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From the Editor's desk

Dengue fever and dengue haemorrhagic fever have emerged as major health problems of the 21st century. The First International Conference on Dengue at Chiang Mai, Thailand, during November 2000, adopted a declaration entitled "Chiang Mai Declaration on Dengue/Dengue Haemorrhagic Fever" for strengthening efforts to control DF/DHF in the new millenium by endorsing the WHO global strategy for prevention and control, increased political commitment, active intersectoral partnerships, strengthening capacity of health systems and by development application and evaluation of new/improved tools.

In the South-East Asia Region, Bangladesh has reported the first ever epidemic of DHF during 2000. Clinical management practices achieved new heights by bringing down case fatality rates below 0.2% in Thailand. Other Member Countries are also following similar trends. Community-based programmes involving intersectoral linkages have yielded good results. Attempts at social mobilization and communication for behavioural impacts (COMBI) are key elements to be pursued by the Member Countries.

The deadline for the receipt of contribution for the next issue of the **Dengue Bulletin** (Volume 25) is 30 April 2002. Contributors are requested to follow the instructions carefully while preparing the manuscript. Contributions must be accompanied by computer diskettes using MS Word for Windows and should be sent to the **Editor, Dengue Bulletin**, WHO/SEARO, Mahatma Gandhi Road, I P Estate, Ring Road, New Delhi – 110 002, India; E-mail: dengue@whosea.org.

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Major Epidemics of Dengue in Taiwan in 1981-2000: Related to Intensive Virus Activities in Asia

By

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Abstract

Major epidemics of dengue fever/dengue haemorrhagic fever/dengue shock syndrome (DF/DHF/DSS) in Taiwan in the last 20 years were strongly associated with imported cases. The data analysis showed that three major epidemics including the first outbreak of DF in Hsiao-Liu-Chiu in 1981 since 1950s, the largest epidemic of DF in Pingtung and Kaohsiung in 1987-1988, and the most important epidemic of DHF in Tainan in 1998, had statistically significant association with the increasing numbers of dengue cases in several Asian countries before or during the epidemic ($p=0.028$, $p=0.043$, $p=0.08$, respectively). Imported dengue cases in Taiwan were primarily travellers who had come from Thailand, Indonesia, the Philippines, Myanmar and Malaysia. The earlier indigenous dengue cases in Taiwan always used to appear later than the peaks of monthly-distributed dengue cases in those countries. On the other hand, active surveillance, epidemiological investigation, mosquito control activities and effective public health administration at various levels indeed reduced the number of confirmed indigenous dengue cases in 1996 and 1997. Taiwan's experience in surveillance further proves the feasibility of avoiding a large-scale epidemic of DHF/DSS as well as hyper-endemicity of dengue viruses. International collaboration in surveillance, epidemic information exchange, and statistical analysiscan play an important role in the prevention and control of DF/DSS in the future.

Key words: Dengue, Viral haemorrhagic fever, surveillance, imported case, Epidemiology Taiwan

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Introduction

Dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the most important re-emergent arbovirus diseases of humans[1]. The epidemiological activities have intensified in the past 20 years because of rapid population growth, uncontrolled and unplanned urbanization with inadequate systems of water and solid waste management, increased frequency of air travel and usage of artificial containers, which provide excellent breeding sites for the mosquitoes[2]. Most importantly, epidemics of the severe form of DHF/DSS occur where increased transmission of multiple serotypes of dengue viruses becomes hyper-endemic and has resulted in more fatal cases[3]. In recent years, the expanding geographical spread of dengue viruses and their mosquito vectors has facilitated a dramatic increase in the frequency of epidemics of DF/DHF/DSS in the Western Pacific, South-East Asia and South American regions[4]. Dengue will continue to be a growing public health problem in most tropical and subtropical regions of the world in the 21st century, unless more effective measures are taken to control the main vectors, *Aedes aegypti* and *Aedes albopictus*[5]. Therefore, the World Health Assembly urged Member States to strengthen their national and local programmes for the control of DF/DHF. WHO has published guidelines on different aspects of dengue[6,7]. International efforts in formulating collaborative prevention and control strategies among dengue endemic countries become more and more important and necessary in the future.

Dengue surveillance, the most cost-effective prevention and control approach, is generally neglected as it is difficult to maintain with continuous enthusiasm by public health professionals at both local and national levels[8]. Surveillance is particularly useful in identifying index cases early, followed by prompt mosquito control activities. In countries without intensive dengue control activities and where imported dengue cases can spread rapidly because of the presence of *Aedes aegypti* and *Aedes albopictus,* surveillance of febrile patients returning from these areas provides valuable epidemiological information for future planning, implementation and evaluation. In fact, active surveillance and monitoring of indigenous transmission of dengue is a crucial step to avoid a large-scale epidemic. Unfortunately, difficulties in dengue surveillance are multi-factorial, including: (i) a high proportion of mild and asymptomatically-infected individuals in areas where dengue is endemic or sporadic; (ii) a complex disease whose symptoms/signs are not distinguishable from other common febrile illnesses; (iii) unrecognized disease by physicians in many places where DF and/or DHF cases rarely occur; (iv) few or inappropriate specimens collected promptly for laboratory confirmation and haematological tests; and (v) insensitive clinical surveillance because most Chinese patients prefer to be self-treated with non-prescriptive drugs rather than visit doctors.

Historical epidemics of dengue in Taiwan had been documented in 1902, 1915 and 1922 in Penghu Islet, in 1924 and 1927 in southern Taiwan, 1931 in Tainan, and 1942-43 in island-wide Taiwan[9], partially because the dengue cases came from epidemic/endemic countries in South-East Asia, and the high prevalence of water storage tanks among households during wartime. The virus was silent for almost 37 years until 1981, when the DEN-2 epidemic

of DF recurred on the islet of Hsiao-Liu-Chiu, which belongs to the Pingtung County administratively and located off southern Taiwan[10]. Fortunately, it did not result in an epidemic in main Taiwan Island. The DEN-1 epidemic of DF then exploded in 1987-1988 in southern Taiwan, particularly Kaohsuing and Pingtung. The first DHF epidemic due to DEN-3 appeared in l994. Four years later, the largest epidemic of DHF caused by DEN-3 occurred in Tainan. Up to now, Taiwan is a very unique dengue epidemic country where the total number of reported DHF/DSS cases have remained below 30 for 57 years since 1943, even though many tourists travel between Taiwan and other Asian countries where the numbers of DHF/DSS were very high. The specific aim of this study was to analyse the trend of major dengue epidemics in Taiwan and whether it was related to the status of dengue in other Asian countries, imported cases, and success and failure in the surveillance system.

Materials and methods

Study area

Taiwan, located 160 km from the south-east coast of mainland China, is about 392 km long and 143 km wide. The population was over 21 million during 1999-2000, with a very high population density. Nearly all Taiwanese are graduated from elementary school and the GNP for the year 2000 was more than US$ 14,000.

Sources of data

Surveillance of dengue in Taiwan

The most unique feature of dengue surveillance in Taiwan is the active epidemiological investigation of each suspected case reported to the Department of Health. This active surveillance system was established in 1988 by the Division of Epidemiology, National Institute of Preventive Medicine. Once physicians at local clinics or hospitals reported suspected dengue cases with persistent fever and one of dengue-like symptoms, the neighbouring 50 households were interviewed to determine the possible source of infection. More than 100 of their blood specimens were mandatory to be collected regardless of their febrile history by local public health personnel after informed consent for dengue-specific IgM test. All DHF cases were evaluated by experienced physicians who had been trained at the Children's Hospital in Thailand. According to the revised guideline by WHO[6], all confirmed dengue cases were defined by positive virus isolation, reverse- transcriptase polymerase chain reaction (RT-PCR), dengue-specific IgM, 4fold serotiter rise or fall by ELISA IgG, or haemagglutination inhibition (HI) antibody. Data of active and passive surveillance were pooled to analyse the trend of the epidemic, the role of imported cases, monthly distribution of indigenous cases, and the effectiveness of surveillance and control.

In addition, the epidemiology unit of infectious disease laboratory at the Institute of Epidemiology, National Taiwan University (NTU), also established 'active sentinel-physician surveillance' by collaborating with several enthusiastic physicians with a public health bent of mind and having experience in reporting dengue cases, in Tainan, Kaohsiung and Pingtung. In early July of each year, special visits to sentinel physicians and medical technologists of the sentinel clinics/hospitals were made for increasing awareness. Suspected blood samples were

delivered to NTU by express mail within eight hours on the same day for RT-PCR test. Once the positive dengue cases were identified by RT-PCR, blood samples from the family members were encouraged to be collected. The national surveillance data were obtained from the National Institute of Preventive Medicine (renamed as the Center for Disease Control after reorganization since July l998).

Other sources of data

Cases of dengue in respect of other Asian countries were obtained from the WHO website[12]. In addition, Dr Sai-Kit Lam provided several annual reports on dengue from Malaysia. Data on dengue in Thailand were also obtained from published papers and government reports in Thailand, Indonesia, the Philippines and Myanmar.

Statistical analysis

All data were entered into the database and analysed by SAS (Statistical Analytical System, Wisconsin, 6.12 version). Due to the small sample size, the Wilcoxon signed ranks test was performed to compare the trend in the increasing number of dengue cases between two consecutive years in those Asian countries that the Taiwanese people liked to visit. The p-values were calculated to test for statistical significance.

Results

A. Recent major epidemics of dengue in Taiwan

Trends of dengue epidemics in Taiwan

In the last 20 years, the epidemic patterns of dengue in Taiwan remained cycled with small-scale outbreaks occurring almost every three years and large-scale epidemics occurring nearly every ten years (Figure 1). The number of reported dengue cases was much higher in 1981, 1987-1988, 1991, l994-l995 and l998 than in other years[11]. Whenever mosquito control activities were not effective enough, the epidemic continued into the following years as was the case with the DEN-1 epidemic in l987-l988. On the other hand, problems in control were also detectable when the total number of the confirmed indigenous dengue cases was higher than that of the confirmed imported cases in 1991, l994, l995, 1998 and 2000. However, when active surveillance was started early enough and multiple channels of reporting were implemented, the total number of confirmed indigenous dengue cases became less than the confirmed imported dengue cases in the years 1996-1997. In other words, the effectiveness of surveillance and control of dengue was easily reflected in whether the number of confirmed indigenous cases increased during that period of time.

Statistical association between Taiwan's major epidemics and dengue activities in Asian countries

To investigate the possible relationship between major dengue epidemics in Taiwan and the status of dengue in other Asian countries at around those times, we reviewed the number of dengue cases reported to WHO from Asia during 1955-1998. A detailed analysis of those cases made it evident that increased numbers of dengue cases were statistically significant in

six countries from 1979 to 1980 (p=0.028), in five countries from 1986 to 1987 (p=0.043), and in nine countries from 1997 to 1998 (p=0.08) (Figure 2).

Prior to the first epidemic of DF caused by DEN-2 in Hsiao-Liu-Chiu in l981, after the long silence of dengue activity since World War II, the Philippines, Thailand, Viet Nam, Indonesia and the Lao People's Democratic Republic had reported increased numbers of dengue cases (p=0.028) (Figure 2). In particular, a series of clusters of the imported cases of this epidemic were fishermen who came from the Philippines, which was also consistent with the striking increase in the number of reported dengue cases from 392 in 1979 to 968 in 1980 in that country.

A similar trend was noticed before the DEN-1 epidemic in Pingtung and Kaohsiung during 1986-1987 when the dengue status in Asia was also statistically high in five countries, including Indonesia, Thailand, Malaysia, Myanmar and Viet Nam, when the most number of imported dengue cases were reported in Taiwan (p=0.043) (Figure 2). This was the time when many Taiwanese businessmen and visitors went to Thailand after rapid increase of the GNP in l987, which was on parallel with the fact that dengue cases had a striking 6.25-fold increase in Thailand from 27,837 in l986 to 174,285 in l987. In addition, Viet Nam also faced a severe dengue problem in 1987 with 354,517 reported cases, a figure much higher than the 46,266 cases reported in l986.

In 1998, the pandemic of dengue spread in many countries. The majority of the confirmed imported dengue cases in Taiwan in l998 primarily came from five countries: Thailand, Indonesia, Malaysia, the Philippines and Myanmar[20] where the number of dengue cases had a statistically significant increase from l997 to l998 (p=0.043) (Figure 2). Of these countries, Indonesia reported the highest increase from 30,730 cases in l997 to 71,087 in l998, with many DEN-3 viruses isolated in 1998.

With the opening of the south-east Asia business policy advocated by the Government of Taiwan since 1996, more commercial exchanges between Taiwan and Indonesia took place. Interestingly, DEN-3 was also isolated from the imported cases of two brother businessmen, who returned from Indonesia through an active surveillance system established at the National Taiwan University. Subsequently, the predominant serotype of dengue virus isolated in most of the confirmed indigenous dengue cases, including DF and DHF/DSS, was also DEN-3 during this largest epidemic of DHF in Tainan since the 1950s.

Similarities and differences in dengue epidemic patterns in five major Asian countries related to Taiwan dengue epidemics

A comparison of the dengue epidemic patterns in these five countries from where Taiwan's imported cases primarily originated, revealed that Thailand also had a 3-year cycle of small-scale epidemics in the years 1987, 1990, 1993 and 1997 (Figure 2). The years of 1987 and 1998 when Thailand

reported the highest number of dengue cases also witnessed the largest epidemic of DF (1987) and of DHF (l998) on the main Taiwan island. However, the peaks of Thailand's dengue cases in those important years were much higher and earlier than the peaks in Taiwan. On the other hand, the time intervals of dengue epidemics in Indonesia were much longer, with the highest number of cases reported in l998. The cycling of dengue epidemics in the Philippines and Myanmar was not clear. All these five countries had almost the highest number of dengue cases in 1998 as compared to other years during the last 20 years of the 20th century.

B. Contribution of imported cases and monthly distribution of dengue cases in major epidemics in Taiwan

(1) **1981**. The dengue fever epidemic was attributed to four successive groups of a total of 71 fishermen, who crossed the country's fishing border, were arrested and detained in the Philippines for some time, and acquired dengue the infection there[10].

(2) **1987-88**. The DF epidemic in l987 resulted in 1387 reported and 488 laboratory-confirmed DF cases, with most of them distributed in Kaohsiung city, Kaohsiung county and Pingtung county[9]. The Government officials at the Bureau of Communicable Disease Control received the first reported case of one female patient with persistent fever, rash, itching and red swelling of the feet and hands on 19 November l987 from a physician at the Mackay Memorial Hospital in Taipei[9] (Figure 3). The epidemic became known only after two months during which it spread affecting a total of 152 cases in Pingtung, and even more (225 cases) in Kaohsiung, because most of the physicians there were not aware of dengue since there had been no activity for almost 44 years.

(3) **1998**. The imported dengue cases in 1998 appeared even during winter months (January-February) and then continued to increase during summer months (July-September). The indigenous dengue cases began to rise in August but peaked in November. Finally, the epidemic resulted in 1430 reported and 348 confirmed dengue cases, which included 334 cases of DF and 14 cases of DHF (Figure 4).

C. Monthly distribution of dengue cases in some Asian countries

Malaysia and Viet Nam, where many Taiwanese prefer to visit, witnessed rising trends of dengue cases about 2-3 months earlier than in Taiwan in l998 (Figure 5). A similar pattern was also noticed in the distribution of dengue cases in Thailand, with DHF cases peaking in June-August of that year (data not shown). On the other hand, indigenous dengue cases in Taiwan began to rise towards the end of summer and after the peak months of DF/DHF in those Asian countries which have a close relationship with Taiwan.

Figure 1. Number of reported, confirmed imported and indigenous dengue cases in Taiwan in 1981 and 1987-2000

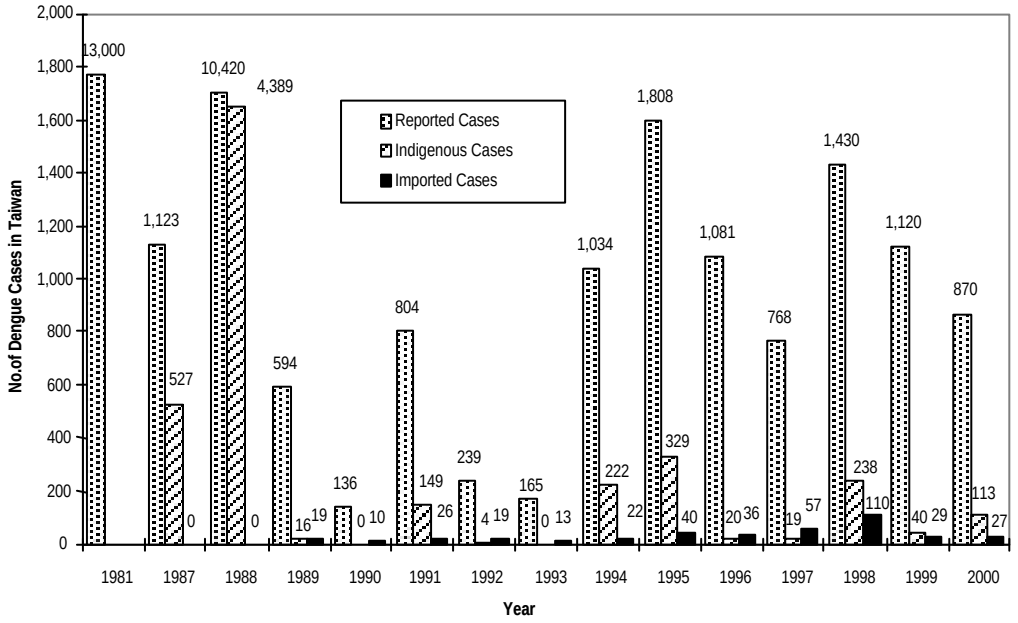


Figure 2. Number of dengue cases reported in five countries, including the Philippines, Thailand, Viet Nam, Indonesia, Lao People's Democratic Republic, 1979-1998

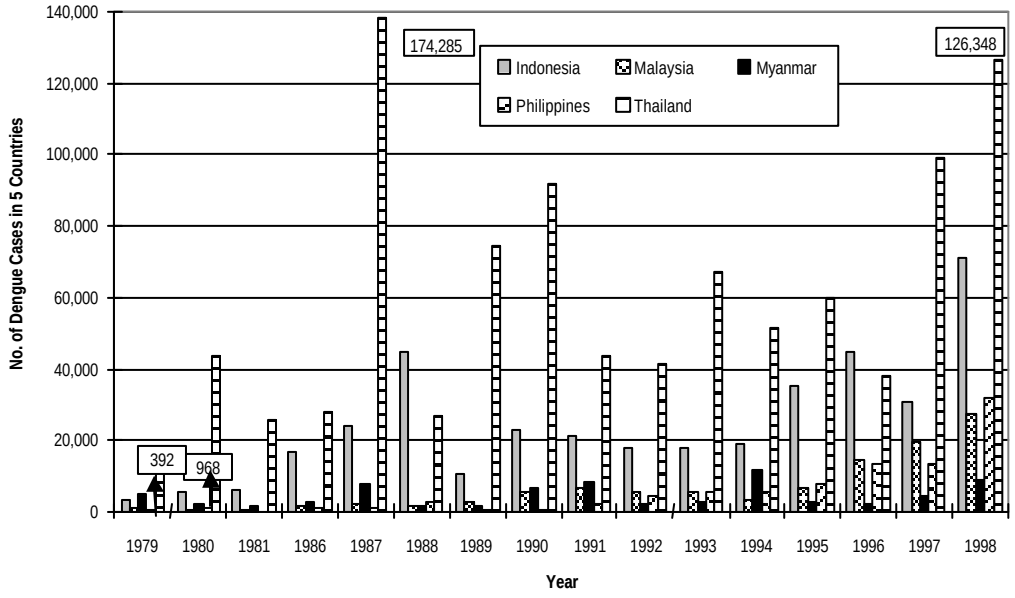


Figure 3. Number of dengue cases and incidence rates in October l987- December l988 in Taiwan

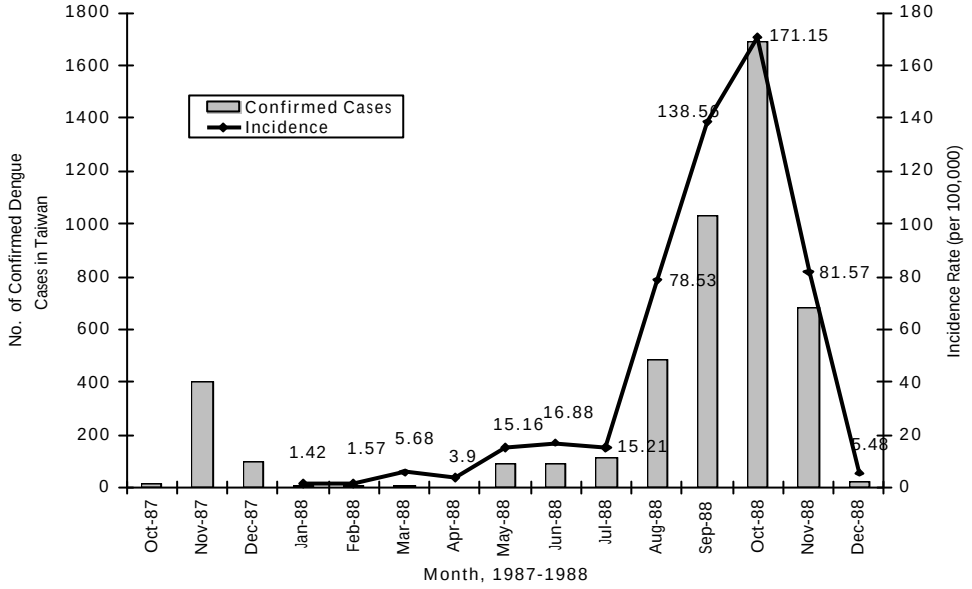


Figure 4. Monthly distribution of dengue cases in 1998 in Taiwan

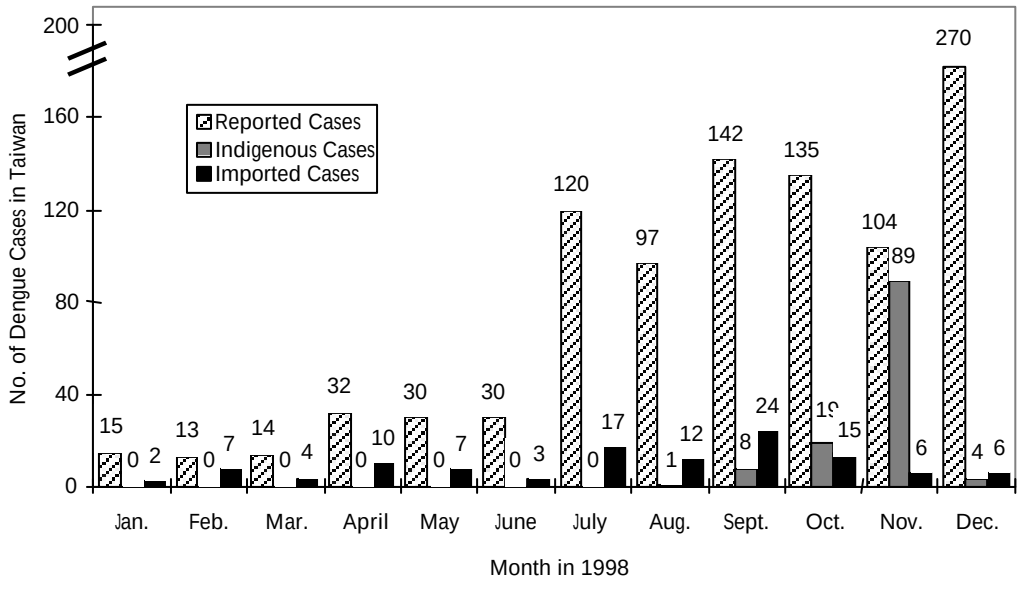
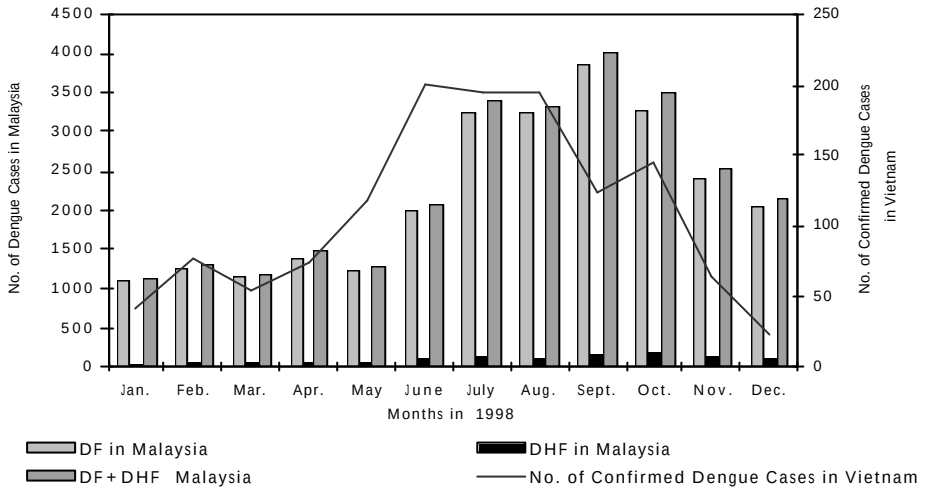


Figure 5. Monthly distribution of dengue cases in Malaysia and Viet Nam in 1998



Discussion

According to WHO reports of dengue cases and deaths during 1995-1998, many countries and areas such as Malaysia, Cambodia, Viet Nam, Thailand, the Philippines, Indonesia, Myanmar in the south-east Asia; Guam, Cook Islands, Fiji, New Caledonia, Kiribati in the western Pacific, and Brazil, Venezuela and Colombia in Latin America had experienced unusually higher levels of dengue/dengue haemorrhagic fever activity in l998 than in previous years[12]. Therefore, a future pandemic of dengue is possible. Countries, which are located in areas neighbouring those where the transmission of dengue virus is intensive, should use surveillance as the most effective prevention and control strategy for dengue.

Dengue epidemics always happen when the chain of transmission cannot be interrupted because of under-diagnosis, incomplete or delayed reporting, and lack of specimens for laboratory diagnosis. Laboratory surveillance of dengue confirms DF and DHF cases, but it is also most helpful in monitoring serotypes and strains circulating in the population. For example, the introduction of a new serotype, which has not been conferred enough high-herd immunity, may serve as an important indicator of future epidemics of DHF/DSS. Therefore, strengthening surveillance of imported cases at local level, coupled with international collaboration on exchange of epidemiological information, will upgrade and improve the efficiency of dengue control.

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Dengue Control in Vanuatu: Towards an Integrated Vertical and Horizontal Control Programme

By

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Abstract

Like in most Pacific island countries, dengue is not endemic in Vanuatu, and so, dengue transmission begins with the introduction of the virus via infected humans or mosquitoes into the country. The Ministry of Health, Vanuatu, has been successful in containing dengue transmission whenever the virus has been introduced, through an integrated approach using vertical and horizontal components. The identification and containment of dengue cases is dependent on both active and passive surveillance of the human population coupled with larval surveys. During epidemics, control measures emphasize clinical case-management, health education and mosquito control (larval breeding source reduction, larviciding and indoor focal house spraying in the homes of cases). During non-transmission periods, an active mosquito larval source reduction programme with community participation is emphasized along with training for health-care providers and health education of the public.

Key words: Dengue, active-passive surveillance, source reduction, Vanuatu.

Introduction

The Republic of Vanuatu is located in the Western Pacific. The archipelago that constitutes the republic contains over 80 islands and is located between Australia to the west and Fiji to the east, with the Solomon Island to the north-west and New Caledonia to the south-west. The population of Vanuatu is 186,678, with major concentrations of 29,356 and 10,738 people in the capital, Port Vila, and Luganville, respectively. Around 79% of the population lives in rural areas. Annual maximum temperatures range from 28° C in February to 23° C in July, with an annual average rainfall of 200-300 cm. The wet season occurs from December to March.

Brief history of dengue in Vanuatu

The first known cases of dengue in Vanuatu occurred in 1971-72 when dengue serotype-2 was found. Since then, all four dengue serotypes have been found in Vanuatu, with transmission occurring in 1975, 1980, 1985, 1989 and 1998. The first known cases of DHF/DSS were described in

1989 when infections with serotypes-1 and 3 were introduced after the dengue-2 outbreaks in 1971-72 and 1975 as well as the dengue-4 outbreak in 1980. The largest epidemic to date, with 3,300 suspected cases, occurred in 1989 (Table 1).

Table 1. *The recent history of dengue transmission in the Republic of Vanuatu*

Year	Number Cases	Serotype	DHF/ DSS	Fatalities
1971-1972	NA	2	No	NA
1975	NA	2	No	NA
1980	16	4	No	NA
1989	3.300	1,3	Yes	12
1998	120	2	No	0

NA = not available

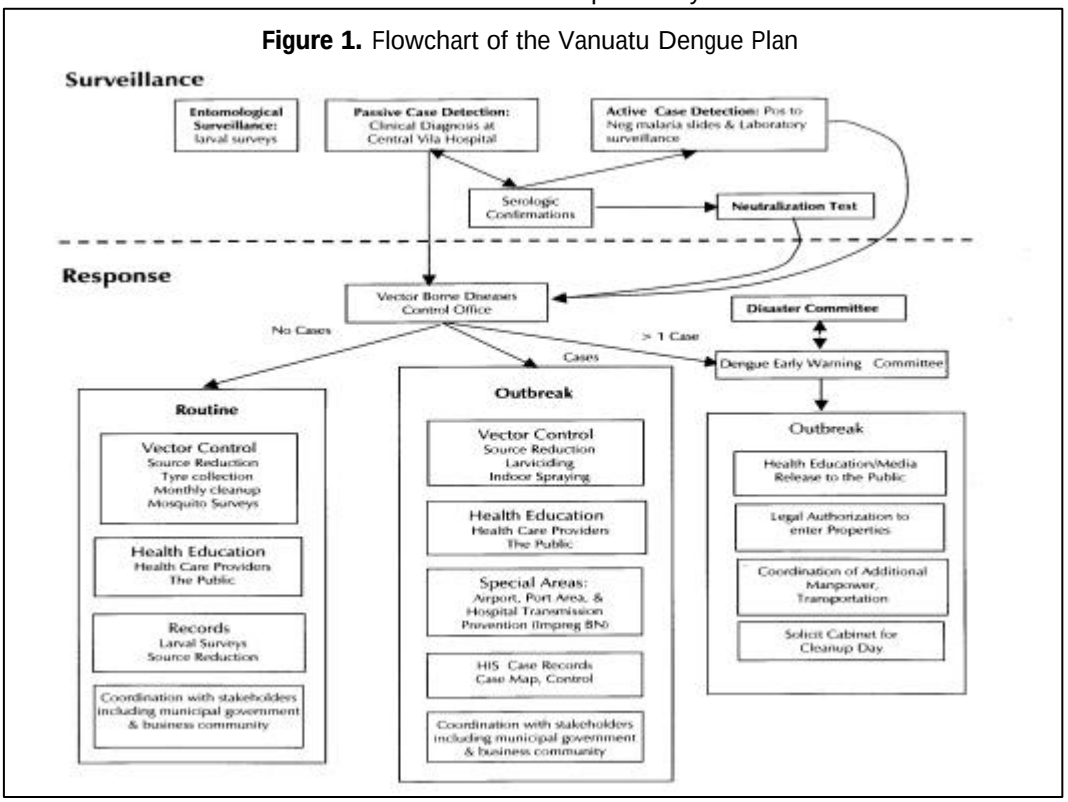
Vectors

Unlike the Solomon Islands to the north and Fiji to the east, *Aedes albopictus* is not found in Vanuatu. *Aedes aegypti*, the primary dengue vector remains, with *Aedes hebrideus* of secondary importance[1]. Primary breeding sites for *Aedes aegypti* are tyres, water drums and discarded refrigerators which are used to hold water.

National dengue plan

The Vanuatu National Dengue Plan (Figure 1) relies on the rapid detection of introduced dengue cases through active and passive surveillance and an immediate response to suspected dengue cases to limit outbreaks. Mosquito surveillance and control is the responsibility of the Malaria and Other

Figure 1. Flowchart of the Vanuatu Dengue Plan



Vector-Borne Diseases Control (VBDC) Programme, which also reviews on a weekly basis information obtained from the active and passive surveillance systems.

Surveillance

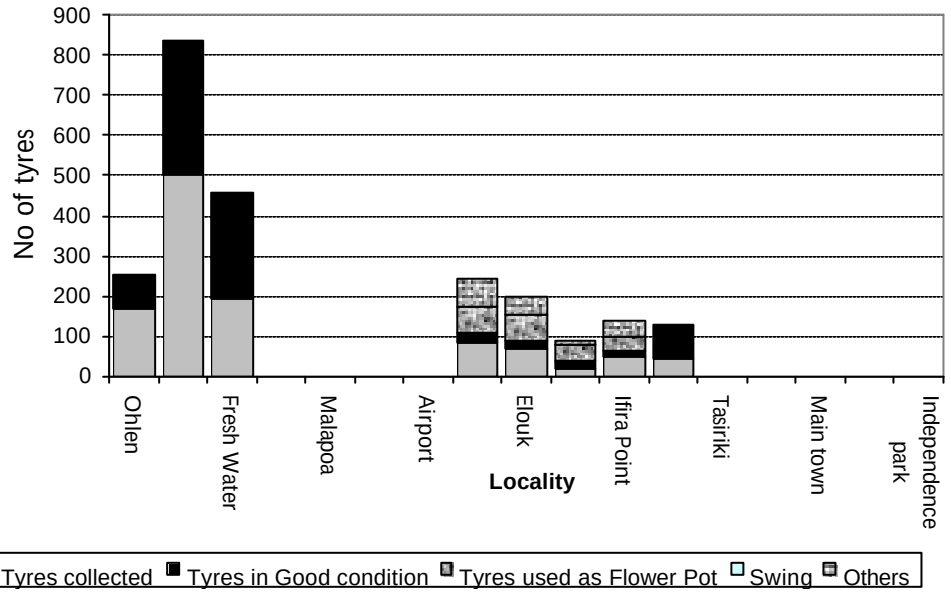
There are three components of the surveillance system in Vanuatu: entomological, passive case-detection, and active case-detection.

An *entomological larval survey* of 100 households is carried out three times a year. Larval samples are brought back to the Ministry of Health (MOH) and identified. Data on mosquito surveys are entered into an Excel file; Breteau Indices are calculated and the results tabulated as graphs. Information on the types and numbers of breeding sites as well as on the uses of tyres by households are collected (Figure 2).

