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Original Article

Initial Investigation of Indigenous Dengue Fever Situation and Efficacy of Prevention Measures in Kaohsiung and Pingtung Areas in Taiwan, 2009

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Abstract

833 indigenous dengue fever cases were recorded from January 1, 2009, to January 2, 2010, in Kaohsiung and Pingtung areas. 630 cases, 127 cases and 76 cases were noted in Kaohsiung City, Kaohsiung County and Pingtung County, respectively. The epidemic peaked in the 46th week and the median age for all positive cases was 48 years. The average duration from the onset of clinical signs to reporting date was 4.4 days and the average frequency of visiting clinics was 2.3 times. Dengue fever pathogen was isolated from 373 cases and was mainly type 3 virus. This type of virus was mostly found in Kaohsiung City and Kaohsiung County. Dengue fever virus type 2 was mainly distributed in Pingtung areas. Up to 90% of household were intvestigated within 2 weeks since the first Dengue fever positive case was recorded in Gangming borough and Gangchien borough of Siaogang District in Kaohsiung City. The number of water-filled containers and dengue fever pathogen positive containers was over 150 and over 13, respectively, per 100 household. Dengue fever case number was significantly decreased 4 weeks after the first dengue fever case occurrence. Similar investigation was also conducted in Tsaoyai borough of Cianjhen District in Kaohsiung City, but only 9% of the households were intvestigated within 2 weeks since the first Dengue fever positive case was recorded. The number of water-filled containers and dengue fever pathogen positive containers was 7.7 and 0.5, respectively, per 100 household. The disease situation in this borough persisted for over 10

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weeks and was extended to surrounding boroughs. The dengue fever situation in Kaohsiung and Pingtung areas in 2009 constituted the third most severe epidemic in the recent 10 years till 2009. Although dengue fever endemic events of different magnitudes were recorded in these areas every year, medical diagnosis and reporting have not significantly improved. Furthermore, different boroughs had different capacities in dengue fever prevention. Thus, further improvement of dengue fever prevention ability of local people, disease prevention personnel and medical staffs was recommended to decrease the impact of Dengue fever on the health of the residents.

Keyword: Dengue fever, Kaohsiung and Pingtung areas, pathogen source elimination

Introduction

Dengue fever has been a transmissible disease that has received wide attention around the world. About 2.5 billion people live in dengue fever risk areas. Dengue fever epidemics have been recorded in over 100 countries in tropical or sub-tropical zones; occasionally, dengue hemorrhagic fever (DHF) may occur [1]. According to WHO, about 500 million people are affected by dengue fever each year, among which 400 thousand cases are DHF that is the main cause of death in children in dengue fever epidemic areas [2].

At present, no effective medicine for this disease has been developed and vaccination is still in progress. Thus, prevention methods for this disease include chemical insecticide and pathogen source elimination. The most effective measure is to eliminate the vector mosquitoes [3]. Many countries, including Singapore [4-5], Republic of Trinidad-Tobago [6] and Brazil [7], have focused on pathogen source elimination. However, disease prevention measures are different in countries and the efficacy is also different. In recent years, disease prevention strategies against dengue fever in Taiwan have focused on pathogen source elimination, supplemented by chemical insecticide application. The area south to the Tropic of Cancer in Taiwan is a tropical climate area and is also the main distribution area of *Aedes aegypti* [8]. Local dengue fever epidemic events were occasionally recorded in this area during summer season. 5336 cases (242 DHF and 21 fetal cases), 965 cases (19 DHF and 4 fetal cases), 2000 cases (12 DHF and 0 fetal case), and 488 cases (5 DHF and 4 fetal cases) were noted in dengue fever epidemics that occurred in 2002, 2006, 2007 and 2008, respectively. The 2002, 2006 and 2008 epidemics took place in Kaohsiung areas, while the 2007 occurred in Tainan areas [8-11].

Based on the “Guidelines for Dengue Fever Prevention” and control edited by Taiwan CDC [12], 3 levels of disease situation, A (2 cases concentrating in one area), B (3-5 cases concentrating in one area), and C (6 cases or more concentrating in one area), were defined according to the case concentration situation. Public health authorities of county/city government should proceed with disease prevention or pathogen source elimination measures immediately in a 100-meter circumference around the household of the patient in degree A and B situations. The range and frequency of pathogen source elimination may also be adjusted by governmental authorities according to the disease situation. Staffs should search for pathogen sources in indoor and outdoor areas, attics, basements and gutters, as well as catching adult vector mosquitoes by net. In level C situation, authorities may delimit areas for blanket elimination for pathogen sources. In order to estimate the speed and capacity of pathogen source elimination in each borough, the impact of these measures on disease prevention was evaluated. Although all dengue fever prevention measures were carried out properly and thoroughly in 2009, the overall disease situation was still intense until the end of 2009. However, different developments of disease situation were recorded in different borough.

In this report, we analyzed information about the dengue fever epidemic in Kaohsiung and Pingtung areas, descriptions of DHF cases, and information on the time lapse between seeking medical assistance and reporting among patients. This research also provides an initial conclusion about the effect of different disease prevention and pathogen source elimination capacities in different boroughs on the magnitude of local outbreaks (Siaogang District and Cienjhen District).

Materials and Methods

A. Research targets of indigenous dengue fever epidemic: the target cases of this investigation were reported and confirmed dengue fever cases in Kaohsiung and Pingtung areas in the database of the Communicable Disease Reporting System, Taiwan CDC. The time frame of this research was from January 1, 2009 (1st week), to January 2, 2010 (53th week). Information about patients and disease investigation was downloaded from the disease situation database and disease investigation system, Taiwan CDC.

1. Definition of reported case:

a. **Dengue fever:** hyperthermia (≧38℃) associated with 2 or more of the following clinical signs: headache, pain on caudal orbital area, muscle/joint pain, cutaneous rash, hemorrhagic manifestation and leucopenia.

b. **Dengue hemorrhagic fever:** 4 criteria must be met:

i. Hyperthermia

ii. Hemorrhagic manifestation: 1 or more of the following clinical signs

(1) Tourniquet test positive

(2) Petechiation, bruising, suggillation
(3) Bleeding on mucosa, gastrointestinal membrane, IV catheter spot or other areas
(4) Hematochezia, hematemesis

iii. Thrombocytopenia (lower than 100,000)

iv. Plasma leakage: caused by increased capillary permeability, 1 or more of the following criteria must be met:
(1) PCV increased over 20%
(2) PCV decreased of 20% after fluid therapy
(3) Pleural effusion, ascites or low ALB (≦3gm/dl)

2. Laboratory examination:

a. Blood serum sample (acute phase serum) was collected within 7 days after clinical signs occurred and was examined by the following methods:

i. Real-Time reversetranscription-polymerase chain reaction (Real-Time RT-PCR): to examine dengue fever virus RNA in early stages and to analyze the type of virus

ii. Virus isolation

iii. Non-structural protein 1(NS1) antigen screening test (BioRad DENV NS1 Ag strip rapid test kit)

iv. Serum antibody test: IgM/IgG capture enzyme-linked-immunosorbent assay (ELISA) using dengue fever virus and Japanese encephalitis virus envelop

b. Gene sequence analysis was conducted in the dengue fever-positive samples:

i. DENV-3 gene analysis: sequencing whole structural gene (C-pM/M-E; 2319 nt)

ii. DENV-2 gene analysis: sequencing Partial NS5 gene(153 nt) and whole structural gene (2325nt)

3. Criteria for laboratory confirmation of positive cases: positive cases were determined when any of the following criteria was met:

a. Dengue fever virus was isolated from serum sample

b. Dengue fever viral RNA was isolated from serum sample

c. NS1 antigen positive

d. IgG or IgM of dengue fever was positive and IgM for Japanese encephalitis virus was negative

B. Analysis of reporting of dengue fever positive cases and medical treatment records:

In order to understand the effectiveness of medical facilities in reporting dengue fever cases, we analysis the average time frame from the onset of clinical sign to reporting date, and medical treatment records of dengue fever-positive cases from January 1, 2009 (1^{st} week) to January 2, 2010 (53^{th} week).

