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TWO-YEAR FOLLOW UP FROM BIRTH OF THAI CHILDREN FOR ROTAVIRUS INFECTION: DETECTION OF ROTAVIRUS IN FECES AND DIARRHOEAL SYMPTOM

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Abstract: Twenty-eight newborns delivered in Chiang Mai, Thailand in October and December, 1988 were followed up for rotavirus infection for two years after birth. Sixty-eight percent (19/28) of the index children discharged group A human rotavirus (HRV) antigen and/or viral genomic RNA in their feces during the two years, and 7.1% (2/28) discharged twice during the period. Seasonal distribution of HRV -positive fecal specimens was 29% (6/21) in January to April, 1989, 9.5% (2/21) in August and September, 1989, 57% (12/21) in December, 1989 to April, 1990, and 4.8% (1/21) in June, 1990.

Eighty-six per cent (24/28) of the index children developed diarrhoeal symptom and the total number of the episodes was 67, i.e., 2.39 for one child during the two years. One severe episode, 20% (3/15) of moderate and 12% (6/51) of mild diarrhoeal episodes were correlated to the rotavirus infection.

INTRODUCTION

Rotavirus is one of the most important aetiologic agents of gastroenteritis among infants and children causing many cases of severe disease and death worldwide (Davidson *et al.*, 1975; Kapikian *et al.*, 1980, 1990). Hospital based and community based studies in different geographic areas of Thailand during 1975-1983 showed that rotavirus was most common cause of diarrhoea during the cold months (November-February) (Puthavathana *et al.*, 1981, Jayavasu *et al.*, 1982, Louisirirotchanakul *et al.*, 1984, Varavithya *et al.*, 1988). Rotavirus was identified in 44% of acute diarrhoeal patients aged 1-23 months admitted to Siriraj Hospital, Bangkok (Phijaisanit *et al.*, 1988), and similar figures were observed also in Japan (Kariyasono *et al.*, 1993) and in Western Europe and the United States during the winter season (Davidson *et al.*, 1975).

We made a seroepidemiological survey of rotavirus in Thailand in 1978 (Maneekarn *et al.*, 1980). In 1984 and 1985, we detected rotavirus antigens in 46% out of 188 diarrhoeal patients by ELISA in Chiang Mai, Bangkok and Khon-Kaen (Yamazi *et al.*, 1988), and made some preliminary work on the fecal IgA antibody responses to rotavirus infection of the patients in Chaiang Mai (Supawadee *et al.*, 1988). In this report, 28 newborns delivered at a hospital in Chiang Mai in October and November, 1988 went followed up from birth for two years for the rotavirus infection. The objective is to know frequency, time and severity of the human rotavirus infection of Thai infants during the first two years of their life.

MATERIALS AND METHODS

Index children and collection of feces

Fourty newborns delivered in the Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Chiang Mai, Thailand in October and November, 1988 were scheduled to be followed up for two years. This time schedule covered two cycles of dry-cool and rainy seasons, so that the distribution of rotaviral infection and

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the response to the infection of Thai newborns in each season were studied. They were selected to cover a wide range of environmental and socioeconomical standards, as far as convenience of the follow-up work allowed. This study was approved by the Human Experimentation Committee, Faculty of Medicine, Chiang Mai University (No.9, 1988) and by the World Health Organization (HQ/89/060317). Parents of the children gave informed, signed consent. Twelve children did not complete the study because their families moved away. The data presented refer to the remaining 28 children.

A Well Baby Clinic was set up by the Department of Family Medicine and Pediatrics, Chiang Mai University. Contact persons with sufficient training visited the volunteers' homes every week, recorded the clinical symptoms, especially gastrointestinal disorders of the children, and collected fecal specimens. Extra fecal samples were collected during diarrhoeal episodes. Parents were requested to bring their index children every two months to the Well Baby Clinic to be examined by a pediatrician (P.R.). During the study period, all participating children received the standard immunization to diphtheria, pertussis, tetanus, polio, measles and BCG at the Well Baby Clinic. Fecal specimens were stored at −40°C until tested.

Detection of rotavirus antigen and determination of electrophenotypes

Fecal specimens were tested for group A rotavirus antigens by a sandwich ELISA and for rotavirus genomic RNA by polyacrylamide gel electrophoresis (PAGE) at Department of Microbiology, Chiang Mai University, and double checked at N.I.H., Tokyo. The ELISA was performed according to the WHO standard method with slight modifications (Inouye *et al.*, 1989). The fecal specimens were tested as 10% suspension in phosphate-buffered saline. Hyperimmune rabbit antisera to Wa strain of HRV serotype 1 was used as capture antibody, and guinea pig antiserum to Wa strain and rabbit-anti guinea pig IgG antibody conjugated with horseradish peroxidase were used as detection reagents. In a preliminary experiment (Yamazi *et al.*, 1988), rotavirus was not found with electron microscopy in all of the fecal specimens negative for rotavirus antigens by this ELISA method. For PAGE of rotavirus RNA, a minor modification of the method of Herring *et al.* (Herring *et al.*, 1982) was used. Double stranded viral RNA was extracted with phenol and chloroform directly from 10% fecal suspension. RNA bands were detected by silver staining.

Table 1 Rotavirus discharge in feces and diarrhoeal symptom

Volunteer No.	Rotavirus in feces	Diarrhoeal symptom		
		Mild	Moderate	Severe
31	—	—	—	—
4	—	5	—	—
9	—	5	30	—
37	—	16, 19, 42	—	—
13	—	30, 379	—	—
27	—	42	—	—
36	—	54	65	—
30	—	78	—	—
15	—	85	61	—
14	11	—	—	—
34	14	15, 33, 34	—	—
21	15	—	68	—
40	18	17, 37	—	—
11	19, 64	66, 95, 96	59, 64, 65	—
38	20	23, 26, 62	24	—
24	40	12, 39	—	—
16	44	7, 38	—	—
5	61	—	—	—
35	63	—	—	—
23	66, 72	6	8, 26	—
39	67	8, 17, 18	—	—
8	69	34, 39, 55, 60	59, 67	69
10	70	2, 34, 42	3	—
25	70	18	—	—
7	72	59, 72, 89	—	—
1	74	11, 41, 70	—	—
6	80	23, 71, 80	79	—
2	90	34, 71, 81, 91	70	—

Figures in the Table are weeks after birth. Underlines show weeks within one week of rotavirus discharge in feces.

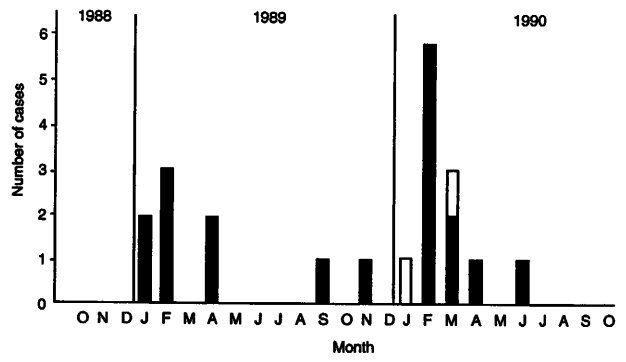

Figure 1 Monthly distribution of fecal discharge of group A rotavirus-antigen and/or RNA. Fecal samples collected weekly from newborns delivered in October and November, 1988 were tested with an ELISA and PAGE. Closed bar, primary infection. Open bar, reinfection.

Table 2 Correlation between HRV-discharge and diarrhoeal symptom

Diarrhoeal symptom	HRV in feces (%)		
	Positive	Negative	Total
Severe	1(4.8)	0(0)	1
Moderate	3(14)	12(0.4)	15
Mild	6(29)	45(1.6)	51
No	11(52)	2,835(98)	2,846
Total	21(100)	2,892(100)	2,913

Twenty-eight children were studied for fecal HRV-discharge and diarrhoeal symptom every week for two years after birth. Correlation between HRV-discharge and the symptom was refered as positive if the virus was found at the same and/or successive week of the diarrhoea.

RESULTS

Rotavirus-Ag and/or -RNA were detected in 19 (68%) among the 28 index children from their stool specimens collected weekly during the two years, and were found twice in two (7.1%) children in separate discontinuous weeks. HRV was not found in any fecal samples from the other nine children (32%) throughout the two years after birth (Table 1).

As for the seasonal incidence, six (29%) out of the HRV-positive fecal specimens were obtained between the 11th and 20th weeks after birth (Jan. to Apr., 1989), two (9.5%) were at the 40th and 44th weeks (Aug. and Sep., 1989), 12 (57%) were between the 61st and 80th weeks (Dec., 1989 to Apr., 1990), and one (4.8%) was at the 90th weeks (June, 1990). From two children who shed HRV twice, the virus was found at the 19th and 64th weeks (Mar., 1989 and Jan., 1990), and 66th and 72nd weeks (Fed. and Mar., 1990), respectively (Table 1, Fig. 1).

Diarrhoeal symptoms were reported in 24 (86%) out of the 28 index children and total number of the episodes was 67, i.e., 2.39 for one index child during the two years. Ten (15%) out of the 67 diarrhoeal episodes were observed at the same or successive weeks of the fecal HRV-discharge. Severe intestinal symptome of diarrhoea was found only in one case (No.8) at the week of the fecal HRV-discharge, and HRV was detected in 20% (3/15) and 12% (6/15) of moderate and mild diarrhoeal episodes, respectively (Table 1). Only 10 out of 21 HRV-positive fecal specimens were obtained at the same or successive weeks of diarrhoeal episodes (Table 1 and 2).

Two children (No.11 and 23) shed HRV twice during the two years after birth. No.11 experienced an asymptomatic primary infection with RNA-electropherotype S and a symptomatic secondary infection with RNA-electropherotype L of the virus. No.23 had two asymptomatic infections with RNA-electropherotype L and with RNA-electropherotype S of the virus, respectively.

DISCUSSION

According to surveys in Thailand in 1984 and 1985, children aged 0-5 years yielded 1.5 diarhhoea episoedes per child per year (Vathanopas *et al.*, 1986, Varavithya *et al.*, 1988). These figures are not so different from our finding. But Thongkrajai *et al.* (Thongkrajai *et al.*, 1988) reported that only 13% (44/339) of children in four villages in Khon-Kaen developed diarrhoea in 16 months. Reason for the discrepancy are not clear.

HRV was detected in 19 (68%) among the 28 index children from their stool specimens collected weekly during the two years, and twice in two (7.1%) children in separate weeks, which indicates that 68% of the newborn babies of this study group were infected, 7.1% experienced reinfection but 32% were not infected by rotavirus during the first two years after birth. Seroepidemiological data in 1978 showed that 80% of children under two years of age had anti-rotavirus antibody (Maneekarn *et al.*, 1980).

Seasonal incidence of HRV-positive fecal specimens from newborn babies delivered in October and November indicated that 29% of the index children experienced primary infection of rotavirus in the first dry-cool season, 9.5% in the first rainy season, 57% in the second dry-cool season and 4.8% in the second rainy season of their life. In tropical areas, rotavirus infections are common year round, but increase during periods of low rainfalls or low humidity and decrease during periods of high rainfalls or high humidity, or both (Hieber *et al.*, 1978, Black *et al.*, 1980, Suanarto *et al.*, 1981, Paul *et al.*, 1982). On the contrary, it is well known that many infectious diseases including bacterial gastroenteritis and even influenza prevail in tropical area in the rainy season as shown in the Thai Government statistics. The reason why rotavirus prevails in tropical area in dry-cool season has been not clearly explained.