Surveillance using *passive case detection* is based on clinical diagnosis at the Vila Central Hospital in Port Vila, on Efate, as well as in the Northern District Hospital in Luganville, on Espiritu Santo, Lenakel Hospital on Tanna, Lolowai Hospital on Ambae, and Norsup Hospital on Malakola.

Separate case definitions for adults and children are used. In adults, a patient with high fever (>38°C) for more than two days, plus at least two of the following: severe headache and/or pain behind eyes, bone and/or joint pain, rash and/or flushing, nausea and/or vomiting and/or dizziness, is considered as a suspect for dengue fever. In

Figure 2. Tyre operation in Port Vila in 1999-2000 showing the number of tyres collected and the uses of tyres not collected



children the definition is: high fever (>38°C) for more than two days plus at least one of the following: poor drinking and/or poor urine output, rash and/or flushing, nausea and/or vomiting and/or dizziness.

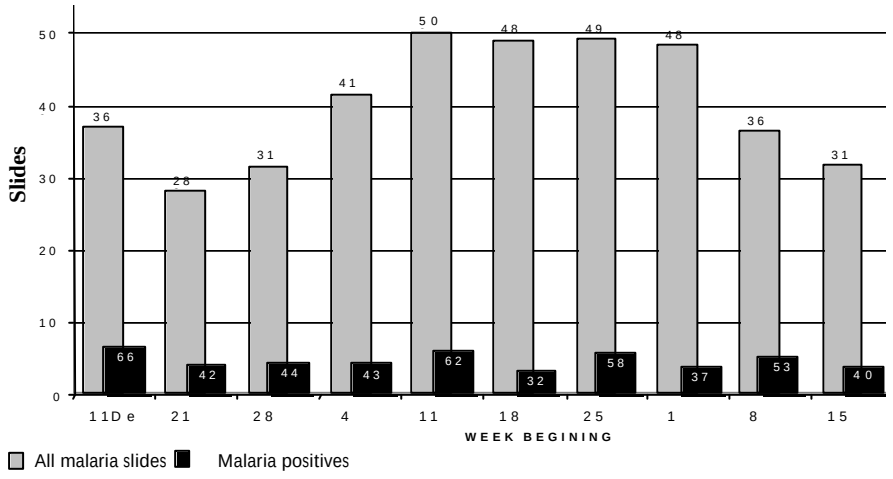
An ongoing programme of refresher training of health-care providers (both nurses and clinicians as well as village health workers) in both dengue case recognition and treatment has been supported by the Pacific Regional Vector-Borne Diseases Programme. Suspected cases based on clinical observations are immediately reported by telephone to the office of the Malaria and Other VBDC Programme, entered into the Vanuatu Health Information System and analysed serologically with a rapid diagnosis test. Prior to 2000, the Dengue Fever IgM and IgG rapid immunochromatographic test (Panbio, Australia)[2] was used and sera positive in the rapid test were sent to the WHO Collaborating Centre for Arbovirus Reference and Research, Queensland Health Scientific Services, Brisbane, Australia, for confirmation by neutralization assays.

Active dengue case detection is by analyses of the ratio of positive to negative malaria slides. An increase in the number of slides taken (indicative of fever), with no increase in the number of malaria positives, would indicate the possibility of a cause of fever other than malaria. The numbers of slides that were malaria negative and malaria positive during the 1998 dengue outbreak are shown in Figure 3. Increases in requested malaria slides require further testing to incriminate dengue as the cause of fever. The change in the number of malaria slides requested and the lower proportion of such slides that were malaria positive indicates an increase in fever cases, which could be caused by dengue. Laboratory testing of serum from persons who were malaria slide negative is necessary to confirm dengue cases.

Routine preventive measures

All information from the surveillance systems goes to the Malaria and Other VBDC office, which coordinates routine anti-mosquito

Figure 3. Malaria-negative and malaria-positive slides during the 1998 dengue outbreak, Vila Central Hospital Laboratory, December-February 1999. *(Note the large increase in negative slides compared to positive slides during January.)*



activities in the absence of dengue. These activities consist of vector control through source reduction including regular tyre collection. Another routine activity to diminish container-breeding mosquitoes is through monthly clean-ups. For these activities, community involvement is essential. Furthermore, larviciding is carried out on mosquito-positive containers at the time of the routine larval surveys. In addition, ULV spraying of malathion using a truck-mounted Leco sprayer is undertaken fortnightly at the international airport and wharfs during the peak *Aedes aegypti* breeding season.

Manples – Community-based control project

The Manples project utilizes community involvement in removing breeding sites. This community-based project, inaugurated in 1999, is supported and supervised by the Ministry of Health through the VBDC office. The purpose of this project is to both educate the people in the Manples area (in a suburb of Port Vila) on vector-borne diseases and mosquito control and to use this knowledge to reduce mosquito breeding sites by community participation.

The Manples project evolved from the routine larval collection team who noticed that a lot of tins, drums and tyres accumulated in this area because there was no established disposal facility. Once every three months the staff of the VBDC office distributes one plastic bag to each household in the Manples area. Each plastic bag costs 100 vatu (US$ 0.71). All tins and water containers are collected in this bag by people living in that household. Every three months the Malaria and Other VBDC office collect the bags and distributes new ones. Financial expenses are low, even when including the cost of petrol to reach the Manples area, and are charged to the recurrent budget of the Ministry of Health.

Education of the Manples residents is based on two activities. First, the staff of the Malaria and Other VBDC office do house-to-house talks each time they distribute the bags. Secondly, trained volunteers then do continuous education in the Manples community. The community is very active with nearly all the Manples population actively participating in the project.

The success of the Manples project can be gauged by the fact that it is in the process of extension to the northern suburbs of Port Vila: Blacksands, Ohlen, Tagabe and Malapoa. A one-day meeting is organized for the initial training of new volunteers and a one-week workshop was organized in June 2001 for all the volunteers.

Awareness of the population

Many kinds of IEC (information, education and communication) materials about dengue are available and are used in Vanuatu. Educational posters on mosquito control are distributed in hospitals, health centres, dispensaries and communities on a regular basis. In case of suspected cases of dengue, booklets in Bislama, the most common of the three official languages of the country, are copied and distributed in the communities. Each booklet gives very simple and basic information about dengue fever, the main symptoms and the way to prevent the disease.

One video in Bislama on mosquito control entitled "*One present long niufala Bebe*", produced by a local theatre group, "The Wan Smal Bag Theatre", is broadcast on TV during the wet season. Another video, in English, *"It can't happen here*", is focused on dengue disease and is shown on TV when suspected cases of dengue are found in the country. Finally, during the "at dengue risk season", messages are displayed at the national radio and TV to remind everyone to clean their gardens and to destroy all potential mosquito breeding sites.

Response

Upon evidence of a suspected case of dengue, anti-vector activities are undertaken at houses within 200 metres of a case house. The control teams consist of 3-4 individuals. In addition to indoor spraying with Hudson backpack sprayers, larviciding with Abate is undertaken and educational materials on dengue distributed. Severe clinical dengue cases are admitted to the medical ward. The medical ward is screened and, in addition, dengue patients are required to sleep under insecticide-treated mosquito nets.

When evidence of more than one suspected dengue case is reported to the Malaria and Other VBDC office, the Dengue Early Warning Committee is convened. Members of this committee are: Manager of the Malaria and Other VBDC Programme, the MOH Director-General, the six directors of the MOH, physicians from the Vila Central Hospital, representatives of the World Health Organization and the Secretariat of the Pacific Community. The Dengue Early Warning Committee discusses plans for controlling a dengue outbreak as presented by the Manager of the Malaria and Other VBDC Programme and approves a plan for control. This committee has the added responsibility of informing the public and soliciting additional resources from supporting institutions including the business community and the municipalities. This support could include additional manpower and transportation. This committee can also request the Cabinet to authorize the public to undertake larval source reduction activities.

One other committee that may become involved during a dengue outbreak is the Disaster Committee. Dengue is officially considered a disaster in Vanuatu. It is the responsibility of the Disaster Committee to coordinate efforts should a dengue outbreak occur concurrent with another disaster (e.g. earthquake, Tsunami, etc.).

Epidemic plan

In a large-scale dengue outbreak, control efforts shift from treatment of homes in the immediate vicinity of a case to treatment of "hotspots" (e.g. areas with significant numbers of cases), as well as hospitals, airports and seaports. Furthermore, greater emphasis is placed on larval source reduction rather than on indoor space spraying for adult mosquitoes. Manpower to supplement the strength of the workers from the Malaria and Other VBDC Programme comes from 40 previously trained volunteers. The Dengue Early Warning Committee would be responsible for coordinating the provision of additional manpower and transportation. Furthermore, this committee may solicit the Cabinet to organize national clean-up days, thereby enlisting the entire community in the dengue outbreak control effort.

The future

Vanuatu, like many other island countries, is constrained by a small number of vector-borne disease specialists in the MOH. A relatively small population also means a limited public health budget. By necessity, Vanuatu must rely on public education, community participation, a small well-trained team of professionals in the MOH, rapid communication within Vanuatu and with its Pacific island neighbours as well as cooperation with its neighbours for laboratory confirmation of suspected dengue cases.

Most importantly, Vanuatu relies on a vigilant surveillance system to rapidly identify suspected cases before they become outbreaks. Cooperation of the community is essential for outbreak prevention through larval source reduction programmes. The Manples project has attracted the interest of other communities in Vanuatu and is being expanded to the main suburban

communities. Successful expansion of the community-based projects will rely on an educated community that realizes that dengue is often a problem generated by communities themselves and, as such, the solution is in their hands.

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Virological Surveillance of Dengue Haemorrhagic Fever in Viet Nam, 1987-1999

By

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Abstract

In Viet Nam during 1987-1999, the virological surveillance offered important information about circulating serotypes of dengue viruses. The activity cycle of DEN-1 was from 1989 to 1996. The DEN-2 activity cycle started in 1987 and remained active into 1997. DEN-3 first appeared in 1995 and continues to be active. DEN-4 has strongly emerged since 1999. In southern Viet Nam, cases of dengue haemorrhagic fever are always confirmed in the first quarter of every year. Therefore, active surveillance and dengue control activities must be initiated during this period.

Key words: Virologic surveillance, viral activity, dengue control, Vietnam.

Introduction

Since 1963, there has been a steady increase in the incidence of dengue haemorrhagic fever (DHF) in Viet Nam, which has been a major health problem and a leading cause of hospitalization and death of children. In northern Viet Nam, DHF was identified for the first time in 1959, where a major epidemic occurred in 1969. In the south, DHF first appeared in 1960, followed by an outbreak in 1963, resulting in 331 hospitalized children with severe haemorrhage, of whom 116 died. With the critical appearance of DEN-2 in 1987[(1)], the disease has been spreading with continuous increase in the number of cases and deaths. DEN-1, DEN-3 and DEN-4 closely followed the circulation of DEN-2. Data collected during 1987-1999 on the viral investigation of these DHF epidemics are presented here.

Materials and methods

Virus isolation and identification

Blood samples collected from acute DHF cases were stored at -20°C or -70°C until tested. The undiluted blood specimens (of 0.05 ml) were inoculated into duplicated 1 ml tubes of C6/36 (*Aedes albopictus*) tissue cells. The tubes were incubated at 28°C for 7-10 days. Infected cell cultures were

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harvested and assayed for dengue virus by the direct and indirect fluorescent antibody technique, using the monoclonal antibody (MAB) SLE 6B6C-1/FITC conjugate and the serotype-specific MAB: DEN-1 (Hawaii 15F3-1-15 and D2-1F1-3), DEN-2 (NGC 3H5-1-21), DEN-3 (H87 5D4-11-24), DEN-4 (H241 1H10-6-7) and Japanese encephalitis (JE) (Nakayama 14H5)[1] All[2] hese MABs were supplied by the Centers for Disease Control and Prevention, Fort Collins, Colorado, USA.

Results and discussion

Virus isolation

The details of DEN serotypes for the period 1987-1999 are given in Table 1.

From Table 1, it is evident that DEN-1 was active during 1989-1996, DEN-2 from 1987 to 1997, DEN-3 came on the scene in 1995 and continues to be active, and DEN-4 has emerged in 1999.

Dengue viruses isolated by months

In Viet Nam, especially in the south, as previously reported, dengue viruses were always isolated in the first quarter of the year, which consistently corresponds to the period in which DHF outbreaks did not occur (see Table 2). It is imperative to implement in this time frame active surveillance and an effective prevention programme for DHF.

Year-to-year variations of dengue serotypes in Viet Nam from 1987 to 1999

During 1987-1999, DHF epidemics occurred in Viet Nam every year, but the two biggest DHF outbreaks were recorded in 1987 and 1998. The circulation of the dengue epidemic serotypes has been changed, as shown in Table 1. The epidemic dominant serotype of 14% was chosen as the threshold for viral activity (see Table 3).

Table 1. *Dengue viruses isolated from DHF patients' blood during 1987-1999*

Year	DEN-1	DEN-2	DEN-3	DEN-4	No. (+) / No. specimens (% +)
1987	1	79	3	3	86 / 548 (15.7)
1988	1	6	0	0	7 / 291 (2.4)
1989	2	1	0	0	3 / 66 (4.5)
1990	18	34	1	3	56 / 796 (7.0)
1991	21	53	4	3	81 / 335 (24.2)
1992	16	17	1	1	35 / 329 (10.6)
1993	22	9	0	0	31 / 268 (11.6)
1994	28	22	5	0	55 / 263 (20.9)
1995	100	51 (6*)	21	0	172 / 640 (26.9)
1996	42 (6*)	79 (9*)	42 (16*)	0	163 / 1 328 (12.3)
1997	9	27	39	1	76 / 768 (9.9)
1998	42	29	263	4	338 / 1 670 (20.2)
1999	22	20	24	23	89 / 696 (12.8)

***** Detected by RT/PCR

Table 2. *Dengue serotype viruses isolated in southern Viet Nam, by month, in 1998*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
No. isolates and serotypes	1D1 3D2 12D3 1D4	3D1 6D2 6D3	 2D3	 3D3	 7D3	5D1 3D2 19D3	2D1 7D2 21D3	10D1 4D2 48D3	3D1 16D3	 4D2 17D3 1D4	2D1 11D3 2D4	 	26D1 27D2 162D3 4D4
No. (+) / No. specimens	17/90	15/142	2/17	3/59	7/113	27/253	30/187	62/239	19/114	22/167	15/70	0/16	219/1,467

D1 = DEN-1, D2 = DEN-2, D3 = DEN-3, D4 = DEN-4

Table 3. *The activity cycle of dengue serotype viruses in Viet Nam during 1987-1999*

Virus	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
%	91.9	85.7	33.3	60.7	65.4	48.6	29.0	40.0	29.7	48.5	35.5	8.6	24.7
DEN-2	15p.			11 p.	8 p.	7 p.	4 p.	5 p.	9 p.	9 p.	7 p.	9 p.	7 p.
%	1.2	14.3	66.7	32.1	25.9	45.7	71.0	50.9	58.1	25.8	11.8	12.4	22.5
DEN-1	1 p.			4 p.	4 p.	4 p.	6 p.	5 p.	12 p.	10 p.	3 p.	15 p.	6 p.
%	3.5	0	0	1.8	4.9	2.9	0	9.1	12.2	25.8	51.3	77.8	26.9
DEN-3	2 p.			1 p.	3 p.	1 p.		2 p.	6 p.	9 p.	8 p.	32 p.	10 p.
%	3.5	0	0	5.4	3.7	2.9	0	0	0	0	1.3	1.2	25.8
DEN-4	3 p.			1 p.	2 p.	1 p.					1 p.	2 p.	7 p.

p: province

- DEN-2 represented 91.9% of the viral isolates in 1987; 60.7% in 1990; 48.6% in 1992; and 48.5% in 1996, then decreased to 8.6% in 1998, with a continuous activity cycle of 11 years.
- DEN-1 emerged post-1987 in Ho Chi Minh City and soon after in 1990, this dengue serotype spread to four provinces (Ho Chi Minh City, Dong Nai, Long An and Tra Vinh) in southern Viet Nam. In 1995, DEN-1 was isolated from 12 provinces (11 in the south and one in the north), representing a viral activity of 58.1%. This activity decreased in 1997 and 1998 to

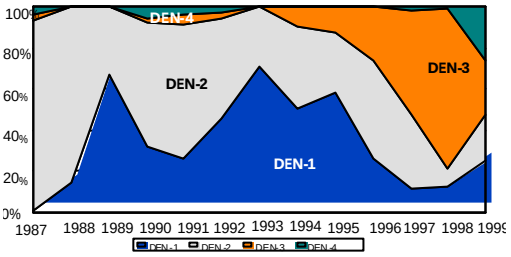
11.8% and 12.4% respectively, representing a viral active cycle of 8 years from 1989 to 1996.

- DEN-3 had the lowest activity during 1987-1994. This serotype was first isolated in 1987 in Ho Chi Minh City and Hanoi, thereafter it was found in 1991 in Ho Chi Minh City, Tien Giang and Hanoi, with a 4.9% of the viral activity. In 1995, DEN-3 cases were confirmed in six provinces: Ho Chi Minh City, Tien Giang, Ben Tre, Vinh Long, and Soc Trang in the south and Nam Dinh in the north, representing 12.2% of the dengue activity. From 1996 to 1998, DEN-3 activity increased to 77.8% and was found in 32 provinces, in which 15/19 are located in southern Viet Nam, 10/28 in the north, 4/11 in the central part and 3/3 provinces in the highland. This was the predominant serotype of the biggest DHF epidemic in 1998. The activity cycle of DEN-3 decreased in 1999 to 26.9% and was only found in nine southern provinces and one province in the central part of the country.

- DEN-4 was first detected in 1987 in three provinces (Hanoi, Song Be, Dong Thap), with 3.5% activity. In 1990, it was detected in Tien Giang; in 1991 in Hanoi and Ho Chi Minh City; and in 1992 in Tien Giang (Tables 2, 3). After a 4year absence, this serotype re-emerged in three provinces in the Mekong delta: Vinh Long in 1997 and Dong Thap and Tra Vinh in 1998. In 1999, this serotype strongly increased to 25.8% and spread to seven provinces (five in the south, two in the north). This will continue to be a health threat in future years, because most children under 12 years of age have had no previous exposure to DEN-4 virus and will thus be susceptible to DEN-4 infection.

The above data is summarized in Figure 1.

Figure 1. Year-to-year variations of dengue serotypes in Viet Nam, 1987-1999



Age and sex distribution of virologically confirmed cases

The age- and sex-wise distribution of positive virus isolation in north Viet Nam and south Viet Nam is given in Tables 4 and 5.

Table 4. *Positive virus isolation rate by age group in north Viet Nam during 1986-1998*

Age group	No. cases (n = 268)	Positive rate %
0-5	17	6.34
6-10	40	14.93
11-15	56	20.90
16-20	**51**	**19.03**
≥ 20	**104**	**38.81**

Table 5. *Positive virus isolation rate by age group and sex in South Viet Nam in 1998*

Age-group	Male	Female	No. (+%)
<1	2	2	4 (1.86)
1-4	2	6	8 (3.73)
5-9	**37**	**26**	**63 (29.43)**
10-14	**36**	**30**	**66 (30.84)**
15-19	17	12	29 (13.55)
≥20	25	14	39 (18.22)
NR	2	3	5 (2.33)

NR: not reported.

Dengue viruses isolated from different age groups of patients are shown in Tables 6 and 7. In the north, the positive virus isolation rate was the highest in adults (57.84%), while in the south, dengue viruses were most often discovered in children in the age group 5-14 (60.28%).

Characteristics of DEN-1 isolates in Viet Nam

As mentioned earlier, in southern Viet Nam, complete and incomplete virions have been isolated from patients, of which the results are shown in Table 6.

Table 6. *Some characteristics of DEN-1 strains isolated in Southern Viet Nam, 1990-1999*

No. of DEN-1	MABs of DEN-1		
Isolates	1F1	15F3	1F1+15F3
234	134	18	82

(Note: The MAB DEN-1 1F1 directed against structural viral antigen; the MAB DEN-1 15F3 directed against non-structural viral antigen.)

During 1990-1999, we isolated 234 strains of DEN-1 in which 134 strains had only structural antigen, 18 strains had only non-structural antigen, and 82 strains were complete virions.

Concomitant infections of dengue during 1992-1998

During 1992-1998, 13 concomitant infection cases caused by 2 or 3 dengue viruses were detected by IFA using MABs (see Table 7).

Table 7. *Cases of concomitant infection of dengue, 1992-1998*

Viruses detected by MABs	No. of cases
DEN-1 & DEN-2	4
DEN-1 & DEN-3	4
DEN-2 & DEN-3	3
DEN-3 & DEN-4	1
DEN-1 & DEN-3 & DEN-4	1

As the number of these cases was small, we could not identify the degree of severity caused by the combination of different serotypes.

Conclusion

(1) The virological surveillance during 1987-1999 offered the circulation patterns of the predominant dengue serotype by year as well as the viral activity cycle, e.g. DEN-1, was prominent during 1989-1996 (8 years); DEN-2 from 1987 to 1997 (11 years) and DEN-3 from 1996 to 1999, and this serotype is still active. DEN-4

emerged strongly in 1999 and will be a health threat in the future.

(2) The emergence of DEN-3 in 1995 provoked the largest DHF epidemic in 1998, and this emerging trend has been on the decrease since 1999.

(3) A large dengue haemorrhagic fever outbreak will occur after a period of 3-4 years since a new serotype has been introduced.

(4) Three populations of virions have been isolated from DEN-1; one population having a structural antigen, another having only a non-structural antigen, and the third a complete virion.

(5) In northern Viet Nam, dengue viruses were most often recovered in adults, while in the south, they were most often recovered in children between the age group 5-14 years.

(6) Dengue haemorrhagic fever cases are confirmed through virological surveillance in the first quarter of every year. Therefore, it is a reminder that active surveillance and effective national control programme must be implemented during this period.

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Dengue, Japanese Encephalitis and West Nile Flaviviral Infections Detected during a Dengue Outbreak in Sonepat District, Haryana State, India

By

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Abstract

During October 1997, three cases of encephalitis were admitted in Bara Hindu Rao Hospital, Delhi, from Murthal village in Sonepat district of Haryana state. In a follow-up study, out of the 30 serum samples collected, eight were found positive for dengue IgM antibodies. Two samples were positive for all the three infections, viz. dengue (DF), Japanese encephalitis (JE) and West Nile (WN) virus, while one sample was positive for two infections, viz. Japanese encephalitis and West Nile. Three male and 10 female cases showed past flavivirus infection. During the *Aedes* survey, house and container indices were estimated at 31.4% and 13.0% respectively. The *Aedes aegypti* landing rate was observed to be 36 per person per hour. The current episode was confirmed to be due to dengue infection. However, JE and WN virus infections were also present in the area.

Key words: Dengue ferver, Japanese Encephalitis, West Nile, Haryana, India

Introduction

India is endemic for three important mosquito-borne viral diseases, viz. dengue haemorrhagic fever (DHF), Japanese encephalitis (JE) and West Nile virus (WN). The first outbreak of DHF was recorded in 1963 in Kolkata (previously known as Calcutta)[1]. Since then dengue has spread to all parts of India. The latest serious outbreak to hit the Capital city of Delhi was in 1996 when 10,252 cases with 423 deaths were recorded[2]. The first outbreak of JE occurred in Pondicherry and Vellore (Tamil Nadu)[3] in south India in 1955 and later spread all over the country, including Haryana state in north India[4]. The West Nile virus transmitted by *Cx. quinquefasciatus* is endemic in India. These three infections maintained specific distribution patterns. While DHF was confined to urban areas, JE and WN recorded rural distribution. However, over the past two decades, DHF has penetrated into rural areas[5,6] along with piped water supply, while JE and WN have hit the peri-urban areas of urban centres, thus increasing the chances of concomitant infections in the human population. During October 1997, a few cases of encephalitis originating in a

village in district Sonepat, Haryana state, were admitted in Bara Hindu Rao hospital in Delhi. A follow-up study was undertaken by a team from the National Institute of Communicable Diseases (NICD), Delhi, in the affected village. The findings are presented in this paper.

Study area

The study area comprised the affected village, Murthal, situated in district Sonepat of Haryana state on National Highway No. 1, about 45 km from Delhi, the Capital city of India. It is a big village with a population of about 20,000. The houses are mainly made of concrete bricks. There is an intermittent tap water supply in the village and the villagers are forced to store water in different types of containers such as cement tanks, clay jars, drums, etc. The surrounding areas have marshes/swamps which are conducive to the breeding of vectors of JE and WN.

Methods and materials

The methodology included rapid sero-epidemiological and entomological surveys as per standard technique in the affected village, particularly in and around the locality from where the encephalitis cases were reported. Serum samples were collected from fever cases and tested at NICD by haemagglutination inhibition test[7], using antigen and antisera received from the Centers for Disease Control, USA, and the dengue IgM immunoblot commercial kit (Gene Labs, Singapore). These samples were also confirmed by the National Institute of Virology, Pune, using Mac Elisa, HI and CF tests. Landing collections of adult *Aedes aegypti* were carried out on human bait inside a room having a positive cement tank, and the landing rate per bait per hour was calculated.

Results

i) Sero-epidemiological

A rapid fever survey was carried out in the affected village and 30 blood slides and 30 serum samples were collected from cases having fever, or a past history of fever, for screening for arboviral infections and malaria parasite. The patients whose samples were taken had symptoms of fever with chills or without chills, cough and vomiting. The age-wise and sex-wise break-up of patients from whom the samples were taken, and the results of the tests are given in Table 1.

From Table 1, it is evident that out of the 30 serum samples collected from Murthal village, eight samples were found to be positive for antibodies due to the recent dengue infection. Two females in the age group of 17 years and 35 years were found to be positive for all the three infections, viz. dengue, Japanese encephalitis and West Nile. One male aged 19 years showed mixed infection of Japanese encephalitis and West Nile. Three males and 10 females showed past flavivirus infection. None of the slides was found positive for malaria parasite.

ii) Entomological

The *Aedes* survey was carried out in and around the houses of the fever cases for the detection of *Aedes* breeding in various water containers.

Table 1. *Age-wise and sex-wise break-up of patients from Murthal village and frequency of positivity for DF, JE and WN infections detected during October, 1997*

Age group (years)	Sex	No. of samples collected	Positive for antibodies					
			DF*	JE**	WN***	Mixed DF, JE and WN	Mixed JE and WN	Past lavivirus
0 to 5	Male	0	0	0	0	0	0	0
	Female	0	0	0	0	0	0	0
6 to 10	Male	2	1	0	0	0	0	1
	Female	0	0	0	0	0	0	0
11 to 15	Male	1	1	0	0	0	0	0
	Female	2	1	0	0	0	0	0
16 to 25	Male	2	1	0	0	0	1	0
	Female	10	2	0	0	1	0	5
26 to 35	Male	2	0	0	0	0	0	2
	Female	1	0	0	0	1	0	0
36 to 45	Male	2	1	0	0	0	0	0
	Female	5	1	0	0	0	0	4
> 45	Male	0	0	0	0	0	0	0
	Female	3	0	0	0	0	0	1
Total		30	8	0	0	2	1	13

* DF= Dengue; ** JE = Japanese encephalitis; *** WN = West Nile

Table 2. *Containers index for Aedes aegypti breeding in Murthal village*

S. No.	Name of container	Number checked	Found positive	Container index	Per cent positive out of total containers checked
1.	Clay jar	67	7	10.4	8.3
2.	Drum	11	2	18.1	2.3
3.	Cement tank	5	2	40.0	2.3
4.	Bucket	1	0	0	0
TOTAL		84	11	13.0%	12.9%

During the survey, a total of 35 houses/premises were checked for *Aedes* breeding and 11 were found to be positive, thereby giving the house index as 31.4%. Similarly, a total of 84 containers were searched for *Aedes* breeding and 11 were found to be positive, thereby giving the container index as 13.0% (Table 2). The most preferred containers for *Aedes* breeding were clay jars followed by cement tanks and drums. *Aedes* breeding was found to be maximum in clay jars which were the primary breeding containers found during the survey month[8].

Landing collections

Adults of *Aedes aegypti* were collected from inside the room and the landing rate per human bait per hour was recorded as 36.

The entomological investigations in this village revealed a significantly higher house index and container index for *Aedes aegypti* mosquito, a proven vector of dengue/DHF.

Discussions and conclusions

The sero-epidemiological and entomological findings indicated that the present outbreak in Murthal village was of dengue, and that the indigenous transmission may have flared up due to high densities of *Aedes aegypti.*

There is no report of the concomitant circulation of two or three viruses in the same individuals. The only report of concurrent circulation relates to the Kolkata outbreak of DHF, when both DEN and Chikungunya virus (alphavirus) were detected to be circulating in the community. Antibodies to more than one flaviviruses in the individual have been detected in the studies conducted in and around Delhi[9]. The detection of mixed infections of Dengue, JE and West Nile fever in the village is a matter of great public health importance, and in case of any future outbreak of encephalitis in the area, the possibility of the circulation of more than one arboviruses may be kept in mind.

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Sero-diagnosis of Dengue Infections in Four Metropolitan Cities of Bangladesh

By

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Abstract

A sero-diagnostic study of dengue infection was undertaken in four large cities of Bangladesh during September–October 1999. Selected patients suffering from viral fever, attending the outpatient and inpatient departments of medical colleges of the four selected cities, i.e. Rajshahi, Khulna, Sylhet and Chittagong, were the sample units. The study population was selected following case inclusion and exclusion criteria. The samples were tested for anti-dengue IgM. A total of 200 blood samples were collected from the four hospitals. Among them, 107 were male and 93 female. A total of 35 (17.5%) samples were interpreted as reactive. Chittagong topped the list with 12 (34.3%) reactive samples. Khulna came second with 11 (31.43%) reactive samples. Out of the 35 reactive samples, males contracted dengue virus more frequently (62.86%) than the females (37.14%). Again, 5-9.9-year-old children were found to be most vulnerable as 16 (45.7%) reactive samples belonged to them, followed by 15->-year age group (8, 22.86%) and 1-4.9-year-old children (6,17.14%). This study proved that many dengue cases go unreported and our physicians obviously are not very much acquainted with this disease of public health importance.

Key words: Serodiagnosis, INDX DIP-S-TICK ™, 1gM, dengue antibody, Bangladesh

Introduction

It has been increasingly recognized that the incidence of DF/DHF has increased dramatically in all major tropical areas of the world in recent years. The frequency of the epidemic activity is increasing with a trend toward larger epidemics and more severe cases. For most of the countries, the number of cases reported during 1981-1990 equalled or exceeded the total number reported in the previous 25 years (1956-1980).

Bangladesh is not spared by the onslaught of dengue infections. After many years of endemicity, dengue activity in 1999 marked a new high, affecting more than

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10,000 people and claiming 35 deaths as of August 24, 2000. (Communicable Disease Control, DGHS, Dhaka).

The present study is a hospital-based descriptive cross-sectional survey, and is aimed at identifying dengue fever patients from cases suffering from viral fever. It was conducted during September-October 1999 when we saw a marked increase in the number of cases clinically diagnosed and serologically proved to be of dengue in Dhaka.

Methodology

Study population and sample size

Selected patients suffering from viral fever attending the outpatient and inpatient departments of medical colleges of the four selected cities, i.e. Rajshahi, Khulna, Sylhet and Chittagong, were the sample units. Fifty blood samples were collected from each of the four hospitals. The study population comprised of individuals of all age groups.

Case inclusion criteria

High fever, with/without a biphasic curve; no focal signs of bacterial infections; case clinically suggestive of viral infections.

Exclusion criteria

If routine lab tests, i.e. platelet count, complete blood count, malarial parasite, chest X-ray, etc., were already done and those reports suggested bacterial infections or any clinical diagnosis other than viral infections; if clinical features pointed a finger at diseases other than dengue infection; and if the patient or his/her guardian refused to participate in the survey.

INDX Dip-S-TicksTM Dengue Fever IgM test

We used the INDX Dip-S-Ticks dengue fever test, which is a semi-quantitative enzyme immunoassay for the detection of IgM antibodies to dengue, for the serological confirmation of dengue in samples of serum, plasma or heparinized whole blood. It utilizes an enzyme-linked immunoassay (ELISA) dot technique for the detection of IgM dengue antibodies. Comparison data with the established reference methods show that the overall sensitivity for the comparisons was 89.6%, and the overall specificity was 94.3%.

Results

A total of 200 blood samples (50 from each of the four designated hospitals) along with data related to the clinical and epidemiological aspects was collected from among the viral febrile patients. Among them, 107(53.5%) were male and 93(46.5%) female. The age structures of the selected patients showed that the 5-9.9-year age group dominated with 36.0% of the total samples collected, followed by the older ones, 15-> years, with 28.5%, 10-14.9 years, with 25%, and the younger ones, 1-4.9 years, with 10.5%, were the least number enrolled.

Degree of reactivity

A total of 35 (17.5%) samples were interpreted as reactive. Chittagong topped the list with 12 (34.3%) as far as the number of reactive cases is concerned. Khulna was second with 11 (31.43%) reactive samples (Table 1).

Table 1. *Cross-tabulation: Districts and test results*

District	Test results		Total
	Reactive	Negative	
Khulna	11 (22.0%)	39	50
Sylhet	8 (16.0%)	42	50
Rajshahi	4 (8.0%)	46	50
Chittagong	12 (24.0%)	38	50
Total	35 (17.5%)	165	200

Out of the 35 reactive samples, males contracted dengue virus more frequently (n=22, 62.86%) than the females (n=13, 37.14%). Again, 5-9.9-year-old children were found to be most vulnerable as 16(45.7%) reactive samples belonged to that age group, followed by 15-> -years age group (8, 22.86%) and 1-4.9-year-old children (6, 17.14%) (Table 2).

Table 2. *Cross-tabulation of age group and test results in four districts*

Age group (years)	Test results		Total
	Positive	Negative	
1 – 4.9	6 (17.14%)	15	21
5 – 9.9	16 (45.7%)	56	72
10 – 14.9	5 (14.3%)	45	50
15 - >	8 (22.86%)	49	57
Total	35 (100.0%)	165	200

Reactivity according to districts

Khulna: Out of the 50 samples collected from this district, 22% were reactive. Of the reactive ones, 7(63.6%) were from females and 4(36.4%) from males. As many as 5(45.45%) patients were 15 years or older and 3(27.27%) fell within the 5-9.9-year-old category (Table 3).