C. Evaluation of the effectiveness of pathogen source elimination:

1. In order to avoid the confounding influence of climate, we selected 2 districts in Kaohsiung City (Siaokang District and Cienjhen District) only as our target areas. Prospective evaluation was also conducted based on pathogen source information (including number of households investigated and number of fluid-filled containers and pathogen-positive containers in indoor and outdoor areas) provided by public

health authorities in each administrative area and borough.

2. In order to evaluate the speed with which pathogen source elimination was carried out within 2 weeks, main evaluation items included investigated households/total households within 2 weeks of the reporting of the first dengue fever case (index A-1), number of fluid-filled containers*100/total households (index A-2, unit: number of containers per 100 households), and pathogen-positive containers *100/total households (index A-3, unit: number of containers per 100 households).
3. The indicator for the effectiveness in controlling disease situation in each district was based on the number of newly-found dengue fever cases between 28 days after the first reported case and January 2, 2010.

Results

A. Description of indigenous dengue fever situation and case analysis

1. Disease severity: 1,359 patients were reported and 833 cases were confirmed as dengue fever cases from January 1, 2009 to January 2, 2010 (53th week). Only 1 dengue fever-confirmed case was recorded in the 12th week (3/21/2009) and the other 832 cases were found after the 31st week (beginning of summer season). The first dengue fever case since the beginning of summer season was recorded in Kaohsiung City. The disease onset date was July 27, and this case was confirmed on August 4.
2. Case distribution: 630 dengue fever-confirmed cases were distributed in Kaohsiung City (260 cases in Cianjhen District, 149 cases in Siaogang District, 79 cases in Lingya District, 48 cases in Sanmin District, and 41 cases in Cijin District), 127 cases were found in Kaohsiung County (107 cases in Fongshan City), and 76 cases were recorded in Pingtung County (64 cases in Pingtung City). Furthermore, 29 dengue fever cases were detected sporadically in Kaohsiung City and County from January 3, 2010 (1st week) to February 12, 2010 (6th week).
3. Development of disease situation: the development of dengue fever situation in Kaohsiung and Pingtung areas could be divided into 2 stages:

a. First stage: 61 cases were found from the 31st week to the 35th week (July to August), mainly in Siaogang District (48 cases). There were 6 cases, 5 cases and 1 case were recorded in Cianjhen District (Kaohsiung City), Fongshan City (Kaohsiung County) and Cijin District (Kaohsiung City), respectively.

b. Second stage: 771 cases were recorded from the 36th week to the 53rd week (September to December), mainly in Cianjhen District and Fongshan City. The first case in Fongshan City was found in the 38th week (9/17/2009). Sporadic cases were then recorded in Gushan District and Nanzih District in the beginning of October, followed by Lingya District, Zuoying District and Sanmin District. Dengue fever epidemic in Pingtung areas started in October, and the severity of disease situation had dramatically increased since the 43rd week. The peak of disease situation was in November (354 cases) while 98 cases were recorded in the

46th week. By then, dengue fever cases were found in all 11 districts in Kaohsiung City, with most of the cases taking place in Fongshan City of Kaohsiung County. The overall disease severity gradually decreased after the 50th week (Figure 1).

4. Demographic characteristics:

a. Dengue fever positive cases: In 833 patients, 383 were male and 450 were female. Age distribution was from 2 years old to 91 years old (average 45.7 years old, median 48 years old, and mode 58 years old). Average age of male patients and female patients were 44.5 years old and 46.8 years old, respectively. No statistical difference was found between male and female patients. The incidence of this disease per 100,000 people was highest in the age group of 65 years old or older (34.52 persons), followed by the 19-64 years old group (24.89 persons), 5-19 years old group (12.75 persons) and 0-4 years old group (3.70 persons). These findings indicated that the incidence of this disease increased with age. As for incidence by gender, the incidence per 100,000 people was 25.0 among female patients and 20.7 among male patients (Figure 2).

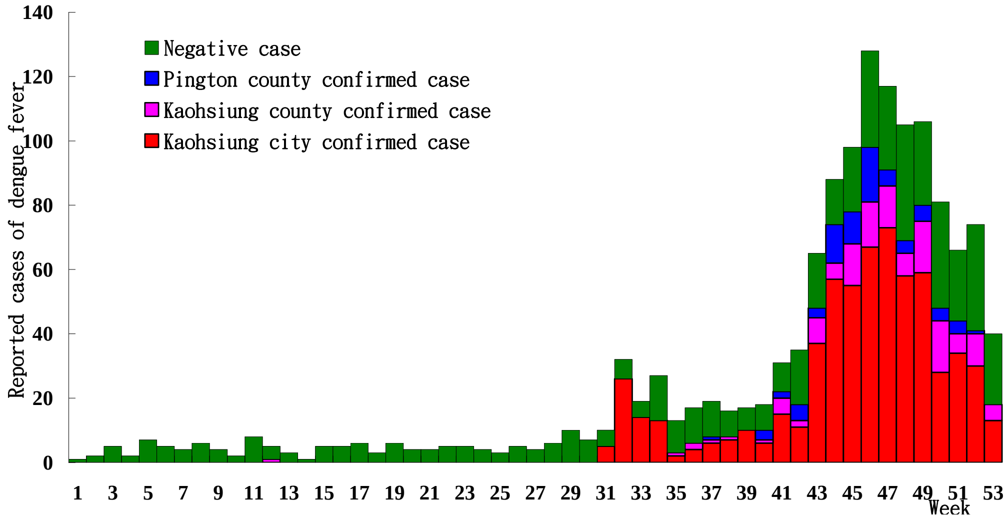

Figure 1. Epidemic curve of local dengue fever events in Kaohsiung and Pingtung areas in 2009.

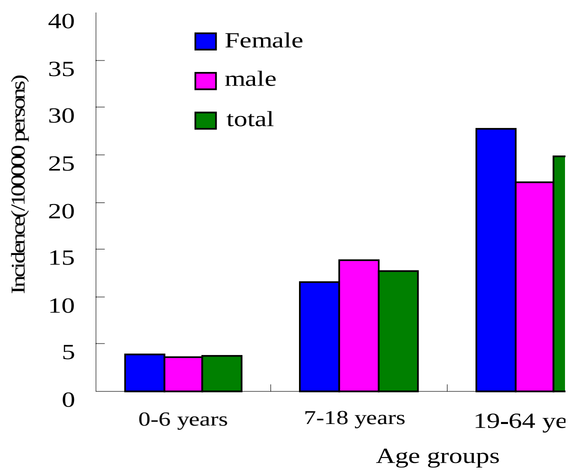

Figure 2. The incidence of local dengue fever epidemic in different agegroups and gender in Kaohsiung and Pingtung areas in 2009.

b. DHF case: In all 833 cases, 11 cases were confirmed as DHF cases (1.3%). The first DHF case was revealed in the 33rd week and the other 10 DHF cases were recorded in the peak time frame (43rd to 49th week). 6 patients were male and 5 were female. The age of these patients ranged from 16 to 81 years old (average 62 years old). This disease caused 4 deaths (3 male and 1 female) and resulted in a mortality rate of 36.4%, 2 of which were found in Cijin District, 1 was in Siaogang District and 1 was in Pingtung City. The age of these patients was 52, 67, 70 and 81 years old, respectively.