Results of this report suggest that most rotavirus infections during two years after birth did not cause severe diarrhoea, as 52% (11/21) of rotavirus infections which were indicated by the HRV discharge into the feces were asymptomatic and only one case showed severe diarrhoea. Asymptomatic infections in full time

newborns have been reported (Chrystie *et al.*, 1978, Bishop *et al.*, 1979). Araki *et al.*, who examined fecal specimens from 69 diarrhoeal and 46 non-diarrhoeal babies aged in average 11.9 months in a Baby Home in Tokyo during winter seasons from 1988 to 1991, found that 74% (45/61) of HRV-positive babies had diarr hoeal symptom but the rest 26% (16/61) were of non-diarrhoea (Araki *et al.*, 1993). Results of our study could not directly be applied to the entire population of Chiang Mai, because the number of index children was small and some inevitable selection of volunteers might be done, i.e., monthers of good health bahavior would be interested in and attend this follow-up study. However, considerably high rate of the inapparent infection can not be neglected and asymptomatic carrier state could be a dangerous sourse of the infection.

In our survey, severe diarrhoea was observed in only one child and rotavirus was responsible for the disease, and 20 and 12% of the moderate and mild diarrhoeal episodes, respectively, could be correlated to the rotavirus infection. In other words, correlation between the severity of diarrhoeal symptom and the fecal HRV-discharge increased in accordance with the severity of the symptom. According to Thongkrajai's report (Thongkrajai *et al.*, 1988), in children aged one month to two years rotavirus was responsible for 7/55 (13%) of diarrhoea cases in villages and 79/201 (39%) of cases at hospital.

For rotavirus gatroenteritis outbreak, high secondary attack rates have been reported (Fonteyne *et al.*, 1978). Chiba *et al.*, (1986) observed that immunity to repeated infections appears to be serotype dependent, but Imamura *et al.* (Imamura *et al.*, 1993) followed seven cases of rotaviral diarrhoea in 1986-1992 and found that one of them was infected twice by type 1 virus. Araki *et al.* (1993) reported a baby who had an asymptomatic infection with serotype 4 virus followed by a symptomatic infection by serotype 1 in the next year.

Prevalence and severity of infectious diarrhoeal diseases differ in time and areas, i.e., in accordance with the climate, level of public health and medical care and some times with virulence of the pathogen. In Thailand, National Control of Diarrheal Disease Programme (CDD) was initiated in 1979 including the use of oral rehydration therapy (ORT). Diarrhoeal diseases over the country ranked as the 2nd leading cause of death prior to the implementation of the CDD programme, but in 1985 it was the 5th leading cause of death in infant under one year (Varavithya *et al.*, 1986). On the other hand, the National Diarrhoea Annual Workshops by Mahidol University and Ministry of Public Health held from 1981 to 1986 concluded that there had been no change of diarrhoeal morbidity, although the mortality and severity of the disease had satisfactorily reduced (Varavithya *et al.*, 1988). Rotaviral diarrhoeal disease was estimated to be responsible for 5,000,000 death annually in less developed countries, and rotaviral gestroenteritis is associated with high morbidity in developed countries (Kapikian *et al.*, 1980). Hence, the situation in Thailand is approaching to these of devel oped countries. This report, results of two-year follow-up after birth of 28 newborns in Chiang Mai might contribute to the understanding and control of rotaviral diarrhoea not only in Thailand but also in another part of the world. Typing of isolated viruses, serum antibody and secretory fecal IgA responses of the index children in this study will be described in a further report.

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SEROEPIDEMIOLOGICAL STUDIES OF HEPATITIS VIRUSES IN THE DOMINICAN REPUBLIC

I. THE PREVALENCE OF MARKERS OF HEPATITIS A, B AND C VIRUSES

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Abstract: A total of 408 subjects (Group I) visiting the center of gastroenterological diseases, Aybar Hospital, Santo Domingo, Dominican Republic and 2,000 apparently healthy individuals (Group II) living in 6 different cities in the Dominican Republic were tested for antibodies against Hepatitis A virus (anti-HAV), Hepatitis B surface antigen (anti-HBsAg) and Hepatitis C virus (anti-HCV). The presence of HBsAg in their sera was also tested. The study demonstrated that most of the Dominican population are infected with HAV by the age of 15 years and that HBV infection is still prevalent in the country. The overall prevalences of HBsAg and of anti-HBs were 3.2% (Group II) to 4.7% (Group I) and 18% (Group II), respectively, although HBsAg prevalence rate in young children was very low. In contrast to the predominant HAV and HBV infections, most of the anti-HCV-positive individuals were concentrated in the age groups of higher than 40 (Group II) and 50 (Group I) years old and only a few sero-positive individuals were observed in the younger population, suggesting that there have been few transmissions of HCV in the Dominican population in the past 40 to 50 years.

INTRODUCTION

Hepatitis A is highly endemic in many tropical and subtropical areas and occasionally causes large epidemics (Szmuness *et al.*, 1977; Gust & Feinstone, 1989; Hadler, 1991; Yap & Guan, 1993). However, in these economically developing countries hepatitis A virus (HAV) infection occurs primarily in young children and almost all have antibodies for HAV by the age of 10 (Tsega *et al.*, 1986). Hepatitis A in adults tends to be more severe than in children. The most effective way of reducing HAV infection is to improve standards of hygiene and sanitation, especially by providing adequate supplies of clean water and proper disposal of fecal material.

Hepatitis B virus (HBV) infection is a disease of the utmost public health importance for Latin America (Fay *et al.*, 1990). Infection with HBV is known to cause significant morbidity and mortality resulting from hepatitis, cirrhosis of the liver, or hepatocellular carcinoma. HBV infection is highly endemic in many developing countries, including those in Asia, Africa and the Western Pacific (Hawkes *et al.*, 1981; Hyams *et al.*, 1989; Boutin *et al.*, 1990; Bile *et al.*, 1991; Tan *et al.*, 1991). In such a setting, most persons are infected in early childhood though both perinatal transmission (mother to infant at birth) and horizontal transmission (close contact with other infected persons). However, the spread of HBV inection is now preventable by the use of passive and active immunization with a safe and cost-effective vaccine. To plan for large-scale HBV vaccination strategies, it is essential to know the picture

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of HBV endemicity and transmission in the region.

It has become feasible to detect the antibody to HCV (anti-HCV), and there have been many epidemiologic studies on the prevalence of HCV infection in blood donors and patients with various liver diseases in the USA, Europe and Japan (Kuhnl *et al.*, 1989; Tanaka *et al.*, 1992). However, there is no information available in the Dominican Republic. In the present paper, we report the results of seroepidemiological study on HAV, HBV and HCV infection for the purpose of obtaining information on the current endemicity of these viruses in the Dominican Republic. The Dominican Republic is bounded on the north by the Atlantic Ocean and on the south by the Caribbean sea and lies within the tradewind belt. The inhabitants are partly of Spanish and French descent, but the majority are of mixed African Negro and European blood. Voodoo is practiced, but to a lesser extent than in neighboring Haiti. They appear to have no special customs affecting the transmission of hepatitis viruses.

Materials And Methods

Subjects and serum samples

Two groups were evaluated. Group I was composed of 408 serum samples which were picked up at random from the serum stocks of the outpatients visiting the center of gastroenterological diseases, Santo Domingo, Dominican Republic, during the period of August to October, 1993. The sera consisted of almost equal numbers from 13 different age groups and both sexes. Age distribution and sex are shown in Table 1. These sera were tested for the presence of antibodies to hepatitis A virus (anti-HAV), HBV surface antigen (HBsAg) and anti-HCV.

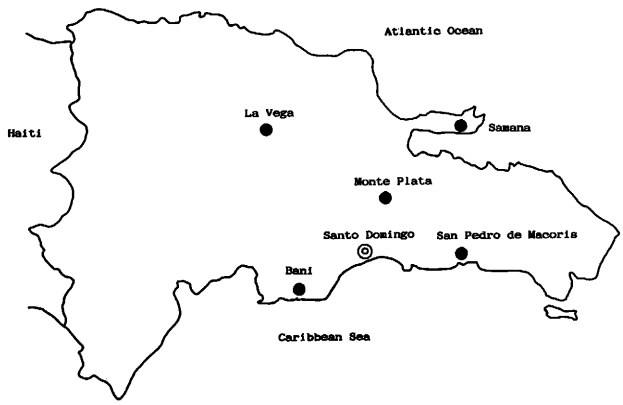

Figure 1 Map of the Dominican Republic and the locations where the sera were collected.

Group II was composed of 2,000 apparently healthy individuals of both sexes and different age groups living in 6 cities in the Dominican Republic (Fig. 1). These consisted of 202 subjects from Bani, 192 from La Vega, 211 from Monte Plata, 201 from Samana, 198 from San Pedro de Macoris and 991 from Santo Domingo, but did not include individuals less than 19 years old. Sera were taken during the period between November 20 and December 17, 1993 and stored at −75°C until the tests for the presence of HBsAg, anti-HBs and anti-HCV were performed.

Methods of serological tests

(*a*) *Anti-HAV*

Total (IgG and IgM) anti-HAV was tested for by enzyme-linked Immunosorbent assay (ELISA) method using a commercially available test kit (HAVAB, Abbott Lab., North Chicago, Illinois, USA).

(*b*) *Hepatitis B serological tests*

(1) In the case of Group I sera, the presence of HBsAg was tested for by ELISA method using a commercially available test kit (HBsAg, Abbott Lab., North Chicago, Illinois, USA).

(2) In the case of Group II sera, HBsAg was tested for by reversed passive hemagglutination (RPHA) (Aucell, Abbott Lab., North Chicago, Illinois, USA), and by passive hemagglutination (PHA) for anti-HBsAg (Sero-clit-anti-HBs, Sankojunyaku, Japan).

(*c*) *Anti-HCV*

Group I sera were tested for anti-HCV by an anti-HCV second generation enzyme immunoassay (recombinant C100-3, HC-31, HC-34, Abbott, Lab., North Chicago, Illinois, USA). In the case of Group II sera, this was performed using the second generation particle-

Table 1 Sex and age distribution of the subjects (Group I) tested for anti-HAV, HBsAg and anti-HCV

Sex	Age groups													Total
	0-5	~10	~15	~20	~25	~30	~35	~40	~45	~50	~55	~60	61~	
Male	19	17	16	16	17	16	17	15	15	15	15	15	15	207
Female	13	18	15	14	15	15	15	16	16	17	16	15	16	201
Total	32	35	31	30	32	31	32	31	31	32	31	30	31	408

agglutination test (PA test) (FUJI REBIO, INC., Japan). In this case, the titers more than 1:32 were taken as sero-positive.

Statistical analysis

Statistical analysis was performed using the X^2 test by Mantel-Haenszel method. P values less than 0.05 were considered significant.

RESULTS

Prevalence of anti-HAV

Positive rates of anti-HAV in Group I are shown in Fig. 2A. Anti-HAV was detected in 64.7% of those in the 6 to 10 year old age group. The prevalence then increased progressively with increasing ages from 96.8% in the age group of 11 to 15 years to 100% in the age group of 16 to 20 years and essentially 100% positive rates continued thereafter. It is clear that HAV is very widespread in the Dominican Republic. No significant difference was observed in the prevalence of anti-HAV between sexes.