Table 3. *Cross-tabulation of age group and test results in Khulna*

Age group (years)	Test results		Total
	Positive	Negative	
1 – 4.9	1 (9.1%)	3	4
5 – 9.9	3 (27.27%)	3	6
10 –14.9	2 (18.2%)	4	6
15 - >	5 (45.45%)	29	34
Total	11 (100.0%)	39	50

Sylhet: Positive ELISA results for dengue infection were lower than in other districts surveyed. A total of 8(16.0%) samples came out reactive out of which 7(87.5%) patients belonged to the 5-9.9 years age group (Table 4). Male patients (7, 87.5%) overwhelmingly outnumbered the females (1, 12.5%) as far as the reactive outcome of the test is concerned.

Table 4. *Cross-tabulation of age group and test results in Sylhet*

Age group (years)	Test results		Total
	Positive	Negative	
1 – 4.9	.00	1	1
5 – 9.9	7 (87.5%)	26	33
10 –14.9	1 (12.3%)	15	16
15 - >	8 (100.0%)	42	50
Total	35 (100.0%)	165	200

Rajshahi: Rajshahi recorded the lowest percentage of reactive samples (4, 8%), of which both sexes were equally reactive for two samples each. Here, 50% of the reactive samples were from the youngest age group (Table 5).

Table 5. *Cross-tabulation of age group and test results in four districts*

Age group (years)	Test results		Total
	Positive	Negative	
1 – 4.9	2 (50.0%)	10	12
5 – 9.9	1 (25.0%)	17	18
10 – 14.9	.00%)	7	7
15 - >	1 (25.0%)	12	13
Total	4 (100.0%)	46	50

Chittagong: The highest number of reactive samples (12, 24%) came from this city. Males constituted the bulk among the infected patients. The age-group structure of the reactive samples showed that 5 children out of total 12 were from the 5-9.9-years age group, followed by 1-4.9-year-old kids (Table 6).

Table 6. *Cross-tabulation of age group and test results in Chittagong*

Age group\ (years)	Test results		Total
	Positive	Negative	
1 – 4.9	3 (25.0%)	1	4
5 – 9.9	5 (41.67%)	10	15
10 – 14.9	2 (16.67%)	19	21
15 - >	2 (16.67%)	8	10
Total	12 (100.0%)	38	50

Discussion

So far, dengue has not been considered a public health threat in Bangladesh as we never experienced any outbreak in the true sense of the term. Only sporadic cases were diagnosed through small-scale surveys that actually failed to unearth the real situation in Bangladesh. The first scientifically-composed survey was conducted in Chittagong in 1996-97, and with a positive rate of 7.1% among the selected patients.

This survey shows an average ELISA positive rate of 17.5% that heralds a public health warning in Bangladesh in the near future. Chittagong being the second largest and most industrialized city of Bangladesh was found to be worst affected, with 34.3% of all reactive samples. Khulna, another industrialized city, presented the second highest number of reactive samples (11, 31.43%).

The age-group structure of the dengue IgM-positive patients showed that the 5-9.9-year-old children were the most affected. Sixteen (45.7%) children from this age group contracted dengue virus, with the patients aged 15 years or above far behind. In Khulna, patients aged 15 years or more, and in Rajshahi, 1-4.9-year-old children were found to be most vulnerable. Males (22, 62.86%) were more vulnerable than females (37.14%). This was reflected in every district, except Khulna, where females (63.64%) were more affected than males.

The subtyping of dengue virus is an important aspect that could not be performed due to the non-availability of reagents, equipment, etc. But subtyping is necessary to understand the progression of

the disease to the epidemic level and more serious forms, i.e. DHF/DSS.

This survey actually highlights the gaps in research with respect to the serological as well as entomological aspects of dengue infection in Bangladesh.

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Analysis of some Socio-demographic Factors Related to DF/DHF Outbreak in Dhaka City

By

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Abstract

A knowledge, attitude and behaviour (KAB) survey was conducted among residents of Dhaka regarding dengue (DF) and dengue haemorrhagic fever (DHF) from August to October, 2000, during the first recognized outbreak of DF/DHF in Bangladesh. A random sample of more than 9,000 houses was visited by survey teams throughout the city. More than 99% of people living in the city had heard about dengue and 95% knew that the disease was transmitted by mosquito bites; 93.5% knew that the dengue-transmitting mosquito bit during daytime, and 52.1% knew that this mosquito bred in containers. Nearly 60% of slum-dwellers could not spend any money to buy commercially-available aerosols/coils for their houses, while the rest 40% could spend very little money for this purpose. About 10% of people living in independent houses and multistoreyed buildings spent more than US$ 10 for mosquito control gadgets per month (equivalent to a week's salary for most workers in Bangladesh). In the slum areas and in semi-permanent (semi-pucca) houses, earthen jars and drums, common sources of *Aedes aegypti* breeding, were frequently used for storing water. In more upscale, independent houses where mosquito density was higher, rooftop concrete water tanks were more common. Two-thirds of city-dwellers thought that both government and citizens should be responsible for mosquito control.

Key words: Dengue, DHF, Socio-demographic factors, Bangladesh

Introduction

While dengue haemorrhagic fever (DHF) was suspected to be the cause of 'Dacca fever' in 1964[(1)], no outbreaks of DHF were recognized and confirmed before July 2000. Between July and December 2000 more than 4,000 hospitalized cases in Dhaka

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(earlier named as Dacca) were reported with more than 80 deaths.

Aedes aegypti and *Aedes albopictus*, the two known vectors of dengue (DF), breed in and around human dwellings[2].Therefore, for *Aedes* control, community participation is a necessary prerequisite[3,4]. A participatory mosquito control campaign could not be successful without assessing and modifying the attitudes and behaviours of members of the community. So, we conducted a knowledge, attitude and behaviour (KAB) survey among some of the residents of Dhaka on (DF) and (DHF) from August-October 2000, during the first recognized dengue outbreak in the city.

Materials and methods

We conducted comprehensive surveillance of the breeding sites for *Aedes* in Dhaka from August-October 2000. As part of the *Aedes* surveillance in Dhaka city, we developed a questionnaire to assess the knowledge, attitude and behaviour of residents regarding DF/DHF. After pretesting, a final version of the questionnaire was developed, which was used throughout the survey period. The questionnaire had two parts. The first part contained information about the address, type of house and occupation of the household head. The second part of the questionnaire gathered information about the knowledge, attitude and behaviour of the residents. The surveyors administered the questionnaire form, printed in Bengali (the national language).

Survey teams, composed of two or three members, visited at least 100 houses in each of all the 90 wards (administrative units) in Dhaka. The surveyors were either current or former students of the Department of Zoology of various universities in Bangladesh.

In each ward, various types of houses were identified. The households were classified into independent, multi-storeyed, semi-pucca and slum. The semi-pucca houses have concrete floors, brick walls, and corrugated tin-sheet roofs; they are semi-permanent houses, found mostly in the peripheral expanding areas of the city. The slums are purely temporary accommodations (made of split bamboo walls and roofs) for very poor, mostly landless people, who migrate from rural areas to the city in search of work. These people usually occupy government-owned barren land within the city, kept for future construction purposes. The inhabitants are rickshaw-pullers, day-labourers and workers of garment and other factories (in this paper all these categories of people are classified as workers). In slum areas, one family often lives in a very small thatched house (usually less than 100 square feet area). There is no water supply or proper sanitation system. In contrast, affluent people occupy independent houses with sizes ranging from 4,000 to 15,000 sq. ft. area.

In consultation with city officials, local ward commissioners and ward secretaries, an estimated proportion of each type of house in each ward was determined. The surveyors also estimated the approximate number of each type of house in each ward. On the basis of these two methods, a final set of proportions of different types of houses for each ward was generated, and

this list was used to select a representative sample of house types in each ward.

In each ward, 10 geographical "centres" were identified where the surveyors started their day's work. Survey "centres" were selected so that they were evenly distributed throughout each ward. Thus, 10 "centres" were pinpointed on the map of the ward before starting the survey work. Around each "centre", 10 househods, adjacent to the "centre", were surveyed. The surveyors introduced themselves to the residents of each selected house, explained the reason for their visit, and finally one of the surveyors interviewed a responsible person, preferably the household head, using a standardized data collection form. Information gathered was entered into SPSS for analysis.

Results

The largest number of houses surveyed were multistoreyed buildings (39.5%), followed by semi-pucca house (30.5%), and independent houses (20.6%). Only 8.3% of the houses were slum-type (Table 1). Overall, each residence housed an average of 6.5 persons.

Table 2 shows that more than 99% of the respondents had heard about DF/DHF during the survey period. More than 90% of the respondents knew that dengue was transmitted by mosquito bites. While it was commonly recognized that the dengue-transmitting mosquito bites during daytime, only 52% of the people overall knew that this mosquito breeds in containers; 23.5% of workers were aware of this fact as compared with 64% of the professionals.

Table 1. *Different types of houses surveyed with information about the number of occupants in each house*

Type of houses	Number of house-holds	Percen-tage of house-holds	Average number of occupants per house
Independent	1945	20.6	7.16
Multistoreyed	3736	39.5	6.33
Semi-pucca	2884	30.5	6.63
Slum	787	8.3	5.69
Others	93	1.0	5.33
Total	9462	100	6.54

Table 2. *Knowledge of city-dwellers regarding transmission of DF/DHF*

Occu-pation	Total number respon-ded	Percentage of people who had the following knowledge[1]		
		Dengue trans-mitted by mosquito bites***	Behaviour of *Aedes aegypti*	
			Bites during daytime ***	Breeds in containers ***
Business	3577	95.1	93.3	50.3
Service[2]	3119	95.5	94.8	54.5
Retired	765	97.3	95.2	63.7
Worker[3]	519	89.6	85.9	23.5
House-wife	507	93.5	94.9	50.5
Profess-ional	505	97.4	93.7	63.8
Student	42	95.2	92.9	69.0
Others	249	91.2	92.8	48.6
Total	9283	95.0	93.5	52.1

***Chi-square test by occupation: $P < 0.001$

[1]More than 99% of the city-dwellers had heard about DF/DHF

[2]Including officials, clerks and other employees

[3]Including factory workers, drivers, day labourers, etc.

About 50% of the residents thought that destroying the breeding places was important in controlling dengue (Table 3). More than 52% of the people surveyed thought that the use of insecticide-treated nets could be helpful, even though 93.5% knew that the mosquito vector, *Aedes aegypti,* bites during daytime.

More than 70% of the residents purchased commercially-available aerosols/ coils to kill or drive away mosquitoes in their houses (Table 4). Among the inhabitants of independent houses and multistoreyed buildings, the insecticide gadgets purchase rate was more than 75%, while among the slum-dwellers, the rate was only 40%. About 10% of the people living in independent houses and multistoreyed buildings spent more than US$ 10 per month for mosquito control, while another 50% spent US$ 2-10 per month for this purpose. Among the slum-dwellers, 40% spent less than US$ 10; which included 28% spending less than US$ 2. Nearly 60% of the slum-dwellers did not spend any money for mosquito control even during the peak *Aedes* season.

Table 3. *Knowledge of residents concerning methods for prevention and control of DF/DHF*

Occupation	% of people having some knowledge of mosquito control	Proportion (%) of people who think that dengue/DHF can be prevented by					
		Using mosquito net[1]	Spraying aerosol[2]	Using coil[3]	Using smoke[4]	Destroy-ing breeding habitat[5]	Other means[6]
Business	86.8	49.7	35.5	45.4	3.5	47.1	6.9
Service[†]	90.6	57.3	37.6	47.3	4.7	52.0	5.3
Retired	93.6	51.4	41.9	43.4	4.6	55.2	6.4
Worker[‡]	69.9	65.2	8.6	52.5	4.4	35.6	11.3
Housewife	90.1	53.9	37.3	43.6	6.1	52.2	5.9
Professional	93.9	41.4	42.2	33.5	6.5	61.4	9.9
Student	100	40.4	35.7	42.9	7.1	81.0	0
Others	86.7	50.5	35.2	48.1	1.4	42.1	10.6
Total	88.3	52.9	36.1	45.5	4.4	50.2	6.7

Chi-square test by occupation: [1] = $P<0.001$, [2] = $P<0.001$, [3] = $P<0.001$, [4] = $P<0.005$, [5] = $P<0.001$, [6] = $P<0.001$

[†]Including officials, clerks and other employees

[‡]Including factory workers, drivers, day labourers, etc.

Table 4. *Measures taken by city-dwellers to kill mosquitoes in their houses within one month prior to the survey*

House type	Proportion (%) of people who took the following measures:					
	Purchased insecticides for mosquito control***	Sprayed insecticides ***	Spent >US$ 10 for insecticides ***	Spent US$ 2-10 for insecticides ***	Had no expenditure on insecticides ***	Took measures other than the use of insecticides ***
Independent	76.1	78.5	10.9	49.5	23.3	37.0
Multi-storeyed	78.1	80.6	9.4	51.8	20.6	31.9
Semi-pucca	67.4	70.2	3.1	38.2	30.2	30.9
Slum	40.5	41.5	0.1	12.2	59.2	19.8
Others	52.7	53.8	11.8	26.9	50.5	29.0
Total	71.1	73.5	7.8	49.6	27.6	31.6

***Chi-square test by house type: P<0.001

More than 75% of the residents had piped water facilities (Table 5). A water storage system was present in 77% of the houses. Overhead water tanks were present at 72% of the multistoreyed buildings and 47% of the independent houses. Earthen jars were used in 34% and 17% of the slum houses and semi-pucca houses respectively. Drums were used in 20.5% and 23% of the slum houses and semi-pucca houses respectively. In independent houses, earthen jars (6.8%) and drums (12.5%) were seldom used.

The residents were asked whom they thought to be responsible for mosquito control in the city. Most of the respondents (66.5%) replied that both the government and city residents were responsible (Table 6).

Table 5. *Water storage system in different types of houses in Dhaka city*

House type	% of houses having water storage system***	Proportion (%) of houses having the following types of water storage system				
		Underground ***	Overhead tank***	Earthen jar***	Drum ***	Others ***
Independent	77.7	37.2	46.5	6.8	12.5	4.6
Multistoreyed	88.6	51.0	71.8	3.6	11.0	3.7
Semi-pucca	65.5	19.6	12.0	16.7	23.4	14.9
Slum	62.3	6.0	0.8	34.3	20.5	21.9
Others	61.3	18.3	34.4	7.5	9.7	12.9
Total	76.8	34.5	42.0	10.9	15.9	8.9

***Chi-square test by house type: P<0.001,

Note: 77.3% respondents reported that they had piped water facilities in their houses.

Table 6. *Attitude of residents towards responsibility for mosquito control*

Occupation	Responsible agencies or people (%)				
	Government[1]	People living in the city[2]	Both Govt. and people[3]	NGO[4]	Others[5]
Business	22.7	11.8	65.1	0.2	0.7
Service[†]	18.7	11.2	69.1	0.3	1.4
Retired	22.6	8.0	68.8	0.1	1.3
Worker[‡]	32.0	16.4	49.9	0	1.7
Housewife	21.5	7.9	71.2	0.2	0.2
Professional	18.8	7.1	73.1	0	1.8
Student	9.5	9.5	81.0	0	0
Others	27.3	14.5	56.2	0	2.4
Total	21.6	11.1	66.5	0.2	1.1

Chi-square test by occupation: [1] = $P < 0.001$, [2] = $P < 0.001$, [3] = $P < 0.001$, [4] = $P > 0.05$, [5] = $P < 0.01$

[†]Including officials, clerks and other employees

Discussion

In order to cope with massive urbanization, Dhaka is expanding both vertically and horizontally, resulting in a large number of multistoreyed buildings. On the other hand, in the peripheral expanding areas of the city, there are a lot of temporary "semi-pucca" houses.

A large number of seminars, symposia and TV programmes about dengue were organized in the city before and during the time of our survey. Newspapers published features about dengue almost every day. Apparently, the mass media was effective at transmitting key information to the public.

Residents were reasonably knowledgeable about the mechanism of the transmission of dengue. In contrast, in the surveys conducted in Myanmar, Indonesia and Brazil, 75%, 65% and 61% of the populations, respectively, knew that dengue was transmitted by mosquito bites[5,6].

Most residents were aware about the daytime biting behaviour of dengue-transmitting mosquitoes. By contrast, the public was not as knowledgeable about the mosquito's breeding behaviour. The most common mosquito in Dhaka, *Culex quinquefasciatus*, bred mostly in drains, and most people seemed to be aware of that fact. When our surveyors explained (after the survey was completed) that *Aedes* mosquitoes bred in containers, residents hesitated to believe this. Only when the surveyors collected mosquito larvae from drums and put them in small containers covered with a piece of mosquito net so that the residents could see emerging adult mosquitoes in a few days, did the residents believe what the surveyors were reporting. So, it may be a challenge to change the attitudes about *Aedes* breeding among all residents.

People who think that destroying of mosquito breeding places is the way to

control DF/DHF is related to their knowledge about mosquito breeding behaviour. The highest proportion (69%) of the students and the lowest proportion (23.5%) of the workers knew that dengue-transmitting mosquito breeds in containers (Table 2). Similarly, 81% of the students and 35.6% of the workers replied that destroying the breeding places was the way to control DF/DHF (Table 3). Very few low-wage earners (workers) thought that the use of aerosol insecticides would prevent dengue. This opinion probably reflects their economic status. Spraying aerosol is an expensive operation, which is beyond the capacity of a worker, who hardly earns more than US$ 40-50 per month. While we did not ask about the income of the respondents, we could reliably assume what the family earned from the type of the house they lived in.

Among water storage containers, earthen jars and drums act as good breeding grounds for *Aedes aegypti*[7]. Among slums and semi-pucca houses, earthen jars and drums were frequently present, because there are no piped water facilities serving poorer areas. As semi-pucca houses are temporary structures, people do not construct underground or rooftop water tanks, so they store water in temporary containers like drums and earthen jars.

Based on our vector survey, independent houses in Dhaka were most likely to have high densities of *Aedes* mosquitoes[8]. It appears that rooftop concrete water containers are one of the main breeding sources in independent houses. In addition, almost all independent houses have empty backyards where coconut shells, broken bottles, tin cans, discarded utensils, etc., also provide very good breeding sources for *Aedes* in this type of house.

Most *Aedes* mosquitoes breed within houses where the reach of government interventions is limited. The participation and cooperation of the public with government agencies is essential for *Aedes* control programmes. It was apparent from this study that the residents understood this dual responsibility. Thus, a carefully planned effort that includes education of residents on behaviours to reduce the breeding of mosquitoes and disease transmission, along with community control efforts, could be successful, assuming an effective intervention strategy could be identified. Novel approaches for effective vector control are needed. Community participation will be a crucial component for achieving success[9,10].

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Demographic Features of Imported Dengue Fever Cases Serodiagnosed in Japan during 2000

By

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Abstract

Serum specimens were collected from dengue-suspected cases for laboratory diagnosis in Japan. The demographic features of the dengue cases confirmed by serodiagnosis at the National Institute of Infectious Diseases, Japan, during 2000, were reported. Dengue virus infections were diagnosed by IgM-capture enzyme-linked immunosorbent assay (ELISA), IgG-ELISA, Rapid immunochromatographic test, haemmagglutination inhibition (HI) test, and reverse transcriptase-polymerase chain reaction (RT-PCR). Nineteen cases were confirmed to be of dengue fever. Nine cases were male and 10 were female. The youngest case was six-year old and the eldest 58-year old. Most of the dengue patients developed illness after visiting countries in South-East Asia and South Asia. Five cases had visited Thailand, three had been to Indonesia, three to the Philippines, three to India, two to East Timor, and one each to Cambodia, Bangladesh and Sri Lanka. In addition, one patient each had visited South America and the Caribbean, Brazil and the Dominican Republic before developing dengue fever.

Key words: Dengue fever, imported cases, serodiagnosis, Japan

Introduction

Dengue virus infections are a serious cause of morbidity and mortality in most of the tropical and subtropical countries in the world[1,2]. The areas where dengue is a health problem have been expanding. It is estimated that up to 100 million cases of dengue fever (DF) and 250,000 cases of dengue haemorrhagic fever (DHF) occur annually worldwide[3]. Thus, dengue is one of the most important infectious diseases in the world today.

Dengue outbreaks occurred in Osaka, Kobe, Hiroshima and Nagasaki from 1942 to 1945, and dengue virus type 1 was responsible for the outbreaks[4]. The dengue

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virus infection has never been epidemic in Japan since then, and there are no domestic dengue virus infections today. There have been, however, imported dengue cases. The serodiagnosis of dengue virus infection has been performed at the Department of Virology 1, National Institute of Infectious Diseases (NIID), upon requests from hospitals and clinics. The features of imported dengue cases that were serodiagnosed at the NIID from 1985 to 1999 have been previously reported [5,6,7].

Methods and Materials

Serum specimens were collected from dengue-suspected cases in clinics and hospitals, and sent to the Department of Virology 1, National Institute of Infectious Diseases, for laboratory diagnosis of dengue. In the present study we report the features of the dengue cases confirmed by serodiagnosis in 2000. Dengue virus infections were diagnosed by IgM-capture enzyme-linked immunosorbent assay (ELISA), IgG ELISA, Rapid immunochromatographic test, haemmagglutination inhibition (HI) test, and reverse transcriptase-polymerase chain reaction (RT-PCR) as previously reported[6,7].

In-house IgM-capture ELISA was performed as previously reported[8]. Commercial IgM-capture ELISA and IgG ELISA (MRL, California, USA) and rapid immunochromatographic test (PanBio, Brisbane, Australia) were also used. RT-PCR was performed as previously reported[9,10]. The primer sequences used to amplify each serotype of dengue viruses and target size were reported[9,10]. HI tests adapted for microtiter plate were performed, using four haemagglutinin units of dengue-2 viral antigen as previously reported[5].

Results

The table below shows the summary of dengue cases confirmed by laboratory tests. Samples from 44 suspected cases were tested and 19 were confirmed to be of dengue. All the cases were DF, and there was no DHF case during this period. Out of the 19 DF cases, nine were male and 10 female. The youngest case was a child of 6 years and the oldest was 58 years. As shown in the table, most of the Japanese dengue patients developed illness after visiting countries in South-East and South Asia; five cases had visited Thailand, three had been to Indonesia, three to the Philippines, three to India, two to East Timor, and one each to Cambodia, Bangladesh and Sri Lanka. They included one person who had visited both Thailand and Bangladesh before developing dengue fever, and one case who had visited both Cambodia and India. In addition, one patient each had visited South America and the Caribbean, Brazil and the Dominican Republic before developing dengue fever.

Table. Demographic information on 19 dengue cases

No.	Age (years), Sex	Country
1	32, M	East Timor
2	58, M	Brazil
3	29, M	Thailand
4	23, F	East Timor
5	33, F	Indonesia
6	37, F	Thailand
7	46, M	Indonesia
8	28, F	Dominica

No.	Age (years), Sex	Country
9	33, F	Indonesia
10	44, M	Thailand Bangladesh
11	21, F	Cambodia India
12	6, F	Philippines
13	22, M	Philippines
14	24, F	Philippines
15	23, F	India
16	37, M	Thailand
17	40, F	Thailand
18	52, M	India
19	36, M	Sri Lanka
Case No.10 visited both Thailand and Bangladesh.		
Case No.11 visited both Cambodia and India.		

Discussion and conclusion

We previously reported that approximately 50% of the Japanese DF cases were between the ages of 21-30 years[6]. This probably reflects the number of travellers who visited dengue epidemic areas. Furthermore, it is important to note that the number of cases who visited Oceania, Central America and Africa before developing dengue illness has been increasing[5,6,7]. Thus, dengue is not a disease to be considered for a differential diagnosis only for patients who come back after visiting Asian countries.

We believe that these dengue cases account for only a small portion of the imported cases, although the exact number of dengue cases in Japan is not known. According to the Japanese new infectious disease control law, which became effective on 1 April 1999, DF/DHF is one of the infectious diseases which all physicians are required to report. Nearly five million Japanese visit countries in the tropical and subtropical areas of the world annually and two million people visit Japan from these areas. Therefore, DF/DHF is an infectious disease which should attract more attention in Japan.

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Predisposing Factors of Dengue Cases by Random Effect Model in the Largest Dengue Haemorrhagic Fever Epidemic in Taiwan in 1998

By

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Abstract

In November 1998, the largest officially-documented dengue haemorrhagic fever (DHF) epidemic since 1943 occurred in Taiwan. This epidemic resulted in more than 110 dengue fever (DF) cases and at least 36 DHF cases. A case-control study was conducted to explore the risk factors of dengue infection. Thirty-four cases and 68 matched controlled cases were included for statistical analysis. After further adjusting the confounders and intra-household correlation by random effect model, three distinctive risk factors were identified. These were: the presence of empty houses, spare tyres, neighbourhood ponds or temples (OR = 3.17, 95% CI: 0.95-10.63) which was first identified in the papers, water containers with covers in the house (OR = 5.77, 95% CI = 1.08-30.8), and screened windows and doors (OR = 0.71, 95% CI = 0.32-0.89). Control measures were aimed at these risk factors and the epidemic subsided in January 1999 when the last dengue case was reported.

Key words: Dengue, predisposing factors, Arbovirus, Epidemiology, Taiwan.

Introduction

The dengue virus infection is the most common arthropod-borne disease worldwide, with an increasing incidence in the tropical regions of the Americas and Asia, including Taiwan. The spectrum of dengue diseases ranges from the febrile flu-like illness, DF, to the severe forms of DHF and dengue shock syndrome (DSS) with high morbidity and mortality. The virus is primarily transmitted by mosquitoes, *Aedes*

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aegypti and *Aedes albopictus*. It is estimated that around 100 million cases of DF occur worldwide and over 200,000 DHF/DSS cases are reported to the World Health Organization (WHO) every year[1-4].

Following a nearly 40-year silence of dengue epidemic since 1942-43, the dengue virus serotype 2 (DEN-2) was introduced into Hsiao-Liu-Chiu, an islet off southern Taiwan in 1981, which caused over 1,700 dengue cases[5]. Since then, different sizes of outbreaks have been reported from different locations in Taiwan. The Department of Health established several surveillance systems to detect dengue patients, including doing active and passive surveillance. In 1998, Tainan, an urbanized city in southern Taiwan, exploded with the largest epidemic of DHF in Taiwan in the last 30 years. This epidemic resulted in more than 110 DF cases and at least 36 DHF cases. A case-control study was conducted to investigate the predisposing factors for the risk factors for the infection.

Many epidemiological studies of dengue epidemics have focused on using a statistical method of logistic regression for independent measures on cases and controls. However, dengue cases usually cluster in certain local areas, and this type of clustering reflects common environment in the sampling block or other unknown factors. A unique feature of those clustering dengue cases was that they exhibited intra-cluster and inter-cluster correlation. Therefore, we used the random effect model with the generalized estimation equation (GEE) approach developed by Liang and Zeger (1986)[6] to analyse the possible correlation of the clustered data collected after our outbreak investigation.

Materials and methods

Study populations

Tainan city is located in tropical southern Taiwan. Our study populations involved laboratory-confirmed dengue cases. The case group recruited at least one confirmed dengue case within one house unit while the control group recruited local residents who were both dengue-IgM sero-negative and without febrile illness, within one month of our interview during the epidemic period. This neighbourhood control group selected those who lived in different *lings* (the official name of sub-district area) from the cases but in the same *li* (the official district covering areas larger than ling). One case was matched with two neighbourhood controls. If the possible source(s) of infection for cases were judged from the working or school sites, the controls were then also selected from the same *ling* of the working or school sites of the cases.

Household interview and environmental inspection

All cases and controls were interviewed blindly by the interviewer, and standardized on the appraisal of environmental conditions such as vector-breeding sites, screens and sanitary conditions in the subjects' living places, including markets, open sewers or ditches within 50 metres. The questionnaire was designed as structured and pre-tested. The variables in the questionnaire included general demographical characteristics, past

or present existence of mosquito-breeding sites on the premises (including water-storage tanks, flower vases, tin cans, containers, unused tyres, flower vases, water trays of the refrigerator), use of screens on windows and doors, types of screen, travel history, presence of pets, indoor spray of insecticides, use of traps or mosquito coils, size of household, type of housing and population density in each house. Both the medical history and blood samples were taken from all family members in each household.

Table 1. *Univariate analysis of dengue virus infection by predisposing risk factors in case and control groups in 1998 in Tainan, Taiwan*

Risk Factors	Cases	Controls	Odds Ratios	95% Confidence Intervals
Empty house, spared tyres, pond or temple around				
Yes	24	37	2.011	0.835-4.841
No	10	31		
Water containers around the house				
Yes	7	27	0.409	0.156-1.074
No	26	41		
Water containers without covers around the house			0.373	0.127-1.098
Yes	5	22		
No	28	46		
Planting at backyard				
Yes	21	47	0.722	0.305-1.709
No	13	21		
Planting with plates at backyard				
Yes	1	7	0.272	0.032-2.311
No	32	61		
Water containers inside house				
Yes	11	18	1.329	0.541-3.261
No	23	50		
Water containers with covers inside the house				
Yes	19	40	0.887	0.386-2.037
No	15	28		
Discarded trash				
Yes	15	34	0.789	0.345-1.805
No	19	34		
Refrigerator with plates				
Yes	6	21	0.497	0.179-1.384
No	27	47		

Laboratory diagnosis

Acute-phase serum samples were collected from patients within seven days after the onset of fever and stored in –70ºC freezer for reverse transcriptase-polymerase chain reaction (RT-PCR) and virus isolation[7]. If the results were negative, the convalescent serum was withdrawn for antibody confirmation by the dengue virus specific IgM-ELISA method[8].

Statistical analysis

Data were entered in Epi-Info 6.0 and double validated. For better correlation analysis, a household was used as the analysis unit. Dengue cases which flocked together were classified as a cluster, and seven clusters in total were determined because each cluster potentially shared the same environmental factors attributed to the transmission of dengue virus. The odds ratio and 95 per cent confidence interval were calculated by univariate analysis. The random effect model with GEE was used for adjusting the confounders by using the statistical analysis system computer package (SAS release 6.12). For hypothesis testing, a α-level of 0.05 was chosen.

Results

Of the 141 confirmed dengue cases, 81 cases agreed to participate and were interviewed. However, some of them were from the same household or no compatible controls were found. Therefore, 34 cases in total were included for data analysis. None of these cases had travelled abroad during the year of the epidemic, nor had they been diagnosed as dengue patients before. Sixty-eight geographically-matched and selected neighbourhood controls were interviewed for analysis.

Table 2. *Univariate analysis of dengue virus infection by predisposing protective factors in case and control groups in 1998 in Tainan, Taiwan*

Protective Factors	Case	Control	Odds ratio	95% confidence interval
Screened windows				
Yes	18	41	0.89	0.56-1.34
No	16	27		
Screened doors				
Yes	16	33	0.77	0.61-1.28
No	18	35		
Screened Windows and doors				
Yes	22	46	0.85	0.64-1.19
No	12	23		
Use mosquito coil, insecticide, repellent or sleeping net				
Yes	18	49	0.436	0.185-1.028
No	16	19		

The univariate analysis showed that the risk of contracting dengue virus infection was not associated with factors such as empty house, spared tyres, presence of a pond or a temple around the house or water containers in or around the house, water containers with or without lid, planting at the backyard with or without water plates, discarded trash, presence of water plates at the bottom of refrigerators, and the size and number of residents in each house (Table 1). In other words, all those major predisposing risk factors were found to be not statistically significantly associated with the dengue virus infection in l998 in Tainan. On the other hand, protective factors such as screened doors, screened windows or the use of mosquito coils, insecticides, mosquito repellents or sleeping nets were also not significantly associated with the dengue virus infection (Table 2).

Table 3. *Multivariate analysis of dengue virus infection by random effect model in case and control groups in 1998 in Tainan, Taiwan*

	Coeffi-cient	Standard deviation	OR (95%CI)[a]
INTERCPT	-0.6273	0.7434	
With empty house, spared tire, pond or temple around	1.1544	0.6168	3.17(0.95-10.63)*
Use mosquito coil, insecticide, repellent or sleeping net	-0.7794	0.6145	0.46 (0.14-1.53)
Water containers around the house	0.4926	0.6585	1.64 (0.45-5.95)
Water containers in the house and with covers	1.7534	0.8542	5.77 (1.08-30.8)*
Discarded trash	0.2208	0.4619	1.25(0.5-3.1)
Water containers around the house and without covers	0.3164	0.4225	1.37(0.6-3.14)
Screened Windows and doors	-0.3436	0.1143	0.71(0.32-0.89)*
Doors open frequently	0.0903	0.0709	1.09(0.95-1.26)

[a] CI: confidence interval

*p<0.05 with statistical significance

After further adjusting for confounders by multivariate analysis of the random effect model, we found that the presence of empty house, spared tyres, neighbourhood pond or temple odds ratio (OR) = 3.17, 95% confidence interval (CI: 0.95-10.63), water containers with covers in the house (OR= 5.77,95% CI = 1.08-30.8) and screened windows and doors (OR = 0.71, 95% CI = 0.32-0.89) were statistically significantly associated with dengue infection (Table 3).

Discussion

The risk factors of the dengue virus infection that had been documented previously were wooden housing, absence of screens on windows or doors, existence of mosquito breeding sites on the premises, and presence of domestic animals[9-13]. Our case-control study demonstrated that the three major risk/protective factors associated with contracting the dengue virus infection were: (1) empty containers in the house; (2) empty houses or ponds around the dwelling; and (3) screen on both windows and doors. Since dengue virus is primarily transmitted by mosquitoes, empty containers serve as the best breeding places close to local residents in epidemic areas. On the other hand, screened windows and doors acted as protective shields when the mosquito tried to fly into the house from outside. Therefore, these two factors were consistent with previous findings[10-13]. In addition, we first identified the emergent factor (our third factor) of empty houses or ponds around living premises which were associated with the dengue virus infection in Tainan [odds ratio = 3.17 (95%CI: 0.95-10.63)]. In fact, Tainan city is an old city with many old buildings, many of them being empty. Those old buildings which were filled up with trash provided the best breeding sites for mosquitoes and usually were neglected by public health practitioners and

environmental inspectors. Therefore, mosquito density around empty houses should also be monitored for better prevention and control of dengue.

Dengue fever is a mosquito-borne disease and the risk of a person contracting the disease is largely determined by individual attributes, household conditions and environmental factors[14]. The transmission of dengue virus by mosquitoes, particularly *Aedes aegypti,* tends to be localized and clustered in the same household. Previous studies separated the risk factors at individual level from environmental level to avoid intra-household correlation because more than one case per household may have occurred. However, this kind of approach may neglect inter-household correlation, especially considering environmental factors among households as independent variables. In this study, we took the inter-household correlation into consideration by using the random effect model and showed a better statistical method to search for environmental factors contributing to the transmission in different localities. Future studies should also pay attention to the intra-household correlation for a better understanding of the dengue virus transmission at three major different levels: individual, household and environment.