5. Laboratory examination for viral typing and gene sequence analysis:

a. Among the 833 cases, 373 were able to proceed to the viral typing examination. 341 cases (91%) were identified as type 3 dengue fever virus, in which 280 cases were found in Kaohsiung City, 48 in Kaohsiung County and 5 in Pingtung County. 32 cases (9%) were diagnosed as type 2 dengue fever virus (1 in Kaohsiung City, 1 in Kaohsiung County and 30 in Pingtung County). Virus typing examination was not carried out in 460 cases.

b. Based on viral gene sequence analysis, there were 2 strains of virus detected. 1 strain was diagnosed as type 2 dengue fever virus and bore high resemblance (>97%) to the strain isolated in Vietnam. The other strain was recognized as type 3 dengue fever virus and had high similarity (>97%) to the one found in the Philippines.

B. Analysis of reporting dates of dengue fever positive cases and medical treatment records

1. Time frame between onset of clinical signs and reporting date:

a. During the 31st week to the 53rd week, 706 dengue fever-confirmed cases were reported by hospitals and local clinics. The average time frame was 4.43 days between clinical sign onset and reporting date, with the average being 4.36 days, 4.91 days and 4.19 days in Kaohsiung City, Kaohsiung County and Pingtung County, respectively (Table 1). The average time frame in Pingtung areas was shorter than that in Kaohsiung City and County, but no statistical difference was noted. However, this average time frame increased to 6.91 days when public health stations expanded sample collection and examination (127 cases), and statistical difference was revealed. In summary, there were 272 cases (33%) with a time frame of 6 days or longer more, which indicated that viremia stage had passed in many cases when reported.

Table 1. Average time frame of clinical sign onset and reporting date of local dengue fever cases in Kaohsiung and Pingtung areas

City/County	Cases	Week no.			
		31-35 week	36-48 week	49-53 week	31-53 week
Kaohsiung City	520	4.85	4.34	4.26	4.36
Kaohsiung County	118	--	5.16	4.56	4.91
Pingtung County	68	--	4.41	3.36	4.19
Total	706	4.85	4.47	4.27	4.43

b. Furthermore, the average time frame was 4.85 days in the 31st-35th week, while it was 4.47 days and 4.27 days in the 36th-48th week and 49th-53rd week, respectively. These findings showed a shortening of average time frame with time increase. However, no statistical significance was detected.

2. Medical treatment record:

a. The average time of visiting clinic/hospital for 706 cases was 2.25 after the 31st week (beginning of summer season). The average 2.54, 2.20 and 2.23 for the 31st-35th week (137 cases), 36th-48th week (395 cases) and 49th-53rd week (227 cases), respectively. These findings revealed that the average time for visiting clinic/hospital did not increase with time.

b. The average time (1.37) for visiting clinic/hospital in Pingtung areas was shorter than that in Kaohsiung County (2.73) and Kaohsiung City (2.26), and this result was statistically significant (Table 2).

C. Initial evaluation of cleaning efficacy in 7 boroughs in Siaogang District and 7 boroughs in Cianjhen District

In this study, we analyzed disease information collected from 7 boroughs in Siaogang District (including Gangming, Gangcian, Gangkou, Ganghou, Gangxing, Gangnan and Gangjheng borough, with 8,376 household and 23,424 people) and 7 boroughs in Cianjhen District (including Caoya, Pingdeng, Mingxiao, Mingyi, Mingjheng, Mingli and Mingdao borough, with 11,477 household and 29,342 people).

1. 59 dengue fever-positive cases were recorded in 7 boroughs in Siaogang District. The first 2 cases were reported at August 3rd and 6th from Gangcian and Gangming borough. The date of clinical sign onset was July 30th. Within 2 weeks (from July 30th to August 13th), 40 dengue fever-positive cases were revealed mainly from Gangming (9 cases) and Gancian (27 cases) borough, which indicated that dengue fever endemic may had been persisted for a certain time. Thus, local government initiated disease prevention procedures and thorough pathogen source cleaning work immediately. 2486 household (>90%) were investigated within 2 weeks (Aug 4th to Aug 18th). 4400 fluid-filled containers with 366 dengue fever pathogen-positive containers were recorded. The range of average number of fluid-filled container and pathogen-positive container per 100 household was 150-250 and 13.5-19, respectively. Furthermore, similar disease prevention procedures and pathogen cleaning works were also

Table 2. Average time for visiting clinic/hospital in Kaohsiung and Pingtung areas

City/County	Cases	Average time for visiting clinic/hospital			
		31-35 week	36-48 week	49-53 week	31-53 week
Kaohsiung City	520	2.54	2.24	2.24	2.26
Kaohsiung County	118	--	2.78	2.66	2.73
Pingtung County	68	--	1.19	2.07	1.37
Total	706	2.54	2.20	2.33	2.25

conducted in Gangnan and Gangxing borough. Disease severity was significantly decreased within 4 weeks after the first dengue fever case reported and only sporadical cases found. No further community infection event was recorded. The index for pathogen cleaning work was shown in Table 3.

2. 42 dengue fever-confirmed cases were noted in 7 boroughs in Cianjhen District from 31st week to 53rd week, in which 0 case was revealed from Pingdeng and Mingyi borough. The first case was reported from Caoya borough, while the infection site was Ciaogang District. The date of clinical sign onset for the second case was August 22nd (34th week) and the infection site was Caoya borough. 226 household (less than 10%) were investigated within 2 weeks after the first case reported. 317 fluid-filled containers and 20 pathogen-positive containers were found. The average number for fluid-filled container and pathogen-positive container per 100 household was 7.7 and 0.5, respectively. Disease severity in Caoya borough was increasing after the 34th week and 12 dengue fever-positive cases were continually reported within 3 months. The first case in Mingjheng borough was noted in 35th week. Thorough pathogen cleaning works were not simultaneously conducted in other 5 borough, like that in Siaogang District. Thus, the disease situation was expanded to other borough. Dengue fever cases in Mingxiao and Mingdao borough were reported in 43rd week, and that in Mingli borough was reported in 45th week. There were dengue fever-positive cases detected within 3 weeks after the first case reported in Mingjheng, Mingxiao and Mingdao borough. The ratio of investigated household and total household was ranged from 0.09 to 0.47,

Table 3. Capacity and efficacy of pathogen cleaning work in 7 boroughs in Siaogang District and 7 borough in Cianjhen Distric

District	Borough	Household	No. of people	No. of case	First case Date of clinical sign		index for pathogen cleaning work within 14 days			No. of case after 28 days
					onset	Reporting date	A-1	A-2	A-3	
Siaogang	Gangming	1511	4238	10	7/30	8/4	0.91	150.89	13.96	1
	Gangcian	827	2432	31	7/30	8/6	1.34	256.35	18.74	1
	Ganghou	1037	3144	7	8/3	8/10	1.05	176.18	11.19	4
	Gangjheng	1195	3487	3	8/8	8/14	1.28	156.07	4.94	2
	Gangkou	1559	4340	4	8/13	8/17	1.27	162.41	3.40	2
	Gangnan	1107	2615	3	8/19	8/22	0.70	81.75	0.72	0
Cianjhen	Caoya	4100	11679	15	7/27	8/03	0.06	7.73	0.49	12
	Mingjheng	1950	4990	12	8/26	9/10	0.47	66.97	10.00	9
	Mingxiao	1096	3147	6	10/18	10/23	0.09	9.31	0.27	2
	Mingdao	596	1444	5	10/22	10/26	0.34	37.08	2.01	1
	Mingli	816	2005	4	10/26	11/3	0.12	12.75	0.37	1

Note： A-1 investigated households/total households
A-2 number of fluid-filled containers*100/total households
A-3 pathogen-positive containers *100/total households

and the fluid-filled container and pathogen-positive container per 100 household was 7.7-66.9 and 0.3-10, respectively. At the end of disease situation 4,253 household were investigated and 5,657 fluid-filled containers and 410 pathogen-positive containers were found in these 3 boroughs (Mingjheng, Mingxiao and Mingdao). In summary, the number of investigated household, detected fluid-filled container and pathogen-positive container was 7,219, 7,219 and 673, respectively, in Cianjhen District. The index for pathogen cleaning work was shown in Table 3.