Prevalence of HBsAg

Fig. 2B shows the prevalence of HBsAg in 13 different age groups in Group I. The overall prevalence of HBsAg was 4.7%. The rate (6.3%) for HBsAg in males was significatly higher than that in females (1.5%) ($P< 0.05$). However, no HBsAg-positive individuals were detected in children under 5 years of age or in males between 6 and 10 years of age. HBsAg-positive rate was maximum (13.3% in total, 18.8% in males, 2.4% in females) in the age group from 6 to 20 year old. Table

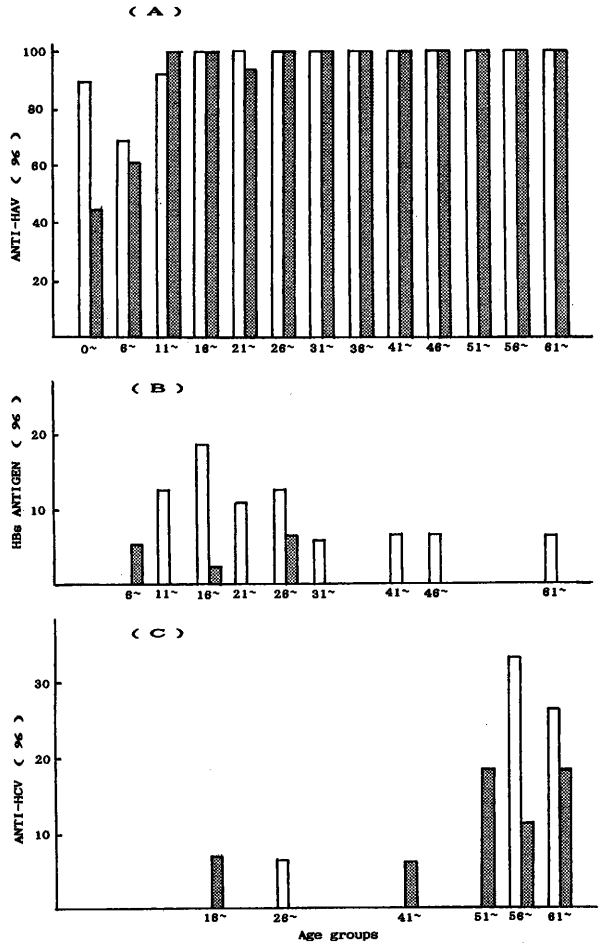

Figure 2 Prevalence by age and sex of anti-HAV (A), HBsAg (B) and anti-HCV (C) of Group I subjects. White column and dotted column indicate males and females, respectively.

Table 2 Prevalence of HBsAg and anti-HBs by age and sex in the Dominican healthy individuals (Group II)

Sex	Age groups	20~	30~	40~	50~	60~	70~	Total
Male	No. of tested	253	206	157	107	54	33	810
	No. of HBsAg-positive	10 (4.0)	10 (4.9)	9 (5.7)	6 (5.6)	1 (1.9)	3 (9.1)	39 (4.8)[a]
	No. of anti-HBs positive	44 (17.4)	38 (18.5)	31 (19.8)	27 (25.2)	14 (25.9)	8 (24.2)	162 (20.0)
Female	No. of tested	430	350	208	109	69	24	1190
	No. of HBsAg-positive	6 (4.0)	9 (2.6)	7 (3.4)	0 (0)	1 (1.5)	1 (4.2)	24 (2.0)[b]
	No. of anti-HBs positive	50 (11.6)	59 (16.9)	40 (19.2)	23 (21.1)	14 (20.3)	6 (25.0)	192 (16.1)

Figures in parenthesis indicate per cent.

a : b, $P<0.05$ by the X^2 test with Mantel-Haenszel method.

2 shows the prevalence of HBsAg in Group II by age and sex. The overall prevalence of HBsAg was 3.2% (63/2, 000). The rate observed for HBsAg was again higher in males (4.8%) than in females (2.0%) and this sex difference was statistically significant ($P<0.05$). The prevalence of HBsAg continued to increase gradually with age and reached 5.7% in males and 3.4% in females in the 40 year old age group. The HBsAg-positive rates once decreased in older age groups (60-69 years in males, 50-69 years in females) but increased again in individuals more than 70 years old, although the reason is not clear. This tendency was also observed in Group I.

The prevalence of HBsAg in 6 different districts was compared. The rate ranged from 1.5% in Samana to 5.6% in Monte Plata. Although the prevalence of HBsAg in Monte Plata (5.6%) was statistically higher than that in La Vega (2.0%) or in Samana (1.5%) ($P<0.05$) (Data not shown), the factors responsible for this difference in the districts were not clarified in the present study.

Prevalence of anti-HBs

The prevalence of anti-HBs in different age groups and sex is shown in Table 2. The prevalence continued to increase gradually with age, reaching 25.9% in males in the 60-69 years old group and 25.0% in females in the 70-79 years old group. However, no significant difference was observed in the overall prevalence between males (20.0%) and females (16.1%). When the prevalences of anti-HBs by age and districts were compared, the highest incidence for anti-HBs was found in Monte Plata population (25.9%), which was statistically higher than those in other areas except for that of San Pedro de Macoris (21.2%). The prevalence rate of anti-HBs was lowest in Bani (13.4%), followed by La Vega (16.2%) and Samana (16.9%). This difference of anti-HBs rates by districts appeared to correspond to the HBsAg-positive rates in those districts.

Prevalence of anti-HCV

As shown in Fig. 2C., the overall prevalence of anti-HCV in Group I was 4.7%. All young children under 15 years old were sero-negative. However, there was an abrupt change in the prevalence in individuals more than 50 years old, the group in which the sero-positive individuals were concentrated. A part (1,401 serum samples) of Group II was also tested for anti-HCV by PA test (Table 3). The overall prevalence was 2.6% in males and 2.2% in females. Although Group II was younger than Group I, there was again an abrupt increase in the prevalence in individuals more than 40 years old. The average titer of anti-HCV was significantly higher in these individuals than that in individuals under 39 years of age (Data not shown).

DISCUSSION

In the present study, it was demonstrated that most of the Dominican population is infected with HAV by age of 15. This pattern of infection with HAV is consistent with the findings observed in other developing countries where the infection occurs primarily in young children (Gebreselasse, 1983; Craig *et al.*, 1993). In the developed world, with improved standards of hygiene and sanitation, the rate of infection declines and a significant proportion of the population escapes infection in early childhood and the age of primary infection gradually increases (Hadler, 1991; Yap and Guan, 1993). In other words , the rate of infection in early childhood reflects the sanitary environment in the country. Thus, the rate of HAV infection in the Dominican population would decrease through further efforts to improve the system of clean water supply and proper disposal of fecal material.

The prevalence of 3.2 (Group II) to 4.7 (Group I) % of HBsAg carriers in the population and as high as 25% anti-HBs seropositive rates, which increase with age,

Table 3 Prevalence of anti-HCV by age and sex in the Dominican healthy individuals (Group II)

Age groups	20~	30~	40~	50~	60~	70~	Total
Males							
No. of tested	166	129	96	78	41	24	534
No. of positive	1 (0.6)	0 (0)	7 (7.6)	2 (2.5)	3 (7.3)	1 (4.2)	14 (2.6)
Females							
No. of tested	316	254	149	79	53	16	867
No. of positive	4 (1.3)	3 (1.2)	5 (3.4)	4 (9.8)	1 (1.9)	2 (12.5)	19 (2.2)

Figures in parenthesis indicate per cent.

indicated that HBV infection still prevails in the Dominican population. However, the HBsAg prevalence rate in young children was very low and began to increase only in late childhood. According to the classification by WHO (Zuckerman, 1987), the above HBsAg positive rates in the Dominican Republic are classified as intermediate prevalence region type (2 to 7%). The rates are higher than those in central Europe and North America (0.2 to 0.5%) (Zuckerman, 1987) and Japan (0.8%) (Tanaka *et al.*, 1993), but apparently lower than those in Asian and African countries (Kiire, 1993; WHO, 1992). In Latin America, the rates are different from country to country, but appear to be higher in rural areas with different natives than in urban areas (Vanderborght *et al.*, 1993; Craig *et al.*, 1993; Kenneth *et al.*, 1993; Blitz *et al.*, 1994; Pujol *et al.*, 1994).

Although further studies on HBV transmission are necessary, transmission presumably occurs through percutaneus or permuscosal exposure to infected blood or other secretions in children as suggested by Fay *et al.*, (1990) or still through infected needles, in addition to perinatal infection from HBeAg positive mothers to newborns. In Japan, passive immunization and vaccination have been successfully carried out in newborns from HBeAg-positive mothers to block HBV infection (Yano, 1986). Thus, HBV vaccination strategies in the Dominican Republic should be directed with a priority toward the prevention of perinatal and late childhood transmission and also aimed at individuals belonging to high-risk groups.

HBsAg prevalence rates were statistically higher among males than females in both groups of individuals in the Dominican Republic. This higher prevalence among males has been observed in other countries (Gebreselassie, 1983; Fonseca *et al.*, 1988). Although there have been some explanations, such as sex-specific behaviour increasing the chance of exposure to the virus, or some immunological deficiency in males or lower titer of HBsAg in female carriers, which in turn results in a higher detection rate in males (Szmuness, 1978), the underlying mechanism of the sex difference remains obscure.

HCV infection in the Dominican population showed a specific picture. Only a very small proportion of younger people are infected with HCV, whereas seropositive prevalence in people higher than 40 to 50 years old exhibits an abrupt increase, suggesting that there has been few transmission of HCV in the Dominican population in the past 40 to 50 years. This abrupt increase of prevalence in adults of HCV infection has been reported from Japan (Tanaka *et al.*, 1993) and several other countries (Darwish *et al.*, 1992; Thuring *et al.*, 1993). Recent studies on the transmission of HCV have suggested that the major source of transmission is by blood transfusion and not by perinatal infection (Thaler *et al.*, 1991; Price *et al.*, 1993; Ohto *et al.*, 1994). The fact that the infectivity of HCV in blood is 10^6 times lower than that of HBV (Brady *et al.*, 1983) would facilitate HCV-specific transmission pattern. Thus, it could be presumed that HCV infection in the Dominican population might be eliminated in the near future by establishment of a complete screening system for HCV infection before blood transfusions.

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VIROLOGICAL STUDY ON DENGUE EPIDEMIC IN VIENTIANE MUNICIPALITY, LAO PDR, 1994

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Abstract: In May 1994, an epidemic of dengue fever (DF)/ dengue hemorrhagic fever (DHF) was reported in Ban Hom commune in Vientiane municipality. This was after a six-year period of non-epidemic DF/ DHF in the municipality. In early June, we collected 81 serum specimens from children with fever of unknown origin (FUO) in the commune. Of 81 specimens, 48 were taken from children within 5 days after the onset of FUO and subjected to dengue (DEN) virus isolation. Four strains of DEN-1 and 12 strains of DEN-2 viruses were isolated from 15 serum specimens (31.3%). A case of dual infection with DEN-1 and DEN-2 viruses was detected by enzyme antibody method using type-specific monoclonal antibodies and reverse transcription polymerase chain reaction (RT-PCR). The percentage incidences of the positive virus isolation from the serum specimens taken on the second and the third day of fever were 41.8% and 21.1%, respectively, and no virus was isolated from the specimens other than those from these two days. All the specimens were examined by the IgM-capture ELISA. Thirty-two specimens (39.5%) were positive for DEN IgM antibody.

INTRODUCTION

Lao PDR, a land-locked country, located in the Indochina Peninsula, has a total population of about 4.2 milion with an area of 236.8 thousand square kilometers. In 1985 and 1987, large outbreaks of dengue fever (DF)/ dengue hemorrhagic fever (DHF) occurred in the urban areas of the Vientiane municipality. In these outbreaks, 1076 and 5232 DF/DHF hospitalized cases were reported to the Vientiane Municipality Health Service, respectively (Bounlu *et al.*, 1992; Fukunaga *et al.*, 1993). From 1988 to 1993, DF/DHF epidemic did not occur, though several sporadic cases were reported in these areas. In 1994, an epidemic took place again, expanding to the rural areas of the Vientiane municipality, and a total of 1590 hospitalized cases were reported by the middle of October.

We conducted a survey of the epidemic in a rural commune, called Ban Hom, where the DF/DHF epidemic was the first report in the Vientiane municipality in 1994. Serum specimens were collected from children with fever of unknown origin (FUO) in the commune, and virological and serological examinations were carried out on the specimens. This is the first report of dengue virus isolation in Lao PDR.

MATERIALS AND METHODS

Study area

The study area, Ban Hom commune, is located south-west of Hatxayfong district, about 15 km south-east of the center of Vientiane municipality and has a total population of 968 residents (Fig. 1). It is primarily an agricultural commune. In May, the DF/DHF outbreak was first reported in this commune. Severe DF/ DHF cases were sent to the hospitals in Vientiane municipality. The data of hospitalized DF/DHF patients were collected at the Vientiane Municipality Health Service.