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Clinical and Laboratory Presentations of Dengue Patients with Different Serotypes

By

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Abstract

A five-year retrospective review of confirmed dengue patients admitted to the Children's Hospital, Bangkok, during 1995-1999 was undertaken to compare the clinical and laboratory presentations between DEN-1, 2, 3 and 4. Dengue serotype could be identified in 50.6% (2,398 cases) of all the confirmed cases (Total: 4,743 cases) during this period. DEN-3 was the predominant serotype found in 50.6%, followed by DEN-1 (25.8%), DEN-2 (20.9%) and DEN-4 (2.7%). There was no gender difference. The mean age of patients was significantly higher in DEN-4 (9.8 years) while in DEN-1, DEN-2 and DEN-3 it was 8.2, 8.8 and 8 years, respectively. The peak incidence of DEN-1, DEN-2 and DEN-3 was among children between the age of 5-9 years while in DEN-4 it was between 10-14 years. From this study it was concluded that 91.8% of DEN-2 infections were secondary and it presented with more severe disease as demonstrated by more degree of plasma leakage, more shock and more complication of fluid overload cases. DEN-3 and DEN-4 tended to have more degree of liver involvement as demonstrated by more patients with abnormal elevation of liver enzyme AST and ALT and higher mean value of both AST and ALT. Because 29.6% of DEN-3 presented as primary infections and DEN-4 was the rare serotype, so that hepatic dysfunction/encephalopathy was not a big problem during this study period. DEN-1 seemed to have mildest clinical presentation with 29.6% presented as primary infections. With this knowledge, if the circulating dengue serotype in the area is known, a better plan for case management can be established: preparation for more colloidal solutions and probably blood transfusion in DEN-2 and DEN-1 outbreaks and preparation for the management of DHF patients with hepatic dysfunction/encephalopathy if there is DEN-3 or DEN-4 outbreak.

Key words: DEN-2, Plasma leakage, Shock, DEN-3, DEN-4, elevation liver enzyme, AST, ALT.

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Introduction

Dengue infection is the most important mosquito-borne viral disease known to mankind. There has been a dramatic increase in the number of cases and the severity of the disease in the past 30 years. Currently about 2.5 billion of the world's population, primarily in tropical developing countries, is at risk. There are tens of millions of cases of dengue which are estimated to occur every year. Hundreds of thousands of these cases are of the more severe form of dengue - dengue haemorrhagic fever (DHF) - which is a leading cause of childhood hospitalization and death in many countries[1].

Some epidemiological, serological and virological studies have shown that DEN-2 is the most common serotype associated with the occurrence of the most severe forms of the disease, DHF and dengue shock syndrome (DSS)[1,2,3]. So far, there has been no clinical study undertaken concerning the clinical and laboratory presentations of the different dengue serotypes. Hence, this study was undertaken in order to compare these factors among each dengue serotype, i.e. DEN-1, DEN-2, DEN-3 and DEN-4.

Materials and methods

Hospital records of DF and DHF patients who were admitted to the Children's Hospital (Queen Sirikit National Institute of Child Health), Bangkok, with confirmed dengue infection by viral isolation and/or polymerase chain reaction (PCR) between 1995-1999 were reviewed. The Armed Forces Research Institute of Medical Sciences (AFRIMS) performed all these laboratory confirmations.

Most of the diagnoses of DF and DHF patients at the Children's Hospital were based on WHO clinical criteria[4]. Most doctors did not use the serological and virological confirmations for diagnosis routinely. The DHF severity was also classified by using WHO guidelines[4].

Clinical and laboratory data comparison between patients with each dengue serotype were analysed using SPSS programme.

Results

Between 1995-1999, there were 17,908 dengue patients diagnosed at the OPD of the Children's Hospital; 4,595 of these patients (25.7%) were admitted as inpatients. The serological and/or virological studies were performed in 4,743 patients (89.0%). Dengue viruses were identified in 2,398 patients (50.6%). There were 618 cases of DEN-1 (25.8%), 501 of DEN-2 (20.9%), 1,213 of DEN-3 (50.6%) and 64 of DEN-4 (2.7%). One dengue virus could not be classified and one patient (DHF grade IV) had evidence of two dengue virus infections (DEN-2 and DEN-1).

Primary infections were found in 27.5% and 29.6% of DEN-1 and DEN-3 cases while only 5.8% and 4.7% primary infections were found in DEN-2 and DEN-4 cases respectively (Table 1). Corresponding with these findings, DEN-1 and DEN-3 infections presented themselves as DF in 19.9% and 23.4% of the cases respectively (Table 2).

Table 1. *Primary and secondary dengue infections*

	DEN-1 (%)	DEN-2 (%)	DEN-3 (%)	DEN-4 (%)	Total (%)
Primary	27.5	5.8	29.6	4.7	23.4
Secondary	69.4	91.8	66.6	90.6	73.2

Table 2. *Clinical presentations of dengue infections*

	DEN-1 (%)	DEN-2 (%)	DEN-3 (%)	DEN-4 (%)	Total (%)
DF	19.9	12.8	23.4	15.6	20.1
DHF	62.5	54.3	55.8	65.6	57.4
DSS	17.6	32.9	20.8	18.8	22.5

Secondary infections were found in 91.8% and 90.6% of DEN-2 and DEN-4 infections and the clinical presentations of DHF/DSS in DEN-2 and DEN-4 were 87.2% and 84.4% respectively (Table 2). DEN-2 significantly ($p<0.000$) presented itself as DSS (32.9%), more than DEN-1 (17.6%), DEN-3 (20.8%) and DEN-4 (18.8%). DEN-2 had the highest percentage of patients who still had fever while in shock (22.4%).

There was no sex difference in all DEN serotypes. The mean age of patients was significantly higher for DEN-4 (9.8 years), while in DEN-1, DEN-2 and DEN-3 patients it was 8.2 years, 8.8 years and 8 years respectively ($p<0.000$). The peak incidence of infections of DEN-1, DEN-2 and DEN-3 was in children aged between 5-9 years, while for DEN-4 it was between 10-14 years.

The clinical presentations of all four dengue serotypes: the duration of fever (4.2 days); bleeding manifestations [including patechiae (49.4%), epistaxis (21.4%), haematemesis (19.8%), melena (8.6%), gum bleeding (3.1%) and menstruation (1.6%)]; maculopapular rash (5.9%); and tourniquet test (90.5%) were the same ($p>0.05$) (Table 3).

Table 3. *Haemorrhagic manifestations of dengue infections*

	DEN-1 (%)	DEN-2 (%)	DEN-3 (%)	DEN-4 (%)	Total (%)
Petechaie	51.9	50.6	47.9	42.6	49.4
Epistaxis	21.8	20.8	20.8	31.5	21.4
Hematemesis	19.8	22.2	18.3	27.8	19.8
Melena	8.9	8.5	8.5	9.3	8.6
Gum bleeding	3.0	3.9	2.7	5.6	3.1
Menstruation	1.6	2.5	1.2	1.9	1.6

Liver enlargement was found to be more in DEN-2 (92.7%) and DEN-3 (93.3%) patients [DEN-1 (88.9%) and DEN-4 (85.2%)].

Unusual manifestations were not common for all the four dengue serotypes. Patients who presented with encephalopathy were the same for all four serotypes (0.8%): DEN-1 (0.6%), DEN-2 (1.2%), DEN-3 (0.7%) and DEN-4 (1.6%). Associated infections (3.9%) and conditions (3.3%) were found to be the same for all dengue serotypes (Table 4).

Table 4. *Unusual manifestations of dengue infections*

	DEN-1 (%)	DEN-2 (%)	DEN-3 (%)	DEN-4 (%)	Total (%)
Encephalopathy	0.6	1.2	0.7	1.6	0.8
Associated infections	4.0	3.4	4.2	0	3.9
Associated conditions	4.4	2.3	3.3	0	3.3

The highest mean rising haematcrit (Hct) was the highest in DEN-2 (21.5%) and the lowest in DEN-3 (17.8%). The mean WBC was the highest in DEN-2 (4,440 cells/cumm.) and the lowest in DEN-1 (3,585 cells/cumm.). The mean platelet count was the lowest in DEN-2 (62,178 cells/cumm.) and the highest in DEN-1 (72,078 cells/cumm.) (Table 5).

Table 5. *CBC in dengue infections*

	DEN-1 (%)	DEN-2 (%)	DEN-3 (%)	DEN-4 (%)	Total (%)
Mean Hct maximum	43.3	45.0	42.7	43.8	43.4
Mean Hct minimum	36.4	36.9	36.1	36.7	36.4
Mean rising Hct	18.8	21.5	17.8	20.1	18.9
Mean WBC (cells/cumm)	3,585	4,440	3,992	3,991	3,984
Mean PMN	52	48	41	49	45
Mean AL	7	7	8	8	7
Mean Plt count (cells/cumm)	72,078	62,178	75,502	66,565	71,592

The mean AST is the highest in DEN-3 (272 U) as compared to DEN-1 (195 U), DEN-2 (184 U) and DEN-4 patients (248 U) while the mean ALT was the highest in DEN-4 (122 U) as compared to DEN-1 (85 U), DEN-2 (77 U) and DEN-3 patients (114 U). AST elevation of >200 U was found in 30.2%, 19.3%, 36.4% and 37.1% of patients with DEN-1, DEN-2, DEN-3 and DEN-4 infections respectively. ALT elevation of >200 U was found in 9.2%, 6.3%, 13.7% and 20.4% of patients with DEN-1, DEN-2, DEN-3 and DEN-4 infections respectively (Table 6).

Table 6. *Percentage of dengue patients with elevation of AST/ALT > 200 U*

	DEN-1 (%)	DEN-2 (%)	DEN-3 (%)	DEN-4 (%)	Total (%)
AST	30.2	19.3	36.4	37.1	30.9
ALT	9.2	6.3	13.7	20.4	11.2

The mean amount of IV fluid, crystalloid solution, received during the critical period in DEN-1, DEN-2, DEN-3 and DEN-4 were 71, 83, 71 and 66 ml/kg respectively, while the mean amount of Dextran-40, colloidal solutions, received was 17, 19, 18 and 19 ml/kg respectively.

Blood was transfused in 5.3%, 6.7% and 5.6% of DEN-1, DEN-2 and DEN-3 patients. DEN-4 patients in this study did not require blood transfusion.

Complications of fluid overload were found in 4.4% of patients of DEN-1, 9.6% of DEN-2, 5.4% of DEN-3 and 1.9% of DEN-4 patients.

The case-fatality rate (CFR) was not different between all the four dengue serotypes ($p=0.981$). The CFR for DEN-1, DEN-2 and DEN-3 was 0.4%, 0.5% and

0.3% respectively. There was no death among DEN-4 patients.

Discussion

The dengue serotype could be identified more between 1995-1999 - 50.6% as compared to about 20-30% in the previous year[1]. This was partly due to the improvement in the technique used in identifying the viruses. Previously, only virus isolation by the mosquito inoculation technique was performed. Since 1995 when the PCR technique was introduced, the identification of viruses improved significantly. In the past few years, even a more sensitive PCR technique was applied. Another major factor which contributed to the increase in the percentage of viral isolation/identification was likely due to the early admission of suspected dengue patients during their febrile phase when they had viremia.

All the four dengue serotypes were present in Bangkok during this study period. DEN-3 was the predominant serotype while DEN-4 was very rare. DEN-1 was more common than DEN-2. The changing pattern of the predominant dengue serotype circulating in this area may determine the overall severity of the disease. A study to compare the disease severity with the period when different serotypes of dengue were predominant will help answer this question.

DEN-2 was confirmed to cause a more severe form of the disease, i.e. more plasma leakage as reported previously[3]. This implies that if there is a DEN-2 outbreak, we have to be well prepared to face a more severe form of the disease when possibly more colloidal and blood transfusion would be needed. Early detection of plasma leakage and early administration of colloidal solution if there is evidence of massive plasma leakage, and early blood transfusion if indicated, are essential to prevent complications and death[4].

A previous report had revealed that about 80% of dengue patients had elevation of enzyme AST and/or ALT which may be used as an early indicator of dengue illness[5]. In this study, DEN-3 and DEN-4 were more likely to have elevation of AST and/or ALT. About 30% of DEN-3 infections presented with primary infection and 23.4% resulted in DF, which is a mild form of the disease. These findings can explain that in some DF patients, hepatitis may be found if DEN-3 or DEN-4 was the cause of their illness. Other DEN-3 patients who presented with DHF had an increased tendency for severe disease because they tended to have elevation of liver enzyme, AST and/or ALT, especially in cases with shock. If there is a DEN-3 outbreak, we have to be well prepared for possible more patients with liver dysfunction/failure. Early attendance at hospital, blood screening for AST/ALT and early admission are recommended if a patient has abnormal elevation of these enzymes. Drug usage should be strictly minimal for such patients.

DEN-4, like DEN-3, had a higher tendency of involvement of the liver. Most DEN-4 infections were secondary (90.6%); thus, most patients were likely to present with DHF. Therefore, if there is DEN-4 outbreak, more DHF patients with liver impairment can be expected than in a DEN-3 outbreak.

DEN-4 infected older patients as compared to other serotypes. These older patients could escape the DEN-4 infection earlier in life because this serotype was rarely found in the past. DEN-4 came and disappeared in a cycle of 23 years[1]. The occurrence in older children and adults can be expected if there is a DEN-4 outbreak.

DEN-1 had no special characteristic, except that we found that more DEN-1 patients developed shock after admission than patients with other serotypes.

WBC at the time of shock seemed to correlate very well with the disease severity. DEN-2 patients had a higher mean WBC and DSS patients had a higher mean WBC. The higher the WBC, the more severe is the disease. Further study of WBC and differential counts is another area of interest because it may be used as a simple indicator for disease severity.

This study included only those dengue patients who came to the hospital rather early in the course of their illness so that the virus could be identified. Those patients who had a very severe disease and came to the hospital rather late were not included in this study because the virus could not be identified after defervescence. A technique to identify viruses after the period of viremia should be developed so that the viruses that cause more severe forms of the disease could be identified. The inclusion of patients coming late to hospital will further characterize the severity of each dengue serotype.

Conclusion

All the four dengue serotypes were found in dengue patients seen at the Children's Hospital during 1995-1999. DEN-3 was the predominant serotype (50.6%), followed by DEN-1 (25.8%) and DEN-2 (20.9%). DEN-4 was the rare serotype (2.7%).

This 5-year retrospective clinical and laboratory review of confirmed serotypes of dengue patients suggests that DEN-2 is the most severe serotype which is usually associated with secondary infections (91.8%) and the occurrence of DHF (87.2%), especially DSS (32.9%). Another supporting evidence of the severity of DEN-2 was that these patients needed a larger amount of IV fluid (both crystalloid and colloid solutions) and more blood transfusion. Also, complications of fluid overload were found more in DEN-2 patients (9.6%).

DEN-3 and DEN-4 had more degree of liver involvement as demonstrated by a higher percentage of patients with elevation of liver enzyme (AST and ALT) of more than 200 U. These might predispose DHF patients to have an early liver failure if they had a prolonged shock. In DF cases, they are likely to have hepatitis.

DEN-1 seemed to be the mildest serotype, except that DEN-1 patients were most likely to develop shock after admission.

The nature and severity of each dengue outbreak can be predicted from the above findings. If there is a DEN-2 outbreak, it is likely to have more severe DHF cases with shock. If there is a DEN-3 or DEN-4 outbreak, clinicians have to be cautious about patients with liver involvement and should prepare themselves to manage patients with hepatic dysfunction/failure with or without hepatic encephalopathy.

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Successes and Failures in Dengue Control - Global Experience*

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Abstract

Despite the admirable achievement of the control and near eradication of *Aedes aegypti* in the Western hemisphere in the mid-twentieth century, today this species is more abundant and widespread than at any time in human history. As a result, dengue viruses have spread to be among the most common pathogens on earth. This paper reviews the history of the success and then the failure to control *Aedes aegypti*. Two crucial failures contributed to the dismal story of dengue control: (1) no major educational institution has ever committed itself to solve the problem; and (2) there are no outraged and vocal citizen groups. At least 12 sectors of society have some interest in or responsibility for the control of dengue: national health, city health, environment, urban planning, justice, education, science and technology, the media, private sector and people themselves. The current roles of these sectors in the control of dengue are given. This is followed by a description of the more active roles that might be adopted by each of these sectors. Finally, advocacy methods are described for use by physicians, public health workers and scientists who face the problem of dengue on a daily basis.

Key words: *Aedes aegypti,* DF/DHF, global experience

Introduction

This is not a technical paper. This is a discussion of a framework for approaching the environmental control of dengue. The most important elements of any such framework are a sense of moral indignation at unnecessary suffering and for the leadership to mobilize human will power and resources to take on the task of controlling dengue. In this context, I am pleased to acknowledge the initiative of His Majesty King Bhumipol of Thailand whose sensitivity to the problem of dengue and

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whose leadership has made inevitable the First International Conference on Dengue and Dengue Haemorrhagic Fever, Chaing Mai, Thailand, November 2000 .

Successes

The 20th century has witnessed great triumphs with respect to the control, even eradication, of *Aedes aegypti*, the principal urban cycle mosquito vector of dengue viruses. In the very first year of the 20th century, a team led by Major Walter Reed of the US Army, tested the mosquito transmission hypothesis put forward by Dr Carlos Finley, a Cuban private physician, with supportive data from Dr Henry Carter, US Public Health Service[1]. By December 1900, as a result of Reed's experiments, *Aedes aegypti* had been convincingly shown to be a yellow fever vector, the intrinsic incubation period in the mosquito was proven, and yellow fever virus had been shown to be of such a small size that it could pass through a porcelain filter. But, the truly inspiring story occurred immediately thereafter. Using source reduction methods made up on the spot, Maj. William Gorgas successfully eradicated urban yellow fever from Havana, a city that had reported cases for over 300 years[2]. Gorgas' improved methods were applied in Panama, permitting the successful construction of the Canal. In 1919, the Rockefeller Foundation funded a Yellow Fever Commission that soon eliminated urban yellow fever throughout the American hemisphere. In Brazil, with the strong support from its President, Dr Fred Soper, of the Rockefeller Foundation, applied imagination, a variety of methods, a disciplined force, meticulous records and sheer force of personality to discover that persistent source reduction could lead to species eradication[3,4].

In 1947, when Soper was appointed Director of the Pan American Health Organization, *aegypti* eradication campaigns were organized throughout the hemisphere. By 1960, using DDT to supplement Soper's well-described methods[5], a*egypti* were certified as eradicated from all but four of 27 American countries. Sadly, one of these four countries was the United States. From the late 1960s, when the US adopted a surveillance programme instead of species eradication, *aegypti* eggs were exported in used automobile tyres to Central and South America. Today, the distribution of the species exceeds the maximum range recorded earlier in the 20th century.

Success did not entirely cease with Soper's retirement from PAHO. Using similar methods, Cuba came very close to eradicating *aegypti* following the 1981 epidemic of dengue haemorrhagic fever[6], and Singapore has maintained very low *aegypti* populations for more than 30 years[7]. The dengue control movement is gaining some new ground: new, more sensitive and specific measures of entomological surveillance have been proposed[8,9], improved community-based methodologies are available[10], and several pilot or small-scale successes have been obtained with effective community participation[11,12]. Most DHF endemic countries have made substantial investments in dengue control. Undoubtedly, at this meeting we shall learn about numerous improvements that have been made in mosquito control programmes.

Failures

The remorseless progression of the 20-21st centuries dengue pandemic tells us that large-scale control of *Aedes aegypti* has been among their most conspicuous public health failures. Why is this?

There are many reasons. Among them, limited national and global resources, failure of planners to recognize that dengue imposes an important financial and social burden, the regional nature of the dengue problem, absence of proven and sustainable vector control methods, the confusion caused by the squabble between the proponents of "eradication" and "control" strategies, indecisiveness because of waiting for a dengue vaccine, and the generally doleful support for and leadership by the public health sector. In short, dengue is a paradigm for unsolved 21st century health problems, a subject dealt with at length in a new book by Garrett[13].

The way forward

My intention is not to dwell on the reasons for the failure to control *Aedes aegypti*, although a great deal can always be learned by examining the past. Instead, I would like to direct a few personal observations to the following question: what can be done to improve the chances of success of controlling dengue in the new century?

My thinking is framed by four precepts:

- The transmission of dengue viruses is basically an urban environmental problem.
- Human behaviour contributes importantly to creating and sustaining *Aedes aegypti* breeding sites.
- With respect to people and mosquitoes, our scientific and management knowledge base is insufficient to design and sustain successful programmes to control *Aedes aegypti*.
- The dengue problem is global and immense.

It follows that successful control of dengue will require a major, sustained, cooperative and well-funded effort. We cannot hope to approach dengue control except by re-prioritizing society's social goals, creating new partnerships and developing new tools through an imaginative programme of enhanced research.

Social organizations

Educational institutions: A 1992 study commissioned by the Rockefeller Foundation could find no major university in any dengue-endemic country that offered graduate programmes in *Aedes aegypti* bionomics or control. Many national vector control programmes do not have PhD medical entomologists in leadership positions. Similar voids can be found in programmes designed to modify human behaviour and in the graduate training needed to supply researchers. Those working in the field with graduate degrees usually have obtained them abroad, focusing on topics of little relevance to endemic areas. Thus, there is no academic and

research base to supply personnel or complement government interventions.

Citizen groups: In Western societies, it is common for aggrieved citizens to form groups to protest against harmful conditions and to fight for the actions and reforms needed to change them. These kinds of groups have been conspicuously absent in the face of the dengue problem. While the absence of such groups may reflect Asian "values" or differences in civil society in the Asian cultural milieu, scholarly and political attention needs to be paid to these social phenomena and the gaps that they leave.

Partnerships

At the very least, each of the following sectors or agencies have an important role to play in dengue control:

(1) *Public health ministries*: Ministries of public health define the size and impact of dengue as a health problem, provide facilities and manpower for coping with its clinical burden and many provide the manpower and resources to combat *Aedes aegypti*[14]. Instead of sustained mosquito control programmes dengue is controlled through mosquito abatement mounted in response to reported cases, e.g. adulticide sprays. Budgets are often inadequate to support either control or a meaningful research effort.

(2) *City health departments:* The modern megacity is often autonomous or semi-autonomous[15]. Its health department may organize dengue vector control programmes that are independent of national authorities.

(3) *Environment ministry:* In many countries, environment ministries have assumed responsibility for source reduction and the application of pesticides against *Aedes aegypti.* This is consistent with the reality that environmental programmes are needed for dengue control.

(4) *Urban planning:* It is not at all clear that past or present generations of urban planners or architects have included as a goal the reduction of breeding sites for *Aedes aegypti* in public or private spaces.

(5) *Justice sector:* The design and/or enforcement of laws that regulate human behaviour are usually the responsibility of the justice sector. Sanctions and fines discouraging the breeding of vector mosquitoes by householders have been the key to historically successful *A. aegypti* control programmes. Such laws continue to be used effectively in modern-day Singapore, Malaysia and Cuba.

(6) *Education sector:* A number of countries have designed, implemented and evaluated curricula for schoolchildren that teach the biology of *Aedes aegypti* and its control, including laboratory and field work. Universities, envisioned by most national leaders as institutions crucial to national development, have failed to respond to the dengue problem[16].

A recent survey of Asian universities found almost no graduate education in medical entomology, especially on the bionomics and control of *Aedes aegypti.* Little attention is paid by academia to the human behavioural aspects of dengue control[17].

(7) *Science and technology:* Most large dengue-endemic countries offer only limited support to research on vector control and almost none to develop the technical and scientific manpower that such research requires[17]. Large industrialized countries do support modest programmes for basic and vaccine research on dengue, but this is not an effort commensurate with the size of the problem.

(8) *The media:* The media everywhere make efforts to educate the public on health issues and to be a part of the solution of public health problems. A dramatic example is Ted Turner's gift to the UN of CNN time dedicated to health and children's issues. In my own experience, I have noted that the *Bangkok Post* has highlighted dengue and DHF outbreaks in Thailand for more than 40 years. I am not aware that anyone in the dengue control community has acknowledged this contribution publicly.

(9) *Private sector:* A significant, but largely unmeasured percentage of effective control of adult *Aedes aegypti* can be attributed to the use of commercial products, e.g. aerosol insect sprays. In some developing countries small private vector control firms have come into existence. In the United States there are many large private firms that provide a wide range of control services against nuisance or vector mosquitoes[18,19]. Private foundations, Rotary Clubs and Rotary International have and will continue to support research, training and pilot community-based dengue control programmes[14,17].

(10) *People themselves*: A number of pilot studies have explored and demonstrated effective ways to interest and educate people on the problem of dengue and A*edes aegypti* control. Efforts have been made to encourage people to take greater responsibility for mosquito source reduction. Much has been learned, but, much remains to be learned.

Each of the above sectors might and can play a different and more constructive role in the control of dengue:

(1) *Public health ministries*: Because dengue is a human health problem, health ministries should serve as coordinator when multiple partners are involved in vector control programmes. Health ministries must maintain essential services such as the care of the sick, surveillance on the vector and on dengue infections and promote research on improved treatment, dengue surveillance and better methods of mosquito control. But, dengue transmission is largely an environmental problem. The

responsibility for vector control must be shared by appropriate agencies.

(2) *City health departments*: Because large cities are a major milieu for the transmission of dengue viruses, city health departments must play a central role in dengue control. But, city and national programmes must be tightly coordinated. For example, cities might design, conduct and evaluate pilot control programmes using laboratory support provided at the national level.

(3) *Environment ministries*: Several important responsibilities usually delegated to environment ministries are critical to the control of dengue: solid waste management, drainage, regulation of construction sites, distribution of safe drinking water and the management of rainwater and gray water drainage in underground culverts.

(4) *Urban planning*: Urban planners should assume the central responsibility for creating master plans that include comprehensive dengue control. Crucial elements include the distribution of ample and safe drinking water, the construction of buildings and building codes designed to minimize sites for mosquito breeding, the regulation of construction sites to prevent mosquito breeding and the coordination of these components[15].

(5) *Justice sector*: Source reduction requires human behaviour change resembling programmes such as seat belt use or smoking cessation. National legislatures must write laws that provide incentives and disincentives that promote source reduction behaviour; the justice sector should enforce them.

(6) *Education sector:* The needed manpower and the intellectual, scientific, research and technical underpinning for dengue control must come from the education sector. In the large dengue endemic countries, universities must adopt affirmative programmes to provide a cohort of leaders in virology, vector bionomics, behavioural sciences, as well as in environmental, legal and architectural fields. As a global problem, major universities in industrialized countries also should assume a prominent role.

(7) *Science and technology:* Science and technology funding for the research enterprise must be forthcoming. Where education and science funding come from separate appropriations, coordination between ministries is crucial to attract high-calibre people to the field and to sustain a quality dengue control effort.

(8) *The media:* There are many opportunities for explicit partnerships between the media and agents of change. In Puerto Rico, TV telenovellas have told the dengue story. A Rockefeller Foundation pilot project in Mexico worked with a local television station to commission puppet shows

that dramatized the problem of *Aedes aegypti*.

(9) *Private sector:* The private sector is perhaps the greatest untapped resource that can help with dengue control efforts. Vector control programmes are frequently planned by government workers who are unknowledgeable or uncomfortable with the private sector. The private sector can provide help at any scale, from neighbourhood mosquito abatement to the national level. If work contracts are written carefully and the rewards for success are sufficient, the private sector is capable of delivering sustained mosquito abatement and source reduction imaginatively and competently[18,19].

(10) *People themselves:* The biggest lesson of modern history is that effective national development depends upon the strength of a civil society. Authentic participation in decision-making and individual "ownership" of a healthy environment are crucial to bring into existence an informed citizenry who expect much of themselves and make appropriate demands on government. Community-based disease control programmes contribute constructively towards the emergence of civil societies.

Research

Allocations of funds and scientific manpower are simply not commensurate with the size or complexity of the dengue control problem. Some pressing research topics can be listed:

(1) **Bionomics**: Although this mosquito is one of the best-studied, the flight range, survival, biting and breeding behaviour of *Aedes aegypti* need to be studied in many different sites. What is lacking is essential information on the size of mosquito populations that are needed to sustain dengue virus transmission.

(2) **Vector control methods**: Many methods are available to reduce or destroy *Aedes aegypti*. Nonetheless, since the days of Dr Fred Soper, no one has found the right mix of methods that matches the resources available and is compatible with today's legal systems. Are currently available methods simply too labour intensive and expensive? Or, is the failure of *aegypti* control an example of systems failure? Basic research is needed on how to reduce populations and destroy vectoral capacity.

(3) **Control – serological surveillance**: Great emphasis has been given to syndromic surveillance in detecting and controlling dengue. It is abundantly clear, however, that the majority of dengue infections are inapparent. The implications of this observation must be just as clearly understood. The success of the control of dengue transmission must be monitored by measuring dengue infection rates in control areas. This can be done simply by assaying antibodies obtained from finger-tip blood samples, use of statistics to minimize sample size by selecting

random populations and use of a well-described single dilution neutralization test[20-23]. An automated and inexpensive antibody test with serotype specificity is an urgent research goal.

(4) **Control – human behaviour**: *Aedes aegypti* breeding sites are largely man-made; humans provide the blood essential to the survival of both mosquito and virus. Mosquito breeding is not linked to emotion-laden customs or behaviours. As is the case with malaria, ignorance, traditional practices and carelessness must be overcome to promote appropriate behaviours. Stratagems to motivate behaviour changes need to be devised and tested. What role can schools play in changing behaviour? What messages are appropriate for various target age and sex groups?

(5) **Control-systems development**: Above all, dengue control is a test of a system. Disease surveillance and awareness of illness and cost burden are needed to motivate and inform educational interventions and to design interventions in specific localities. Tests need to be made of ways in which the legal system can enforce and re-enforce desired behaviour. Studies need to be made of materials and devices that can be used by householders that will provide passive prevention of mosquito breeding or killing of larvae or adults.

(6) **Control-private/public partnerships**: With sufficient funds, it can be predicted that the private sector will be able to provide effective control of *Aedes aegypti.* Pilot-scale projects are needed to test the ability of the private sector to control *Aedes aegypti* with and without the public sector.

Discussion

The difficulty of improving collective efforts to control dengue raises important questions:

Why control dengue? Dengue is a major disease problem with a moderate death toll. The 1993 World Bank's World Development Report ranked dengue control as a low priority health programme for two reasons: (1) Annual deaths due to dengue are 1000-fold lower than such diseases as diarrhoea and malaria; and (2) Dengue control methods are both unproven and expensive[24]. Recent efforts to re-calculate the cost of dengue place the dollar burden in the same league as hepatitis B and C or the tropical disease cluster (trypanosomiasis, schistosomiasis, filariasis, leishmaniasis and onchocerciasis)[25]. These diseases command considerably more investment in research, control and prevention worldwide than does dengue. In calculating DALYs, the authors made a decimal error in estimating DHF/DSS deaths. From reliable annual country reports to WHO for the period 1996-1999, deaths due to dengue average 2584 per year worldwide, not 25,000. While admitting under-reporting in some countries, it is clear that the reported figure is not wrong by 900%! I suspect the real cost of dengue/DHF is much higher than the global high-end estimate of 1289

DALY's/million population (malaria median – 6020 DALY's). Measurement of the cost of dengue requires a different scale. Dengue conveys a sense of disorder not measured in dollars. It is the health equivalent of street crime or grafitti. These disorders create visually and emotionally contaminated environments in which people feel unsafe and lose their enjoyment of life. Dengue, as a house-borne disease, makes many people feel unsafe. Millions of parents in dengue endemic countries lose sleep worrying about the safety of their children. This is the conclusion emphasized by Sornmani and colleagues in their unique study on the social and economic impact of DHF in Thailand[26]. Fear and worry are the enormous and largely unmeasured burdens of dengue.

Why not use a vaccine and forget mosquito control? Dengue vaccine development has proven a formidable challenge. Yet, there can be no doubt that a successful tetravalent dengue vaccine can and will be developed. But, many scientific and legal complexities will accompany the administration of dengue vaccines. Also, it is important to remember that dengue viruses are maintained in African and tropical Asian sub-human primates. This means that under the best of circumstances, dengue vaccines may not eradicate dengue viruses. While it is not certain that monkey-adapted dengue viruses will infect humans, it is negligent to base public policy on the probability that dengue viruses may not emerge from sylvatic to urban cycles. *Aedes aegypti* also transmits two other viruses of pandemic potential, yellow fever and chikungunya. No matter what vaccine choices become available, public health policy must also commit to the long-term control of *Aedes aegpyti.* Mosquito control is a societal responsibility, similar to providing safe drinking water or waste management.

Can a competent mosquito control programme have unexpected results? Some countries have provided excellent *Aedes aegypti* control services. But, paradoxically, a sustained period of successful control has resulted in high levels of susceptibility leading to disease in adults. In settings in which the importation of dengue viruses continue, limited vector populations may persist and small outbreaks may occur. Large numbers of adults, who for the first time are susceptible to both primary and secondary dengue infections, become clinically ill. In Singapore, a few thousand dengue infections may have sent a few thousand adults to their doctors' offices with dengue[26]. With an excellent reporting system, in societies with low tolerance for sickness, these small outbreaks have had a big impact. In Cuba, reduced transmission uncovered another paradox: at long intervals from first to second infections, classical DHF/DSS occurred in adults with higher case-fatality rates than the disease at short intervals[27,28]. Without global eradication of dengue, those countries that make a strong effort to control *aegypti,* will remain permanently at risk.

How can we build partnerships for dengue control? For partnerships to succeed, we should try to identify the vested interests or benefits that the prospective partners will derive from working toward a common goal. It is particularly important to try to find financial incentives for working in partnerships, or, financial liabilities for failing to do so. An excellent partner may be the local and global tourist industry. Dengue is

among the leading diseases of tourists. In some parts of the world, hotels themselves provide breeding sources for *Aedes aegypti* and are sites of dengue transmission.

Let's get started

(1) Agitate! Move the dengue problem from the health to the political sector.

(2) Teach! Help decision-makers to learn more about dengue.