Discussion

Dengue fever epidemics have been recorded in Kaohsiung and Pingtung areas every year in the recent 10 years. In 2009, 833 cases were detected, which was the third highest number after 2002 (5,203 cases) and 2006 (952 cases) since 2001 to 2009. These cases were mainly found after July, and this was similar to previous records [9-10]. Disease situation peaked in November and then gradually decreased. There were more adult and female patients. In 2009, type 3 dengue fever virus was distributed in Kaohsiung City and Kaohsiung County, while type 2 virus was mainly found in Pingtung areas. 11 DHF cases were noted during this epidemic. Most of which were 65 years old or older, and this disease caused 4 deaths. The average time frame between clinical sign onset and reporting date was 4.43 days, and the average time of visiting clinic/hospital was 2.25. Over 30% of the cases were reported 5 days after onset of clinical signs.

The wide range of clinical signs of dengue fever, from subclinical to severe DHF/Dengue Shock Syndrome, was the biggest challenge for dengue fever monitoring and diagnosis. Different clinical signs may be observed in patients of different age. In this report, the incidence of this disease increased with the age of patients. The age distribution of this disease in Taiwan was mainly in adults, which was similar to that in Singapore and Cuba. However, this result was different from that in countries with high risk of dengue fever epidemics, such as Vietnam, Thailand and the Philippines [13]. In these countries, most patients of DHF were under 15 years old and adult patients presented more obvious clinical signs. According to studies in Singapore, this contradiction may be explained by the fact that primary dengue infection in children was mostly subclinical. The ratio of DHF case decreased with aging of patients. The mortality of DHF cases in 2009 in Taiwan was 36.3%, which was higher than that in high risk of dengue fever epidemic areas (<3%). This result may be due to the fact that DHF patients in Taiwan were mainly elder people with chronic diseases. Thus, further effort should be put into clinical/medical treatment and care of DHF cases decreasing mortality.

Prompt response is most important for transmissible disease prevention. If disease cannot be properly diagnosed and disease prevention procedures are not punctually carried out, more efforts and material resources are needed to prevent further damage caused by the disease. The viremia stage of dengue fever is from 1 day prior to till 5 days after clinical sign onset. Prompt reporting of the case and proper prevention procedures are very helpful in decreasing the risk of disease expansion. We analyzed the medical records of reported dengue fever patients and found that the average time frame between clinical sign onset and reporting date, and the average time of visiting clinica/hospital was 4.43 days and 2.25, respectively. However, the average time frame increased to 6.9 days when expanded dengue fever screening tests were conducted. In one previous study in 2006, the average time frame and average time of visiting clinic/hospital which could decrease disease spread was 4.42 days and 1.89, respectively [10]. Compared with these studies, the reporting of dengue fever cases and delayed diagnosis by medical staffs have not improved, although the channel of reporting system has become more convenient, and governmental effort and public health education have been conducted more frequently. Local public health authorities reported that some clinics or local hospitals did not report dengue fever cases in order to avoid people complaining. Thus, in order to enhance the awareness and reporting motivation of medical staffs and to improve dengue fever prevention efficacy, enhancing health education for medical staffs and investigating concealment of disease situation for administrative sanction are extraordinarily important.

According to the results of viral gene analysis in 2009, this epidemic was caused by at least 2 types of dengue fever virus. Type 2 virus was imported from Vietnam and caused the epidemic in Pingtung areas, while type 3 virus was probably imported from the Philippines and resulted in the dengue fever epidemic in Kaohsiung areas. The isolated viral gene types from southern Taiwan were similar to those causing dengue fever epidemics in Southeastern Asia countries. One study, conducted by Dr. Shu et. al. from 2003 to 2007, analyzed dengue fever virus gene sequences collected from imported dengue fever patients with travel history within 2 weeks before disease onset. The results revealed that 17 countries with dengue fever epidemics were the major areas visited, mainly Southeastern Asian countries including Vietnam, the Philippines, Indonesia and Thailand [14]. This may have resulted from increased frequency of visiting these areas due to recreational travel, visiting relatives or business purposes. In order to increase the efficacy of disease prevention, body temperature of passengers coming back from dengue fever epidemic areas was monitored in the airport. Disease prevention staffs collect sample from passengers with fever or those who voluntarily inform of fever history for laboratory examination. Disease prevention procedures are carried out immediately after a dengue fever positive case is detected. Fever screening exam in the airport was initiated in 1998 and NS1 screening test has also been included since July 1, 2008. In recent years, imported dengue fever cases have gradually increased to over 40% of all cases of imported diseases [15]. This suggests that the screening procedure provides certain level of efficacy in disease prevention and avoiding local epidemics through early detection of imported patients.

However, fever screening examination cannot detect all infected patients due to subclinical or minor clinical symptoms. People with subclinical or minor symptoms may have decreased willingness or even be unwilling to visit the clinic/hospital. Thus, it is necessary to enhance related health education for public about self-protection measures when travelling in dengue fever epidemic areas and to encourage people to provide travel history to medical staffs voluntarily. It is also important that medical facilities inquire about travel history from patients to detect imported patients at an early date in order to avoid viral transmission and an epidemic.

Dengue fever is an environmental, community disease. The major vectors are *Aedes aegypti* and *A. albopictus*. People are exposed to the risk of dengue fever infection once the dengue fever virus has been brought into an environment where vectors exist. The more crowded a place is with people or buildings and the dirtier an environment, the easier it is for vectors to propagate. The disease prevention efficacy of emergency insecticide spray is still controversial. Vector mosquito group may restore 1-2 week after insecticide dosing while pathogen sources are not thoroughly eliminated. Furthermore, people may mistake that all vectors are eliminated after insecticide dosing and the neglect the importance of pathogen source cleaning. “Guidelines for dengue fever prevention” was re-edited in early 2010 by Taiwan CDC in accordance with relative disease prevention experience from local record and from other countries. Besides investigation for disease situation of reported case, major prevention measures also include pathogen source elimination associated with chemical insecticide dosing. Public health authorities should proceed vector and pathogen source elimination procedures at the resident areas, work areas or places where the patient stayed for 2 hours (or longer) during viremia stage. These procedures should be completed within 48 hours and the range for these measures should be at least 50-meter circumference centered by possible infection sites [16].

Pathogen source elimination is the radical method for dengue fever prevention, which is also recommended by WHO in blocking virus reproduction and transmission route, and in decreasing the risk of transmission [17]. Thus, possibility of dengue fever epidemic may persist when proper pathogen source are still existed in the environment. In our research we analyzed the information collected from this epidemic and revealed significant difference in efficacy of pathogen source elimination in 2 districts in Kaohsiung City. In the early stage of this epidemic, delayed diagnosis and report of the first dengue fever case in Gangming and Gangcian borough (Siaogang District) caused dramatically increased in dengue fever-positive cases within 2 weeks. However, disease situation was under control within 4 weeks through general pathogen source elimination measures, cleaning of vector-positive or fluid-filled containers, and thorough elimination of pathogen source in adjacent boroughs to decrease the vector mosquito density. On the other hand, the speed and efficacy of pathogen source elimination in 7 boroughs in Cianjhen District was much less than that in Siaogang District. Thus, the epidemic was prolonged for several weeks and was also extended to adjacent boroughs from Caoya borough. This result indicated that enhancing the speed and efficacy of pathogen source elimination may help in controlling disease situation in epidemic areas within few weeks. However, previous researches (including this report) did not indicate clearly about the relation between standard time frame and efficacy of pathogen source elimination. Further studies are needed for evaluation and application of “golden time frame” and “standard pathogen source elimination efficacy” for dengue fever prevention.