Serum specimens

On June 1st, 1994, 81 serum specimens were collected from children 1-16 years old, with FUO at the Ban Hom Health Center. The serum specimens were

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stored at -70°C in the National Institute of Hygiene and Epidemiology (NIHE) until transported to the Dept. of Virology, Faculty of Medicine, University of the Ryukyus.

Virus isolation

Serum specimens taken within 5 days after the onset of FUO were subjected to virus isolation. The serum was diluted 1 : 30 with Eagle's MEM containing 2% fetal calf serum and antibiotics and inoculated onto C6/36 cells cultured in 25-㎠ flasks (Igarashi, 1978).

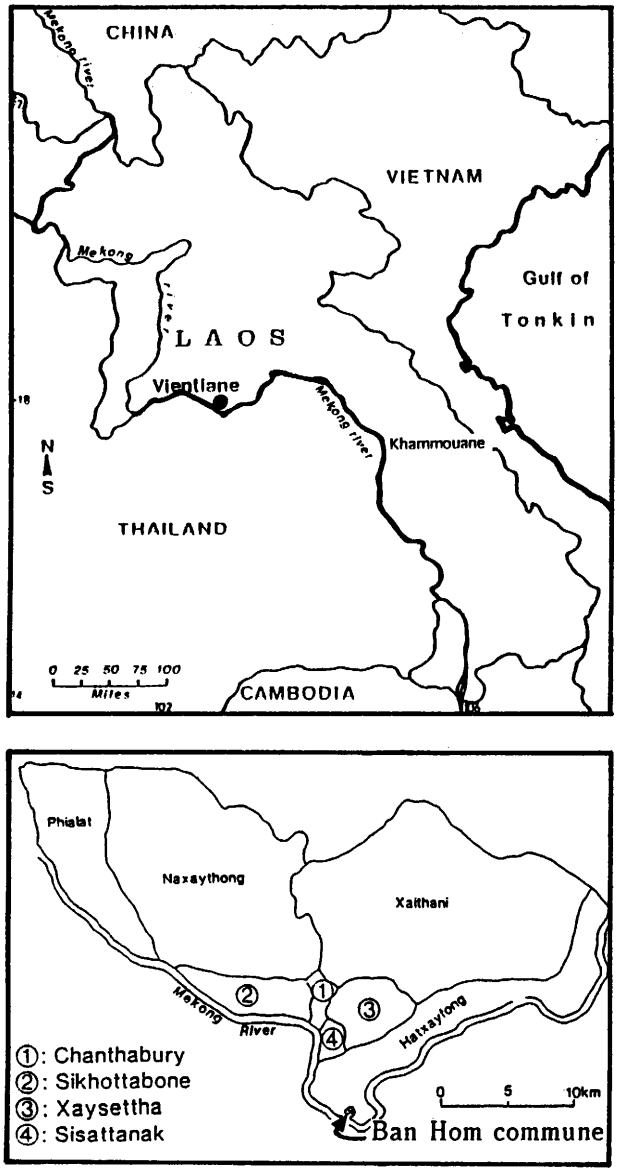

Figure 1 Maps of Lao PDR (upper panel) and Vientiane municipality (lower panel). Vientiane municipality consists of 8 districts. Ban Hom commune is located in the south-west of Hatxayfong district.

The infected cells were incubated at 28°C for 7 days. The culture fluid was then inoculated on to BHK-21 cells and cultured at 37°C for 3 days. The infected BHK-21 cells were screened for the presence of DEN virus antigens by peroxidase-anti-peroxidase (PAP) staining method using hyperimmune rabbit anti-DEN serum (Ishimine *et al.*, 1987; Okuno *et al.*, 1978). The DEN antigen positive cells were further examined for the serotype by PAP staining with type-specific monoclonal antibodies. The serotype was also confirmed by reverse -transcription polymerase chain reaction (RT-PCR).

RP-PCR

The primers for the detection of DEN virus genome are described elsewhere (Morita *et al.*, 1991; Morita *et al.*, 1994). Amplification of the fragments of the virus genome was carried out by programmed incubation : 10 min at 53°C for RT once, followed by 35 cycles of PCR, consisting of 92°C for 1 min, 53°C for 1.5 min and 72°C for 2 min. The amplified DAN was run in 2% agarose gel and visualized by ethidium bromide staining.

DEN antibody assays

For the neutralization test, a fifty percent focus reduction neutralization test in BHK-21 cells, using PAP staining method, was employed (Ishimine *et al.*, 1987; Okuno *et al.*, 1978; Vongxay *et al.*, 1995). A neutralizing antibody titer less than 1 : 20 was defined as negative.

IgM-capture enzyme-linked immunosorbent assay (IgM-capture ELISA) was essentially the same method as reported elsewhere (Bundo and Igarashi, 1985; Innis *et al.*, 1989; Ruechusatsawat *et al.*, 1994). Human IgG was purified from the pooled DEN patient sera by DEAE-column chromatography and labeled with horseradish peroxidase (Wilson and Nakane, 1978). An ELISA titer less than 1 : 100 was defined as negative.

RESULTS

In the Ban Hom commune, the DF/DHF epidemic started in May, reached a peak in June and subsided by August, 1994. Fig. 2 shows the numbers of the monthly hospitalized DF/DHF cases in the commune. During the epidemic, a total of 61 patients were admitted to the hospitals in the central area of Vientiane municipality. Most of them were children under 15 years old.

Of 81 serum specimens from children with FUO, 48 were considered as acute phase sera (i. e. within 5 days after the onset of fever), and subjected to virus isolation. DEN viruses were isolated from 15 (31.3%) specimens

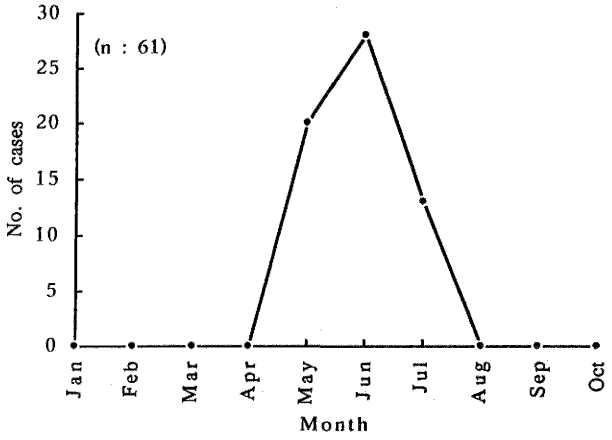

Figure 2 Monthly hospitalized DF/DHF cases in Ban Hom commune in 1994.

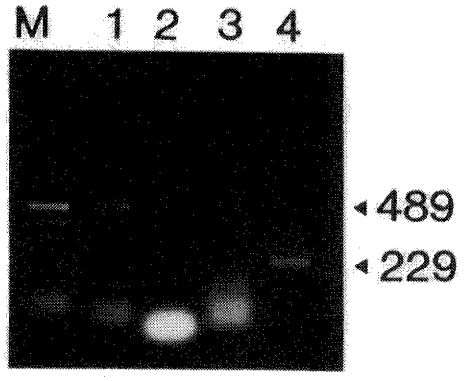

Figure 3 Agarose gel electrophoresis profile of RT-PCR products from B52-infected culture fluid using DEN-1 primers (lane 1), and DEN-2 primers (lane 2), from DEN-1 infected culture fluid using DEN-1 primers (lane 3) and from DEN-2 infected culture fluid using DEN-2 primers (lane 4). M: DNA size marker. Expected DNA sizes are indicated on the right side.

using a mosquito cell line (C6/36) (Table 1). Four strains of DEN-1 and 12 strains of DEN-2 viruses were identified by PAP staining method using the type-specific monoclonal antibodies and RT-PCR method. PAP staining of BHK-21 cells infected with the virus(es) recovered from B52 serum specimen showed positive reaction with both DEN-1 and DEN-2 type-specific antibodies. The RT-PCR products from the B52 specimen also showed two bands, identical to those from DEN-1 and DEN-2 prototype viruses (Fig. 3), indicating that the patient, B52, was a case of dual infection with DEN-1 and DEN-2 viruses. A follow-up study revealed that the child, B52, did not develop severe enough symptoms to require admission. It would be noted that the child, B52, had the pre-existing neutralizing antibody to DEN-3 virus (Table 1).

Fifteen serum specimens, from which DEN viruses were recovered, were taken on either the second or the third day of FUO. No virus was recovered from sera taken on other than these 2 days in this study. Among these 15 serum specimens, 10 had no neutralizing antibody to DEN virus, 4 had heterologous antibody and one had homologous antibody to the isolated virus serotype.

All the serum specimens were examined for DEN

Table 1 Results of virus isolation, neutralization test and IgM-capture ELISA

Serum code	Days of fever*	Virus isolation	Neutralization titer to				IgM-capture ELISA
			DEN-1	DEN-2	DEN-3	DEN-4	
B6	2	DEN-1	—	56	—	—	—
B25	2	DEN-2	—	—	—	—	—
B27	2	DEN-2	—	—	27	—	—
B52	3	DEN-1 & DEN-2	-	-	86	-	-
B64	3	DEN-2	-	-	-	-	-
B73	2	DEN-2	-	-	-	-	-
B76	2	DEN-2	-	-	-	-	-
B80	2	DEN-2	-	-	-	-	-
B96	2	DEN-2	-	-	-	-	-
B98	2	DEN-1	-	-	-	-	-
B99	2	DEN-2	-	-	-	-	-
B101	3	DEN-2	-	24	-	-	-
B102	3	DEN-2	-	-	-	-	-
B110	2	DEN-1	-	28	-	-	120

*Days after onset of fever, -Neutralization titer <20, or ELISA titer <100

IgM antibody by IgM-capture ELISA. Of 81 serum specimens, 32 (39.5%) were positive for DEN IgM antibody, indicating confirmed DEN virus infection. Among 15 serum specimens with positive DEN virus isolation, only one (6.7%) specimen was positive for the IgM antibody (Table 1).

Discussion

In 1994, the outbreak of DF/DHF occurred for the first time in a rural commune, Ban Hom, located in Hatxayfonhg district, in Vientiane municipality. By the middle of October, the number of hospitalized cases with clinical diagnosis of DF/DHF was 431 in the district, including 61 cases in Ban Hom. During the epidemics in 1985 and 1987, most of the hospitalized cases were residents of the urban areas of Vientiane municipality. Thus, DF/DHF seems to be expanding to the rural areas in Vientiane municipality.

The sera taken from the children with FUO were studied in the outbreak of Ban Hom commune by performing virus isolation and IgG and IgM antibody assays on the serum specimens collected. Thirty-two (39.5%) of 81 cases were confirmed as DEN infection by the DEN-specific IgM antibody detection, indicating the usefulness of the IgM-capture ELISA. DEN-1 and DEN -2 viruses were isolated from 15 out of 48 serum specimens, indicating that the main causative viruses were those two serotypes in the Ban Hom commune. All 15 cases did not develop severe hemorrhagic manifestation and recovered in several days. Four of the 15 cases were identified as having the preexisting antibody to the heterologous DEN serotype by IgG antibody assay. It should be noted that the case B52, who had had preexisting antibody to DEN-3, was dually infected with DEN-1 and DEN-2 viruses during the outbreak, but did not develop a severe form of DEN infection. This kind of serological and epidemiological approach on a larger scale with both mild and severe cases of DEN infection would give important information on the pathogenesis of DHF.

Acknowledgment

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A NEW BLACK FLY SPECIES OF *SIMULIUM* (*NEVERMANNIA*) FROM SUMATRA, INDONESIA

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Received February 13, 1995/Accepted March 6, 1995

Abstract: *Simulium* (*Nevermannia*) *glatthaari* sp. nov. is described based on the pharate female and male, pupal and mature larval specimens collected from Sumatra, Indonesia. This species is assigned to the *ruficorne*-group of the subgenus *Nevermannia*, and is distinguished from *S.* (*N.*) *aureohirtum* Brunetti, the only known species of this species-group in south-east Asia, chiefly by the number of pupal gill filaments, and the arrangement of the anterior teeth of the larval hypostomium.

