DISTRIBUTION. China (Fujian), Hong Kong (new record), Japan, Taiwan and Thailand.

6. ***Simulium (Simulium) nodosum* Puri, 1933**

Simulium (Simulium) nodosum Puri, 1933: 813-17; Takaoka, 1984:33; An, 1989: 187.

The pharate female and pupa agree with the original descriptions given by Puri (1933) and the larvae also conform to the redescription given by Datta (1988). This species belongs to the *nobile*-group and is separated from other related species by the simple female claw, and three inflated pupal gill filaments per side. The pupa of *S. (S.) shirakii* Kono and Takahasi, 1940 from Taiwan is similar to that of this species (Takaoka, 1979), but a detailed comparison is impossible due to the lack of a larval stage of *S. (S.) shirakii*. Future studies may clarify the identity of the Taiwanese species.

SPECIMENS EXAMINED. Pharate ♀ with pupal skin, 8 immature larvae (in alcohol), collected from a stream, HONG KONG: Tai Po Kau Forest Stream, 28. X. 1976, by D.Dudgeon; 2 mature larvae (in alcohol), Lam Tsuen River, by D.Dudgeon, 28. X. 1976.

DISTRIBUTION. China (Fujian, Yunnan), Hong Kong (new record), India, Thailand.

7. ***Simulium (Simulium) quinquestriatum* Shiraki, 1935** *Stilboplax 5-striatum* Shiraki, 1935: 27-33.

Simulium (Stilboplax) 5-striatum: Ogata and Sasa, 1954: 330-31; Ogata et al., 1956: 77-78; Orii et al., 1969:1-13.

Simulium (Simulium) quinquestriatum: Anonym., 1974: 192; Takaoka, 1977: 205-09; Takaoka, 1979: 396-99; An, 1989: 187.

The adult female, male, pupal and larval specimens from Hong Kong almost agree with the redescription given by Takaoka (1979). This species belongs to the *striatum*-group redefined by Takaoka and Davies (1995a). The haired basal section of the radial vein in the female separates this species from most other related species of the *striatum*-group.

SPECIMENS EXAMINED. 2 ♀, 10 ♂, HONG KONG: New Territories, Sai Kung, light trap, by W. J. Voss & Hui Wai Ming, 2. IV. 1965; 1 ♀, 7. VII. 1964; 1 ♀, 18. VII. 1964; 1 ♀, 18. VII. 1964; 1 ♂, 1. IX. 1964; 1 ♂, 8. X. 1964; 1 ♀, 2 ♂, 30. XI. 1964; 1 ♀, 15. I .1965; 1 ♂, 15-16. I. 1965; 1 ♀, 20-21. I. 1965; 1 ♂, 25. I. 1965; 2 ♀, 4 ♂, 30. I. 1965 (all pinned, Bishop Museum). 1 ♀, 1 ♂, Tai Po Kau, light trap, by Lee Kit Ming & Hui Wai Ming, 1. IX. 1965; 13 ♀, 39 ♂, 2. IX. 1965; 2 ♂, 3. IX. 1965 (all pinned, Bishop Museum); 1 ♀, hand net, 3. VI. 1965; 1 ♂, 28.VI. 1965; 1 ♀, 3-4. VII. 1965; 1 ♀, 1 ♂, 12. XII. 1965; 2 ♂, 15-17. VII. 1965; 2 ♀, 27. XII. 1965; 1 ♀, 29. VII. 1965; 1 ♂, 9. VIII. 1965; 1 ♀, 13. VIII. 1965; 1 ♀, 19. VIII. 1965; 1 ♀, 20.VIII. 1965; 2 ♂, 23. VIII. 1965; 1 ♀, 4 ♂, 27. VIII. 1965; 1 ♂, 31. VIII. 1965; 1 ♀, 5 ♂, 1. IX. 1965; 13 ♀, 41 ♂, 2. IX.1965; 1 ♀, 3 ♂, 3. IX. 1965; 2 ♂, 4. IX. 1965; 1 ♂, 15. XII. 1965; 1 ♀,

20. XII. 1965 (all pinned, Bishop Museum). 1 ♀, 3 ♂, Tai Po Kau, light trap, by W. J. Voss & Hui Wai Ming, 4. IV. 1965 (all pinned, Bishop Museum). 5 ♂, Tau Po Kau Forest Stream, light trap, by D. Dudgeon, 2. VI. 1983; 1 ♀, 2 ♂, 17-18. I. 1965; 1 ♀, 1 ♂, 14-15. II. 1984; 14 ♂, 12-13. IV. 1984 (all in alcohol). 6 pupae, 3 pupal exuviae and 2 mature larvae (all in alcohol), Bride's Pool, by D. Dudgeon, 22. IX. 1977.

DISTRIBUTION. China (Fujian, Yunnan), Hong Kong (new record), Japan, Korea, Taiwan.

8. ***Simulium (Simulium) suzukii* Rubtsov, 1963**

Simulium Susukii Rubtsov, 1963 : 525-26.

Simulium (*Simulium*) *suzukii* : Takaoka, 1977 : 209-13 ; Takaoka, 1979: 395-96.

Simulium ryukyuense Ogata, 1966: 123-30. Syn. by Takaoka (1977).

The two males collected from Hong Kong agree with the description given by Takaoka (1977). This species belongs to the *tuberosum*-group. This is the first record of this species from Hong Kong.

SPECIMENS EXAMINED. 1 ♂, HONG KONG : Tai Po Kau Forest Stream, light trap, 14-15. II. 1984, by D. Dudgeon; 1 ♂, same data as the former except 12-13. IV. 1984 (both in alcohol).

DISTRIBUTION. China (Yunnan), Hong Kong (new record), Japan, Korea, Taiwan.

ACKNOWLEDGEMENTS

We are grateful to the Bishop Museum, Hawaii, U. S.A. for loaning specimens additional to those collected by one of us (D. Dudgeon). Mrs. Helen Györkös made preliminary identifications of the pinned and alcohol-preserved specimens collected before 1983. We thank to Miss C. Aoki, Division of Medical Zoology, Oita Medical University and to Dr. Denis Hill, formerly of the Department of Zoology, The University of Hong Kong, for their help. DMD is grateful for space and copying facilities provided by the Department of Biology, McMaster University.

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