Regular monitoring of vector mosquitoes and pathogen source elimination are routine work for dengue fever prevention. Related disease prevention procedures should be initiated when vector density increases. Containers should not be piled up or discarded freely. Regularly used, fluid-filled containers should be cleaned at least once per week. People should be encouraged to clean public places regularly to decrease vector mosquito propagation and to eliminate pathogen sources. Public health authorities should provide correct information about dengue fever prevention to advance public understanding and to encourage people to maintain environmental cleanness. Dengue fever was an important cause of death among children in the 1960s in Singapore. A nation-wide vector mosquito control program was conducted in 1968-1973, and the disease situation was brought under control through this program. Besides continuing elimination of pathogen sources, public education, restructuring of the laws and public power enforcement are also important factors for dengue fever prevention [16]. Thus, it is important to cultivate the idea of dengue fever prevention deep into the public’s daily life through cooperation of central and local public health authorities as well as non-governmental resources.

Conclusion

The climate in Kaohsiung and Pingtung areas is warm and rainy, which is suitable for dengue fever vector propagation. Dengue fever epidemics have been recorded in every year in Taiwan, although there is no evidence that dengue fever has become endemic in Taiwan. Pathogen sources may increase if a clean environment is not carefully maintained. The interaction between Taiwan and Southeastern Asian countries is increasing and the risk of local dengue fever epidemic has also increased due to imported cases becoming the epidemic source when the patients are not detected timely. Thus, pathogen source elimination is the ultimate method for dengue fever prevention. People should be encouraged to maintain the cleanness of community environment and to eliminate all pathogen sources in order to avoid or decrease the transmission of dengue fever. When an epidemic occurs, the public, medical facilities and local disease prevention staffs are part of the disease prevention chain, and dengue fever epidemic transmission can be restrained through fast epidemic source detection as well as pathogen source and vector mosquito elimination.

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Preliminary Review of Risks for Community Dengue Fever Outbreaks - Using Kaohsiung City as an Example

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Abstract

In comparison with the distribution of types of Aedes mosquito in other counties/cities, Kaohsiung City has a higher ratio of *Aedes aegypti* than *Aedes albopictus*. Since the biological characteristics of *Aedes aegypti* and the high demographic density of Kaohsiung City, the outbreak of dengue fever has been in constant rotation or repeatedly occurring. To understand the risk factors of dengue fever outbreaks in the community, neighborhood magistrate visits were conducted in addition to ecological environment observations. The results showed several risk factors in the community, including old buildings, narrow alleyways, longer eaves that block sunlight, frequent interactions between residents, often under the eaves that may lead to a dengue outbreak. These factors are statistically significant if they hold a P value of 0.04 when paired with the factor of being located within 4 meters of the road. The demographic structure of the old communities has a higher age span with lower educational attainment and socioeconomic status; this limits access to personal hygiene education and increases the interactions between residents which result in the increasing of risk of a dengue fever outbreak. If a floral shop street is one of the characteristics of a community and residents are unable to distinguish potential breeding containers for mosquitoes, a dengue fever outbreak can easily occur. It is extremely important for community leaders to lead or take part in community activities; however, the work of an individual or a few residents can only aid in limited results of effective prevention of dengue fever. The fact that there is a shortage in help, the higher age of neighborhood magistrates, and the inability promote community awareness all are potential community health risks. Within the community, only those residents who identify with their community participate in maintaining their community's environment for the well-being of themselves and the community. The measures need to be taken in prevention of dengue fever is to conduct community education for the residents to elevate awareness in maintaining hygienic environments not only at their own homes, but also within the community.

Keywords: dengue fever, community

Introduction

Dengue fever is an environmental and community disease. The risk of a dengue fever outbreak occurs when infected mosquitoes appear and appropriate breeding sites exist [1]. Dengue fever is a mosquito-transmitted viral contagious disease, the pathogen being the dengue virus, a flavivirus of the Flaviviridae family with 4 different antigens. Currently, it is estimated that local dengue hemorrhagic fever epidemics have occurred in at least 100 dengue fever prevalent countries and approximately 40% of the world population (250 million people) live in risky tropical or sub-tropical regions. According to estimates, over 5 billion people have contracted dengue fever, among which 400 thousand people were infected with hemorrhagic dengue fever; thus averaging to approximately 50 million cases of dengue fever infection each year [2-3]. In the recent years, transportation has become ever more convenient and through business relations, visitations, and studying, the number of more confirmed cases brought into the country has increased. Gene sequence analysis data has shown that local dengue fever outbreaks in Taiwan are caused by imported viruses [4]. In the southern districts of Taiwan, especially in high densely populated communities in Kaohsiung City, where dengue fever outbreaks have often been in continuous rotation or reoccurring. Since *Aedes aegypti* are in greater numbers than *Aedes albopictus*, and the fact that *Aedes aegypti* has different biting and indoor habiting than those of *Aedes albopictus*; in addition to the existence of breeding containers in homes which lead to the breeding of mosquitoes often result in outbreaks of dengue fever within the community.

The cause of dengue fever outbreaks in the community is closely linked to the community environment, and thus is considered a community disease. “Community” as a sociological terminology, mainly refers to a fixed group of people living within a geographical area because of their common geographical, demographic characteristics, and living together in close relationship between social life and other factors, resulting in common interests, common problems, and common needs, and thus have a common sense, seek common development of collective action to achieve common goals. The maintenance of the community environment not only needs the participation of the entire community, but also the cooperation between the residents which is the prerequisite for the development of the community [5]. According to past experience, when hospitals reported suspected cases of dengue fever, outbreak investigation found the cases notified lived in communities where dengue fever rarely or never occurs of which city and county officials may carry out routine emergency control strategies; whereas if the reported cases lived in communities with a high occurrence of dengue fever outbreaks, a larger scale of preventive measures would be activated. The nature of residents, environmental, demographic mix, community volunteers, and other input mechanism may be different in communities with high outbreak numbers and those with little or no outbreaks; therefore the differences are something to be further studied and understood. Few researches have investigated the dengue fever outbreak factors from studying the community; thus this report attempts to target communities at risk of dengue fever by observing community patterns, living environment, humanities, etc., combined with interviews with the neighborhood magistrates in understanding their views and opinions on dengue fever and resident interaction. It is expected that the data found in this investigation can be used in the prevention and control of dengue fever outbreaks, and in hope "the community mobilization in prevention can be conducted before epidemics occur" to prevent one case of dengue from leading to an outbreak and ensure community health.

Study Method

A. Environmental observation

1. Investigating jurisdiction and dates: using the infectious disease storage BO system of Taiwan Centers for Disease Control (TCDC) as data origin, this study chose three districts in Kaohsiung City, of which 2 neighborhoods were selected (one with outbreak(s) and one with no outbreaks. The neighborhood with outbreak(s) (Case neighborhood) was chosen with dengue fever outbreaks that lasted at least 4 weeks or more during the years 2003 to 2010. The neighborhood that had no outbreaks (Control neighborhood) had infections but no outbreaks occurred. The investigation duration was December 1st to 10th of 2010.
2. Investigating the community and environment: the selected neighborhoods are as following: Wang-chung from the San-ming district, Jhen-chang from the Cianjhen district, and Pu-chao from the Ling-ya district for the Case neighborhoods, and Bao-hua from the San-ming district, Ping-deng from the Cianjhen district, and Ho-Shiu from the Ling-ya district for the Control neighborhoods. Confirmed cases from the Case neighborhoods were pin-pointed through GIS, and if the area exceeds 200 meters, it is viewed as within another community; a total of 16 communities were selected from the areas mentioned above. The community environmental data were collected and recorded, including those of the width of the alleyways, the amount of daylight, and types of housing. Approximately 50 to 60 situations of the living conditions were collected from each community along with the demographic data from each neighborhood for reference.