In south-east Asia, a black fly species *Simulium* (*Nevermannia*) *aureohirtum* Brunetti has long been the only species belonging to the *ruficorne*-group defined by Crosskey (1969) (e.g. Takaoka and Davies, 1995). In the course of taxonomic studies on the Simuliidae of the Oriental Region, we found another species assignable to the *ruficorne*-group, judged by the genitalia of both adult sexes, which was collected from North Sumatra by Dr. R. Glatthaar. Although adult specimens available are only a few pharate individuals, this species is readily distinguished from *S.* (*N.*) *aureohirtum* by the number of the pupal gill filaments and the arrangement of the anterior teeth on the larval hypostomium, and is here described as the second member of the *ruficorne*-group in south-east Asia.

DESCRIPTION

Simulium (_Nevermannia_) _glatthaari_ Takaoka and Davies, sp. nov.

FEMALE (dissected out of pupa). *Head.* Narrower than thorax. Frons and clypeus densely covered with pubescence. Frontal ratio 1.6:1.0:1.8. Fronto-ocular area well developed. Frons-head ratio 1.0:4.3. Antenna composed of 2+9 segments. Maxillary palp with 5 segments, 3rd segment (Fig. 1) somewhat enlarged, with elongate sensory vesicle, 0.47 × length of 3rd segment. Maxilla with 10 inner teeth and 13-15 outer ones. Mandible with ca. 20 inner teeth and 10 outer ones. Cibarium lacking denticles. *Thorax.* Scutum densely covered with pubescence. Scutellum with pubescence and several erect long hairs. Postscutellum bare. Pleural membrane bare. Katepisternum longer than deep, bare. *Legs.* Fore basitarsus slender, cylindrical. Hind basitarsus parallel-sided. Calcipala well developed (Fig. 3). Claws (Fig. 4) each with a large basal tooth, 0.35 × length of claw. Color markings not yet developed. *Wing.* Costa with 2 parallel rows of short spines as well as hairs. Subcosta fully haired. Basal portion of radius fully haired. *Genitalia* (Fig. 7). Sternite 8 bare medially, furnished with numerous short and long hairs on each side. Anterior gonapophysis thin, membranous, rounded, with ca. 10 short setae; inner borders sowewhat separated from each other. Genital fork with slender stem and wide arms. Paraproct depressed anterolaterally. Cercus much wider than long. Spermatheca not yet sclerotized.

MALE (dissected out of pupa). *Head.* Holoptic; upper eye consisting of large facets in 22 vertical columns and 22 horizontal rows. Antenna composed of 2+9 segments; 1st flagellomere elongated, ca. 2.0 × as long as 2nd flagellomere. Maxillary palp with 5 segments; sensory vesicle (Fig. 2) small, globular. *Thorax.* Nearly as in ♀. *Legs.* Fore basitarsus slender, cylindrical. Hind basitarsus narrow, parallel-sided. Calcipala well developed. Color markings not yet developed. *Wing.* As in ♀. *Genitalia* (Figs. 5 and 6). Coxite large, much longer than wide. Style shorter than coxite, broad in lateral view, with apical spine on posterodorsal edge.

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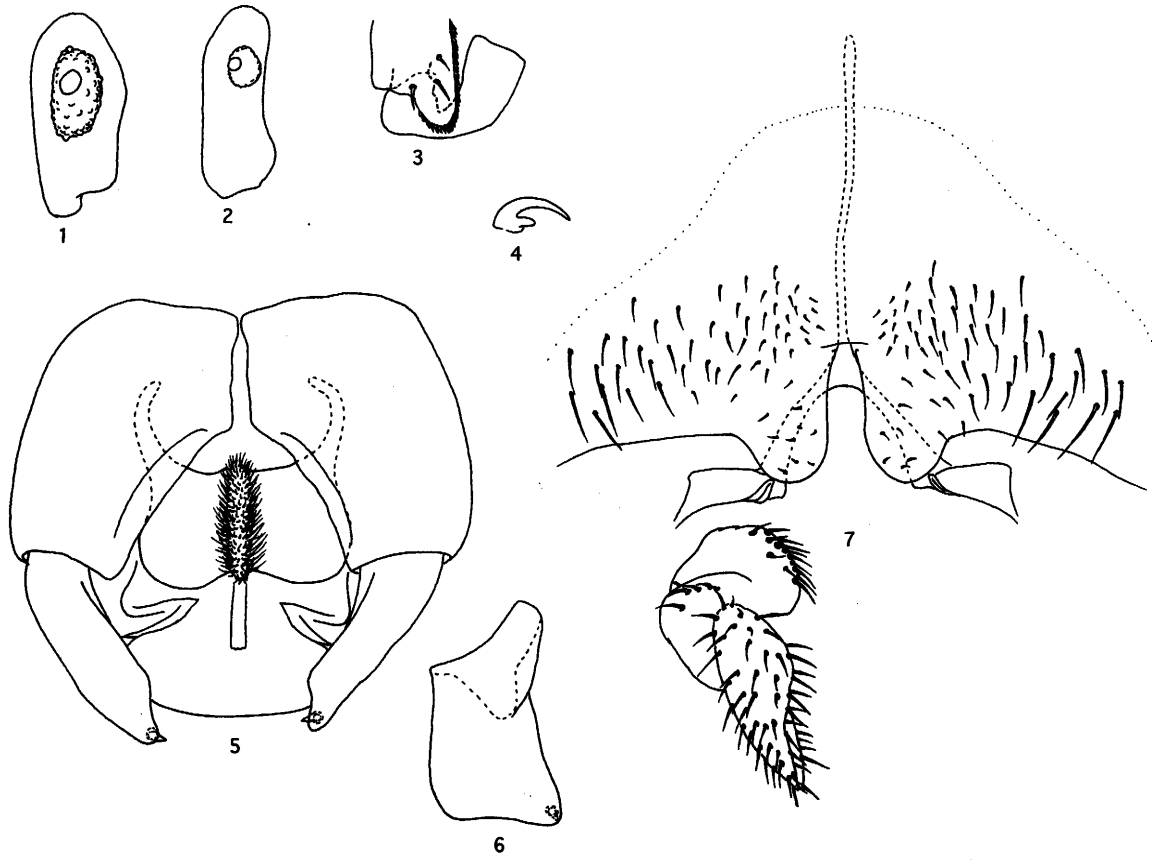
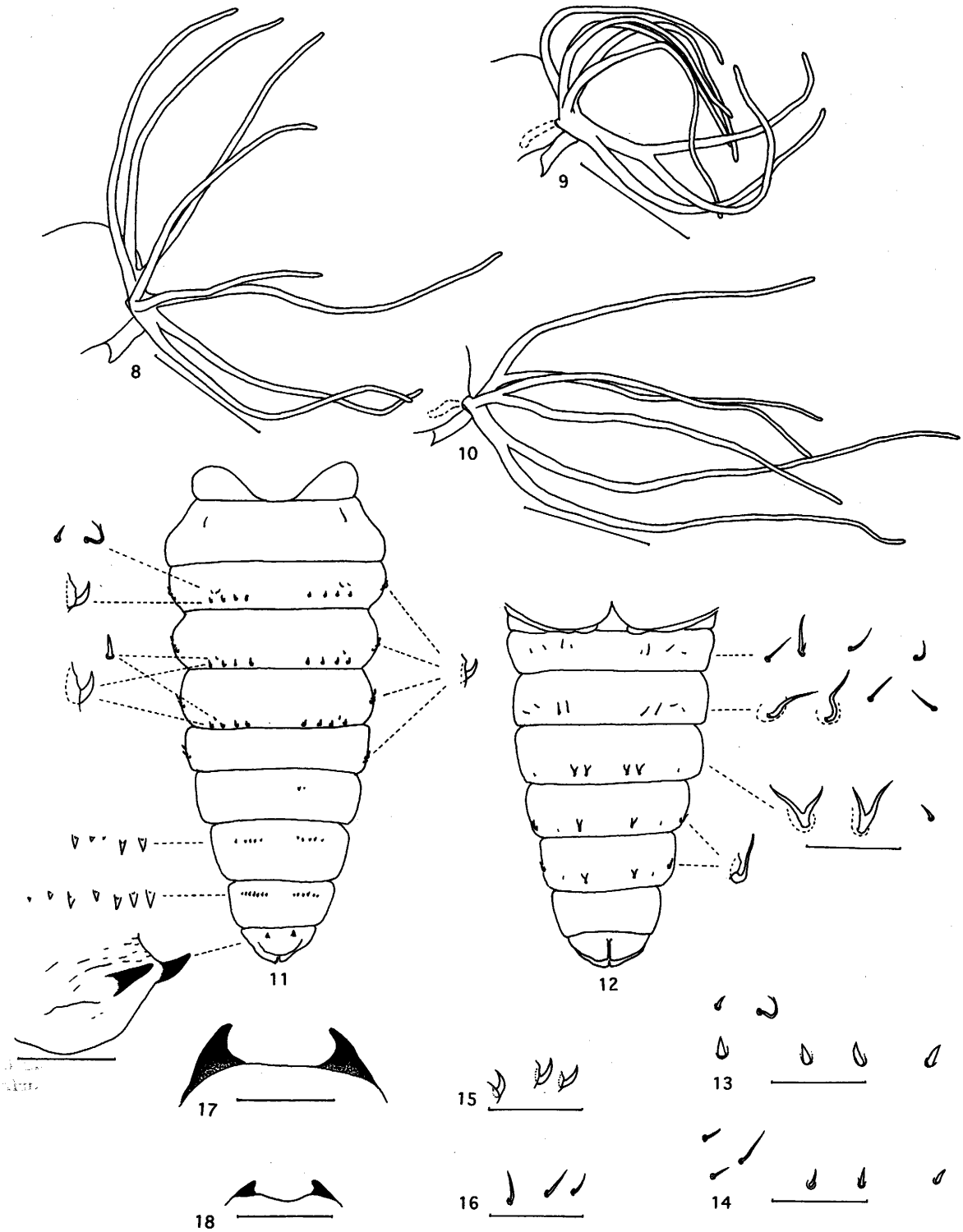