(3) Build a global alliance for dengue control that includes agencies or organizations representing the sectors identified above. This might include: WHO and other health-oriented UN agencies, Global Forum of Mayors, UN and global environmental agencies, international urban planners, international associations of lawyers and legal scholars, UNESCO, international education and universities associations, international research administrators, foundations and international development agencies. The purposes of this meeting are to raise awareness, raise funds, enlist the support of partners to work on a set of mutually established goals and prepare for partnership formation on the national level.

(4) Promote country-level public/private vector control partnerships. Build the leadership required to forge partnerships.

(5) Design interventions for public/private vector control partnerships and find the funds to support demonstration projects.

(6) Fund a dynamic and innovative programme of social science research/capacity-building on dengue control.

(7) Fund a dynamic and innovative programme of vector control research and capacity-building.

(8) Encourage, support and reward innovation. Why not give Prizes for good ideas and accomplishment?

Dengue is working its way to the top of the health priority list. When it arrives, we must be prepared.

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Development of Dengue Vaccine

By

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Abstract

The dengue viruses are estimated to cause several hundred thousand cases of dengue fever, dengue haemorrhagic fever and dengue shock syndrome annually. Attempts to prevent the infection focus on the development of a vaccine that would protect against all four serotypes of the dengue virus. Various biotechnological approaches are being explored, including the use of live attenuated or inactivated viruses, infectious clone-derived vaccines, immunogens vectored by various recombinant systems, subunit immunogens and nucleic acid vaccine. Three candidate vaccines are undergoing clinical evaluation and several are at the stage of pre-clinical evaluation. A WHO steering committee is conducting activities aimed at accelerating the development of vaccines against dengue and Japanese encephalitis.

Key words: Dengue vaccine, clinical evaluation, preclinical stage

Introduction

Dengue viruses are the most widespread arthropod-borne viruses. They are members of the flaviviridae family, which includes more then 70 related but distinct viruses. Among these are important aetiological agents such as those of yellow fever (YF), Japanese encephalitis (JE), West Nile encephalitis and tick-borne encephalitis. Dengue is one of the most important tropical infectious diseases. It is estimated that there are some 100 million cases of dengue fever, 500 000 cases of dengue haemorrhagic fever (DHF) and 25 000 deaths attributable to dengue annually[1]. In recent decades the transmission of dengue viruses has intensified in many countries and the disease has extended its geographical range to previously unaffected areas of the South-East Asia Region, the Western Pacific Region and the Region of the Americas of the World Health Organization. In the past, the African Region and the Eastern Mediterranean Region were considered to have low incidences of dengue, but there was an upsurge of the disease in these regions

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during the early 1990s. Dengue has grown dramatically as a health, environmental and economic problem, now occurring in most countries. More than half the Member States of the United Nations, with a population of some 2500 million, are at risk.

Dengue viruses are classified antigenically into four serotypes. Infection with one serotype results in lifelong immunity to it but there is no cross-protection against the others. Persons living in areas of endemicity can be infected with two, three and, probably, four dengue serotypes during their lifetime. Infection with any serotype can produce clinical illness, ranging from a non-specific febrile syndrome to severe and fatal DHF/dengue shock syndrome. An immunopathological response following secondary infection of humans with a heterologous serotype of dengue virus can be a risk factor for the more severe forms of the disease. This was recently confirmed in Cuba, where an 18-year interval between a dengue virus type 1 outbreak in 1977/1978 and a dengue virus type 2 outbreak in 1997 provided an opportunity to evaluate risk factors[2]. All patients with severe forms of dengue, including cases of DHF and deaths, were born before the dengue virus type 1 epidemic, and nearly all experienced the secondary dengue virus infection. In contrast, almost all those who sero-converted without illness experienced the primary dengue virus infection. These observations could have implications for the development of a dengue vaccine because they suggest that a safe vaccine should be polyvalent to avoid inducing monotype-enhancing immune responses that may lead to severe manifestations of the disease.

No effective vaccine is available. Research into dengue vaccines focuses on the use of live attenuated or inactivated vaccines, infectious clone-derived vaccines, immunogens vectored by various recombinant systems, subunit immunogens, and nucleic acid vaccines.

Tetravalent live attenuated vaccine

The most advanced live attenuated tetravalent vaccine was developed in Mahidol University, Thailand, with the support of WHO's South-East Asia Regional Office. Attenuated viruses of all four serotypes were developed by serial passage of wild-type viruses in primary dog kidney (PDK) cells or other cell types[3]. After intensive and stringent laboratory studies, including evaluation in animal models, the vaccine underwent clinical trials in Thailand in mono-, di-, tri- and tetravalent formats, which proved safe and immunogenic in adults and children. The vaccine proceeded to commercial development by agreement with Aventis Pasteur. A randomized, controlled, double-blind study was carried out to determine the safety and immunogenicity of batches of the vaccine produced by this company[4]. All formulations were safe and tolerated in humans. Vaccines immunized with tetravalent vaccine gave multivalent antibody responses, the highest antibody titres being against dengue virus type 3. A phase 1 clinical trial of Aventis Pasteur vaccine was recently completed in Thailand. After two doses, seroconversion to all four serotypes was demonstrated in most vaccinated volunteers and antiviral activity remained quite stable for at least a year.

Various reformulations of the tetravalent vaccine are being evaluated in an attempt to obtain a similar immune response to each serotype. Vaccine strains developed at Mahidol University are characterized by lower infection, dissemination rates and transmissibility in *Aedes aegypti* mosquitoes than those of the parent viruses[5]. Moreover, the phenotypes of the vaccine strains were stable and unchanged by passage in humans and mosquitoes.

Serial passages of dengue viruses in PDK cells were used for the development of dengue vaccine at the Walter Reed Army Institute of Research (WRAIR) in the USA. All four monovalent formulations elicited seroconversion in humans. The vaccine was well-tolerated, caused no clinically serious adverse events and induced the production of neutralizing antibodies to all four serotypes. Tetravalent formulations were prepared and evaluated in a monkey model. Challenge studies in rhesus monkeys demonstrated that most animals seroconverted after two doses of the vaccine. After virus challenge, viremia was measurable in 4 of 20 monkeys. In pilot studies in humans, three doses of tetravalent vaccine induced 50% and higher seroconversion to all four dengue serotypes. The dissemination rates of WRAIR vaccine viruses in mosquitoes were low and it is unlikely that these viruses would be transmitted under natural conditions[6]. The next stages of the clinical trials are in progress.

Chimeric vaccine

Several research groups are successfully exploring infectious clone technology for the development of a dengue vaccine. The ChimeriVax*TM* system, originally developed to construct JE vaccine, has now been applied to dengue viruses by Acambis in the USA. A chimeric YF-dengue type 2 virus (D2) was prepared, using a recombinant cDNA infectious clone of a YF vaccine strain (YF17D) as a backbone, into which the premembrane (PRM) and envelope (E) genes of dengue 2 virus were inserted[7]. YF vaccine was selected as a backbone because of its excellent safety record during a long period of practical use. All monkeys vaccinated with ChimeriVax-D2 virus developed neutralizing antibodies and were protected against challenge with a wild-type dengue-2 virus. The high replication efficiency, attenuation phenotype in animal models, immunogenicity and protective efficacy, and genomic stability of ChimeriVax-D2 justify it as a novel candidate vaccine for evaluation in humans. YF/dengue viruses for three other serotypes have been constructed and are undergoing laboratory analysis and evaluation in animal models.

Another approach is based on the use of a dengue type 4 mutant containing a deletion in non-coding regions as a genetic background for the construction of a dengue chimeric vaccine[8]. Viruses with deletion mutations are genetically more stable than the ones with point mutations and are less likely to revert to the genotype of the parent virus when propagated in vaccinees. On the basis of laboratory tests and work with a monkey model, some deletion mutants were defined as attenuated viruses. Phase 1 clinical trials of a 3' deletion mutant were carried out in adult humans. The results indicated that this dengue 4 deletion mutant was safe and immunogenic. It is planned to use this attenuated virus as the backbone for

the construction of chimeric dengue viruses of serotypes 1, 2 and 3. The ultimate aim is to develop a tetravalent vaccine.

Work at the Centers for Disease Control and Prevention in the USA showed that attenuation markers of dengue 2 vaccine strain PDK-53 were encoded by genetic loci outside the structural gene region[9]. On this basis, chimeric dengue type 2/type 1 viruses were constructed which contained the non-structural genes of PDK-53 and structural genes of the dengue 1 strain[10]. Chimeric virus retained the attenuation *in vivo* and *in vitro* markers and was immunogenic in mice, inducing the production of neutralizing antibodies against dengue 1. It is considered as a potential dengue 1 candidate vaccine. The results also suggest that the infectious clones from the PDK-53 vaccine are promising attenuated vectors for the development of chimeric flavivirus vaccines.

DNA vaccines

A candidate DNA vaccine expressing dengue virus type 1 PrM and E proteins was developed and used for the immunization of different kinds of monkeys[11,12]. The candidate vaccine induced the production of virus-neutralizing antibodies and gave partial protection against challenge with homologous dengue virus. Intramuscular immunization of rhesus macaques was more immunogenic than intradermal immunization. Another study focused on the construction of a dengue vaccine containing PrM and E genes of the Guinea C strain of dengue type 2 virus[13]. In immunized mice the candidate vaccine induced neutralizing antibody production and strong anamnestic responses to challenge. Further extensive preclinical and clinical trials are required before a decision can be made on the acceptability of DNA vaccine for practical use.

Inactivated and subunit vaccines

The success of inactivated flavivirus vaccines against JE in Japan and tick-borne encephalitis in Austria and Russia led to attempts to develop a killed dengue vaccine. However, early work in this area was unsuccessful because of difficulties in growing high titres of dengue virus in cell lines. It was recently shown that flaviviruses can grow to high titres in Vero cells[14]. Dengue virus type 2 was grown in Vero cells and, after inactivation, purification and concentration, was used for the immunization of laboratory animals[15]. The experimental vaccine induced the production of a protective level of antibodies in monkeys. This approach will probably allow the development of an effective inactivated dengue vaccine.

Recombinant DNA techniques provided the possibility of cloning specific genes encoding for protective antigens and of expressing them in other host cells, including *E.coli*, yeast and insect cell systems. This technology has been used by several researchers for the development of subunit vaccines. Recombinant E protein of dengue 2 virus, produced in a baculovirus vector system, induced neutralizing antibody production and partial protection of immunized monkeys[16]. Products from *Drosophila* cells appeared to be promising in

the early stages of testing in animals[15]. Further efforts are required to increase the immunogenicity of subunit vaccines by incorporating them into adjuvants or other systems for stimulating immune responses.

Vaccinia virus as vector for dengue vaccine

The use of genetically-modified vaccinia virus as a vector for genes encoding flavivirus vaccine antigen could have broad application for the genetic engineering of viral vaccine. Modified vaccinia Ankara (MVA) vector with a restricted host range was developed for the construction of recombinants[17]. The safety of this vector was demonstrated in a large number of volunteers. MVA and recombinants derived from this virus do not replicate efficiently in human and most other mammalian cells, and this character is genetically stable. Monkeys repeatedly immunized with MVA recombinant expressing dengue 2 E protein have virus-neutralizing antibodies and are fully protected against challenge with homotypic dengue virus[18]. Work is planned on constructing MVA recombinants expressing immunogenic E protein of other dengue virus serotypes.

WHO activity in the development of dengue vaccine

WHO has designated the dengue viruses as a high-priority target for accelerated vaccine development. This work is conducted by a steering committee on dengue and JE vaccines, established in 1984. In the area of dengue vaccine, the main purpose of the steering committee is to promote and facilitate the development of candidate vaccines with a view to expediting their introduction in developing countries. This involves the evaluation of new biotechnological approaches, active participation in clinical trials of candidate vaccines, and the facilitation of vaccine introduction through the planning and assessment of low-cost vaccination schedules[19]. The steering committee has supported some research projects that have led to the development of candidate vaccines now undergoing clinical evaluation.

In order to promote the evaluation of live attenuated vaccines in clinical trials, a group of WHO experts has been developing guidelines for the safety of dengue vaccine. These guidelines could help public health officials to make decisions about conducting dengue vaccine trials in their countries. They could also help researchers to arrive at technical decisions before designing trial protocols. The steering committee on dengue and JE vaccines supports research projects aimed at standardizing immunological methods, including the neutralization test for dengue viruses, to be used by laboratories involved in evaluating the immunogenicity of dengue vaccine.

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Global Perspectives on Dengue Research

By

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Abstract

Dengue viruses infect nearly 100 million human beings each year living in 110 countries spread over all tropical areas on earth. Tens of millions of dengue illnesses occur annually including the hundreds of thousands of children who are hospitalized for dengue haemorrhagic fever. A health problem of this scope should be regarded as high priority and should have attracted ample funding from donors and national authorities. But such is not the case. A brief historical review reveals that there was a greater number of laboratories and a greater allocation of resources to dengue research 30-50 years ago than there is today. WHO needs to provide leadership in promoting dengue research. Each and every dengue-endemic country should realize that a sustained research capability is crucial to solve the long-term problem of dengue control. This paper provides a brief review of the history of dengue research. For several disciplines, key scientific questions are listed. Answers to these research questions are urgently needed to cope with the dengue problem.

Key words: DF/DHF, global perspective, research, control

Global status of dengue and dengue haemorrhagic fever

Dengue viruses circulate in nature as four antigenically-related serotypes, the only such group among the arthropod-borne viruses. Each of the four serotypes have evolved into multiple genotypes. The viruses are maintained in nature in two cycles, a jungle cycle (presumably older) in which several sylvatic mosquito species transmit viruses to several species of sub-human primates, and an urban cycle in which the virus is transmitted predominantly by *Aedes aegypti* to human beings. The dengue viruses are unique in that a single dengue infection may "sensitize" individuals to severe and fatal disease accompanying infection with a second serotype.

In response to a number of 20th century phenomena, the distribution and population

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of *Aedes aegypti* and the global burden of dengue have grown dramatically in recent decades. With burgeoning human populations, urbanization and the development of rapid transport systems, dengue fever and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) now occur in over 100 countries and territories. They cause an estimated 50-100 million infections among the more than 2.5 billion people at risk in urban, peri-urban and rural areas of the tropics and subtropics. While the full burden of dengue infections is not known, each year it is estimated that some tens of millions of persons experience classical dengue fever and another 500,000, mainly children, are hospitalized for DHF/DSS. Death rates are as high as 5% in some areas. Dengue is now endemic in the American, Western Pacific and South-East Asian regions of WHO while some parts of the African and Eastern Mediterranean regions are affected. Prior to 1970 only nine countries in the world had experienced DHF/DSS epidemics; by 1995, the number had increased more than four-fold. In the 1950s, an average of 908 DHF/DSS cases were reported each year. For the period 1990-1998, this average had increased to 514,139 cases. In 1998, a total of 1.2 million cases of dengue and DHF were reported to WHO, including 3442 deaths.

Global status of dengue research and control

History: Dengue research began early in the 20th century before the virus was isolated. The clinical and laboratory features of dengue, the viral status of the agent, the susceptibility of monkeys and the vector status of *Aedes aegypti* were established in a series of well-designed human volunteer studies. Dengue viruses, types 1 and 2, were isolated in suckling mice and characterized during World War II.

Research milestones: In the late 1950s, the clinical syndrome, DHF/DSS, was described and attributed to dengue infection. A decade later, it was recognized that DHF/DSS accompanied second dengue infections and, unique in human medicine, during initial dengue infections in infants who were born to dengue-immune mothers. Subsequent epidemiological studies, a monkey model and numerous *in vitro* observations provided an explanatory mechanism, antibody-dependent enhancement (ADE) of dengue infection. This is based upon evidence that dengue viruses replicate in cells of mononuclear lineage in human beings. These Fc-receptor-bearing cells are efficiently infected following attachment of complexes of dengue virus and non-neutralizing IgG antibodies. From the 1970s, with the emergence of modern immunology, the role of cellular and humoral immunity, the molecular mechanisms of inflammation and the control of dengue infections were studied. During and subsequent to the 1980s, full-length sequences of the dengue genome have been described for multiple strains of all dengue serotypes. Recently, molecular genetic research has yielded engineered vaccines and rapid, highly sensitive methods to detect and study viral infections.

Facilities: During the 1950s dengue studies were performed in field laboratories maintained by the Rockefeller Foundation in Trinidad, Brazil, Africa and India, and by

colonial research institutes located in South and south-east Asia. A decade later many of these research networks were phased out and replaced by national public health laboratories, a network of U.S. military infectious diseases research laboratories (Thailand, Malaysia, Philippines, Indonesia, Peru, Brazil) and for a brief time, WHO vector research units in Thailand, Indonesia and India. Today, dengue research is supported by intramural funds in government research institutes of the larger developed countries. The biggest cadre of scientists is in the U.S. public health service (CDC and NIH) and military research laboratories. As compared with chronic diseases, dengue is a low-priority health problem for developed countries. Comparatively modest support for dengue research is provided on a competitive basis to a small number of university scientists in developed and some of the relatively affluent developing countries.

Control Programmes: During the 1940s, a historically unique mosquito control programme was initiated. By 1960, under the leadership of the Pan American Health Organization, *Aedes aegypti* had been eradicated from most major South and Central American countries. After this achievement, however, many of these programmes were dismantled and within two decades, the vector regained its former range.

Research questions and WHO's role

A comprehensive research programme on all aspects of dengue and its control will entail a vast, multidisciplinary effort. In the view of the author, some of the key research questions and the role for WHO in answering them include:

Basic virology: Progress in basic virology is moving very quickly. The flavivirus field is diverse, small and dynamic. Compared with other genera, disease-producing flaviviruses are little used in the study of basic virology.

Questions: What is the function of the proteins translated from the dengue genome? Do functions differ by serotype? Genotype? What amino acids, proteins and three dimensional conformations participate in the cell entry process? What are the mechanisms of entry? Do these differ by serotype? Genotype? How do dengue viruses replicate, assemble and release from relevant human target cells?

WHO role: Use convening function of WHO to focus research on important disease or vaccine-related questions.

Viral evolution: Questions: What is the global extent of contemporary zoonotic cycles of dengue viruses? Did dengue evolve into four distinct serotypes in geographically isolated zoonotic cycles? If so, when? When did each dengue serotype escape from sub-human primates to human beings? And, how many times? Are or were dengue viruses transmitted from humans to monkeys? Are monkey dengue viruses currently being transmitted to humans?

WHO role: Jungle dengue could threaten long-term efforts to control urban dengue. If WHO committees highlight the importance of this question, it may stimulate

interest and promote and coordinate efforts to recover dengue viruses from zoonotic cycles.

Host-virus interactions: Questions: What cells in human beings serve as important/essential sites of dengue virus infection (target cells) during the course of dengue fever? DHF/DSS? Do target cells differ with different serotypes? Genotypes? Infection sequence? What cells contribute to the signs and symptoms of classical dengue fever, DHF and aberrant dengue? What is the mechanism of entry of dengue viruses into non-Fc-Receptor-bearing target cells? Why are some dengue genotypes associated with severe secondary dengue infections and others are not? Can mice or other small laboratory animals be used to study key virus-host interactions? Can *in vitro* systems be used to study virus-host interactions? How does antibody promote severe infections? What are the characteristics of inapparent dengue infections? Can such infections be recognized in nature? Are blacks genetically resistant to dengue infection or disease? What human gene(s) control response to dengue infection?

WHO role: This is difficult research. Direct funding by WHO and use of the convening function may promote and accelerate research in this area. Discovery of the human gene that controls response to dengue could lead to new drugs or powerful new methods of dengue control.

Pathophysiology/treatment of dengue/ DHF: Questions: What are the mechanisms (e.g. target cells affected) and effector molecules that mediate major and minor clinical phenomena observed during classical dengue fever, classical DHF and aberrant dengue? Can these mechanisms or effector molecules be identified at an early stage of dengue infection permitting specific treatment to prevent onset of severe disease? Can dengue infections be identified early enough to permit early interventions? Can the treatment of DHF/DSS be improved, simplified or made more cost-effective? Can dengue antivirals be developed?

WHO role: Very few well-funded, high-quality research groups in the world are studying dengue pathophysiology. WHO may be able to support the creation of new interdisciplinary groups. Capable clinical researchers may be able to work on dengue if they can have access to research-level virological support.

Vaccine development: Current dengue vaccines under development include: two sets of tetravalent live attenuated viruses (LAV) (Mahidol/Merieux and WRAIR), a genetically-altered LAV (NIH), a dengue-dengue chimera (CDC/Mahidol), a yellow fever-dengue chimera (Acambis), an alphavirus replicon (USAMRIID), two naked DNA vaccines (Naval Medical Research Center/USAMRIID), a formalin-inactivated tetravalent vaccine (WRAIR) and numerous sub-unit vaccines prepared by commercial, university or government laboratories. Some of these vaccines are or have been tested in human volunteers.

Questions: What are the immunological goals of dengue vaccination? What is the herd immunity of different dengue serotypes? How do immune responses in laboratory animals compare with those in human beings? What

laboratory methods can be used to measure and predict solid protection of vaccinated humans? Which of the above vaccines (or others) meet dengue vaccination goals? What is the efficacy of candidate dengue vaccines? What are the safety concerns of dengue vaccines? What laboratory methods can be used to measure and predict safety of dengue vaccines?

WHO role: WHO should directly address safety issues and laboratory markers of protection. WHO should monitor dengue vaccine research and promote the phase I and phase II testing of attractive vaccine candidates, particularly in children. WHO and its children's vaccine allies should raise the funds needed to assist in scale-up, testing, production and distribution of safe and effective dengue vaccine(s).

Control – vector bionomics: A 1992 study commissioned by the Rockefeller Foundation could find no major university in any dengue-endemic country that offered graduate programmes in *Aedes aegypti* bionomics or control. Many national vector control programmes do not have Ph.D. medical entomologists in leadership positions. Those with graduate degrees usually have obtained them from abroad. Thus, there is no academic base to complement government programmes or provide the research that is essential to achieve optimal local control of dengue vectors.

Questions: What is the flight range, survival, biting and breeding behaviour of *Aedes aegypti* in different sites? Although this mosquito is one of the best-studied, important information relevant to mosquito population survival and to sustained dengue virus transmission is lacking.

WHO role: This is a critical area for WHO capacity-building and networking. Global medical entomology personnel research and personnel resources are at all-time lows.

Control methods: Many methods are available to reduce or destroy populations of *Aedes aegypti*. Nonetheless, since the days of Dr Fred Soper, no-one has found the right mix of methods that matches the resources available or is compatible with today's legal systems.

Questions: Are currently available methods simply too labour intensive and expensive? Or, is the failure of *aegypti* control an example of systems failure? (see below). How can basic research contribute to reducing populations or destroying vectorial capacity?

WHO role: The entire field of mosquito control needs to be energized, even re-born. Leadership and funding are essential.

Control – human behaviour: *Aedes aegypti* breeding sites are largely man-made; humans provide the blood that promotes the survival of both mosquito and virus. The activities that promote mosquito breeding are not usually linked to emotion-laden customs or behaviours. As with malaria prevention, ignorance, traditional practices and carelessness must be overcome.

Questions: Can key attributes of behaviour-promoting mosquito breeding be identified? Can strategems be devised and tested to motivate behaviour changes? What role can education play in changing behaviour? What are the target age and sex groups? What messages work best for each age and sex group?

WHO role: Using its convening function, WHO should attempt to breathe life into this research field. Direct support of research and pilot projects is essential.

Control - systems development: Above all, dengue control is a test of a system. Disease surveillance and awareness of illness and cost-burden are needed to motivate and inform educational interventions and to design interventions at specific localities.

Goals: Tests need to be made of ways in which the legal system can enforce and re-enforce desired behaviour. Studies need to be made of materials and devices that can be used by householders that will provide passive prevention of mosquito breeding or killing of larvae or adults.

WHO role: If WHO doesn't stimulate and support improvement of surveillance and control systems, who will? There is some important research to be designed and supported; largely, this is an area for the Control of Communicable Diseases Division.

Control – private/public partnerships: With sufficient funds, it can be predicted that the private sector will be able to provide effective control of *Aedes aegypti.*

Goals: Devise one or more pilot-scale projects to test the ability of the private sector to control *Aedes aegypti* to an established level. Devise programmes for the public/private sector cooperation in the control of vector and nuisance mosquitoes.

WHO role: WHO leadership is essential. This is part research and part intervention. Cooperative projects supported by WHO's Tropical Diseases Research (TDR) and Communicable Disease Control Programmes are needed.

Building Partnerships for Dengue Control: The Challenges and Opportunities - Experiences from other Disease Control Programmes

By

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Abstract

The private and commercial sectors represent a great force that can help increase the reach and sustainability of preventative and disease management services. Examples are drawn from diarrhoea disease control programmes, contraceptive marketing and insecticide-treated mosquito nets for malaria control in Africa. Care must be taken to "segment" the market so that public sector resources can be targeted to those who really need it in a way that does not disrupt investments from other partners. Often a neutral task force is needed to mediate and articulate the mutual benefits of the partnerships. Steps in the partnership model include an inventory of capacities, consensus meetings, market research, communications, monitoring plans, etc. Protocols are also available for improving diagnosis and treatment in the private sector. Specific to *Aedes* control, these examples suggest novel ways of dealing with non-essential containers, safer water jars, copepods and ovicidal soap. Likewise, for disease recognition and management, there may be ways of improving practices in the vast, and largely unregulated, private sector. In short, we need to expand our definition of "community" to include the private and commercial sectors, who can be mobilized to meet mutual "business" and "public health" objectives

Key words: Dengue, *Aedes* control, Public-Private Partnerships, Community

Dengue control is the epitome of a community-based programme. Since Dr Duane Gubler's 1989 address "*Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: Top down or bottom up?"[1], there has been growing recognition that the public sector cannot "deliver" dengue control to the population, and that "communities" must have more involvement. In the past decade, there has been a great deal of progress in broadening community involvement in *Aedes* source reduction and improving caretaker recognition of dengue disease and

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appropriate response. However, sustainability and scale have plagued many of these "community-based" control programmes. Other disease control programmes may provide examples of how, in dengue, we can overcome the problems of scale and sustainability through partnerships with the private and commercial sectors. We must, in effect, expand our definition of "community" to include not just households and service groups, but the local shopkeeper as well. An expanded definition of "community" to include the shopkeeper (and the supporting commercial networks) may provide the combination of vertically-structured centralized and community-based approaches deemed necessary for short-term success as well as long-term sustainability[2].

The private and commercial sectors represent a great force, for both good and bad, that can be tapped by national dengue control programmes to increase the reach and sustainability of both preventative and disease management services. This working paper will outline first, a model for engaging the commercial sector being used to launch a nationwide insecticide-treated materials (ITM) initiative in Ghana, and a new continent-wide ITM project in Africa. It will also outline how these principles were used for diarrhoea control programsme in Latin America, and for contraceptives in Morocco and Indonesia. For dengue control, these partnerships may involve recycling and source reduction, larvicide distribution, coils and fumigants, safer water jars, improved caretaker recognition and demand for quality services in both the public and private sectors.

Common principles

Mutual benefit, market segmentation, neutral task force, consumer-driven

While the first model deals with large-scale manufacturing, distribution and promotion companies for insecticides, nets and malaria drugs, and the second with individual private providers and their clients, the principles are the same. In order for partnerships to work, they must be mutually beneficial. There are examples of successful partnerships in the areas of contraceptives, ORS and diarrhoea control projects (through the promotion of handwashing with soap), showing that public health objectives can also make good business sense: the greater the coverage, the greater the sales volume. Second is the concept of "market segmentation". Public health delivery systems often serve a disproportionate number of people who can afford to pay. Experiences from ORS and contraceptive marketing show that when these populations are offered convenient, high quality and affordable options through the commercial sector, they are often eager to switch. As those who are able to pay migrate out of the public sector, an opportunity is created to reallocate resources towards the more needy. The commercial partnership can thus be seen as an extension of the national disease control strategy, and not as a competitor or project divorced from the overall programme. Third, partnerships require effort, and often a neutral task force who can work evenly between the ministry and the commercial sector to relieve the ministry of undue pressures or the appearance of favouritism, and to bring

together the needs for equity and public health impact on the one side, and commercial viability on the other. Finally, the marketing plan or health education must be consumer-driven: not based on a prescription of what we think the public should know and do, but what actually motivates families to decide and act, to seek treatment or consume, in one manner or another.

Commercial sector partnerships for insecticide-treated materials in Africa

In January 1998, a global task force was formed with representatives from WHO, UNICEF, the World Bank, and the USAID BASICS project, and representatives from insecticide manufacturers including AgrEvo, Bayer, Zeneca, Cyanamid, the SC Johnson Wax Co. and mosquito net manufacturers including Chemdol, South Africa and the Siam-Dutch Mosquito Net Co. Through a series of discussions, a consensus developed that the public sector and ministry of health cannot on its own adequately promote and distribute ITMs on a large scale. Likewise, the financial risk was too great, and the profit margins too narrow, for the manufacturers to individually create a retail market for their own products. Strategic partnerships between the commercial and public sectors could be formed to share the investments initially needed to research and build a market environment that would result in the mutual benefits of increased product use, access and sustainability. This strategy requires a shift of donor support away from commodity procurement and distribution to sharing the costs of market analysis and the initial health education and promotion to grow the overall market. This donor investment may only be required for the first few years, after which the commercial partners continue promoting the issue of malaria control and marketing their individual products. ITMs are not the first for this type of initiative: successful partnerships for other public health products exist, including ORS, contraceptives, latrines and soap. These other examples show that commercial/public partnerships can provide sustainability (decreased long-term donor dependence); coverage (cost-effective sharing of resources), and equity (market segmentation and decreased burden on the public sector).

Steps in the partnership model[3]

While each partnership is unique, they were established using a common model, with guidelines and protocols available for each of the steps. The steps include, first selecting the relevant public health need, in this case ITMs; then conducting an inventory of company capacities and the competitive market; developing a consensus in the public sector, establishing a commercial/public task force, under the chair of the Ministry of Health; then developing a marketing plan, contracting market research, finalizing marketing strategy, developing promotional materials and launching the campaign. Finally, there are the steps of monitoring and evaluation of public health impact and programme management. After a consensus was built at the global level among insecticide and net manufacturers, donors, UNICEF and the WHO Roll Back Malaria, Task Force for

ITMs, the model was applied by the Ministry of Health in Ghana.

Initial steps

Assessing capabilities, establishing task force, marketing plan and provisional budget

The assessment of November 1998 showed strong technical and financial support in the government and among the major donors, especially DFID, USAID and the World Bank. Among the potential commercial sector partners, AgrEvo, Bayer, Zeneca and the SC Johnson Wax Co., are present and their products are registered. (This includes both bulk and single-dose formulations of deltamethrin, cyfluthrin and lambda-cyhalothrin). In addition, there are a number of world-class consumer research and promotion agencies that can be contracted, and a number of distribution companies that penetrate the 700 pharmacies, 6,000 "chemical sellers" and 130,000 "table top" vendors throughout Ghana.

The Ministry of Health convened a round table with donors and commercial sector partners in January 1999. A provisional budget of $1.2m was developed for market research and a two-year, nation-wide promotional campaign, using radio, TV, "road-shows", print and interpersonal communication was initiated. This budget includes $190,000 for project management, $133,000 for market research, and $770,000 for advertising and promotion, including mass media, grassroots and trade promotion (detailing). The campaign is two-phased: donors and the four commercial partners will together pay for the market research, strategy development and generic "umbrella" campaign for malaria awareness and the importance of ITMs, using an "overbrand" logo for the range of ITM products endorsed by the ministry. The individual companies then will promote their own products under this umbrella campaign and compete for their share of the overall market share using their own brand name and logo alongside the ministry "overbrand" logo. It is expected that as the commercial sector begins to meet the needs of the middle and higher economic strata, the public sector, Ministry of Health and NGOs will be able to focus their resources solely on those at the very bottom, and strike a balance for a commercially viable, non-donor dependent, long-term and equitable access to ITMs in Ghana.

This model is the framework for a new USAID-supported project "NetMark", that began on 1 October 1999, to build a sustainable market for insecticide-treated mosquito nets in Africa. NetMark is a partnership between the US-based NGO, Academy for Educational Development, the SC Johnson Wax Co.(SCJ), a 113-year-old, $5 billion/year company that is a global leader in the marketing of consumer insect control products such as RAID® and OFF!®. SCJ has had manufacturing and marketing operations in Africa for more than 40 years and is present in most countries. The other partners are Group Africa, Ltd. (a grass-roots marketing firm based in South Africa), Johns Hopkins University and the U.K. Malaria Consortium. Again, here the strategy is to work *with* the commercial sector to create awareness and demand for a specific public health issue and product. The

vast majority of the "product" will go through commercial channels and the public sector will concentrate more on sharing in demand creation, policy, monitoring health impact and issues of equity.

Curative services

Working with the pharmaceutical sector and private providers

The model outlined above has also been used successfully to promote contraceptives in Morocco, reproductive health services in Indonesia, handwashing with soap in five Latin American countries and ORS in Bolivia and Côte d'Ivoire. For malaria, a similar approach is being discussed for Kenya, where the Ministry of Health recently changed the first-line treatment from chloroquine to sulfadoxine-pyremethamine (SP). There are potential partnerships with the local pharmaceutical industry to increase public awareness for the need to change from the ubiquitous chloroquine-based "fever powders" and through the pharmaceutical distribution, promotion and detailing network, increase the market share for SP and reduce the share for the chloroquine formulations, as well as other malaria drugs not part of the current national malaria drug policy.

Working with private providers to improve diagnosis and treatment

In many situations, including DHF, private practitioners often provide much or most of the curative care, but are largely ignored in efforts such as the current Integrated Management of Childhood Illness (IMCI) assessment and training activities. But involving private practitioners will require practical quality assessment and improvement methods and tools that can be implemented by peripheral government and NGO organizations. Following is an example of the development and testing of such tools in two sites in Bihar and one in Rajasthan, India, and one site in Java, Indonesia, by Northrup, et al., 1998[4].

Two tools were developed. First was an assessment methodology, the verbal case review (VCR), a delayed household exit interview of mothers who had taken their sick, under-5 child to a practitioner in the previous two weeks. The VCR asks the mother to recall specific actions taken by the practitioner in history-taking, physical examination, treatment and counselling of the child, and in the analysis compares those results to the national and international guidelines. The second tool is an intervention approach called by its acronym, INFECTOM, consisting of four components: Information about national protocols, Feedback comparing practitioners' behaviour with those standards, Contracting with those providers for specific case management actions and Ongoing Monitoring, with VCR results relative to the actual promises and contract.