B. Community interviews

1. Study designers and participants: semi-structural questionnaires were conducted during the dates from December 19th to 23rd of 2010. Through purpose selection from the San-ming, Cianjhen, and Lin-ya districts of Kaohsiung City, five neighborhood magistrates were interviewed (each of which had held the position at least five times). The main overlook of the interviews include dengue fever awareness, normal community resident interaction, volunteer mobilization and status, community structure, related community training and activities, and the risks of community dengue fever outbreaks.

2. Study procedures: the data collection procedure: (1) tools prepared before interview include maps, semi-structural questionnaires; (2) interviewers must understand the purpose of the study, the method of study data collections, and procedures; (3) interviewers contact the interviewees beforehand to confirm interview locations and dates; (4) the collection of the data from the interview is conducted in the neighborhood magistrate's office. The interview lasts approximately one hour; (5) three to four interviewers are present and have many years of work-related experience in dengue fever prevention, among of which one person must participate in the complete procedure.
3. Data analysis: all the collected data are analyzed, categorized, and arranged. All collected data are strived to be neutral and uninfluenced by the interviewee's objective opinion.

Results and Discussion

This study covers the statistical analysis of data and observations and interviews by community acquired qualitative data. The results and discovery of this study will be described and discussed according to community property, community leaders, community participation, and community observation separately.

A. Community traits

The communities selected for this study are from three districts of different statuses which are described as following: San-ming District includes 7 main communities which are San-kuai, Da-gong, Wang-chi Nei, Baou-ju Gou, Lion's Head, Ben-guan, and Fu-ding and is the most populated district in Kaohsiung City, with approximately one-fourth of the total city population. San-ming district mainly houses residential areas with a north-south bound highway and the Chung-Shan Freeway going through. At the same time, there is also a transportation interchange station, making the district transportation convenient. Lin-ya district is made up of the following four tribes: Lin-ya Liao, Guo-tian chi, Wu-kuai, and Lin-de Guan and is the new part of Kaohsiung City housing government, schools, and business facilities. Most of the buildings here are of cultural, business skyscrapers and has become Kaohsiung City's most competitive and potential district due to the government offices located here, the increased development of trade centers, and future planning for recreational docks. The Cianjhen district includes seven tribes: Cianjhen, Chao-wei, Si-shi Jia, Li-chi Nei, Gang-chan Chi, Fo-gong, and Nei Lin-chi Liao and is made up of mainly industrial and fishing businesses. The Cianjhen Port is where most of Kaohsiung's fishing boats are docked. Apart from the fish-freezing industry, a tourist fish market is also in development. In addition, San-duo shopping center is where the most department stores are located in the city. Many city residents shop and watch movies here on the weekends, and it has become the most popular shopping center in Kaohsiung City [6]. Below are the descriptions of each of the communities selected community state, demographic structure, and community characteristics collected from community observations and interviews with neighborhood magistrates.

1. Community status

In the past, studies and theories related to communities, believe that the status of the community differs due to the living habits of the residents and community activities, such as agricultural communities and urban communities [5]. In observing Kaohsiung city communities, we have found some of the communities are of a singular manner, for example the Pu-chao and Ho-shuan communities of Lin-ya district where most of the buildings are a mix of cottages, houses, and tall buildings with much business activity. Some of the communities have both old and new sections, such as the Wan-chung community in San-ming district where the new and old sections each take up about half of the community. The old part consists of old and empty houses with a messier and dirtier environment; yet, the new section consists mainly of tall buildings or houses constructed in the last decade with a planned environment and is cleaner than the older section. The outbreak of single cases that occurred on Ding-shan St. in 2010 belongs to the older section of the Wan-chung community where the alleyways are narrow and where the Ding-shan Wan-chi Nei market is located, thus with puddles and items deposited, etc, all of which are high risk factors in the outbreak of dengue fever (see Picture 1).

Ping-den community of Cianjhen district is another area where new and old sections exist together and the two are significantly different. The old sector of the community is larger and is mostly made up of houses and single buildings on state-owned land, with many houses only sizing to about 10 ping (Ping is an area measure, equal to 3.3057 square meter or 36 square feet), with narrow alleyways, and only 10% as living quarters. The newer sector of the community is smaller, and was only developed in the recent years consisting of mostly mansion-styled single buildings with spacious streets, all of which are private property. In the past, several confirmed cases appeared in Ping-den

Picture 1. Within the old marketplace where alleyways are narrow with many puddles, accumulation of debris, and bad lighting, all of which are factors that may lead to an outbreak.

community, and after investigation were found to be in the older sector of the area. Comparing the community dengue fever outbreaks occurred in 2006 and 2009, the characteristics included being located in older communities, with older buildings and narrow alleyways. This is especially apparent in those cases living in older buildings with longer eaves, leading to the lack of sunlight, in addition with the frequent interaction among residents, often chatting, watching grandchildren, or working under the eaves; therefore making older communities a risk factor in resulting in a dengue fever outbreak. In the past years, the community status described above has often seen with outbreaks of dengue fever. Although this study analyzed the factor of sunlight in relation to the outbreak of dengue fever, the P value is 0.08 (t test) and has not reached statistic significance, this may also be due to the fact that there are not enough community samples (see Picture 2)

This study observed eight case communities and eight control communities (a total of 16 communities). In observing these communities, 50 to 60 household environments near case homes were documented, total 860 households observed. This data includes alleyways between households width measurement, the years of old houses or buildings, and so on. Statistics found that the alleyway width within 4 meters provided the P value of 0.04 (n = 16), which shown to be statistically significant (see Table).

Picture 2. Communities with older buildings, narrow alleyways, especially those with longer eaves hindering sunlight, in addition to frequent interactions between residents are causes of dengue fever outbreaks.

Table . Dengue fever outbreak occurs in Kaohsiung city and whether alleyway width within 4 meters

Variable	community outbreak		no community outbreak		Value	P value
	n	average %	**n**	average %		
Within 4 meters of alleyway width	8	0.47	8	0.02	2.5077	0.04 *

* t test: the test of difference between two average percentages, under unequal variance assumption.

In 2005, a mark-release-recapture study conducted by Russell et al in Queensland, Australia showed that *Aedes aegypti* can easily cross smaller and quieter roads, whereas fewer can cross freeways. The study also concluded busier roads may interfere in the spreading of mosquitoes. These observations show that the possible habitats of the *Aedes aegypti* has been destroyed and large obstructions may influence the migration of the insects [7]. Large and wide road may hinder the outbreak of mosquito diseases (see Picture 3)

In 2010, a study conducted by Ryan et al pointed out the connection between the changes in environment and population density, housing development state, and the influences on dengue fever outbreaks [8]. This result is similar to that of this study, where outbreak communities often include the possible risk factors of narrow alleyways, high population density, lack of sunlight, and old communities.