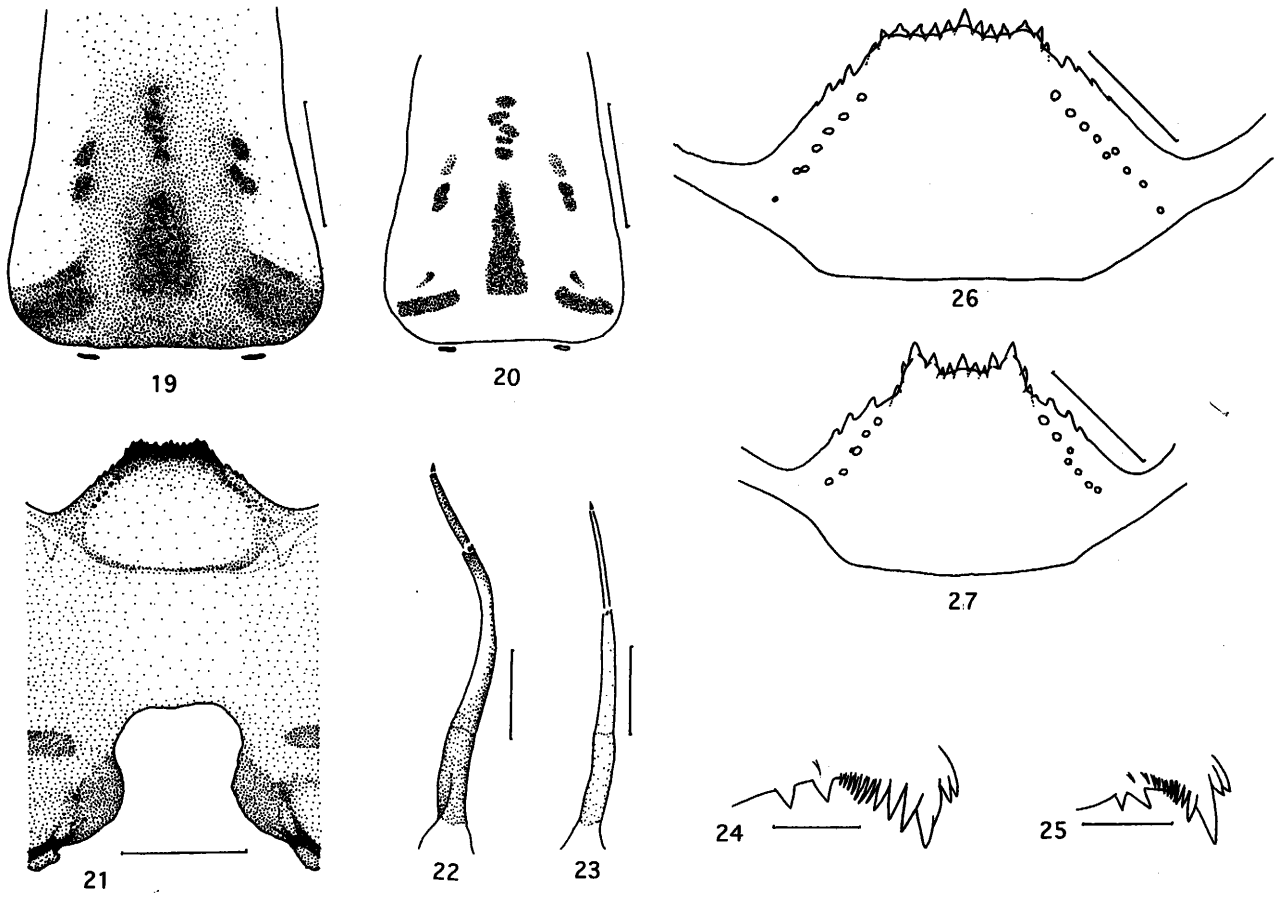
Figures 1-7 Adult of *Simulium* (*Nevermannia*) *glatthaari* sp. nov. 1, ♀ 3rd maxillary palpal segment; 2, ♂ 3rd maxillary palplal segment; 3, distal tip of basitarsus and 2nd tarsal segment of ♀ hind leg; 4, ♀ claw; 5, ♂ genitalia in situ (ventral view); 6, left style (lateral view); 7, ♀ genitalia in situ (ventral view; spermatheca and left paraproct and cercus omitted).

Ventral plate broad, with setose median keel produced ventrally; arms short, curved inward. Paramere with a distinct parameral hook. Median sclerite simple, rod-shaped.

PUPA. Body length (excluding gill filaments) 3.0-3.8 mm. *Head* and *thorax*. Integument dark yellow, moderately covered with small tubercles. Head with 3 pairs of simple, short trichomes (in some pupae frontal trichomes not detected). Thoracic trichomes 6 pairs (3 anterodorsally, 2 anterolaterally and 1 posterolaterally), all simple. Gill (Figs. 8 and 9) with 8 slender filaments, 2.0-3.0 mm long, widely diverged, arranged in 3+2+3 or 2+3+3 filaments on very short stalks in 4 pupae (Fig. 8) but on relatively long secondary stalks in 3 other pupae (Fig. 9), secondary furcation of ventral triplet in 1 of 3 pupae located on distal 2/3 thus showing very long secondary stalk. All filaments tapered apically, with numerous weakly defined transverse ridges, covered with minute tubercles. *Abdomen* (Figs. 11 and 12). Terga 1 and 2 yellow, weakly tuberculate; tergum 1 with a single slender seta on each side; tergum 2 with 4 hooked spines (a little smaller than those on terga 3 and 4) and 2 spinous setae on each side (Fig. 13). Terga 3 and 4 each with 4 hooked spines and 1 small spine on each side. Terga 7 and 8 each with spine-combs and comb-like groups of very minute spines in transverse row. Tergum 5 bare. Tergum 6 occasionally with spine-combs consisting of a few spines. Tergum 9 with comb-like groups of very minute spines and a pair of stout terminal hooks (Fig. 17). Sternum 3 with 4 setae on each side. Sternum 4 with 2 stout spines and 2 slender setae on each side; sternum 5 with a pair of bifid hooks situated close together and 1 seta on each side; sterna 6 and 7 each with a pair of inner bifid and outer simple hooks widely spaced and 1 seta on each side. Lateral surface of segments 2-5 each with 3 hook-like spines on each side (Fig. 15). *Cocoon*. Wall-pocket-shaped, thinly woven, often extending forward and inward, making narrow anteroventral collar, with no anterodor-



Figures 8-18 Pupa of *Simulium* (*Nevermannia*) *glatthaari* sp. nov. except Figs 10, 14, 16 and 18 for *S.* (*N.*) *aureohirtum*. 8, 9 & 10, gill filaments; 11, dorsal surface of abdomen; 12, ventral surface of abdomen; 13 & 14, spinous setae on tergum 2; 15 & 16, lateral spines or setae on segments 2-5; 17 & 18, terminal hooks. Scale 1.0 mm for Figs. 8, 9 & 10; 0.1 mm for Figs. 11-18 (in Figs. 11 & 12 scale applies to enlargements of spines and hooks only).



Figures 19-27 Larva of *Simulium* (*Nevermannia*) *glatthaari* sp. nov. except Figs. 20, 23, 25 and 27 for *S.* (*N.*) *aureohirtum*. 19 & 20, cephalic apotome; 21, ventral surface of head capsule; 22 & 23, antenna; 24 & 25, tip of mandible; 26 & 27, hypostomium. Scale 0.2 mm for figs. 19-21; 0.1 mm for Figs. 22, 23, 26 & 27; 0.05 mm for Figs. 24 & 25.

sal projection.

MATURE LARVA. Body length 5.0-6.0 mm. Body entirely greyish, abdomen gradually widened from 1st to 7th or 8th segment when viewed dorsally. Cephalic apotome brown except narrow pale portion along lateral sides, with distinct dark brown head spots (Fig. 19); ventral surface of head capsule largely darkened. Antenna (Fig. 22) composed of 3 segments and apical sensillum, as long as or a little shorter than stem of labral fan; proportional length of 3 segments from base 2.0:3.1:1.3. Labral fan with ca. 40 main rays. Mandible (Fig. 24) with enlarged comb-teeth decreasing in size from 1st to 3rd tooth; mandibular serrations composed of 2 large teeth somewhat spaced, without supernumerary serrations. Hypostomium (Fig. 26) with row of 9 apical teeth; median tooth longer than others which are subequal in size; lateral margin markedly serrate apically; hypostomal setae 7-9 lying parallel to lateral margins. Postgenal cleft (Fig. 21) small, as long as or a little shorter than postgenal bridge. Thoracic cuticle bare. Abdominal cuticle bare except dorsal surface of posterior segments sparsely covered with minute colorless setae and sides of anal sclerite of last segment covered with numerous colorless setae. Rectal papilla with 3 simple lobes. Anal sclerite of usual X-form, with anterior arms ca. 0.5 × as long as posterior ones; widely sclerotized at base. Ventral papillae absent although there is a large bulge produced laterally on each side. Posterior circlet with ca. 84 rows of up to 22 hooklets per row.

TYPE SPECIMENS. Holotype pharate ♀, associated with pupal skin and cocoon, collected from the foot of Mt. Sibayak, North Sumatra, 13.IV.1977, by Rolf Glatthaar. Paratypes, 1 pharate ♂, 6 pupae (3 of these are

thoracic integument with gill filaments only) and 10 mature larvae, same data as holotype. Types will be deposited in the Natural History Museum, in London, U. K.

ECOLOGICAL NOTES. Pupae and larvae of this new species were collected from trailing grass leaves in a rice-field drainage brook (width 1 m, depth 0.3 m and velocity 10 l/sec), at altitude of 1200 m, at the foot of Mt. Sibayak, North Sumatra. Collected together with this species were mostly pupae and larvae of *S.* (*Gomphostilbia*) *sundaicum* Edwards and *S.* (*Simulium*) *argyrocinctum* de Meijere. One mature larva of *S.* (*N.*) *aureohirtum* was also gathered.

DISTRIBUTION. Sumatra.

REMARKS. This was named after Dr. R. Glatthaar who collected the material.

The present new species is easily assigned to the *ruficorne*-group of the subgenus *Nevermannia* by the unique shape of the genitalia of both sexes, and is distinguished from the other known species of this species-group by the eight pupal gill filaments per side (Figs. 8 & 9) and the arrangement of the anterior teeth of the larval hypostomium (Fig. 26). The pupal gill filaments are six per side in *S.* (*N.*) *aureohirtum,* the only other member of this species-group in south-east Asia, and four in the other known species in the Ethiopian, Palaearctic and Australasian Regions (Crosskey, 1969). All these known species of the *ruficorne*-group have the anterior teeth of the larval hypostomium of which each corner tooth is prominently higher than intermediate teeth, as shown in the Fig. 27.

Apart from these characters, this species differs from *S.* (*N.*) *aureohirtum* by the coloration of the larval cephalic apotome (Fig. 19) and the shape and coloration of the larval body. The latter species shows marked head spots on pale ground color of the cephalic apotome (Fig. 20) and has the pale body color and the abdominal segments 1-4 subequal in size. In addition, there is a difference in the relative size of the mandibular comb-teeth, of which second tooth is larger than the third in this new species (Fig. 24) but is subequal to or shorter than the third in *S.* (*N.*) *aureohirtum* (Fig. 25). The larva of *S.* (*N.*) *glatthaari* is also characterized by the shortened third antennal segment compared to the second one (Fig. 22). The rectal papillar lobes are simple in the new species but usually compound in *S.* (*N.*) *aureohirtum,* though rarely simple as reported by Takaoka (1976), and the new species has more rows of hooklets on the posterior circlet than the latter which has 60 to 70 rows (Puri, 1933; Takaoka, 1979). In the pupa of *S.* (*N.*) *aureohirtum,* spinous setae on the tergum 2 (Fig. 14), those of lateral surface of abdominal segments 2-5 (Fig. 16), and terminal hooks on the tergum 9 (Fig. 18) appear to be less prominent than those of this new species.

As in most other known species of the *ruficorne*-group, adults of *S.* (*N.*) *aureohirtum* were reported to show the characteristic color markings of the legs and the antennae (Puri, 1933). However, such color characters could not be observed in the immature pharate specimens of this new species.

It remains to be studied whether the new species is autogenous, as shown in *S.* (*N.*) *aureohirtum* (Takaoka and Noda, 1979).

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Jpn. J. Trop. Med. Hyg., Vol. 23, No. 2 (1995) pp. 133-137

STUDIES ON ANNUAL CHANGES IN MICROFILARIAL PREVALENCE OF *DIROFILARIA IMMITIS* AMONG HOUSE DOGS FOR 27 YEARS IN NAGASAKI CITY, JAPAN

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Abstract: The positive rate of dogs with *Dirofilaria immitis* microfilariae among house dogs was examined for 27 years from 1968 to 1994 in the southern and northern parts of Nagasaki City. Roles of the number of the main vector, *Culex pipiens pallens* and of RPSS (rate of population utilizing a sewage system) were analyzed statistically in relation to the annual changes in microfilarial prevalence. In the southern and northern parts, there was a clear correlation among three parameters of the positive rate in the dogs, the number of *Cx. p. pallens* and RPSS. The present study proved that the decrease in the positive rate of dogs in both the southern and northern parts is mainly attributable to a decrease in the number of *Cx. p. pallens*, which resulted from the decrease of the breeding places of this mosquito following the spread of the public sewage system and the improvements in the use of roads and open roadside ditches.