The verbal case review was developed because most of the current, quality evaluation tools, which require direct observation and exit interviews with patients immediately after the clinical encounter, are impractical in many settings. For example, it is difficult to observe itinerant private providers or drug vendors, when the busy private provider finds it an intrusion and

refuses to participate, or when it takes a long time to accumulate enough exit interviews to make an adequate sample size of patients. The VCR is a combination of the exit interview methodology of the WHO Health Facility Survey and the Verbal Autopsy. It screens for cases through a household survey to identify under-5 children sick in the last two weeks. The instrument is then used to ask mothers of the sick children to recall the case-management practices that took place during an encounter with a practitioner for a particular illness. Instead of determining through direct observation whether the provider "checks the child's fever with a thermometer" the VCR asks the mother if that action was performed. However, in India it was found that mothers were unable to say what medicines were used other than type (tablet, syrup, ORS, injection, etc.). Thus, for malaria purpose, it is necessary for interviewers to have samples of medicines to show the caretaker. For DHF, questions might also include use of a bloodpressure cuff, tourniquet test, etc.

The second tool, INFECTOM, then uses the VCR data to implement quality improvement. In the first stage, group meetings were held to inform the private providers of national guidelines and the discrepancies with VCR results. In the Bihar study, 44 out of the 67 private providers mentioned by mothers in the VCR study, attended, at their own expense, a two-day seminar. Following the seminar, community health workers (CHWs) visited all 67 providers and negotiated behavioural "contracts" on specific actions in the guidelines. Two weeks later, the CHWs began monitoring the private providers with additional verbal case reviews and provided them feedback on their actual performance towards the "contract". This monitoring was conducted twice before the final survey at seven months, which showed a significant improvement in case-management practices for ARI, diarrhoea and fever. The group sessions had only a moderate impact on quality improvement. Evidence from this and other studies that a multi pronged approach is needed to change clinical practice, and that educational outreach through peers (detailing) is more effective than didactic lecturers or simply providing written guidelines[5].

Another important aspect of this intervention was the information flow facilitated by the CHW, in conducting the VCRs and detailing to the private providers. Numerous studies have shown that patient expectations are an important influence on a provider's case management practices. Through the VCR with mothers and continuous monitoring and feedback to the community on private provider performance, the interventions hoped to create an environment in which the community would have information on expected quality standards, and act upon providers to deliver good quality care: "consumer education" in other words. In adapting these methodologies to quality of care for the management of dengue and dengue haemorrhagic fever, this would include "consumer" information on appropriate DHF management protocols, and the added expense and dangers of "inappropriate" therapy, especially unnecessary injections and infusions. Patients should be encouraged to use (and pay for) appropriate services and avoid the others.

In summary, there are examples, models and protocols for engaging the private and commercial sectors, largely taken from malaria control programmes, which may have some application to DHF control strategies. First, there is great potential for developing commercial sector partnerships for increasing the reach and sustainability of ITMs. Second, there is potential for engaging national pharmaceutical manufacturing and distribution companies for promoting the malaria treatment endorsed by national policy, and reducing the "market share" of those drugs not approved. Finally, there are examples and protocols available for a systematic approach to improving the quality of care in the private sector.

Private and commercial sector partnerships for dengue control

As mentioned in the introduction, partnerships may involve: recycling and source reduction, larvicide distribution, fumigants, safer water jars, improved caretaker recognition and demand for quality services in both the public and private sectors, and other tactics.

Collection and recycling[6] of unnecessary domestic water containers is of course an area that demands commercial sector partnerships. Commercial marketing may also be applied to the distribution of copepods[7] or ovicidal soap[8]. Initial contacts have been made with the Cyanmid International Company in Singapore to explore potential partnerships for retail marketing of Abate® in other countries in the region, but there needs to be further discussion on the advisability of pushing temephos into the mass market. Two immediate dangers could be: pressure for increased insecticide resistance, and a 'chemical dependence', i.e. that householders will rely on a chemical fix, rather than 'home hygiene', source reduction etc.

In Yangon, Myanmar, much of the *Aedes aegypti* breeding was found to be in vases for cut flowers on the household alters, where each morning a family member will also place a bundle of burning joss sticks or incense. The Ministry of Health, Vector-Borne Disease Control Unit considered the idea of encouraging the joss stick manufacturers to incorporate a pyrethroid insecticide in the incense so that every morning the room would be fumigated. The project was never pursued, but offers intriguing possibilities.

Likewise, in Battambang, Cambodia, there has been consideration of commercial partnerships for marketing safer water jars. The current 400- and 600-litre water jars are nearly impossible to drain and clean, and rarely come with well-fitting covers. The concept here was to market a jar with a small bunghole that could be un-stoppered for draining and cleaning and a well-fitting cover. There was a trial of such water jars in the refugee camps on the Thai-Cambodian border in the late 1980's, but the effort was not "marketed" and sustained. In Battambang, the "safer water jar" would be promoted and advertised in collaboration with the larger water jar manufacturing businesses.

Behaviour change for *Aedes* control, caretaker recognition and appropriate treatment-seeking are areas where communication and marketing specialists from the commercial sector could greatly improve the quality of our information, education and communication (IEC) effort. For example, the tourism industry in cities such as Vientiane, can be hurt by a dengue outbreak. Hotels and their marketing agencies can be encouraged to help pay for media placement (as they do in Phnom Penh) and provide the services of consumer research and communications specialists. Camille Saadeof the USAID BASICS Project writes: "Milton Friedman's famous observation that 'the business of business is business' may no longer be as accurate as it once was. There is evidence that a growing number of companies are taking on a broader social role, seeking to be one of service to the communities as they serve their own core business interests[9]". Participation in the "Healthy Cities" and dengue control projects would be a natural fit for the hotel and tourism industry.

Finally, for improved quality of clinical care, there are endless anecdotal accounts of mothers paying exorbitant fees for unnecessary injections and medicines. As we promote IEC campaigns for DHF awareness and prompt treatment-seeking, it is equally important that the consumer understands what is appropriate and what is inappropriate therapy. A recent study from Hanoi showed "an unexpectedly high proportion of customers, being "Tu Lam Bac Sy" (their own doctors), deciding themselves which drugs to buy[10]." That being the norm for most DHF-endemic countries, we need to work much more closely with the private pharmacy network to improve prescribing and referral practices and with the communities and caretakers to improve demand for quality services.

In conclusion, as the epitome of a community-based control programme, by expanding our definition of "community" to include the private and commercial sectors, we can greatly increase the reach and sustainability of dengue control efforts. The private and commercial sectors are an important force, and can be mobilized to meet mutual "business" and "public health" objectives. There are precedents for partnerships in the fields of reproductive health and malaria, and such partnerships can be formed for dengue as well.

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Evaluation of Community-based *Aedes* control programme by Source Reduction in Perumnas Condong Catur, Yogyakarta, Indonesia

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Abstract

Community-based (Desawisma – group of housewives) control of *Aedes* population through source reduction was evaluated in Rukun Wilayah 17, Perumnas Condong Catur, Yogyakarta special province, Indonesia. Source reduction activities included (i) emptying and brushing of positive containers; (ii) providing lids on earthen pitchers/filled containers; and (iii) removal of discarded articles in the experimental areas. Control areas (Rukun Wilayah 13) received regular temephos application four times a year, general outdoors malathion fogging before the rainy season, and malathion fogging indoors/outdoors within 100 metres of DHF case. Ovitrap indices (OI) indoors for *Aedes aegypti*; *Aedes Albopictus* and mixed population in experimental areas at pretest were estimated as 44.3%, 2.1% and 2.1%, respectively in contrast to control areas with similar indices of 28.9%, 2.2% and 3.3%, respectively, inspite of fogging in the area a week earlier. Definite decrease of OI was observed in experimental areas during six weeks of dry season, whereas it increased from 34.4% to 37.2% in the control areas. However, during the rainy season OI did not show any difference in both the areas. Breteau index decreased from 41.2 to 20.7 in dry season and further reduced to 9.8 at the end of 18 weeks in experimental areas, in contrast to increase from 16.7 at pretest to 27.9 at the end of 18th week in the control area. Study concluded that source reduction reduced significantly OI and BI of *Aedes* species in experimental area.

Key words: Source redution, *Aedes* species, Ovitraps, Yogyakarta.

Introduction

Dengue haemorrhagic fever (DHF) is a major public health problem in Indonesia. The first outbreak was recorded in 1968 with 58 cases and 24 deaths (case-fatality rate of 41.5%)[1]. Since then the disease incidence is not only increasing, but has spread geographically to other parts of the country. During 1976-77 an epidemic was reported in rural areas in Bantul, located 12 km away from Yogyakarta, with 1260 cases and 32 deaths[2]. It was estimated that there were 30,730 cases of DHF with 681 deaths (case-fatality rate of 3.05%) in Indonesia by 1997[3].

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History of dengue control has undergone a series of trials starting with larviciding with temephos 1% sand granules @ 1ppm once a year in positive containers before the onset of transmission season supplemented by malathion focus fogging (around DHF case). Since 1992, emphasis shifted to community participation through DHF working groups (DHF/WG) at village level under the supervision of health centre. One of the members of DHF/WG is from Family Welfare Education Women's Movement [PKK – the women movement for family prosperity at village (Desa) level][4]

A study to evaluate the source reduction method through community participation of Dasawisma – a group of 10-15 housewives, was organized in Perumnas Condong Catur, Yogyakarta from 25 June – 4 September 1993. Results of the study are included in this paper.

Study area: For study, two areas with similar endemicity of DHF and *Aedes* breeding potential at least 300 metres apart were selected: Rukun Wilayah 17 Perumnas Condong Catur was designated as experimental area, while Rukun Wilayah 13, Perumnas Condong Catur was taken as control area. Both the areas belong to Sub-district Depok, District Sleman, Yogyakarta Special Province, Indonesia.

Methods and materials

Experimental area: Dasawisma housewives* carried out source reduction activities once a week for 18 weeks. It was started after "larval survey" and raising pre-test data. Housewives carried out three activities, viz. (i) emptying and scrubbing of positive containers, i.e. wash basins, earthen pitchers, animal drinking pans, and flower vases; (ii) covering earthen pitchers and drums with lids, and (iii) eliminating discarded articles. No other control activities were carried out in the experimental areas.

Control area: Routine activities undertaken by health staff included temephos application 4 times a year in selective (+ve) containers, ULV outdoors, general thermal fogging in early rainy season, two times. Malathion fogging indoors and outdoors within 100 metres of a DHF case.

Evaluation

Ovitrap Index: Ovitraps as per WHO manual 1972[5] were placed at pretest, and repeated at 6, 12 and 18 weeks later, both in the experimental and control areas, both indoors and outdoors.

Larval survey: Similarly larval surveys were carried out at pretest, 6 weeks, 12 weeks and 18 weeks' interval to work out Breteau Index (BI).

Analysis of Data: Data from both the experimental as well as control areas was analysed statistically by Mantel-Haensel Chi Square Test.

Results and discussions

Effect of source reduction on the ovitrap index (OI): The data on OI were collected at pre-test, 6 weeks and 12 weeks later only, because there was thermal fogging measure carried out by health worker at 18 weeks later. The effects of source reduction for 12

** Housewives were given information and training about lifecycle of* Aedes *and source reduction techniques. They were also provided with water basin brushes and buckets with lids for removal of discarded articles.*

weeks on the OI indoors are presented in Table 1. It revealed that the OI of *Aedes aegypti*, *Aedes albopictus* and both (mixed) species indoors in the experimental area at pre-test were 44.3%, 2.1% and 2.1 respectively, whereas the OI of *Aedes aegypti*, *Aedes albopictus* and both (mixed) species indoors in the control area were 28.9%, 2.2% and 3.3% respectively, although control areas had been fogged indoors and outdoors with malathion a week before the research study. The total OI (*Aedes spp*) indoors at pre-test between experimental and control areas did not differ significantly ($X^2 = 3.7225$; $P > 0.05$). Table 1 also revealed that the total OI decreased from 48.5% to 23.3% in the experimental area but it increased from 34.4% to 37.2% in the control group. It was concluded that source reduction for 6 weeks in the dry season showed significant decline while there was no decline in the control group (X^2 MH = 7.1181; $P < 0.05$). The total OI indoors in the 12th week between experimental area and control area did not differ significantly in the early rainy season (X^2 = 0.0589; $P > 0.05$). It was concluded that the effect of source reduction on the OI indoors did not differ significantly with control areas in the early rainy season. The effect of source reduction to the OI outdoors is given in Table 2. It revealed that the OI of *Aedes aegypti*, *Aedes albopictus* and both (mixed) species outdoors in the experimental area at pre-test was 32.17%, 17.3% and 4.1% respectively, whereas the OI of *Aedes aegypti*, *Aedes albopictus* and both (mixed) species outdoors in the control group were 28.4%, 4.5% and 2.3% respectively. The total OI outdoors decreased significantly ($X^2 = 8.7931$; $P < 0.05$) from 54.1% to 32.1% in the experimental group, meanwhile it did not decrease significantly in the control areas ($X^2 = 0.5052$; $P > 0.05$). It was concluded that source reduction of *Aedes* for 6 weeks in the dry season were able to bring down significantly the total OI outdoors too, meanwhile there was no decline in the control area (X^2 MH = 6.5323; $P < 0.05$). The total OI outdoors in the 12th week between experimental area and control area did not differ significantly in the early rainy season ($X^2 = 0.1239$; $P > 0.05$). It was concluded that the effect of source reduction of *Aedes* on the OI outdoors did not differ significantly from control areas in the early rainy season.

Table 1. *Effect of source reduction of Aedes sp. to the ovitrap index (OI) of Aedes aegypti (1) and Aedes albopictus (2) indoors in Perumnas, Condong Catur, Yogyakarta, Indonesia*

Area	Weeks	Season	N	1		2		1&2		Total	
				+	OI (%)	+	OI (%)	+	OI (%)	+	OI (%)
Experimental	0*	D	97	43	44.3	2	2.1	2	2.1	47	48.5
	6	D	86	20	23.3	0	0.0	0	0.0	20	23.3
	12	R	85	24	28.2	0	0.0	0	0.0	24	28.2
Control	0	D	90	26	28.9	2	2.2	3	3.3	31	34.4
	6	D	86	28	29.2	4	4.7	0	0.0	32	37.2
	12	R	77	23	29.9	0	0.0	0	0.0	23	29.9

* = Pre-test; N = Number of ovitrap examined; D = Dry season; R = Rainy season

Table 2. *Effect of source reduction of Aedes sp. to the ovitrap index (OI) of Aedes aegypti (1) and Aedes albopictus (2) outdoors in Perumnas, Condong Catur, Yogyakarta, Indonesia*

Area	Weeks	Season	N	1		2		1&2		Total	
				+	OI (%)	+	OI (%)	+	OI (%)	+	OI (%)
Experimental	0	D	98	32	32.7	17	17.3	4	4.1	53	54.1
	6	D	84	20	31.0	0	1.2	0	0.0	27	32.1
	12	R	86	22	25.6	0	0.0	1	1.1	23	26.7
Control	0	D	88	25	28.4	4	4.5	2	2.3	31	35.2
	6	D	86	20	23.3	6	7.0	0	0.0	26	30.2
	12	R	75	22	29.3	0	0.0	0	0.0	22	29.3

Effects of source reduction of *Aedes* for 18 weeks on the BI are given in Table 3. It showed that the BI in experimental area at pre-test and six weeks later in the dry season were 41.2 and 20.7 respectively, whereas the BI in control area were 16.7 and 7.0. Otherwise the BI in experimental area decreased to 1.0 in early rainy season (the 12th week), meanwhile it increased to 21.1 in the control area. According to WHO (1994), BI>20 poses a risk of dengue transmission[6]. It was concluded that the source reduction of *Aedes* for 6 weeks during the dry season was not able to eliminate this risk but was able to reduce it after continuing for 12 weeks. The BI in experimental area decreased significantly in the rainy season, meanwhile it increased significantly in the control area (Fisher exact test = 0.03; P<0.05). It was concluded that the source reduction efforts of *Aedes* for 12-18 weeks were able to reducing the BI, meanwhile there was no decline in the control area, especially in the rainy season.

Table 3. *Effect of Source Reduction of Aedes sp. to the Breteau Index (BI) of Aedes sp. In Perumnas Condong Catur, Yogyakarta*

Weeks	Season	Breteau Index (BI)	
		Experimental	Control
0	D	41.2	16.7
6	D	20.7	7.0
12	R	1.0	21.1
18	R	9.80	27.9

Conclusion

The source reduction of *Aedes* done by community (Dasawisma housewives) for 18 weeks (6 weeks in the dry season and 12 weeks in the rainy season) in Perumnas Condong Catur, Yogyakarta, Indonesia, were able to reduce significantly the OI and BI of *Aedes spp* (*Aedes aegypti* and *Aedes albopictus*).

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Evaluation of Commercial Pathozyme Dengue IgM and IgG Tests for Serodiagnosis of Dengue Virus Infection

By

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Abstract

The commercial pathozyme dengue IgM and IgG (CPD IgM-IgG) tests of the Omega Diagnostics Company were used to detect specific IgM and IgG antibodies produced during dengue infection. The dengue haemorrhagic fever patient sera were collected at the Paediatric Hospital No. 1, Ho Chi Minh City, and tested parallel with in-house Mac-ELISA and HAI. The CPD IgM-IgG showed excellent sensitivity: for IgM the sensitivity was 98.11% (n=53) and for IgG the sensitivity was 94.34% (n=53). The CPD IgM-IgG also showed high specificity: 90.48% (n=42) with IgM and 94.59% (n=37) with IgG, by using single sera collected from patients without dengue infection. The CPD IgM-IgG test kits should prove useful in the clinical diagnosis of dengue infections as these are a very good tool for carrying out laboratory-based active surveillance.

Key words: Dengue diagnosis, Evaluation, EIA kits

Introduction

Dengue is recognized as the most important mosquito-borne disease of humans in the world in terms of morbidity, mortality and economic costs. This flavivirus, which is found in most tropical and subtropical areas, has four serotypes (DEN 1-4) that are closely related but antigenically distinct. The virus is mainly transmitted by the *Aedes aegypti* mosquito. Since 1963, there has been a steady increase in the incidence of dengue haemorrhagic fever (DHF) in Viet Nam, which is a leading cause of hospitalization and death in children[1,2]. In 1998, southern Viet Nam experienced serious DHF epidemics recording 123,997 cases ($455.7/10^5$ population) and 347 deaths ($1.3/10^5$ population), with case fatality of 0.28%. As in other developing countries, uncontrolled urbanization, poor environmental conditions and poor mosquito control in the country contributed to the rising incidence of dengue. Detection

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of specific antibodies is a valuable procedure for diagnosing dengue, particularly for active surveillance and disease control.

Typical dengue serology specifies an incubation period ranging from 3-10 days (mean 4-5 days). In a primary infection, IgM antibodies are produced by the fifth day after onset, rise for 1-3 weeks and generally persist for 30-60 days, while IgG appears by 14 days after the onset of symtoms. The speed at which the IgM develops and the amount of IgM antibody produced can vary between patients. In a secondary infection, some patients may not produce detectable levels of dengue IgM antibodies until 20 days after the onset of infection or produce IgM at lower levels or for shorter periods. However, IgG antibodies rise rapidly 1-2 days after the onset to reach levels above those found in primary or past dengue infection. These high levels of IgG persist for 30-40 days before declining to levels found in primary or past infection and persist for life[3,4].

In this study, the commercial pathozyme dengue IgM and IgG were assayed with DHF paired sera and single negative sera. They were then compared with the results of virus isolation, in-house dengue IgM capture ELISA and haemagglutination-inhibition tests. The results of this evaluation are presented below.

Materials and methods

Serum specimens

Serum specimens from DHF patients were collected in the Paediatric Hospital No.1, Ho Chi Minh City, or retrospectively from a bank of frozen sera of our laboratory. The first serum (S1) was collected during hospital admission of the patient, while the second serum (S2) was collected 3-5 days after S1 or later. Single-patient sera, with clinical diagnosis as measles, were also collected and used as negative control sera. During the DHF epidemic, almost all hospitals in southern Viet Nam were overloaded by DHF patients; therefore, paediatricians could not keep these patients in hospitals more longer and S2 must be obtained before their discharge. For this reason, the interval between the collection of S1 and S2 differed due to circumstances, and these were as follows: 3-5 days: 51.8%; 6-7 days: 3.6%; 8-11 days: 16.1%, not mentioned: 28.6%. This variation prevented the build-up of high titre of antibodies in HAI assays.

Haemagglutination-inhibition assay (HAI)

HAI antibodies against DEN-1, DEN-2, DEN -3 and DEN-4 were determined as described by Clarke and Casals[5], but the assay was modified to a micromethod.

In-house dengue IgM capture ELISA (Mac-ELISA)

The Mac-ELISA assay was performed following the actual technique used at the Centers for Disease Control, Colorado, USA, by using the monoclonal antibody SLE 6B6C-1/HRP conjugate[6].

The Commercial Pathozyme Dengue IgM and IgG kits (CPD IgM-IgG)

The CPD IgM-IgG were supplied by Omega Diagnostics Limited, Omega House,

Carsebridge Court, Whins Road, Alloa FK10 3LQ, Scotland, UK. For reading, calculation and interpretation of the results, the manufacturer's instructions described in the test procedures were followed.

Data analysis

Confirmed dengue diagnosis was based on virus isolation and the Mac-ELISA and HAI test, using WHO criteria[7]. For the evaluation of the sensitivity and specificity of the CPD IgM-IgG, paired sera from DHF patients and negative control sera were tested parallel with the CPD IgM-IgG and our known virus isolation, Mac-ELISA and HAI. The first step of this comparison was based on our primary and secondary infections. In the second step, the CPD IgM-IgG and our assays were tested parallel versus all paired and single sera, then their results were compared step by step with each other.

Results and discussion

Number of sera collected, patient severity grades and intervals between S1 and S2

- Fifty-six paired sera were collected from DHF patients with clinical diagnosis as Grade 2: 38 cases, Grade 3: 12 cases, and Non-classified: 6 cases.
- From paired sera, we isolated four DEN-3 viruses from 23 patients. By combining results of Mac-ELISA and HAI, we could differentiate primary dengue (n=14), secondary dengue (n=39), and no flavivirus infection (n=3).
- Forty-two single sera of measles patients were collected and tested with the CPD IgM-IgG and Mac-ELISA, HAI as negative control sera.

Evaluation of the sensitivity of the CPD IgM-IgG by comparison with the virus isolation, Mac-ELISA and HAI

In this comparative study, we divided the sera into three groups and compared S1 and S2 separately and paired sera by IgM and IgG.

Comparison of the CPD IgM-IgG with the positive virus isolation

Among the 23 DHF patient sera, we isolated DEN-3 virus from four patients, in whom there were two primary and two secondary infections (Table 1).

Table 1. *Comparison of the CPD IgM-IgG with four positive virus isolations*

Serol-ogical Tests	Sera	CPD IgM-IgG		Mac-ELISA	
		Posi-tive	Sensi-tivity	Posi-tive	Sensi-tivity
IgM	S1	2/4	50%	0/4	0%
	S2	4/4	100%	4/4	100%
	Paired sera	4/4	100%	4/4	100%
IgG	S1	2/4	50%		
	S2	3/4	75%		
	Paired sera	3/4	75%	4*/4	100%

* tested by HAI

One negative CPD IgG paired sera (No. 15-16) had index value close to its cut-off; the reason probably was a particular case of primary infection, because even in HAI, the increase of anti-dengue antibodies is too slow.

Comparison of CPD IgM-IgG with Mac-ELISA and HAI in primary and secondary infections (3 negative cases were excluded)

The sensitivity of CPD IgM and of CPD IgG with sera of confirmed DHF cases is given in Tables 2 and 3 respectively.

Table 2. *Sensitivity of CPD IgM performed with sera of confirmed DHF cases*

CPD IgM	Primary inf.		Secondary inf.		Total	
	Positive	Sensitivity	Positive	Sensitivity	Positive	Sensitivity
S1	11/14	78.57%	34/39	87.18%	45/53	84.91%
S2	14/14	100%	34/39	87.18%	48/53	90.57%
Paired sera	14/14	100%	38/39	97.44%	52/53	98.11%

Table 3. *Sensitivity of the CPD IgG performed with sera of confirmed DHF cases*

CPD IgG	Primary inf.		Secondary inf.		Total	
	Positive	Sensitivity	Positive	Sensitivity	Positive	Sensitivity
S1	3/14	21.43%	38/39	97.44%	41/53	77.36%
S2	11/14	78.57%	39/39	100%	50/53	94.34%
Paired sera	11/14	78.57%	39/39	100%	50/53	94.34%

The sensitivity of CPD IgM-IgG assays and of Mac-ELISA, HAI versus 53 paired sera of confirmed DHF patients

The CPD IgM assays agreed with the Mac-ELISA results in 52/52 (100%) of cases (Table 4). Both of these tests detected 52 positive of the 53 paired sera, giving an equal sensitivity of 98.11%.

Table 4. *Comparison of CPD IgM assays and Mac-ELISA**

Sera	CPD IgM		Mac-ELISA	
	Positive	Sensitivity	Positive	Sensitivity
S1	45/53	84.91%	40/53	75.47%
S2	48/53	90.57%	51/53	96.23%
Paired sera	52/53	98.11%	52/53	98.11%

*By the Chi-Squares Mantel-Haenszel, $\chi 2$ was 0.00, P =0.752.

The CPD IgG positive rate of S1 and S2 was 77.36% and 94.34% respectively, and with paired sera its sensitivity was 94.34%; meanwhile, the HAI and virus isolation gave the same rate 94.34% (Table 5). The CPD IgG results agreed with the HAI and virus isolation results in 50/50 (100%) of cases.

Table 5. *Comparison of the CPD IgG assays with the HAI and virus isolations**

Sera	CPD IgG		HAI and Virus isolation	
	Positive	Sensitivity	Positive	Sensitivity
S1	41/53	77.36%	Inconclusive	
S2	50/53	94.34%		
Paired sera	50/53	94.34%	50/53	94.34%

*By the Chi-Squares Mantel-Haenszel, $\chi 2$ was 0.00, P =0.660.

The specificity of the CPD IgM-IgG

Forty-two single sera were taken on the day of admission from children with clinical diagnosis as measles, out of which there were 25 positives and 17 negatives by

measles IgM capture-ELISA. These sera were retested with CPD IgM-IgG and the Mac-ELISA, HAI for dengue. The results are given in Table 6.

Table 6. *Performance of CPD IgM and Mac-ELISA with measles sera**

Measles	CPD IgM		Mac-ELISA	
Mac-ELISA	Negative	Specificity	Negative	Specificity
25 (+)	21/25	84%	25/25	100%
17 (-)	17/17	100%	17/17	100%
Total: 42 single sera	38/42	90.48%	42/42	100%

*By the Chi-Squares Mantel-Haenszel, $\chi 2$ was 4.15, P = 0.058

Among the 42 single sera, there were five sera having dengue antibody (≥ 40 HAI units), so we discarded them and only 37 measles sera without dengue antibody were used for the specificity study of the CPD IgG.

Thirty-seven measles sera without dengue antibody were tested with CPD IgG, from which there were two false positive results and 35 negative. So, the CPD IgG is specific to dengue IgG with the rate of 94.59% (Table 7).

Table 7. *Performance of the CPD IgG and the HAI with measles sera without dengue antibody*

Measles	CPD IgG		HAI	
Mac-ELISA	Negative	Specificity	Negative	Specificity
21 (+)	21/21	100%	21/21	
16 (-)	14/16	87.5%	16/16	
Total: 37 single sera	35/37	94.59%	37/37	Inconclusive

Summation

The total number of samples tested for CPD IgM and CPD IgG and their sensitivity and specificity are given below:

Assay evaluated	Sensitivity	Specificity
CPD IgM	98.11% (52 of 53)	90.48% (38 of 42)
CPD IgG	94.34% (50 of 53)	94.59% (35 of 37)
Mac-ELISA	98.11% (52 of 53)	100% (42 of 42)
HAI (paired sera)	94.34% (50 of 53)	Inconclusive with single sera

The CPD IgM-IgG evaluated in this report are suitable for the detection of anti-dengue IgM and IgG antibodies. They should be useful for routine dengue diagnosis, particularly in developing countries as in south-east Asia where dengue infections are prevalent. For the prevention and control of dengue, the CPD IgM and the Mac-ELISA are good tools for the early detection of dengue cases during the inter-epidemic period, or during the pre-epidemic stage, for carrying out laboratory-based active surveillance.

Conclusion

The Commercial Pathozyme Dengue IgM and IgG are reliable, sensitive and specific diagnostic tests for diagnosing primary and secondary dengue infections based on antibody responses. It should be used in laboratories to support the clinical diagnosis of dengue. The Commercial Pathozyme Dengue IgM is also a very good tool for

active laboratory-based dengue surveillance with the aim of controlling dengue epidemics in the tropical and sub-tropical regions.

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The Role of Vectors in Emerging and Re-emerging Diseases in the Eastern Mediterranean Region+

By

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Abstract

Considerable attention has recently been drawn at the global level to the serious threat to humans caused by new, emerging and re-emerging infectious diseases. Among the infectious vector-borne diseases, dengue, dengue haemorrhagic fever, yellow fever, plague, malaria, leishmaniasis, rodent-borne viruses and arboviruses are considered to be persisting, and sometimes re-emerging, with serious threat to human health. In the WHO Eastern Mediterranean Region, dengue, malaria and leishmaniasis are significant vector-borne diseases. This article discusses the role of vectors in the re-emergence of malaria, leishmaniasis and dengue fever and their control.

Key words: Malaria, Leishmaniasis, Dengue fever, Dengue haemorrhagic fever.

Malaria

Malaria is the most important vector-borne disease in the Eastern Mediterranean Region of WHO (EMR). After a decrease in its incidence globally, and in the Region in the late 1960s, an upward trend of malaria occurrence has been observed, caused by a number of technical and administrative factors. One of the most important factors is the increase in malaria vector mosquito densities due to decreased attention to vector control, leading to a consequent increase in the disease transmission.

In EMR, out of the 70 species of anopheline mosquitoes recorded, at least 18 are confirmed vectors of malaria (Figure 1). It is feared that, in addition to the possible intensification of the malaria problem in endemic countries, countries that are at present free from malaria may not be able to maintain their malaria-free status if the required vector vigilance and control services and activities are not restored and maintained by these countries.

For the control of malaria vectors, the global strategy of malaria control highlights

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Figure 1. **Anopheles mosquito, vector of malaria**

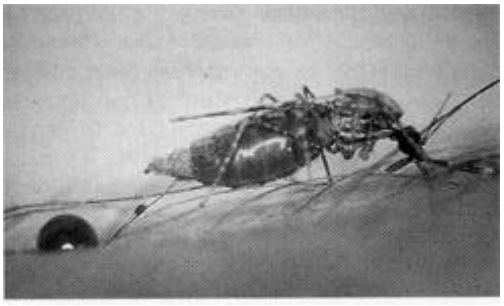

Figure 2. **Sandfly, vector of leishmaniasis**

the need for selective and sustainable control of vector mosquitoes for the prevention of disease transmission and epidemics. The regional strategy on vector control emphasizes the integrated vector control approach, the use of insecticide-treated bed nets and other materials and preparedness for emergencies.

Leishmaniasis

Leishmaniasis is another re-emerging vector-borne disease in the Eastern Mediterranean Region. The well-known vectors are sandflies (Figure 2). They belong to the family *Phlebotominae*, which contains about 700 species, of which about 70 species are proven vectors of leishmaniasis. Of these, about 20 species have been found to play a significant role in transmission in EMR countries. Two species, *Phlebotomus papatasi* and *P. sergenti,* are present in almost all countries of the Region. Visceral leishmaniasis (also known as kala azar), zoonotic cutaneous leishmaniasis and anthroponotic cutaneous leishmaniasis are the three principal forms present in the Eastern Mediterranean Region. The disease is of serious public health concern in six countries and exists to a lesser degree in another 10 countries.

Leishmaniasis is considered to be a re-emerging disease globally and in the EMR. During the past two decades, a rapid increase in its incidence and geographical spread has taken place. For example, in the Syrian Arab Republic, the number of cases of cutaneous leishmaniasis increased from 1650 in 1987 to 9000 in 1992; in Tunisia, cutaneous leishmaniasis increased from 1300 cases in 1983 to 6000 in 1990. In Sudan, the situation is very serious. During the past five years in southern Sudan, over 15,000 cases of kala azar have been treated. During the same period, an epidemic built up in eastern Sudan, with the number of cases increasing from 1100 in 1992 to over 2400 in 1993.

Among the factors responsible for this upsurge, the most important are rapid and unplanned urbanization, mass movements of people, congregation of human populations and implementation of water resource development projects (building dams and irrigation systems) without incorporating safeguards against disease vector

proliferation. Also, the phasing out of vector control activities such as residual spraying of insecticides for malaria and other vector control have contributed to the increase in vector population.

Control of vectors of anthroponotic visceral and cutaneous leishmaniasis is carried out by indoor spraying with residual insecticides and the use of insecticide-treated bednets/curtains and other materials.

The control of zoonotic visceral leishmaniasis vectors is carried out by residual insecticide spraying of houses and animal shelters, especially where the vector, sandflies, are restricted to domestic and peridomestic areas. At the same time, treatment or elimination of dogs, the main domestic reservoir of zoonotic visceral leishmaniasis, should be carried out. In the case of zoonotic cutaneous leishmaniasis, where rodents are the main reservoir, the usual intervention is the application of rodenticides, and destruction of rodent burrows and chenopod plants by deep ploughing. Rodent ectoparasites, such as fleas, are controlled by insecticidal application to rodent burrows before ploughing through them or the application of rodenticides to kill rodents, especially in the vicinity of human inhabitations.

Dengue fever

Dengue fever, commonly known as breakbone fever owing to the characteristic severe pain it can cause in bones and joints, is a viral disease caused by one of the arboviruses (flavivirus), and is transmitted by mosquitoes (Figure 3). In a consultation, "Key issues in dengue vector control towards the operationalization of a global strategy",

Figure 3: ***Aedes* mosquito, vector of dengue**

which was held at WHO headquarters in Geneva from 6 to 10 June 1995, to define a global strategy for the prevention and control of dengue fever and dengue haemorrhagic fever/dengue shock syndrome, it was recognized that dengue fever and dengue haemorrhagic fever outbreaks were increasing in frequency globally. Dengue was recognized to be of public health concern in urban and peri-urban as well as in rural environments. Two thousand million people are estimated to be at risk of dengue fever and dengue haemorrhagic fever.