2. Demographic structure

In community studies, demographic structure is considered an important factor in a community, including the age, occupation, and education level of the residents [5]. When conducting interviews in the community, we found that most of the residents in old communities had middle to high age span, lower education level, and were mostly labor workers with unstable jobs or unemployed. In new communities, the residents mainly consist of young to middle aged, with higher education levels, and steady occupations. For example, when the neighborhood magistrate of Ping-den community of the Cianjhen district mentioned the demographic characteristics of the community, he indicated that the population residing in the older sector totals to approximately three fifths of the entire community, with only two fifths in the newer sector. Most of the resident age spans in the older sector were in the middle to high age span, whereas young to middle aged in the newer sector. In occupation distribution, many residents in the old sector held odd or unstable jobs, whereas most of the newer sector residents were office workers. Therefore, we can see that the demographic structures in the new and old sectors are significantly different. In observing the community state, we can discover that dengue

Picture 3. Wide and busy roads may stop spreading of dengue fever outbreaks.

fever occur more easily in old communities and apart from the types of buildings in old communities, we can also see the significance in the difference of demographic structures. For example, most of the residents in old communities have a higher age span, lower education level and socio-economic status which limit the accessibility to public health education and influence their living habits. In addition, the interaction between old community residents is more frequent which increases the chance of cross infection.

3. Community characteristics

If a community has a certain characteristic that differ it from other communities, and needs large quantities of water containers, this community may have a higher risk of dengue fever outbreak than others, such as Pu-chao community in Lin-ya district. In fact, when observing this community and interviewing the neighborhood magistrate, it was discovered that this community had a clean environment and was a single community manner with no new and old difference, consisting of mainly single building homes and tall buildings, among which tall buildings took up about 20% of the community's buildings. The neighborhood magistrate was very vigorous in conducting dengue prevention measures; however dengue fever outbreaks occurred in 2006 and 2009. After investigation, it was found that the cases live on Xingzhong 1st Rd. which is a famous floral shop street in Kaohsiung City. If residents are unaware to what kind of container may become breeding grounds for mosquitoes, an outbreak of dengue fever may occur (see Picture 4).

B. Community leaders

In community studies and theories believed that a community leader acts an extremely important role in leading the community activities and participation. A community leader can be the local magistrate, official, or even an older and respected member of the community whose actions and words can motivate the community's enthusiasm and willingness to participate.

Picture 4. If residents are unclear as to what kind of container may become breeding grounds for mosquitoes, an outbreak of dengue fever may occur.

The local magistrate would be the leader most residents recognized in the community. In considering the local magistrate's attitudes and actions towards prevention of dengue fever, we can speculate the possible energy shown in dealing with a dengue fever outbreak. The five local magistrates interviewed in this study are all male, between the ages of 44 to 69 and have held the occupation for 8 to 28 years. During the interviews, it was found that the preventive measures taken by the local magistrates consisted mostly passively cooperating with the local district office in maintaining the cleanliness of the community environment. Three of the local magistrates had established an environment cleansing volunteer team which helps in spreading related public health information and cleansing the environment. The other two local magistrates stated that most of the residents in the community are busy with work and are unable to participate in community public activities; therefore, the magistrates themselves have taken up the job of monitoring the community environment. If there were related public health fliers, magistrates and enthusiastic members of the community would help in distribution. For example, the local magistrate in the Pu-chao community in Lin-Ya district who had held the position for 8 years and had lived and grown up in the community for years, knowing that most residents were busy with work and could not care for the community; therefore, as the head of the community, he took the initiative for the benefit of the residents. He stated that after the outbreak of dengue fever occurred on the floral shop street, he himself checked the water containers on the floral shop street every year, asked for preventive drugs from the local health center to hand out to the shops, and even held a dengue fever prevention and public health seminar for the residents using his own money, in hopes that the community residents would have correct dengue fever and personal health habits and concepts. Although the local magistrate put so much effort on such work known to lower the risk of dengue within the community, the work of a single individual or few residents is still very limited in effective prevention of dengue fever. The fact that there is a shortage in help, the higher age of local magistrates, and the inability promote community awareness all are potential community health risks.

C. Community participation

Broadly speaking, community participation refers to the daily lives of people and groups involved in related public affairs through participation in the process put their views, behavior, and other resources in the community and have a certain degree of influence on the community. Community participation can also mean the participation of people in the community through activities and participation in local health administration, not only in maintaining and promoting their own health, but also becoming responsible for their own well-being and of the community's [9-13]. Among the five neighborhoods interviewed, three have established their own volunteer team, of which the environmental volunteer team in Bao-hua community in San-ming district had 50 to 60 volunteers. In cooperation with the local magistrates daily cleansing or active large-scale environment cleanup, this has become a control measure in preventing dengue fever outbreaks. Due to the fact that dengue fever is considered as a community disease, the actions taken in preventing dengue fever can also be seen as a community action. Community status, residential age span, occupation, income, and education level may all be important factors that influence community participation. After interviewing the local magistrate in Bao-hua community in San-ming district, he mentioned that many residents came from other places and the age span was relatively younger, with higher education levels, mostly governmental employees, and higher human quality. Although the participation in regular community activities is not high, specific activities have a higher participation rate, and the residents would actively communicate with the local magistrate when needed. The Pu-chao community magistrate of the Lin-ya district stated that activities held in the community still need more attracting factors in drawing the residents to participate. By examining the descriptions above, those residents with community sense would acknowledge their own community and therefore participate in the maintenance of the community environment for not only their own well-being, but also for the entire community. The prevention of dengue fever is conducted by educating community residents in elevating their community awareness, not only in maintaining a hygienic household environment, but also a clean community environment in order to prevent the dengue fever effectively.

Recommendations and Study Limitations

This study only interviewed five neighborhood magistrates, and was unable to understand more about the status of the communities from multiple viewpoints and evaluate possible problems that may occur. It is suggested that future interviews should be conducted with different subjects, including the neighborhood magistrates, officers, and residents in order to further understand the strengths and weaknesses of the community and evaluate the heath needs for the community; thus to propose a precise and appropriate preventive measure. In addition, when interviewing the neighborhood magistrates, the interviewers introduced themselves as TCDC agents, and when asked about how cooperative the residents are or the difficulties of conduction, the neighborhood magistrates all replied with “agreeable cooperation” and had no difficulties in conduction. It is reasonable to assume that the responses of the interviewees were reserved due to the identity of the interviewers and thus resulted in deviation. Also, during interviews, it was found that the residents had received wrong information when receiving medical care. This may be due to the lack of public health education which leads the public to think that doctor will receive bonuses when they report dengue cases, thus many doctors will report just for the bonus. This has lead to a negative reaction from the residents toward dengue fever preventive measures. Some of the neighborhood magistrates suggest that public health promotion can be held according to the needs of each community, allowing residents to receive correct dengue preventive information and concepts.

In view of the different community cultures, people, and environment, it is advised that more multi-viewed and deeper evaluation should be conducted to understand and propose precise and appropriate preventive measures for each community as needed; thus eradicating the risk of dengue fever outbreaks in the southern region of Taiwan and through interviews conducted in high and low risk communities, understand what environmental factors can be added to the already existing environmental factors in preventing outbreaks of dengue fever. If the case is a single case, as long as community prevention measures are carried out, this may effectively stop further incidents of the dengue fever outbreaks.

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Biosafety and Biosecurity

Introduction to Management of Infectious Biomaterials

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Internationally, infectious biomaterials can be divided into four risk groups (RG) based on the risk assessment of the pathogenicity, transmitting routes, hosts, availability of effective prophylaxes (as vaccine) or treatments (as antibiotics) for microorganisms. These are:

(1)Risk group 1 (RG1) refers to microorganisms which are irrelevant to diseases in healthy adults, denoting infectious but not pathogenic, such as *E. coli* with K-12 antigen and *Bacillus subtilis*.

(2)Risk group 2 (RG2) refers to microorganisms which rarely cause severe diseases and usually with measures of prevention and treatment, such as *Salmonella* and dengue virus.

(3)Risk group 3 (RG3) refers to microorganisms which can cause severe or lethal diseases and probably with measures of prevention and treatment, such as human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis*.