INTRODUCTION

Oda *et al.* (1993) previously reported that the positive rate of dogs for microfilariae of *Dirofilaria immitis* increased from 1968 to 1983 in the eastern, western and southern parts of Nagasaki City, but decreased during the same period in the northern part. Moreover, Oda *et al.* (1994a) collected female *Culex pipiens pallens*, the main vector of *Dirofilaria immitis*, from 1983 to 1989, and found that in 1983 *Cx. p. pallens* was extremely prevalent in the southern part but not in the northern part, and after 1986, the prevalence of this mosquito became low in both parts, in parallel with the spread of sewage system. In this study, we analyzed statistically the annual changes of *Dirofilaria immitis* infection rates among house dogs in southern and northern parts of Nagasaki City over the past 27 years after 1968 to clarify the role of the density of *Cx. p. pallens* and the spread of public sewage systems in reducing *Dirofilaria immitis* infection.

PLACES AND METHODS

The survey was conducted in four districts: a southern district (Tomachi) and three northern districts (Sakamoto, Takao and Yamazato). From 1983 to 1994, blood examination was conducted in April or May from about 400 registered dogs in these four districts at the time of vaccination against rabies. One drop (about 20mm^3) of blood was collected from the ear lobe of

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individual dogs. Blood samples were subjected to Giemsa staining and examined for microfilariae under a stereomicroscope.

Mosquitoes were collected using a light trap (16 watt black light) from 1972 to 1989 in Tomachi and from 1967 to 1994 in Sakamoto. Each trap was usually operated once or twice a week in the May-October period. The details of the sites and methods of mosquito collection were previously reported (Oda *et al.*, 1993; 1994a). The districts in four parts (eastern, western, southern and northern) of Nagasaki City were derived from the residential list used by Suenaga *et al.* (1971).

RESULTS

1. Annual changes in microfilarial prevalence of *Dirofilaria immitis* among house dogs

Table 1 shows the annual changes of the positive rate (percentage of dogs having microfilariae) in each of the four districts during the 1968-1994 period. The annual changes in positive rate were examined by the linear trend test of Armitage (1955). The positive rate of dogs generally decreased in all districts, although some fluctuations were noted. This decrease during the 27 years was significant ($P<0.01$) in all districts.

2. Incidence of new infection among the dogs

The incidence of new infection in each year from 1984 was calculated as in Table 2 by dividing the number of dogs which turned from negative to positive for microfilariae by the number of dogs examined among those which had been negative in the previous year. In each district, this incidence decreased from year to year (Table 2). Fig. 1 shows the relationship between the incidence of new infection and the percentage of dogs having microfilariae for the southern part (Tomachi) and the northern part (data in Sakamoto, Takao and Yamazato were combined) of Nagasaki City. In both parts of the city, there was a significant positive correlation between the positive rate of dogs and the incidence of new infection ($P<0.01$). Thus the percentage of dogs having microfilariae decreased clearly from year to year owing to the reduction of new infection in both the southern and northern parts of the city.

3. Annual changes in positive rates of dogs, the number of *Cx. p. pallens* and the rate of public sewage system

Fig. 2 shows annual changes in the positive rate of dogs, the number of *Cx. p. pallens* females caught and the rate of human population utilizing a public sewage system (RPSS). As described previously (Oda *et al.*,

Table 1 Annual changes in microfilarial prevalence of *Dirofilaria immitis* among house dogs in southern and northern parts in Nagasaki City

Year	Southern part		Northern part							
	District name		District name						Total	
	Tomachi		Sakamoto		Takao		Yamazato			
	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs
1968	100	34(34.0)	42	15(35.7)	64	30(46.9)	123	61(49.6)	229	106(46.3)
1977	120	42(35.0)	33	15(45.5)	43	14(32.6)	41	13(31.7)	117	42(35.9)
1983	149	57(38.3)	32	5(15.6)	134	34(25.4)	32	6(18.8)	198	45(22.7)
1984	137	41(29.9)	63	17(27.0)	161	41(25.5)	46	7(15.2)	270	65(24.1)
1985	107	44(41.1)	52	16(30.8)	74	14(18.9)	55	8(14.6)	181	38(21.0)
1986	129	41(31.8)	76	23(30.3)	57	9(15.8)	30	6(20.0)	163	38(23.3)
1987	127	36(28.3)	73	13(17.8)	103	13(12.6)	36	5(13.9)	212	31(14.6)
1988	137	36(26.3)	70	24(34.3)	177	30(16.9)	39	5(12.8)	286	59(20.6)
1989	155	41(26.5)	46	14(30.4)	148	26(17.6)	57	7(12.3)	251	47(18.7)
1990	125	24(19.2)	74	10(13.5)	164	24(14.6)	59	3(5.1)	297	37(12.5)
1991	51	7(13.7)	86	8(9.3)	166	21(12.7)	61	4(6.6)	313	33(10.5)
1992	145	26(17.9)	83	10(12.0)	154	17(11.0)	56	3(5.4)	293	30(10.2)
1993	74	11(14.9)	32	0(0.0)	78	7(9.0)	30	1(3.3)	140	8(5.7)
1994	137	20(14.6)	56	3(5.4)	149	16(10.7)	54	2(3.7)	259	21(8.1)

Table 2 Number and percentage of dogs infected newly with *Dirofilaria immitis* microfilariae

Year	Southern part		Northern part							
	District name		District name						Total	
	Tomachi		Sakamoto		Takao		Yamazato			
	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.
1984	46	8(17.4)	20	4(20.0)	57	4(7.0)	12	1(8.3)	89	9(10.1)
1985	40	8(20.0)	26	3(11.5)	25	0(0.0)	19	1(5.3)	70	4(5.7)
1986	36	6(16.7)	25	2(8.0)	24	1(4.2)	12	0(0.0)	61	3(4.9)
1987	49	4(8.2)	32	3(9.4)	28	0(0.0)	15	0(0.0)	75	3(4.0)
1988	58	4(6.9)	32	1(3.1)	61	5(8.2)	20	0(0.0)	113	6(5.3)
1989	63	4(6.3)	25	3(12.0)	79	7(8.9)	28	1(3.6)	132	11(8.3)
1990	66	4(6.1)	20	1(5.0)	70	1(1.4)	30	0(0.0)	120	2(1.7)
1991	14	1(7.1)	40	1(2.5)	87	1(1.1)	29	0(0.0)	156	2(1.3)
1992	21	0(0.0)	44	1(2.3)	89	1(1.1)	31	1(3.2)	164	3(1.8)
1993	70	1(1.4)	33	0(0.0)	75	2(2.7)	28	0(0.0)	136	2(1.5)
1994	79	1(1.3)	36	0(0.0)	64	1(1.6)	30	0(0.0)	130	1(0.8)

*No. dogs examined : Number of dogs examined among those which had been negative in the previous year.

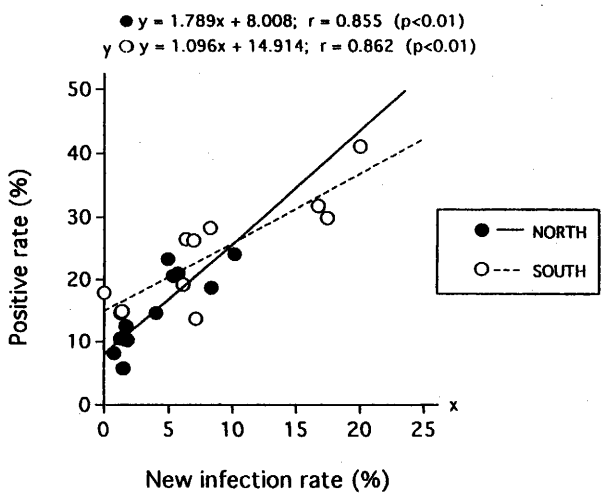

Figure 1 Relationship between positive rate of dogs and new infection rate in southern and northern parts in Nagasaki City.

1994a), the numbers of *Cx. p. pallens* females in southern and northern parts were obtained at Tomachi and Sakamoto, respectively. The RPSS for Tomachi was regarded as the RPSS for the southern part of Nagasaki City, and the RPSS's for the northern part was calculated by averaging the RPSS for Sakamoto, Takao and Yamazato. In the southern part, the positive rate of dogs remained unchanged between 1968 and 1983 but it decreased gradually thereafter, the number of *Cx. p. pallens* was considerably high until about 1980 but it was

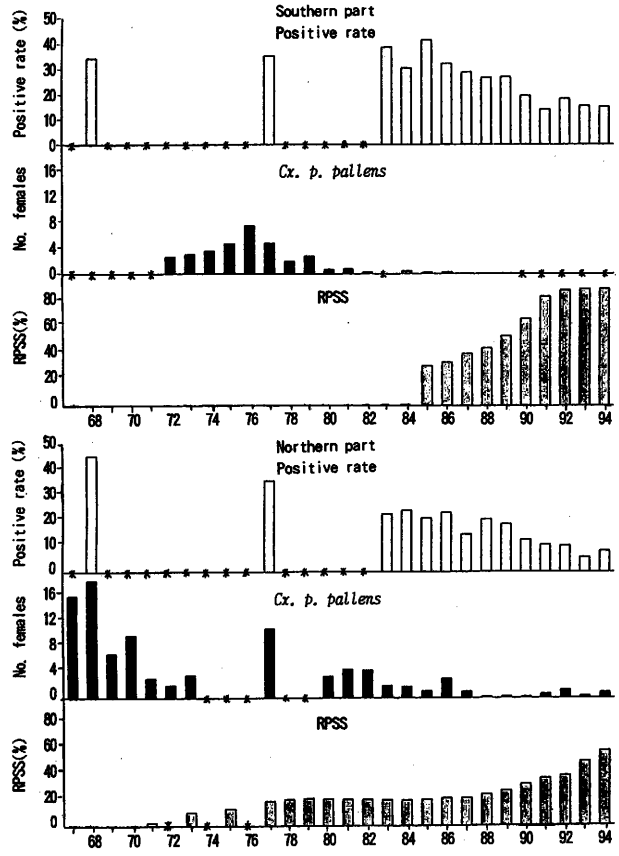

Figure 2 Annual changes in positive rate of dogs, mean number of *Culex pipiens pallens* per night and rate of population utilizing a sewage system (RPSS).
*Data not available

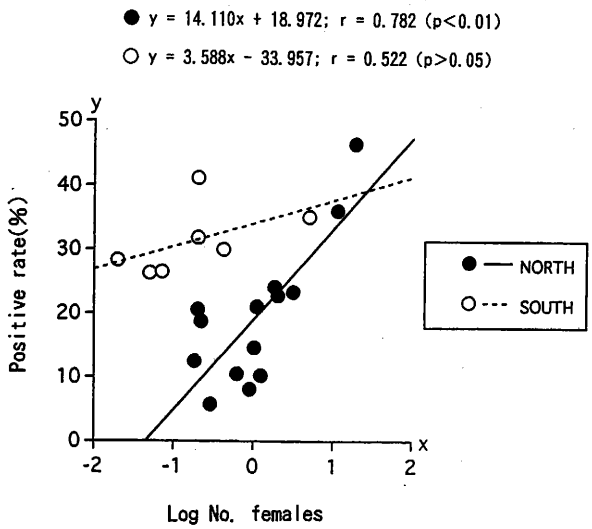

Figure 3 Relationship between positive rates of dogs and number of *Culex pipiens pallens* females.

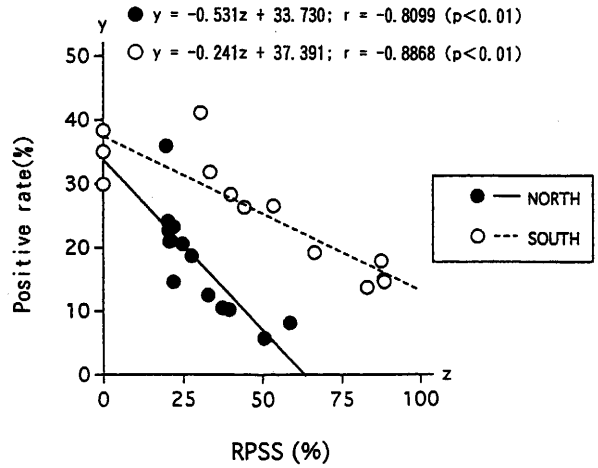

Figure 4 Relationship between positive rates of dogs and RPSS in southern and northern parts in Nagasaki City.
RPSS : Rate of population utilizing a sewage system.