In the absence of any specific treatment and vaccine, the global dengue prevention and control strategy basically depends upon prevention and control measures to eliminate or drastically reduce the population of mosquito vector, *Aedes aegypti*, in a sustainable manner; also, early diagnosis and prompt management of dengue haemorrhagic fever and dengue shock syndrome are vital. To achieve these objectives, it is necessary to integrate dengue vector control with other vector-borne disease control programmes; strengthen technical and institutional resources for vector control at country level; and mobilize all possible resources to involve the community in vector control for sustainability.

Dengue history in the Eastern Mediterranean Region

Dengue fever was widespread in many countries in the Eastern Mediterranean Region during the 19th and the first half of the 20th century. A decline in dengue transmission was recorded in Egypt after 1940. This decline was attributed by Darwish and Hoogstrall[1] to rapid decrease of *Aedes aegypti* populations with the introduction and widespread use of dichlorodiphenyltrichloroethane (DDT) during and after the Second World War.

Dengue activity was reported in Somalia in 1982[2]. Between 1985 and 1987 a serological survey during an outbreak of febrile disease in refugee camps near Hargeisa, northern Somalia, confirmed dengue activity[3]. In Sudan, dengue activity was detected through another serological survey, in which 17 isolates of dengue type 2 and one of dengue type 1 were detected[3]. In 1992, an outbreak of febrile illness in Djibouti was found to be due to a mixture of malaria and dengue[4]. Pakistan first reported an epidemic of dengue fever in 1994, and dengue fever cases were reported from Saudi Arabia in 1994[5,6]. The evidence for local transmission of dengue fever cases in Jeddah, Saudi Arabia, was presented by Ghaznawi in 1995[7], who also confirmed the presence of dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus* in different districts of the city.

In the light of recent history, it can be said that recently persistent and active dengue transmission has not been observed, but sporadic outbreaks are occurring in some countries, and that dengue in this Region appears to be re-emerging after an absence of about half a century. There is good evidence that fresh transmission of dengue through its vector mosquito, *Aedes aegypti*, is taking place.

The main factors behind the recent re-emergence of dengue in the Region are:

- Decreased use of DDT. At present application of DDT is not favoured because of development of insecticide-resistance in vectors or global environmental concerns.
- Rapid urbanization and the resultant development of slum and shanty towns around urban centres. Such unplanned human habitations usually lack civic facilities such as proper sewerage systems, and hence the possibility of vector/pest proliferation.
- The large number of displaced human populations, many of them living in refugee camps (in Afghanistan, Djibouti, the Islamic Republic of Iran, Pakistan, Somalia, Sudan). These populations are more prone to mosquito bites.
- Rapid and increased means of human transport, which have increased the chances of introducing pathogens and vectors.
- Inadequate attention to *Aedes* control in urban areas.
- Community awareness about dengue and vector mosquitoes, which is very low in general.

Dengue control

Aedes aegypti, the main vector mosquito, has been recorded in 13 of the 22 countries of the Eastern Mediterranean Region. It is known as a domestic mosquito, found inside

and near human habitations. It is commonly considered to be an urban mosquito, but it breeds in rural areas with equal ease. In some countries in the Region, *Aedes aegypti* is found to be breeding in natural receptacles such as tree holes, but always near human habitation. The distribution of *Aedes ægypti* in the Region is given in the table 1 below. Classical dengue is believed to have originated in south-eastern Asia, where the mosquito *Aedes albopictus* is the principal indigenous vector[5,6]. In EMR, *Aedes albopictus* is a lesser vector of dengue and has been recorded in Pakistan only. The role of *Aedes albopictus* as a vector of dengue in the Region needs confirmation.

Table 1. *Distribution of important Culex and Aedes mosquitoes in the Eastern Mediterranean Region*

Member State	Mosquito							
	Culex pipiens	*Culex molestus*	*Culex antinatus*	*Culex quinque-fasciatus*	*Culex univitatus*	*Aedes aegypti*	*Aedes caspius*	*Aedes albopictus*
Afganistan	+					+		
Bahrain				+		+		
Cyprus	+							
Djibouti	+			+		+		
Egypt	+		+		+		+	
Iran, Islamic Republic of	+			+	+		+	
Iraq	+							
Jordan	+	+	+					
Kuwait			+	+	+		+	
Lebanon	+							
Libyan Arab Jamahiriya	+					+		
Morocco	+							
Oman				+		+		
Pakistan	+			+		+		+
Qatar				+				
Saudi Arabia	+			+		+	+	
Somalia				+		+	+	
Sudan				+		+		
Syrian Arab Republic	+					+		
Tunisia	+					+		
United Arab Emirates				+		+		
Republic of Yemen	+			+		+		

+ = species present

Progress is being made in developing a pan flavivirus vaccine. Difficulties persist because of lack of appropriate animal models to test the attenuated vaccines and also because of antibody-dependent enhancement of viral growth[2].

In view of the above, elimination or drastic reduction of the population of mosquito vectors *Aedes aegypti* and *Aedes albopictus* remains the main control measure.

Control of dengue vector mosquitoes in EMR

In view of the sporadic nature of dengue outbreaks, vector control programmes that are specifically devoted to eliminating or controlling *Aedes aegypti* or *Aedes albopictus* do not exist. Vector suppression activities are undertaken only in the case of outbreaks, which are mostly limited to ground or aerial application of pesticides.

The *Aedes* mosquito populations are, to some extent, kept suppressed through national malaria control programmes, or, in some malaria-free countries, by disease vector/pest control programmes, as they use chemical pesticides (a wide range of organochlorines, organophosphates, carbonates and pyrethroids are used). Some countries also use biocides. Trials with *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* have been carried out in some countries. Recently, in response to a regional initiative on integrated vector control, considerable effort will be invested in making full use of this method. The integrated vector control approach utilizes the most suitable combination of environmental, chemical and biological control. Biological control of vector mosquitoes is mainly by the use of larvivorous fish. At least, 15 out of 22 countries are using or have used larvivorous fish, such as *Gambusia affinis*, *Tilapia mozambica*, *Aphanius dispar* and *Oreochromis* species, for mosquito larval control. But this method is still far from perfect.

Conclusion

In conclusion, a number of vector-borne diseases are re-emerging in the Eastern Mediterranean Region due to a number of natural and man-made factors. To prevent the emergence of new vector-borne diseases and re-emergence of those already under control, it is essential to strengthen national vector control programmes. Vector control activities must be integrated both by bringing together different vector control methods - environmental, biological and chemical – and by coordinating the vector control activities of various vector-borne disease control programmes (against malaria, filariasis, leishmaniasis, dengue, flea- and rodent-borne diseases and other arboviruses of public health significance). This is a trans-disease control approach for cost-effectiveness and sustainability. It is also very important to have an efficient and sustainable surveillance system for the vectors and their resistance to various insecticides. Those countries that are epidemic-prone must develop and maintain emergency preparedness plans.

In accordance with the regional vector control strategy, an integrated vector control

strategy should be implemented with maximum community participation and by using primary health care as the main vehicle for sustainability of achievements.

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The Use of GIS in Ovitrap Monitoring for Dengue Control in Singapore[+]

By

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Abstract

Ovitraps are used extensively in Singapore as a tool to monitor, detect and control *Aedes* populations. They give an approximate gauge of the adult population in an area and act as an early warning signal to pre-empt any impending dengue outbreaks. A Geographic Information System (GIS) was established in 1998 to develop a real-time *Aedes* mosquito control and monitoring system for spatial epidemiological study. The GIS monitors the network of 2000 ovitraps placed island-wide to better understand vector trends and disease patterns. Analysis is done on the ovitrap breeding data collected weekly to identify hotspots and risk areas where there is a danger of high *Aedes aegypti* infestation. Three ovitrap models had been developed to analyse the ovitrap breeding data collected. The analysis results are used to plan vector surveillance and control operations. This paper reports the experience of this control and monitoring methodology in Singapore.

Key words: GIS, Ovitrap, Dengue control, *Aedes*, Singapore.

Introduction

Singapore is a tropical island with a land area of 682.7 sq km and a population of four million, characterized by uniform temperature (mean daily ≈ 26.8°C), high humidity (mean daily ≈ 84.3%) and abundant rainfall (mean annual ≈ 2346 mm) throughout the year. The city-state lies just north of the equator near latitude 1.5 deg N and longitude 104 deg E.

Dengue is endemic in Singapore and has seen a recent resurgence despite an effective vector control programme based on a three-pronged approach that incorporates source reduction, public health education and law enforcement[1]. Several factors resulted in the resurgence of dengue in Singapore. The immunity level of the population has declined[1] while adult densities of the *Aedes* vectors have

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[+] *As this paper is focusing on the use of GIS for ovitrap monitoring, therefore the author did not elaborate on the other layers that were used in GIS analysis. – Editor.*

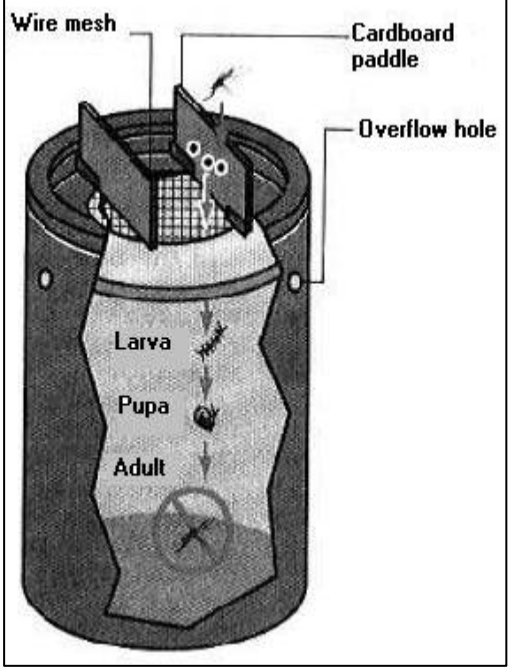
multiplied due to the rise in temperatures and increased rainfall[2]. Climatic changes have been reported to affect the biology and behaviour of the vectors, allowing them to develop higher competence for dengue transmission[3].

Ovitraps

Ovitrap surveys could be considered a sensitive and an efficient technique for detecting and monitoring *Aedes* populations at low densities. They are safe, economical and environment-friendly[4]. With only 10% of the island under active *Aedes* mosquito field surveillance due to limited manpower for vector control, the extensive use of ovitraps is an important resource to help collect data on *Aedes* population on a wider area and gauge the effectiveness of control efforts.

Currently, ovitraps (Figure 1) are used as a means of detecting *Aedes aegypti* presence as well as an approximate gauge of the adult population in an area. It can be used to estimate fairly well the population of adult mosquitoes in the environment by counting the number of eggs laid on the moist paddle. In this way, a sudden increase of mosquito population can be detected. Changes in the species breeding (*Aedes aegypti* to *Aedes albopictus* or vice versa) can also be detected. The ovitraps are checked weekly for breeding and the breeding samples, if any, are collected and identified by the laboratory. The ovitraps are then cleaned to remove any eggs that are stuck on the inner walls of the ovitraps, re-filled with cow grass solution which is much more attractive to the female mosquitoes than just water, and placed back in their positions. The paddles are collected and replaced with new ones. The collected paddles are checked under the microscope for eggs which are also counted.

Figure 1. **Diagram of an Ovitrap**



There are 2000 ovitraps placed on the island currently, 30% are placed inside premises, while 70% are placed outside premises under shade. Most of the premises are residences although there are some which are schools and commercial buildings. The bulk of the ovitraps are placed in dengue "sensitive areas (SAs)" where regular search-and-destroy rounds are carried out. Others are placed in previous SAs that had already been cleaned up, for early detection of any resurgence of the vector. Some are also placed in persistent complainants' homes to determine the mosquito species that cause the nuisance.

Geographic Information System (GIS)

A GIS is an automated computer-based system with the ability to capture, retrieve, manage, display and analyse large quantities of spatial and temporal data in a geographical context. The system comprises hardware (computer and printer), software (GIS software), digitized base maps, information and a whole set of procedures such as data collection, management and updating[5].

Specific diseases and public health resources can be mapped in relation to their surrounding environment and existing health and social infrastructures. Such information when mapped together creates a powerful tool for the monitoring and management of disease[6]. GIS provides a graphical analysis of epidemiological indicators over time, captures the spatial distribution and severity of the disease, identifies trends and patterns and indicates where there is a need to target extra resources.

In 1998, a GIS was established in Singapore to research as well as to support operations on dengue control. Roads, residential buildings and other relevant databases were obtained and mapped to form the base map layer using Arcview GIS 3.2a. Other layers such as *Aedes* breeding sites, dengue case incidences, complainants' addresses, sensitive areas, weather data (rainfall, temperature and relative humidity) and other related information were also mapped into the GIS.

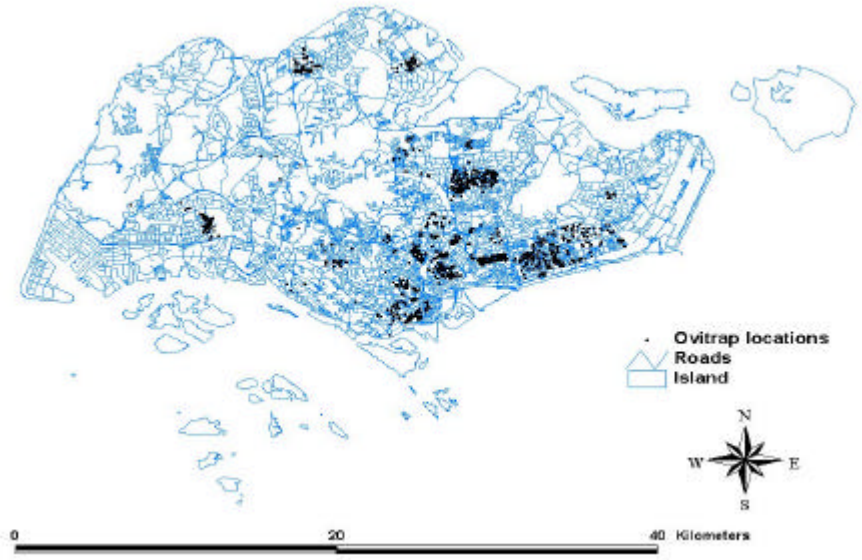
Application of GIS on ovitraps

An ovitrap layer comprising a spatial map and an attribute table was created in the GIS for monitoring and analysing the network of ovitraps placed island-wide to better understand vector trends and disease patterns (Figure 2). Every ovitrap placed is given a unique number for identification and its spatial location is stored in the GIS. The attribute table behind this ovitrap layer stores the ovitrap's identification number, the surveillance team in charge of the ovitrap, the date of the weekly collection, the address of the site, the housing type of the site (e.g. flat, house, school, etc.), the position of the ovitrap (indoor or outdoor), the status of the ovitrap (removed or missing), the species found in the ovitrap, the larval instars and pupal stages, the breeding density, the mixed breeding species types (e.g. *Aedes aegypti* and *Aedes albopictus*, *Aedes aegypti* and *Culex quinfasciatus,* etc.) and the dominant species type breeding in that ovitrap for that week. In the case of a mixed breeding, the species which had a higher density would be the dominant species. Decisions on operations and deployment of manpower are made using the ovitrap information as well as databases on diseases and mosquito surveillance results.

Routine monitoring of *Aedes aegypti*

Analysis is done on the results collected weekly to identify hotspots or risk areas where there is a danger of *Aedes aegypti* infestation, to pre-empt any impending dengue outbreak. As a huge amount of data is collected weekly, there is a need to sieve out only the important and useful information. A query is done to gather all the *Aedes aegypti* breeding ovitrap sites as this mosquito is known to be the primary vector

Figure 2. **Spatial distribution of ovitraps in Singapore**



in the transmission of dengue in Singapore[7]. These ovitrap sites would be sorted by density and addresses to highlight high breeding areas and areas with many breeding ovitraps. These ovitrap sites are also clustered if there are two or more *Aedes aegypti* breeding ovitraps within a distance of 250 metres. This distance is the normal flight distance of the vector, which is not further than 240 metres in Singapore[8]. The distribution of *Aedes aegypti* breeding in the ovitraps for each SA is also summarized weekly to highlight the "hot" SAs where total breeding density in ovitraps remains relatively high for a number of weeks when compared with other SAs. Another important indicator is the change in the dominant species of a particular ovitrap. If other mosquito species had been detected consistently and *Aedes aegypti* is suddenly found in the ovitrap, then that area will be placed on high alert and monitored carefully. This change means that the vector has invaded an area where it was not previously found and an outbreak might soon occur.

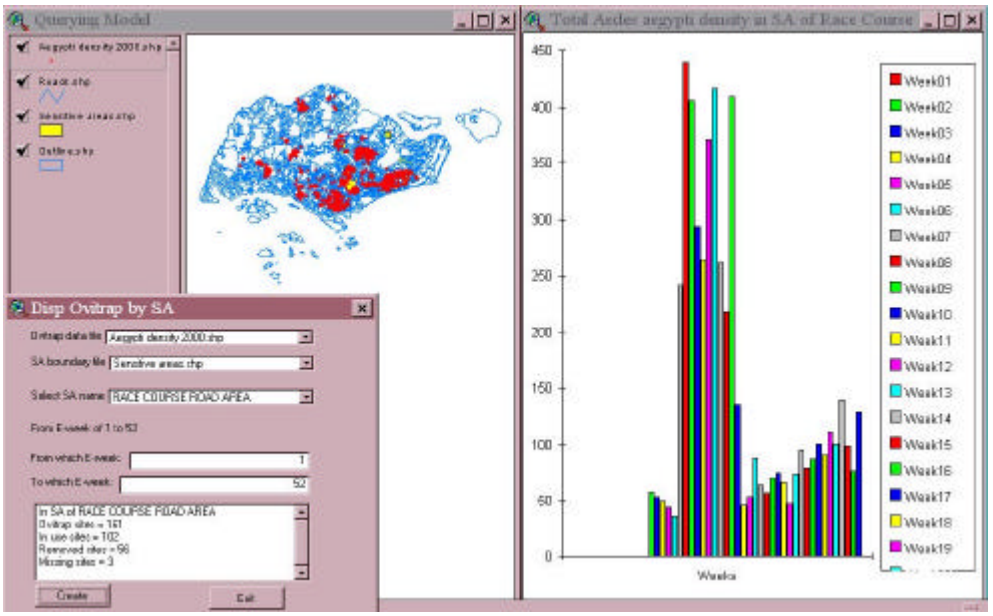
Ovitrap models

Three models had been developed to monitor, analyse and evaluate ovitrap breeding data to better understand the *Aedes* situation on the island for surveillance purposes.

Hotspot model

A hotspot ovitrap model was developed to display, identify and highlight ovitrap sites that have been breeding above a certain density level for a specific number of weeks during a defined time period. For example,

Figure 3



The inset shows the query model where we select the output data file as "*Aegypti* density 2000", SA as "Race Course Road Area" and week 1 to 52 to display total *Aedes aegypti* density by weeks for year 2000 in Race Course Road area. The bar graph is generated for the query, where the y-axis gives the total *Aedes aegypti* density and the x-axis with its different colour bars represents the weeks in the year 2000.

we set the density as greater than one larva or pupa per ovitrap and the time period as four weeks to identify ovitraps that had been breeding consistently for the past four weeks. This model is particularly useful for identifying areas that had been having high *Aedes aegypti* population density consistently for some time and should be paid extra attention. It can also be used to gauge whether control efforts in the area have been effective or successful.

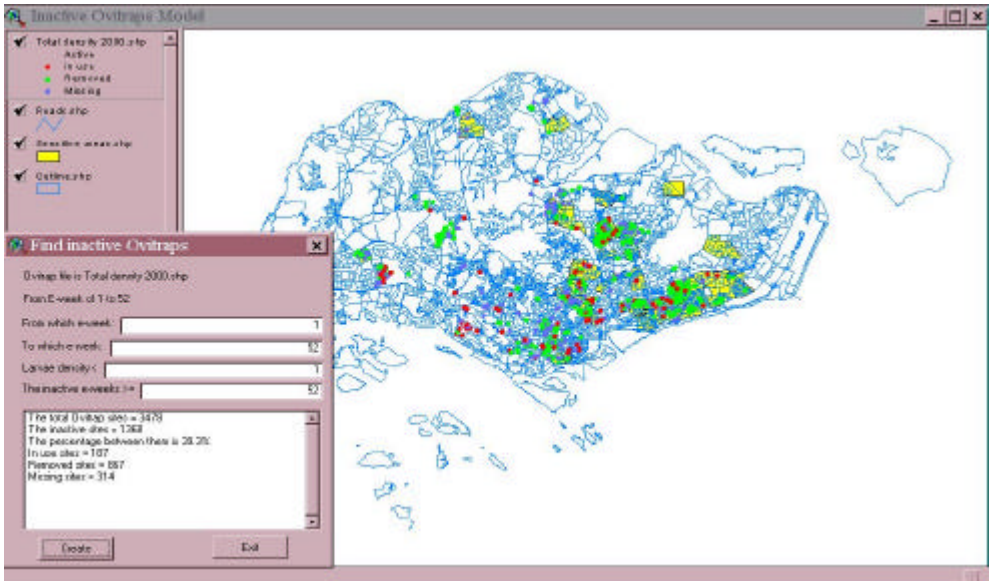
Query model

The query model was developed to generate and display bar graphs of total ovitrap breeding density for a specific SA or any selected area for a defined time period by weeks. With this model, surveillance teams are able to query the ovitrap breeding situation in an area for any defined time period. Figure 3 shows the query model and results displayed for Race Course Road for the year 2000.

Inactive ovitraps model

The inactive ovitrap model was developed to identify ovitrap sites with low or no breeding for a defined time period (Figure 4). These locations are then evaluated to see if there was a need for the ovitraps to be shifted to another location where breeding might be detected to promote more efficient use of the ovitrap.

Figure 4



The inset shows the inactive ovitrap model where we select the output file as "Total density 2000" which stores the breeding data, week 1 to 52, larval density < 1 and inactive weeks = 52 to identify all the inactive ovitraps in the year 2000 which are represented by red dots on the map.

Discussion

With the build-up of epidemiological and entomological databases, the next step would be to develop spatial analytical methods and models to test hypotheses concerning vector and disease relationships and the nature and processes of disease transmission. These modellings will involve the integration of GIS with standard statistical and epidemiological methods. The spatial modelling capacities offered by GIS can help one understand the spatial variation in the incidence of disease and its covariation with environmental factors and public health systems. Important technical and logistical innovations in data and data access for GIS are already available in the market. There has also been greater accessibility to global positioning systems and availability of inexpensive hand-held devices for using the system and the addition of direct-to-GIS data links to these systems[9].

Singapore will start using palmtops to gather field *Aedes* surveillance data in the near future. We are working to develop an optimal ovitrap sampling frame and eventually come up with an accurate ovitrap index as an indicator for actions. With the use of ovitraps and the GIS, the task of vector and disease surveillance will be brought to greater heights. Information will be available in realtime and other discoveries about the relationship between vector and the disease could be made possible through spatial analyses. A greater understanding of the vectors would bring us a step closer to eradicating dengue fever in Singapore.

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Host Feeding Pattern of *Aedes aegypti* and *Aedes albopictus* in Kolkata# India

By

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Abstract

Blood meal analysis of *Aedes aegypti* and *Aedes* albopictus, collected from cattle sheds and human dwellings in the highly congested residential areas of the city, and from outdoor situations in an urban garden, revealed a high bovine blood index of both the species in the former and a high human blood index in the latter two collection areas. The difference in the various blood indices of each of the two species in the same collection areas is statistically significant ($P < 0.05$). The blood-feeding pattern of *Aedes aegypti* and *Aedes albopictus* pooled from the three collection sites revealed that a majority of members of both the species had fed on one host and a small percentage on more than one host, and, that, both the species were mainly anthropophilic in nature.

Key words: *Aedes aegypti*, human blood index, *Aedes albopictus*, Kolkota.

Introduction

Aedes aegypti is the principal vector of dengue and dengue haemorrhagic fever in south-east Asia[1]. *Aedes albopictus* has also been recognized as a secondary vector and is important in the maintenance of dengue viruses[1,2].

In view of the epidemiological significance[3] of the host feeding pattern of vector mosquitoes, and the paucity of information on the subject with respect to *Aedes aegypti* and *Aedes albopictus,* coupled with drastic changes in the ecological scenario of the city, which are likely to affect the feeding preferences of vectors, the blood-meal analysis of both the species was undertaken and the results are presented here.

Materials and methods

A description of how the mosquitoes were collected was needed.

#Earlier known as Calcutta

Study area: Fully-fed *Aedes aegypti* and *Aedes albopictus* were collected from human dwellings (HDs), cattle sheds (CSs) distributed in various parts of the city, and from outdoor situations in an urban garden. The garden has a mini zoo, which is home to a few rabbits and a variety of birds in addition to a few horses and cows under a large shed. A number of one-roomed houses inhabited by the maintenance staff are situated at the periphery of the garden.

Methods: Fully-engorged adult females of *Aedes aegypti* and *Aedes albopictus*, collected in the same number of collections during June 1996 to May 1998 from the above-mentioned collection sites were subjected to Ouchterlony gel immunodiffusion technique[4], using human (Hu), bovine (Bo), avian (Av) and equine (Eq) antisera, and the host feeding pattern of the two species determined. The human-landing periodicity of both the species was also studied.

Statistical analysis: A simple chisquare test (x^2) was done to show the difference in various blood indices of each of the two species in the same collection site.

Results and discussion

Human blood index: Out of the 636 *Aedes aegypti* and 186 *Aedes albopictus* mosquito vectors collected from HDs in the residential areas of the city, 578 (90.88%) and 162 (87.0%), respectively, reacted positively to the test. Of these, 99% of the former and 100% of the latter were positive for human blood, i.e. human blood index (HBI) of *Aedes aegypti* and *Aedes albopictus* from HDs was 98.96 and 100 respectively. None of the two species collected from human shelters were found to be positive for bovine blood.

Bovine blood index: The bovine blood index (BBI) of *Aedes aegypti* and *Aedes albopictus* collected from cattle sheds (CSs) was 82.35 and 100, respectively, but HBI for both the species was 'nil'. A small fraction of *Aedes aegypti* had, however, shown evidence of feeding on more than one host in both types of dwellings (Table 1).

The difference between HBI and bovine blood index (BBI) of each of the two species in the same area statistically is highly significant ($P < 0.05$ at 5% level). In other words, the difference between HBI in human dwellings and BBI in cattle sheds of the same species is statistically not significant ($P > 0.05$ at 5% level), indicating opportunistic feeding behaviour of both the species, an observation which has been made earlier also[5].

In the urban garden, a large proportion of *Aedes aegypti* (75.65%) and *Aedes albopictus* (80.66%) had fed on human blood and a small fraction of both the species on avian blood. Both the species also showed an inclination for multiple feeding (Table 2).

Table 1. *Blood meal analysis of Ae. aegypti and Ae. albopictus collected from human dwellings (HD) and cattle sheds (CS) in Kolkata, India*

Collection sites	Species	Samples tested	No. +ve	No. and % positive for				
				One Host*				Two Host**
				Hu	Bo	Av	Eq	
HD	*Ae. aegypti*	636	578 (90.88%)	572 (98.96%)	-	-	-	6(Hu+Bo) (1.04%)
	Ae. albopictus	186	162 (87.0%)	162 (100%)	-	-	-	-
CS	*Ae. aegypti*	100	85 (85%)	-	70 (82.35%)	-	-	15(Hu+Bo) (17.65%)
	Ae. albopictus	45	40 (88.88%)	-	40 (100%)	-	-	-

The variation in different blood indices of both *Aedes* sp. is significant (P<0.05)
* One host: precipitin band against one antisera.
** Two hosts: precipitin band against two antisera.
Hu = Human, Bo = Bovine, Av = Avian, Eq = Equine.

Table 2. *Blood meal analysis of Ae. aegypti and Ae. albopictus collected from urban garden (outdoor) in Kolkata, India*

Species	Samples tested	No. +ve	No. and % positive for				
			One Host*				Two Host**
			Hu	Bo	Av	Eq	
Ae. aegypti	156	156	118 (75.65%)	-	12 (7.69%)		14(Hu+Bo) (8.97%) 12(Hu+Av) (7.69%)
Ae. albopictus	378	362	292 (80.66%)	-	38 (10.05%)	4 (1.10%)	6(Hu+Bo) (1.65%) 16(Hu+Av) (4.40%) 6(Hu+Eq) (1.65%)

The variation in different blood indices of both Aedes sp. is significant (P<0.05)
* One host: precipitin band against one antisera
** Two hosts: precipitin band against two antisera.
Hu = Human, Bo = Bovine, Av = Avian, Eq = Equine.

The variation between different blood indices of the two species in the urban garden is statistically significant ($P < 0.05$ at 5% level), implying that both *Aedes aegypti* and *Aedes albopictus* are anthropophilic in nature in the said area, irrespective of the presence of other vertebrate hosts in the vicinity. The host-feeding behaviour of the two species in the garden is unlikely to be affected by the presence of visitors, i.e. between 11 A.M. – 5 P.M., since the man-landing activity of the two species is at its peak after daybreak, in the morning for a few hours, and a little before sunset[6].

A majority of *Aedes aegypti* (94.27%) and *Aedes albopictus* (95.03%) had fed on a single vertebrate host, but a small percentage of each of the two species (*Ae. aegypti* 5.73%, *Ae. albopictus* 4.97%) had fed on more than one host during one gonotrophic cycle[7] (Tables 1 & 2) which, while revealing their multiple feeding behaviour[3] is of epidemiological significance.

Of the total *Aedes aegypti* collected, 82.51% were from indoor (HDs and CSs) habitats, indicating the endophilic nature of the species. On the contrary, *Aedes albopictus* is exophilic (62.06%) in nature[8,9] (Tables 1 & 2).

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We wish to express our thanks to Mr Sukumar Das and Mr Manas Pal for secretarial and technical assistance.

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Short Note-1

Aedes aegypti survey of Chennai* Port/Airport, India

By

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Introduction

The International Health Regulations, 1969, enjoin upon national governments to keep international ports/airports and peripheral areas up to 400 metres free of *Aedes aegypti* in its adult and immature stages and the mosquito vectors of malaria and other diseases of epidemiological significance[1]. The Ministry of Health, Government of India, set up *Aedes* control units in all major international ports/airports in the country. Periodic monitoring of the successful implementation of *Aedes* control programme is undertaken by the National Institute of Communicable Diseases (Directorate General of Health Services, Government of India). Present finding relates to an assessment carried out in Chennai port/airport during March 1998.

Port area

The Chennai International Seaport is situated at a latitude 13^0 6N and longitude 80^0 8E in the Bay of Bengal. The total land area of the port is 551 acres, and the water spread area is 420 acres. The port area is divided into north, central and south zones and fishing harbours. Slum colonies surround the port area.

Airport

The airport has both international and domestic terminals, covering an area of 1.5 and 1.8 sq km respectively. The airport is divided into two circles, with five zones each, for administrative conveniences.

Aedes control activities

Port area

Mosquito control in the port area is being undertaken by two agencies; (i) The Chennai Port Trust (CPT); and (ii) the Port Health Organization (PHO). Anti-mosquito measures are undertaken by CPT while the

* *Formerly known as Madras*

issuance of yellow fever certificates, ship deratting and overall supervision of mosquito control is undertaken by PHO.

Airport

At the airport also, mosquito control is the responsibility of the airport health officer, while the fumigation of incoming and outgoing aircrafts is undertaken by the Airport Authority of India.

Survey results

Tables 1 and 2 include data on *Aedes aegypti* index and container index in the airport and port areas respectively.

Table 1. *Aedes aegypti index and container index in different zones of Chennai Airport*

Area	*Aedes aegypti* index			Container index		
	Premi-ses searc-hed	Premi-ses posi-tive	Premi-ses index	Conta-iners search ed	Found posi-tive	Per-cent
Cowl Bazar Area	22	6	27.2	33	7	21.2
Old Airport Area	18	1	5.5	35	2	5.71
New Airport Area	19	1	5.26	49	5	10.20
Total	**59**	**8**	**3.55**	**117**	**14**	**11.96**

In the airport area, the *Aedes aegypti* index was found to be maximum in the Cowl Bazar area (21.2%), which is a residential colony adjoining the airport area, in which breeding was promoted due to high water storage practices compelled by intermittent water supply. The container index in this area varied from 10.20% in the new airport area to 5.71% in old airport area. Used aircraft tyres lying in the open and holding rainwater were again the major breeding sites.

Table 2. *Aedes aegypti index and container index in different zones of Chennai Seaport*

Area	*Aedes aegypti* index			Container Index		
	Premi-ses searc-hed	Premi-ses searc-hed	Premi-ses searc-hed	Conta-iners search ed	Found posi-tive	Per-cent
South zone	18	2	11.1	48	10	20.8
Central zone	17	1	5.8	71	18	25.35
North zone	15	1	6.6	32	1	3.1
Total	**60**	**4**	**6.6**	**151**	**29**	**19.20**

In the Port area, the *Aedes aegypti* index was found to be maximum (11.1%) in the South zone and minimum (5.8%) in the Central zone, whereas the container index was found to be maximum (25.35%) in the Central zone and minimum (3.1%) in the North zone. Used automobile tyres holding rainwater were identified as the key breeding sites.

High *Aedes aegypti* indices observed during the dry months in both the port and airport areas, hint at a worsening situation by the monsoon period. This necessitated the need for more vigilance and further strengthening of ecology-based control measures.

Acknowledgements

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undertake *Aedes* survey at the Chennai air/seaport areas. Thanks are also due to Dr S. R. Reddy, Port Health Officer, Dr Selvaraj, Airport Health Officer, Dr V. V. Sai Ram Babu, CMO, and his staff for their active cooperation and help during the survey. Thanks are also due to Mr N. A. Khan, technician and Mr Subash Chand, Lab. Attendant, NICD, Delhi, for the technical assistance provided by them.

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Prevalence of *Aedes aegypti* at the International Port and Airport, Kolkata* (West Bengal), India

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The International Health Regulations (1969)[1] envisage that every port and its adjoining area within a perimetre of 400 metres should be kept free from immature and adult stages of *Aedes aegypti*, the vector of dengue haemorrhagic fever (DHF) and yellow fever. In order to ensure this, active mosquito surveillance and vector control measures within the prescribed limits are in place at the Kolkata port/airport areas. As a cross check a study was carried out by a team of experts from the National Institute of Communicable Diseases (NICD) in and around the Kolkata international port and airport, in the West Bengal state of India, during September 1997. The findings of the survey are presented in this paper.