(4)Risk group 4 (RG4) refers to microorganisms which can cause severe or lethal diseases and usually without measures of prevention and treatment, such as Ebola virus and variola virus [1].

Current management of infectious biomaterials in Taiwan is based on "Regulations Governing the Infectious Biomaterials and Specimens Collection from Patients of Communicable Diseases" [2], mainly regulates the organizations that possess, store or use infectious biomaterials above RG2. However, it’s difficult to set unitary standards in view of these institutions with different sizes and attributes. Therefore, these organizations are required to set up an institutional biosafety committee (IBC) or designate personnel to be responsible. We expect management spirit of ” autonomous management, focal checking, and fulfilling notification”. Also, the organizations should strengthen the supervision and implementation on operations of possessing, storing, transferring and using infectious biomaterials above RG2.

Besides, several concepts need to be clarified. In principle, the operation of infectious biomaterials should be in the laboratory at the same biosafety level. The laboratory scientist should establish a habit of assessing risks and fully comprehend the factors of microbial pathogenicity, infectious dose, transmission routes, operating concentration or volume and experimental methods to raise or lower the required biosafety level of laboratory operation if necessary. Although taking over-protective measures won't interfere with the safety of staff, the measures may affect the experimental operation and personnel comfort, which probably induce laboratory incidents. For instance, the serum antibody test of HIV, a blood-borne RG3 pathogen, is needed to be laboratory.

Furthermore, laboratories in medical institutions test plenty clinical or outbreak specimens every day. Such samples do not meet the "infectious biomaterials" definition in Paragraph 4, Article 4 of “Communicable Diseases Control Act” (referring pathogens, their infectious derivatives, and substances that confirmedly contain these pathogens or derivatives). And if these samples are administered as infectious biomaterials in the possessing, storing, using and transferring, it will be notably operational besetment for laboratories practically. Therefore, only the clinical or outbreak specimens determined with pathogens by test need to be stipulated by law. As for testing tuberculosis, for example, suspected bacterial strains are regarded as outbreak specimens before sent to contract laboratories for identification, while the samples identified as *Mycobacterium tuberculosis* are required to follow the regulations in subsequent operation (such as storage or sending for drug susceptibility test).

No matter infectious biomaterials or outbreak samples, the packaging and shipping should be in accordance with the "Manual of collecting outbreak samples" announced by Taiwan CDC, "Hazardous material transporting labels" in national standard CNS 6864 Z5071, and refer to WHO's “Guidance on regulations for the Transport of Infectious Substances 2011-2012”, and “Dangerous Good Regulations” of International Air Transport Association (IATA). According to the law, Taiwan CDC is responsible for the infectious biomaterial affairs currently. Despite with no homothetic basis on non-infectious biomaterials, in the reason of serving the public, it provides the consent since the Custom demands competent authorities' consent whenever non-infectious biomaterials are exported or imported.

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Thinking on Constructing an Ideal Negative Pressure System in the Laboratory

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Negative pressure is a pressure within a closed system that is lower than the surrounding environment. The pressure difference makes the airflow moves from higher pressure to lower pressure, which causes the matters suspended in the air move in a certain direction. Negative pressure is applied in the medical field. For example, negative pressure laboratories that are utilized to manipulate high infection risk agents and negative pressure isolation rooms that are utilized to treat highly contagious patients. A negative pressure laboratory is a physical containment space that contains biological safety cabinets and other local exhaust ventilation (LEV) equipment. By adjusting the ventilation system, making supply air greater than exhaustion, a pressure gradient will be created so that the airflow will move from the clean areas to the contaminated ones [1].

Negative pressure is designed to confine the pathogens, which caused by experiments or its accidents, within the laboratory equipment. The discharged air from the laboratory will be filtered by the high efficiency particulate air filter (HEPA filter) before releasing to outdoor environment. To exhaust the airflow and decrease the pathogens, aerosols produced during the process and conducted in biological safety cabinets will be collected by the HEPA filter, while aerosols suspended in the laboratory will be collected by the HEPA filter of the LEV system. According to the concepts above, when contributing an ideal laboratory negative pressure system, the following items are required to be considered.

First, an ideal negative pressure laboratory system should include an anteroom and an operating room, where there is pressure gradient between each room. In general, the pressure in the laboratory should be lower than the adjacent common area. The pressure between the laboratory and the common area should be at least 12.5 Pa (1 Pa is equal to 9.80665 kilogram-force per square meter). The pressure between the anteroom and the operating room should be also 12.5 Pa, while total pressure between the common area and the laboratory should be 25 Pa [2-3]. If there is any pressure change such as insufficient negative pressure or positive pressure in the laboratory, the trouble should be identified and corrected immediately. At the same time, the pressure in the laboratory and every compartment should be re-checked.

Regarding to the physical structure of the negative pressure laboratory system, including the partition wall, the ceiling, and the floor, the followings should be considered: (1) the materials should be durable, water-proof, corrosion-proof, and compatible to other building materials; (2) the material should be able to sustain the setting negative pressure. For example, the materials can endure the pressure change while the open or close the doors or turn on or turn off indoor ventilation facilities such as biological safety cabinets; (3) use materials that will not be deformed or damaged by 1.25 times of the maximum negative pressure; (4) construct the partition wall in a way that can keep its air tightness; (5) the transfixions, holes, and gaps in the laboratory should be stuffed with material that are acid/alkali-resistant and impact-resistant [3]. This item was the worst part in the checklist because 47% (16/34) of *M. tuberculosis* laboratories was found deficiencies in this part when 2009 Taiwan Centers for Disease Control's speculation [4].

Although negative pressure can be adjusted by adjusting the exhaustion and supply of the ventilation system, staff should still consider the following points for designing it. (1) Facilities that deal with exhaustion and filtration should be close to the laboratory to reduce the total length of the pipes to reduce the impedance and energy consumption, and to decrease the infection source in the pipeline. (2) Switch on or off the biological safety cabinet should be taken into consideration when designing the amount of intake air so that the air change will not lead to abnormal situation such as unstable negative pressure or positive pressure. (3) The laboratory ventilation system and the biological safety cabinets should be interlocked controlled. (4) The ACH (air change per hour) in the laboratory should be at least 12 times per hour. However, excessive air change does not provide better efficiency to exhausting the infection source but only waste energy. (5) There should be a backup ventilating fan (*i.e.* totally two sets of fan for exhausting air, while one of which is for operating and the other one is for standing by). When the primary fan is malfunction, the backup one can keep maintaining the exhaustion and the negative pressure. (6) Areas that are close to the outlet or inlet to the ventilation system should be clear; otherwise, it will cause turbulent flow or short circulation of airflow.

In case that the negative pressure system would stop working due to the power outage, there should be uninterruptible power supply (UPS) system and backup power generator sets. The UPS system can provide instantaneous protection from input power interruptions, while stand-by generators can supply power for a longer time and keep the negative pressure before the utility power returns. Laboratory staff should keep an eye on their UPS and stand-by generators to ensure that those facilities can begin supplying power as soon as the unusual events occur. In addiction, pressure surveillance equipment should be set up near the door to the laboratory, and the staff should maintain and correct it regularly so that each room will be under control. Moreover, the staff should keep record of the pressure and observe the change everyday in event of unusual events.

There are multiple ways to construct a negative pressure laboratory. However, excessive negative pressure and ACH should not be over-emphasized; on the contrary, the more important concern is to make sure the stability of the laboratory negative pressure and the efficacy of excluding infectious sources. Under the principle of cost efficiency, energy saving, and carbon reduction, the laboratory can adopt air-conditioning with ACH reduction design to adapt to different usage frequencies. Thus, it will be an ideal negative pressure laboratory that meets the modern needs.

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