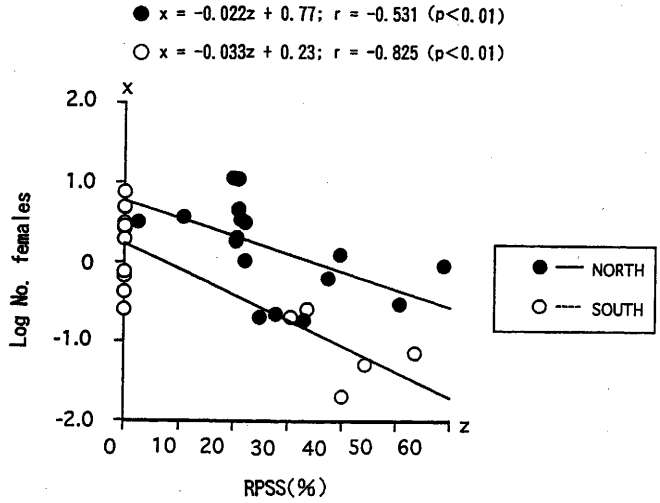

Figure 5 Relationship between number of *Culex pipiens pallens* females and RPSS.
RPSS : Rate of population utilizing a sewage system.

quite low after that year, and the RPSS increased sharply after about 1985. These patterns of annual changes in the three parameters for the northern part were approximately equal to those observed in the southern part, excepting that the percentage of dogs having microfilariae began to decrease earlier in the northern part (around 1977) than in the southern part, and that the number of *Cx. p. pallens* caught in the northern part began to decrease in about 1977. These features of the northern part probably reflect the fact that public sewage systems began to spread in about 1970.

To analyze the relationships among the positive rate of dogs, the number of *Cx. p. pallens* and RPSS, we calculated a correlation coefficient and a regression line between two of the three parameters (Fig. 3-5). In the southern part, the correlation between the positive rate of dogs and the number of *Cx. p. pallens* was not strong, probably because the number of available data in pair was not sufficient. However, it is noteworthy that the percentage of positive dogs in this part had a significant correlation with the RPSS ($P<0.01$) as in Fig. 4. A significant correlation between the number of *Cx. p. pallens* and the RPSS was also noted ($P<0.01$), as shown in Fig. 5.

In the northern part, a clear correlation was noted in any combination of two among three parameters of the positive rate of dogs, the number of *Cx. p. pallens* and RPSS (Fig. 3-5). From these results, it was concluded that the decrease in the positive rate of dogs in both the southern and northern parts is attributable to a decrease in number of *Cx. p. pallens,* which was an outcome of a decrease of the breeding places of this mosquito following the spread of public sewage systems.

DISCUSSION

The present study revealed correlations among the positive rate of dogs, the number of female *Cx. p. pallens* caught and the RPSS, and that the correlation was particularly strong between the positive rate of dogs and the RPSS. In view of these findings, the decrease in the percentage of positive dogs observed apparently in both parts of this city after 1983 may be explained as follows. In public sewage systems, the sewage including all house

sewage but excluding rain water is directly sent to the sewage processing plant. Under this sewage system, clean rain water containing little organic substance in open road side ditches does not constitute significant oviposition sites of mosquitoes. As a result, the sewage system reduced the number of breeding places of *Cx. p. pallens*. The number of breeding places of this mosquito was further reduced by the improvement of roads and open roadside ditches, in addition to the development of public sewage systems. Accordingly the number of female *Cx. p. pallens* decreased, and the transmission of *Dirofilaria immitis* to dogs by mosquitoes was reduced.

Contrarily, the positive rate of dogs increased greatly during the 1968-1977 period, in Koga, Toishi, Fukuda and Hayasaka (the four newly populated residential areas at margins of Nagasaki City) (Suenaga *et al.*, 1980). This is explained by the fact that no public sewage systems were available in any of these four districts at that period. The lack of sewage systems probably resulted in the formation of many breeding places of *Cx. p. pallens*, leading to the increase in the percentage of positive dogs by a large number of vector mosquitoes.

If the number of *Cx. p. pallens* in both the southern and northern parts remains unchanged or decreases from the current level on, the positive rate of dogs will further decrease. The current number of *Cx. p. pallens* may have already approached critical vector density, as pointed out by Wada *et al.* (1984), but this needs to be further studied. As pointed out in a previous paper (Oda *et al.*, 1994a), it is also likely that secondary vectors (*Culex tritaeniorhynchus* and *Aedes albopictus*) will play a relatively more important role in the future, following a decrease in the number of *Cx. p. pallens*.

The number of stray dogs may be another factor influencing the transmission. The number, as given by the number of arrested dogs (unpublished data) decreased in about 1972 and thereafter remained little changed in Nagasaki City. From this, stray dogs are not considered to be an important factor for the decrease of positive rate.

According to our questionnaire survey in 1989 and 1993, the number of households which keep dogs indoors has been increasing (Oda *et al.*, 1994b). It is not clear whether this change contributed greatly to the reduction in positive rate of dogs.

It is known that the preventive drugs of diethylcalbamazine and ivermectin which has recently been developed have the excellent effects against *Dirofilaria immitis*. However, according to our questionnaire survey in 1989 and 1993, the percentage of dog owners who used the preventive drugs was not high (Oda *et al.*, 1994b). Therefore, these drugs do not seem to contribute greatly to reducing *Dirofilaria immitis* infection.

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Short communication

HIGH PREVALENCE OF SEXUALLY TRANSMITTED DISEASES IN AN AREA IN MOZAMBIQUE

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Each year 10 million Japanese travelers and workers visit various foreign countries, including Asia and Africa. Sexually transmitted diseases (STD)s are now more common in many developing countries than in the developed nations (De Schamphelerie *et al.*, 1990; Mosha *et al.*, 1993; Nkowane, 1991). Evidence indicates that the classic STDs facilitate the transmission of human immunodeficiency virus (HIV) infection (Pepin *et al.*, 1989). Particularly compelling is the need to prevent fetal death, prematurity, and neonatal complications by STDs, especially in developing countries (Schulz *et al.*, 1987).

Whereas many studies have been conducted on the prevalence of STDs in urban populations of developing countries, data are sparse on their prevalence in rural areas, there is little reliable information on the risk of STDs especially in the developing countries. This is because most studies on the prevalence of STDs have been conducted in selected populations, such as inpatients or outpatients being administered medical treatment for the infection. The true prevalence of STDs can be derived only from data on unselected populations.

Our objective was to investigate the incidence of STDs in people in rural areas of in Mozambique and to identify factors involved in the dissemination of the infections.

We conducted our investigation in an area around Quelimane in Mozambique in 1991 and 1994. Blood samples were taken from 88 people, 59 males (average age; 32.01±11.65, range; 12-60) and 29 females (average age; 22.61±8.25, range; 11-42) in 1991, and from 38 males (average age; 29.03±8.03, range; 18-52) in 1994. All subjects were socially active and believed themselves to be healthy. Laboratory tests of serum were conducted by a commercial laboratory, BML co. in Tokyo. The methods used were reversed passive hemagglutination (RPHA) for hepatitis B surface antigen and passive hemagglutination (PHA) for hepatitis B surface antibodies, Glass plate tests, agglutinin,

Table 1a Subjects testing positive for STDs in 1991

	STS	TPHA	FTA-ABS*	Syphilis†	HIV‡	HBsAg	HBsAb	Subjects
Male	8	8	4/5	6	1	3	15	59
Female	4	4	0/1	3	1	3	7	29

Table 1b Subjects testing positive for STDs in 1994

	STS	TPHA	FTA-ABS*	Syphilis†	HIV‡	HBsAg	HBsAb	Chlamydia	Subjects
Male	16	17	3/7	14	1	3	12	22	38

* ; Ratio of positive tests to number of people tested
† ; Serologically diagnosed syphilis:
Either findings: 1) STS (+) and TPHA (+)
2) STS (+) and TPHA (−) and FTA-ABS (+)
‡ ; Afterward there was no remnant serum that was positive for HIV-2 using ORTHO HIVCHEK™ System 3 and Labblot-2.

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Table 2a Number of serologically proven STDs among subjects tested in 1991: HBV, syphilis and HIV

	None	Single	Double	Triple	Subjects
Male	36	19	3	1	59
Female	17	11	1	0	29

Table 2b Number of serologically proven STDs among subjects tested in 1994: HBV, Treponema pallidum, HIV and Chlamydia trachomatis

	None	Single	Double	Triple	Subjects
Male	7	12	13	6	38

treponema pallidum hemagglutination (TPHA) and fluorescent treponema antibody absorption (FTA-ABS) for syphilis, enzyme-linked immunosorbent assay (ELISA) (HTLV-3EIA, Dainabbott) and western blot (Labblot-1, Fuji-Rebio) for HIV-1, and fluorescent antibody for IgG against Chlamydia trachomatis.

As shown in Table 1a, in 1991, we found no significant difference between the male and female subjects in incidence of STDs. Nine of the 88 subjects (10.2%) were serologically diagnosed with syphilis; two (one male and one female) were HIV carriers; and 28 were infected with hepatitis B virus (HBV). As shown in Table 2a, 35 subjects (39.7%) had at least one of the STDs we tested for.

In 1994, 14 of 38 subjects were serologically diagnosed with syphilis; one was an HIV carrier; 15 were infected with HBV; and 22 were infected with trachomatis: (Table 1b). A total of 31 (81.6%) either had STDs or a history of STDs and half of the 38 people tested in 1994 had a history of two or three different STDs (Table 2b).

These findings suggest a high prevalence of STDs in the general population of Quelimane. According to Lijestrand *et al* (Lijestrand *et al*., 1990) the prevalence of active syphilis in pregnant women in six of the country's 10 provinces ranged from 1.6% (Muiane, community-based survey) to 9.8% (Maputo, suburban antenatal care unit). Possible explanations for the high prevalence of STDs we found include the collapse of health services during the civil war and changing due to the customs in rural areas.

The incidence of chlamydial infection in Quelimane was higher than the reported global incidence (De Schryver and Meheus, 1990). We consider this result to be due in part to the difference in the test methods employed test methods. We found a higher prevalence of STDs in 1994 than in 1991. This difference may result from the number of tested cases 30 out of 38 in 1994 and 11 out of 88 in 1991, respectively, since public health services who reduced in the rural villages.

We could get little informations on STD symptoms from interviews with the subjects. Thus, the near-total absence of reported symptoms in these cases precludes a screening program for STDs based on symptoms alone.

Vuylsteke *et al* (Vuylsteke *et al*.,1993) have reported a high prevalence of Haemophylus ducreyi antibodies in the Mozambican population. We did not have the proper assay system to test for this classic venereal disease.

As mixed infections are common, syphilis and chlamydia screening for every STD patient should be instituted. Mozambique in the 1990's appears to be facing problems associated with syphilis and other STDs similar in severity and magnitude to those faced by the western world in the early 1900's (De Schryver and Meheus, 1990). We also need to be concerned about other non-tested STDs.

The low prevalence of HIV infection contrasts with the prevalence of the other STDs. Only two males and one female (2.4%) were confirmed as HIV-positive. Because of the civil war, Mozambique has been relatively isolated from neighbouring countries such as Malawi, Tanzania, and Zambia, all of which have a high prevalece of HIV infection. However, the high prevalences of STDs that we found suggests that circumstances in Mozambique are favourable for an explosive HIV epidemic, especially now that the civil war has ended and the roads are again open.

In conclusion, because of the very high rates of STD in Mozambique and the great potential for the spread of HIV infection, STD control and management must assume top priority. Case management at the primary health care level provides an oppotunity to educate people about their high-risk behaviour. Although the acquired immune deficiency syndrome, human papillomavirus infection, and other newly identified STDs, are attracting much attention in Japan, we must remain viligant for the serious sequelae of syphilis and gonorroea, especially in areas where these classic STDs remain uncontrolled.

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