Airport area

The Kolkata International Airport area covers about 12.5 sq kms. Daily, on an average, 75 aircrafts, both national and international, touch the airport. The Airport Health Organization (AHO) undertakes the mosquito control measures in the airport area, covering approximately 14 sq kms. These measures include the application of Baytex (fenthion) in polluted drains and spraying/fogging with natural pyrethrum extract/(malathion 95%) in the main terminal building and other establishments within the airport, including residential staff quarters.

Kolkata port area

The port area comprises two ports, viz. Kolkata port and Budge Budge port. The Kolkata port alone is situated on the bank of Hooghly river at a distance of about 200 km from the sea. An area of about 77 sq kms is covered by the port, including the protective zone, with a depth of 400 metres around it. The operational area of the port comprises Khidirpur (K.P.) dock, Netaji Subhas (N. S.) dock, and Rajabagan Dock. The Budge

** Formerly known as Calcutta*

Budge port is situated 50 km down the river Hooghly.

Antilarval measures at the port include weekly search for detection and elimination of active breeding foci of *Aedes aegypti* and application of fenthion (Baytex) in potential non-potable breeding places. For adult mosquito control, the Kolkata Port Health Organization is undertaking pyrethrum space spray at fortnightly interval. No anti-mosquito measures are undertaken in Budge Budge port.

In the present study, the larval and adult collections were made simultaneously in each locality. Container index (per cent positivity of wet containers) has been used to determine the density of *Aedes* breeding. Samples containing larvae other than *Aedes* species were analysed to detect the co-breeding habits of commonly available species in the area.

In order to ascertain the prevalence of adult *Aedes* species (landing rates), a survey was carried out by spending 15 minutes in each dwelling/outdoor situation found positive for *Aedes* breeding and species-wise per manhour density for *Aedes* mosquitoes was recorded. Tables 1 and 2 contain data on container indices in the airport and port areas respectively.

From Table 1 it can be seen that no *Aedes* larval infestations were found at the international airport, whereas in the domestic terminal, the container index for *Aedes aegypti* ranged from 3.45 in slums to 5.26 in office complexes, including staff quarters. For *Aedes albopictus* a high index of 28.58 was recorded in the workshop area and 8.62 in other areas. The containter index for *Aedes vittatus*, a feral species, ranged from 3.45 to 3.51 in peripheral areas.

Table 1. *Container indices of Aedes aegypti, Aedes albopictus and Aedes vittatus at Kolkata Airport*

Area zone	No. of containers	*Aedes aegypti* index		*Aedes albopictus* index		*Aedes vittatus* index	
		No. found positive	%	No. found positive	%	No. found positive	%
A	30	0	0.0	0	0.0	0	0.0
B	21	1	4.76	6	28.58	0	0.0
C	57	3	5.26	5	8.77	2	3.51
D	58	2	3.45	5	8.62	2	3.45
Total	166	6	3.61	16	9.63	4	2.40

A = International Airport and surrounding area including hangers and cargo

B = Domestic Airport Terminal including workshop, Administrative Building, cargo shed, junk yards, etc.

C = Office Complexes including Staff Quarters and Barrack buildings

D = Urban/slum areas, State Govt. office, etc. around the Airport

From Table 2 it can be seen that *Aedes aegypti* infestation was restricted to Kolkata port only. The highest index (23.08) was recorded from crowded Babu Ghat surrounding slum areas, while in the dock area the indices ranged from 5.55 to 6.32. *Aedes albopictus* in the port area was confined to dock areas with index ranging from 11.11 to 14.74, whereas *Aedes vittatus* showed low prevalence (2.78). In the Budge Budge port only *Aedes albopictus* with an index of 4.35 was recorded.

Table 2. *Container indices* of Aedes aegypti, Aedes albopictus and Aedes vittatus at Kolkata Port and Budge Budge Port*

Area Zone	No. of containers searched	*Aedes Aegypti* index		*Aedes albopictus* index		*Aedes vittatus* index	
		No. found positive	%	No. found positive	%	No. found positive	%
Kolkata Port							
K. P. Dock	95	06	6.32	14	14.74	0	0.0
N. S. Dock	36	02	5.55	04	11.11	01	2.78
Babu Ghat shed	39	09	23.08	0	0.0	0	0.0
Total	170	17	10.00	18	10.59	01	0.59
Budge Budge Port							
Port area	23	0	0.0	01	4.35	0	0.0

*Included pooled data of surrounding slum area

The landing rates of all the three *Aedine* species are given in Table 3.

From Table 3, it is apparent that the adult *Aedes* survey carried out the in domestic airport terminal and surrounding slum areas revealed the landing rates for *Aedes aegypti*, *Aedes albopictus* and *Aedes vittatus* to be 3.0, 6.0 and 0.0 and 3.0, 9.0 and 3.0 respectively. No adult mosquitoes were detected in the standing aircraft. The adult *Aedes* survey carried out in Kolkata port areas revealed the landing rates for *Aedes aegypti* as high as 9 and that of *Aedes albopictus* varied from 6 to 12 per manhour. In the Budge Budge port area, only *Aedes albopictus* with a landing rate of 6.0 per manhour was recorded.

Table 3. *Landing rates of Aedes aegypti, Aedes albopictus and Aedes vittatus at the Ports and Airport of Kolkata and Budge Budge Port*

Area/Airport/ Ports	Landing rates/Per Manhour Density		
	Aedes aegypti	*Aedes albopictus*	*Aedes vittatus*
Kolkata Airport			
Domestic airport (junk yard)	03.0	06.0	0.0
Slum area (around the airport)	03.0	09.0	03.0
Kolkata Port			
K. P. Dock Yard	0.0	12.0	0.0
Administrative building backyard (K. P. Dock)	0.0	08.0	0.0
Transport company (Slum area, K. P. Dock)	09.0	06.0	0.0
Budge Budge Port			
Port area	0.0	06.0	0.0

The results highlight the need for further strengthening of vector control measures in both the port and airport areas.

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All that Cycles may not be Climate-driven*

Hay, S. et al. (2000) Etiology of interepidemic periods of mosquito-borne disease

Proc. Natl. Acad. Sci. U.S.A. 97, 9335-9339

The past five years has seen numerous articles linking mosquito-borne disease epidemics with meteorological perturbations, most notable El Ni o. In addition to the interest of scientists and the public in the effects of weather changes, this work has received attention owing to the possibilities of developing climate-driven models that could predict these disasters. It is anticipated that such early warning systems would prepare governments and other health providers for disease outbreaks.

There is no doubt that mosquito populations are sensitive to changes in temperature and rainfall, what is questioned by a recent article by Hay *et al.* Is whether the response to these environmental variables is sufficient to cause the market increases in disease seen during epidemic periods. Exploring the driving forces behind epidemics of dengue and malaria, they concluded that intrinsic population dynamics are more likely to initiate debate on approaches used for exploring associations between temporal data and the role of climate-driven models as predictive tools.

Hay *et al.* investigated the periodicity in both epidemiological and meteorological data using the time-series technique of spectral density analysis. The epidemiological data referred to hospitalized cases of dengue haemorrhagic fever in Bangkok and malaria in the tea estates of highland Kericho in Kenya over a period of 33 years (1966-1998). The meteorological data comprised coincident monthly temperature and rainfall estimates for both locations, and an additional climate variable, the multi-variate El Ni o Southern Oscillation Index (MENSOI).

The spectral density analysis showed that cases of both dengue and malaria peaked every three years, and the MENSOI (reflecting El Ni o) peaked every three years. By contrast, there was no significant variation in the temperature and rainfall in Kericho and Bangkok beyond an annual cycle. These results are suggested to refute the hypothesis that epidemics of dengue and malaria are determined by climate. Instead, it is proposed that the oscillations in these diseases are natural intrinsic properties of the host-parasite population dynamics.

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Although it is unclear whether these observations are the exception rather than the rule, the implication is that early warning systems for these disease outbreaks might need to look more closely at the population dynamics of these infectious diseases than to the weather.

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Chiang Mai Declaration on Dengue/Dengue Haemorrhagic Fever (Strengthening efforts to control dengue in the new millenium)

International Conference on Dengue/Dengue Haemorrhagic Fever
20-24 November 2000, Chiang Mai, Thailand
Weekly Epidemiological Record 2001, 76(4):29-30

Considering that

Dengue fever (DF) is the most important mosquito-borne viral disease of humans. There has been a dramatic increase in geographic spread, numbers of cases and severity of disease in the past 30 years. Currently 2.5 billion of the world's population, primarily in tropical developing countries, is at risk. Annually, there are estimated to be tens of millions of cases of the disease. Hundreds of thousands of these are of the more severe dengue haemorrhagic fever (DHF) which is a leading cause of childhood hospitalization and death in many countries. The economic impact of DF/DHF is comparable with that of other major infectious diseases such as malaria, tuberculosis and hepatitis.

And that

Tools to reduce dengue morbidity and mortality are currently available for appropriate case management and mosquito control. Dengue is an environmental issue, its prevention and control requires collaboration with many sectors. Several countries have demonstrated that, with strong political commitment, the wide and wise use of these tools can result in successful control.

In recognition

Of the magnitude of this global public health problem, and at the initiative of His Majesty King Bhumibol Adulyadej of Thailand, an international conference with over 700 public health specialists from 41 countries was held in Chiang Mai, Thailand, from 20 to 24 November 2000.

The delegates of the First International Conference on Dengue/DHF in the new millenium recommend that all countries at risk for dengue transmission develop and implement sustainable prevention and control programmes, and

Resolve:

- To strongly endorse the WHO global strategy for prevention and control of DF/DHF;

- To advocate increased political commitment and resources for improved and sustained prevention and control efforts;
- To promote active intersectoral partnerships involving international, regional, national and local agencies, NGOs, foundations, private sector and community organizations;
- To build and strengthen capacity of health systems for DF/DHF treatment, surveillance, prevention and control, and
- To pursue, encourage and support the development, application and evaluation of new and improved tools and strategies for DF/DHF prevention and control.

Chiang Mai, Thailand
24 November 2000

Dengue (DEN) Vaccine Development

Abstracted from 2000 Report of the Steering Committee on Dengue-Japanese encephalitis vaccines

Chiang Mai, Thailand, 18-19 November 2000

Live attenuated DEN vaccines

Immune response following vaccination with tetravalent live attenuated DEN vaccine (Aventis-Pasteur, France)

In Phase 1 clinical trials of monovalent DEN1-4 vaccines developed at Mahidol University, all individuals successfully seroconverted. Monovalent vaccines were able to elicit both CD4+ and CD8+ T-cells, with the response best for DEN1 and lowest for DEN4 viruses. A phase 1 clinical trial in Thailand using a tetravalent formulation has been done. From over 6000 volunteers, 35 individuals were documented to be flavivirus naive, and were used in this study. A two-dose vaccine regimen was used. Twenty-eight days after the first inoculation, seroconversion to each of the four serotypes was observed in a plaque-reduction neutralization test ($PRNT_{50}$) (DEN1=74.2%; DEN2=54.2%; DEN3=100%; DEN4=28.5), however only 22.8% of individuals seroconverted to all four serotypes. At 180 days after the first inoculation antibody levels dropped, so that only 3% of individuals were still positive for all 4 serotypes. Twenty-eight days after a second inoculation, the percents seroconversion to each of the serotypes increased (DEN1=82.3%; DEN2=73.5%; DEN3=100%; DEN4=64.7%) with 64.7% of individuals seroconverting to all four serotypes. Anti-viral activity remained more or less stable for a year. A concern currently being evaluated is whether or not the 35 flavivirus naive volunteers were so because of an atypical immune response. Various reformulations of the tetravalent vaccine are being evaluated in an attempt to level the immune response to each serotype. Individuals pre-immune to flaviviruses responded well to a single dose of vaccine.

Tetravalent live-attenuated DEN vaccine (Walter Reed Army Institute of Research, WRAIR, USA)

The WRAIR live attenuated, serial dog kidney passaged, DEN vaccines have now been tested in humans. All four monovalent vaccines elicited seroconversions (DEN1=100%; DEN2=92%; DEN3=46%; DEN4=58%), however GMT neutralization titers ranged from 668 (DEN1) to 15 (DEN3). Reactogenicity of the four monovalent vaccines was less than that seen with YF 17D

vaccine. Tetravalent formulations have now been prepared and analyzed by pre-clinical testing in rhesus monkeys and Phase 1 and 2 clinical trials in humans. Vaccination-challenge studies in rhesus monkeys, using a tetravalent formulation, demonstrated that most animals seroconverted (DEN1= 100%, DEN2=100%; DEN3=90%; DEN4=70%) after two doses of vaccine. After virus challenge, viremia was measurable in 4 animals (DEN1=1/5; DEN2=0/5; DEN3=1/5; DEN4=2/5). In pilot human studies (n=10) three doses were required to achieve a 50% rate of seroconversion to all for DEN serotypes. Because of this, dose ranging studies for each serotype was performed. Seroconversion rates for dose-optimized tetravalent vaccine approached that of the monovalent formulations (DEN1=94%; DEN2=76%; DEN3=70%; DEN4=47%, n=37). Subsequent efficacy studies will next be done in children (11-14 months).

Chimeric DEN vaccines

Evaluation of DEN 4 virus and its chimeric viruses of type 1, 2, and 3 antigenicity as candidate vaccines (National Institutes of Health, USA)

Three DEN4 mutants that contain deletions in 5' non-coding (NC) region, *i.e.*, 5'd (82-87), 5'd (73-77), and 5'd (76-81), were evaluated in monkeys to determine their infectivity, immunogenicity, and protective efficacy against wild-type (wt) DEN4 virus challenge. The cumulative data from two experiments indicate that there was a reduction of viremia response to each of the deletion mutants, compared to that observed with wt DEN4 virus.

However, this interpretation should be considered with some caution, because the low level of viremia produced by the wt DEN4 virus. It is clear that these deletion mutants did not exhibit an enhanced viremia. Convincing evidence for attenuation of these 5'NC deletion mutants was provided by the analysis of the antibody response. Importantly, each of the monkeys inoculated with the deletion mutants was completely protected against subsequent challenge with the wt DEN4 virus at a dose of $l \times 10^5$ plaque forming units (PFU). These results allowed the identification of additional mutants containing deletions in the 5' NC region of the DEN4 virus genome that could be considered as candidate vaccines for further evaluation in humans. Phase I clinical trials of mutant 3'd (172-143) as an attenuated live DEN4 experimental live vaccine were conducted in three separate experiments on a total of 20 healthy adult volunteers. Following a single inoculation of $l \times 10^5$ PFU, all volunteers remained afebrile and exhibited very few symptoms or clinical signs; except for a transient rash. Three vaccinees showed an elevation of liver enzyme ALT above 45 IU/l and all occurred at day 12-14 after inoculation. With the exception of one vaccinee showing transient rash, the other two individuals were asymptomatic.

Analysis of viremia by direct culture of serum in Vero cell monolayers showed that viremia was detected in 14 of the 20 vaccinees, but the peak virus titer in most cases was low. PCR analysis of the DEN4 genome in the serum samples confirmed the

low level viremia. Importantly, seroanalysis of samples from the first ten vaccinees completed thus far showed that each vaccinee developed DEN4 neutralizing antibodies (GMT=618) at four to six weeks after immunization. These results indicate that this live DEN4 experimental vaccine is safe, attenuated and immunogenic in humans. Thus, this attenuated DEN4 vaccine candidate containing the 30 nt deletion in DEN4 3'NC region can now be used as the genetic background for construction of chimeric dengue viruses of serotypes 1, 2, or 3. In this manner, a novel tetravalent dengue virus vaccine could be developed.

Chimeric live attenuated vaccines utilizing yellow fever 17D as a vector for envelope genes of flaviviruses (ChimeriVax™) (Acambis, USA)

The ChimeriVax™ system used to develop a candidate live attenuated JE vaccine has now been applied to DEN viruses. This approach replaces the E gene of the 17D yellow fever vaccine with the analogous gene of the vaccine-targeted flavivirus. Chimeric YF/DEN viruses have been constructed for all four serotypes of DEN, utilizing the donor genes from DEN1, PUO-359; DEN2, POU-218; DEN3, PaH881; and DEN4, 1228 strains. All YF/DEN chimeras grow to high titer in cell culture (6.3 to 7.2 $\log_{10}$ PFU/0.5ml), and like ChimeriVax™/JE show lower neurovirulence following intracerebral inoculation of mice than the parent YF 17D vaccine. All four DEN/YF chimeras produce short-lived, low level viremia in rhesus monkeys following sub-cutaneous inoculation, and elicit a dose dependent virus neutralizing antibody response. Adjusted dose tetravalent vaccine elicits comparable virus neutralising antibody to all four stereotypes. The DEN2 ChimeriVax™ will protect monkeys from virus challenge. There does not appear to be interference between YF and DEN/YF ChimeriVax™ in the respective immune responses, nor are the ChimeriVax™ vaccines able to replicate in mosquitoes.

Standardization of DEN virus neutralization testing - Phase I evaluation (WRAIR, USA)

A standardized DEN virus PRNT assay has been developed. Two changes from the original protocol have been made: the elimination of guinea pig complement from the assay and replacement of human serum albumin with heat-inactivated fetal bovine serum. In addition, the test is now run in triplicate rather than duplicate. Eleven labs were selected for Phase 1 testing of this protocol. Two labs refused to participate because reagents were contaminated with mycoplasma. Nine labs received protocols, reagents and a serum test panel in August 2000. Results from 4 labs have been received. The preliminary results indicate: 1) positive control serum has good neutralizing antibody against all 4 serotypes, 2) negative control serum has no detectable neutralizing antibody, 3) alternative samples with better neutralizing activity will be needed to comprise the testing panel.

The presentations led to discussion on further activity under the auspices of this project. It was recommended to continue search on Vero cell lines free of mycoplasma and cell line from the European Collection of Cells should be checked.

Dengue/Dengue Haemorrhagic Fever Prevention and Control Programme in Indonesia

Report of an External Review, Jakarta, Indonesia, 5-19 June 2000

SEA-HaemFever-73/SEA-VBC-79

Indonesia recorded the first outbreak of Dengue Haemorrhagic Fever in 1968 in Surbaya and Jakarta. Since then DHF has not only achieved greater intensity but has spread over 16 provinces by 1998. Case fatality rate although brought down from 41% in 1968 to 2% in 1998, through better case management, is still high when compared to Thailand, its neighbouring country, where it has been reduced to 0.5%. The Indonesian DHF control programme has tried different strategies for control of vectors, with varying degrees of success. At the instance of the National Government, WHO arranged an External Review through a group of experts to provide technical advice to improve both the elements of the programme, i.e. case management and vector control. The terms of reference for the external review were:

(1) To review the situation of dengue haemorrhagic fever (DHF) and determine the factors associated with its epidemic transmission and geographic spread in the country;

(2) To review the overall dengue prevention and control programme including policies, strategies, infrastructure and programme delivery at provincial, district, municipality and community levels;

(3) To review the management, administrative and logistical aspects of the programme as well as to identify problems and constraints encountered in implementing dengue prevention and control through general health services, and

(4) To recommend improvements in policies and strategies including resource mobilization, establishment of networks and partnerships to control dengue in the country.

Methodology

The team visited groups, organizations, institutions and agencies responsible for or involved in dengue fever (DF)/DHF prevention and control in Indonesia, critically reviewed approaches, methods, procedures, epidemiologic and economic data and outcomes, and had intensive discussions with numerous individuals on the approaches that could have the greatest

chance of success in Indonesia. While the Indonesian programme has a long history of evolution and change and has focused primarily on larval mosquito control and clinical case management, it has not been effective in preventing major epidemics of DF/DHF. It was concluded that the programme should be formalized as the National Dengue Prevention and Control Programme (NDPCP), with the incorporation of additional essential components. The programme should be founded on a truly community-based, integrated approach to DF/DHF prevention and control with strengthening of existing partnerships that have been developed with NGOs and other organizations and establishment of new ones. The programme should focus first on the urban centres of the county.

Major Recommendations

The major recommendations are as follows:

- An intersectoral National Dengue Task Force should be appointed to oversee and facilitate the implementation of NDPCP. Members of this Task Force should include representatives from most of the agencies/institutions/organizations.
- The NDPCP should be expanded to include the following five basic components: (a) surveillance; (b) emergency response; (c) education of the medical community; (d) community-based, integrated mosquito control, and (e) research.
- The government process of decentralization must be undertaken in such a way as to facilitate the effective implementation of the NDPCP at the local level.
- The NDPCP should be funded at a level that reflects the importance of DHF as one of the eight leading causes of hospitalization and death among children in Indonesia, with a substantial portion of the funds being used to implement the "3M" programme* and to support capacity-building of other components of NDPCP.
- The NDPCP should be piloted in three provinces (Central Java, North Sulawasi, and South Sumatra) where the existing infrastructure and partnerships have good potential to support successful programmes. These demonstration cities should be used as models to train programme staff from other areas.
- The surveillance system for DF/DHF should be expanded to include both passive and active components. The active surveillance system must be laboratory-based and focused on providing an early warning predictive capability for epidemic transmission.
- The ministry of Health (MoH) should develop a central public health laboratory to support surveillance for DF/DHF and other

* Source reduction strategy based on (i) covering water containers (Menutup), (ii) cleaning water containers (Menguras) and (iii) burying discarded containers (Mengubur).

infectious diseases. It should function as the national reference laboratory, providing reference services, standardized protocols and reagents, and quality control to provincial and other laboratories.

- The training programme on clinical diagnosis and case management of DHF should be intensified and expanded to include physicians, nurses and paramedics at all levels from hospitals to health centres in all affected areas of the country.
- For each dengue-endemic locality, a profile of the local *Aedes aegypti* larval ecology should be established and container-specific behavioural messages and control measures developed, that build upon existing household practices and are appropriate to the local socio-cultural settings.
- An analysis of the human resources requirements in vector control and behavioural science should be undertaken at all administrative levels, taking into consideration the implications of the forthcoming decentralization process. Working groups should be established in both areas to develop inventories of existing human resources in universities and other institutions that have expertise in entomology and behavioural sciences, and a plan should be developed to train persons in these areas.
- Formative research on behaviour change and the use of behavioural scientists in developing health education programmes and strategies for social mobilization should be strongly encouraged at the local level.
- It is highly recommended that a formal school curriculum for environmental health that includes vector-borne disease control be developed by the MoH and the Ministry of Education and be incorporated into school programmes nationwide.
- In order to ensure community ownership, broad intersectoral collaboration and support from the private sector, the support and partnership of NGOs and civic organizations, such as Rotary International, should be encouraged and facilitated.
- The MoH should develop and implement a new extramural public health research programme to collect data that will facilitate the implementation and ultimate success of NDPCP.
- Emergency plans, responsive to both seasonal and epidemiological indicators, should be developed to deal with an epidemic of DF/DHF at the local level and include case management, media response and mosquito control components. The mosquito control plan should emphasize larval control using an integrated, community-based approach.

International Conference on Mosquito Control
Controlling Aedes aegypti (Vector of Dengue and Yellow Fever) and general mosquito control

Fort-de-France, Martinique
28 February – 3 March 2000

Weekly Epidemiological Record 2000, 75(21):173-175

For decades, the countries affected by dengue fever have been routinely controlling *Aedes aegypti.* This control has been done mainly by applying insecticides to larval habitats, destroying unwanted containers and educating the population. During epidemics, this has been complemented by insecticide space spraying against adults.

In recent years, no-one has been able to clearly demonstrate that the use of these specific measures prevents or limits dengue epidemics efficiently and on a long-term basis, despite the fact that considerable sums have been invested in the control of dengue vectors.

Larval control is hampered by the multiplicity and inaccessibility of breeding sites, constantly replenished by man. In most cases, space spraying only has a transient and limited impact on adult mosquitoes and on transmission of the virus.

Finally, health education has not had the desired effect. Trying to activate the community only has a limited response and is more effective when there are epidemics, when it is usually too late to have any real impact on transmission.

This situation, even if there are some exceptions, reflects the reality of the problem confronting the affected countries, whatever their level of economic development.

Everyone recognizes that effective, lasting control of *Aedes aegypti* should be based on a multi-sectoral approach. Such control means *inter alia*, the effective participation of the community. However, this participation cannot be obtained if, at the same time, the community continues, despite its efforts, to be bitten by other species of nuisance mosquito and in particular by the urban mosquito (*Culex quinquefasciatus*), which is omnipresent and abundant in most places. *Aedes aegypti* is rarely perceived to be a nuisance and its disappearance often passes unnoticed.

Therefore, only control aimed both at vector mosquitoes and at nuisance mosquitoes is likely to receive the substantial

and sustained support of the community, whether we are talking about private individuals or their political representatives.

Consequently,

- Given the progress of dengue epidemics in most countries
- Given the continuing occurrence of yellow fever in some countries, in particular in South America
- Given that efforts to control vector species, notably *Aedes aegypti*, has not given the expected results.

The International Conference on Mosquito Control organized in Fort-de-France from 28 February to 3 March 2000 recommends:

(1) That anti-*Aedes* control measures be integrated into a general mosquito control programme with cooperation at regional, national and international levels. This approach best suits the expectations and needs of the population for an improvement in their quality of life. It also corresponds to the expectations of political decision-makers concerned about ensuring sustainable development for the populations they represent.

(2) That the training of staff involved in mosquito control be stressed. Given the limited range of active insecticides, the increase in resistance, the importance of preserving zones where mosquitoes have been controlled or will be controlled, all mosquito control requires competent and motivated staff, capable of designing, implementing and evaluating the integrated control that best responds to the circumstances.

(3) That community participation, often indispensable, is real and based on systematic feedback, the results of which are automatically taken into account in the drawing-up of education campaigns and actions in the field.

Meeting of WHO Scientific Working Group on Dengue Recommendations of SWG (TDR)

3-5 April 2000, Geneva, Switzerland

TDR/DEN/SWG/00.1

In recent decades, dengue has grown dramatically as a health, environmental and economic problem. However, the resources needed to cope – material, human and research – have not kept pace. In the coming 10 years, the environmental and social determinants of dengue transmission risks will continue to expand; another billion people will be added to the world population, the process of urbanization will intensify and changes in global climate and local weather patterns are expected. In order to deal with the increasing threat of dengue, the Scientific Working Group (SWG) recommended that a multi-pronged approach be adopted which takes into account these changing social and environmental conditions.

Recommendations

Specifically, the SWG recommended that efforts be focussed on reducing the mortality and morbidity caused by dengue haemorrhagic fever (DHF). This can be done by:

- **Improving case management** using well-established clinical interventions.
- **Developing a whole new class of early prognostic/diagnostic tests**, particularly through research on the pathogenesis of vascular permeability and altered haemostasis.
- **Preventing viral transmission**. Currently, the only way to prevent dengue transmission is by controlling the mosquito vector, *Aedes aegypti.* This will require the development and evaluation of new tools to reduce mosquito populations, including source reduction. Major efforts will be required to increase evidence-based vector control programmes and to support state-of-the-art research on human behaviour and behaviour change in relation to mosquito breeding.
- **Focussing dengue vaccine research** on early evaluation in children and accelerating marketing by setting standards for vaccine efficacy and safety.
- **Research capacity strengthening.** In both industrialized and disease endemic countries, severe shortages in research capacity and capability

have been noted, reflected in a lack of vector control entomologists, field capable research scientists, clinical epidemiologists who can perform studies on improving DHF/dengue shock syndrome (DSS) case management, basic flavivirologists and cellular and humoral immunologists. In fact, dengue biology requires a balance between field capable scientists and molecular and genetic researchers. TDR should make a critical appraisal of resource needs in dengue, then design and follow a strategic plan to address them.

- **Fund raising.** TDR needs to help raise dengue to a new level of recognition as part of a major effort to attract new funds to support basic and applied research and to implement new methods for dengue control, whether vaccines, vector control, or ideally, both.

Revision of the International Health Regulations

Progress report, February 2001

Weekly Epidemiological Record 2000, 76(8): 61-63

There is a continuous evolution in the public health risk posed by infectious diseases related to their causative agents, to their easier transmission in changing physical and social environments, and to their development of resistance to existing antimicrobial agents. In 1995, the World Health Assembly adopted resolution WHA 48.7 on the revision and updating of the International Health Regulations. The Health Assembly was fully aware that the strengthening of epidemiological and laboratory surveillance and of disease control activities at national level (i.e. where the diseases occur) is the main defense against the international spread of communicable diseases.

The main challenges encountered during the revision of the International Health Regulations include: ensuring that only public health risks (caused by an infectious agent) that are of urgent international importance are reported under the Regulations; avoiding stigmatization and unnecessary negative impact on international travel and trade of invalid reporting from sources other than countries, which can have serious economic consequences for Member States; and making sure that the system is sensitive enough to pick up new or re-emerging public health risks. This approach goes beyond notification based solely on specific diseases, though a list of diseases may be provided as a supplementary guide.

The development and field testing of syndromic reporting to replace disease-specific reporting was the first step in the revision process. Five syndromes were initially identified to cover the diseases of potential urgent public health importance, and included diseases that occur naturally well as those that might be caused intentionally. A pilot study in 22 countries in all WHO regions (completed in 1999) field-tested the approach. As a result of an interim review, it was concluded however that syndromic reporting, although valuable within a national system, was not appropriate for use in the context of a regulatory framework, mainly because of difficulties in reporting syndromes in the field test, and because syndromes could not be linked to preset rules for control of spread. It was also recommended that, because trade was often adversely affected when certain public health risks occur, links with the World Trade Organization (WTO) should be investigated. Several meetings to begin this process have already been conducted between WHO and WTO Committee on Sanitary and Phytosanitary Measures.

Since 1996 WHO has sought to strengthen its global alert and response capacity by setting up a mechanism actively to collect information on reported public health risks, to verify it confidentially with Member States, and then to ensure that appropriate containment measures are taken. This mechanism is WHO's global alert and response network. This network, which complements and strengthens existing networks, aims to ensure that the best expertise is harnessed wherever and whenever it is needed, as cost effectively as possible. To maintain global public health security, it provides coordinated mechanisms for epidemic alert and response. A steering committee ensures long-term preparedness for outbreaks, so that acute responses may lead to longer-term technical assistance. International efforts to contain epidemic outbreaks are under permanent evaluation.

A great deal of information on public health risks, originating from formal laboratory and epidemiology networks and from electronic discussion groups and diverse media, has been collected through WHO's global alert and response network. Since 1997 when the mechanism became fully operational in WHO, 745 reports have been investigated in direct collaboration with the countries concerned, and the network is being continually extended to reduce currently existing gaps in coverage, mainly in developing countries where epidemiological and laboratory capacity is being reinforced. In addition to information on public health risks (whether arising naturally or through intentional acts), this network could also provide information on non-communicable diseases and environmental, chemical or nuclear risks. WHO is currently investigating the feasibility of this further application. Work is also being done of developing a decision tree which, once field-tested, could be useful to countries in determining whether a public health risk is of urgent international importance and, if so, in helping decide which public health measures should be applied.

Hence, proposals now being made within the framework of the revision of the International Health Regulations include the use of WHO's global alert and response network as an additional source of information on public health risks of urgent international importance together with reports from countries, and of the decision tree. It is proposed, however, to make only confidential use of the information derived from the network until it has been verified and analyzed by WHO, working with the countries concerned and with WHO collaborating centres. Such collaboration is essential in a world where information is widely available. For example, in two recent instances, unverified public health information published on electronic sites resulted in severe financial losses for the countries concerned. Collaboration between WHO and these countries after the reports appeared resulted in the misleading information being corrected.

Based on experience gained from the operation of WHO's global outbreak alert and response network, it is therefore proposed that the revision of the International Health Regulations should cover:

(1) maintenance of a reliable system to prevent the extension of public health risks through the application of updated and broader routine public health measures for transport of persons and goods; and

(2) reporting of potential public health risks (by both countries and the WHO network), evaluating the information in collaboration with the Member State concerned to establish whether it is of urgent international importance and, if this is the case, ensuring that appropriate international public health measures are recommended by WHO.

The following main next steps are envisaged:

(1) seeking support from the World Health Assembly for continuing work on the revision of the International Health Regulations, including discussions with the WTO Committee on Sanitary and Phytosanitary Measures, the development of a decision tree for determining whether a public health risk is of urgent international importance and field-testing this decision tree in countries (2001);

(2) preparation of a draft revised text of the International Health Regulations (by end 2002);

(3) holding meetings of regional working groups to evaluate the applicability of the new text to Member States (by end 2003); and

(4) submission of the revised text to the World Health Assembly (no later than May 2004).

Instructions for contributors

The *Dengue Bulletin* welcomes all original research papers which have a direct or indirect bearing on dengue fever/dengue haemorrhagic fever prevention and control, including case management. Papers should not contain any political statement or reference. In addition to full papers, the *Bulletin* publishes short notes, review articles and book reviews.

Manuscripts should be typewritten in English in triple space on one side of white A4 size paper, with a margin of at least 4 cm. on either side of the text and should not exceed 15 pages. The title should be as short as *possible.* The name of the author(s) should appear after the title, followed by his or her official position, name of institution and complete address.

References to published works should be listed on a separate page at the end of the paper. References to periodicals should include the following elements: name and initials of author(s); title of paper or book in its original language; complete name of the journal, publishing house, or institution concerned; volume and issue number, relevant pages and date of publication, and place of publication (city and country). References should appear in the text in the same numerical order (Arabic numbers in parenthesis) as at the end of the article. For example:

- Nimmannitaya S. Clinical spectrum and management of dengue haemorrhagic fever. The Proceedings of the International Conference on Dengue Haemorrhagic Fever, Kuala Lumpur, September 1-3, 1983:16-26.
- World Health Organization. Viral haemorrhagic fevers. Report of a WHO Expert Committee, Technical Report Series 721, 1985.
- Jamaluddin M, Jalees S, Sharma RS and Verghese T. Dengue/DHF outbreak in Shahjahanpur, Uttar Pradesh, *India.* Dengue Newsletter, 1993, 18:2.

Figures and tables (Arabic numerals), with appropriate captions and titles, should be included on separate pages, numbered consecutively, and attached at the end of the text with instructions as to where they belong.

Articles should include an abstract of not more than 300 words conveying the content of the paper and its main conclusions; an introduction explaining clearly why the work described was carried out and what it is expected to contribute to scientific and technical knowledge; and conclusions and recommendations, if pertinent.

Articles submitted for publication should be accompanied by a statement that they have *not* already been published, and, if accepted for publication in the Bulletin, will not be submitted for publication elsewhere without the agreement of WHO, and that the right of republication in any form is reserved by the WHO Regional Offices for South-East Asia (SEARO) and the Western Pacific (WPRO).

One hard copy, original and clear figures/tables and a computer diskette indicating the name of the software, of the manuscript should be submitted to:

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