Assessment of Dengue Fever Emergency Control Task Carried Out in Kaohsiung City in 2006

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Abstract

The objective of this emergency control task was to rapidly eradicate local virus-borne female mosquitoes to interrupt the route of transmission for an indigenous dengue fever endemic. All measures taken during this task were centered on this purpose.

This study aimed to evaluate the efficacy of an emergency pesticide spray operation for dengue fever carried out in Cianjhen and Lingya Districts of Kaohsiung City in 2006 using knockdown rate, mortality rate, adult mosquito index, relative control efficiency, and dosage of pesticide used. The results showed that after applying the two commercially available pesticides, "Perdelta E.C." and "Deltamethrin 2.8% E.C.," (both using the same type of thermal fogger (PulsFog K10)) to Cianjhen and Lingya Districts respectively, the knockdown and mortality rate of *Aedes aegypti L.* in either district were above 90%. However, the disease control in a few households was not satisfacfied,

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probably due to inexperienced workers with unfamiliar spraying techniques leading to insufficient pesticides used. In the meantime, within one day of the spray operation, the adult pest index dropped rapidly to a zero reading, making an 100% relative control efficiency. However, if the breeding source was not eliminated after the first two days of spray operation, the mosquito density would rise again, subsequently making the relative control efficiency dropped back to around 65%. Furthermore, there was noticeably a significant difference from the actual flow or output rate of the thermal fogger ($p < 0.05$) to the original machine specification when the real amount of pesticide applied at each household site was measured during the spray. Also, the spray workers tended to make the pesticide solution in somewhat higher concentration than instructed. And to make things worse, some spray workers were not properly trained. These factors resulted in somewhat uneven spray quality across the board, with a common tendency for over spraying (from 1 up to 8 times of the intended amount). In conclusion, despite evidence showing drug resistance has occurred with the local *Aedes aegypti L.* mosquito population in Kaohsiung's Cianjhen and Lingya Districts, with correct decisions made by the city government, right timing of the pesticide spray operation, plus Taiwan CDC's on-site instruction to improve the workers' spray skills, the overall outcome of this operation was seen as successful and effective in preventing the dengue fever endemic from getting worse.

Keywords: Aedes *aegypti L.*, drug effect evaluation, control efficiency, adult mosquito index

Introduction

Dengue fever is a disease of the environment. As long as there are

suitable breeding sources, transmission is always possible. Over the past 30 years, cases of dengue fever have been increasing worldwide. According to a World Health Organization report, currently more than half (2.5 billion) of the global population live in a dengue fever hot zone. Each year, 100 million people are infected with dengue fever, including re-infection cases; among them, approximately 250,000-500,000 patients have dengue hemorrhagic fever. The geographical location, cultural factors and alternating transmission of 4 dengue virus serotypes cause the frequency and strength of outbreaks in Southeast Asia to increase year by year. Since Taiwan is located in the subtropical region, the weather is hot and humid. In addition, the island is densely populated and is extremely suited for the rapid multiplication of vectors. In the past 20-30 years, Taiwan has often had to deal with dengue fever outbreaks. For instance, an outbreak occurred in the Kaohsiung-Pingtung region in 1988. That particular outbreak involved 4,389 confirmed cases. In 2002, an even larger outbreak occurred in southern Taiwan with 5,388 confirmed cases, including 2,832 cases in Kaohsiung City. Another outbreak occurred in 2006 with 965 cases, including 757 in Kaohsiung City. Dengue hemorrhagic fever occurred in 19 cases, leading to 5 deaths. There is currently no vaccine or effective therapeutic drugs for dengue fever, and the only way to interrupt native transmission is to eliminate vectors. Control strategies can be categorized into 2 types: long-term and emergent [1]. For emergent control measures, when a dengue fever outbreak occurs, areas with reported cases or a high vector density are sprayed immediately so that native transmission is stopped. Although frequent use of insecticides will result in the emergence of drug resistance [2-4], once an outbreak occurs, the media, citizens, government officials, and experts all expect spraying activities

to rapidly control the outbreak so that citizens can return to living a normal and healthy life. However, every time an outbreak goes uncontrolledly, most of the blame is placed on the spray workers or the fact that mosquitoes have developed drug resistance.

In order to control the dengue fever outbreak in 2006, Kaohsiung City spent approximately NT$13 million on emergent spraying activities, including NT$7.5 million on worker salaries, NT$3.7 million on insecticides, NT$1.5 million on aerosols, and NT$400,000 on fuel costs. In order to justify this large expenditure, how efficacious were the control measures? Strategies to prevent drug resistance were separated into the early control phase (August) and middle control phase (October) for the Chiencheng and Linya districts, respectively. Factors such as the mosquito knockdown rate, mortality rate, adult index, and dosage were used to evaluate the efficacy of emergent spraying and the skills of spray workers in Kaohsiung City. This method can serve as a model for other local governments to use in improving the spraying quality, reducing citizens' complaints and maximizing the effectiveness of emergent control measures.

Materials and Methods

1. Mosquito Strains and Breeding
   (1) Susceptible strain: *Aedes aegypti* mosquitoes were collected in the Tainan region in 1987 and breeding was continued by the Centers for Disease Control (CDC) Vector Laboratory till the present (more than 500 generations).
   (2) Wild-type strain: *Aedes aegypti* larvae were collected in Kaohsiung City's Chiencheng and Linya districts from August to October, 2006.

They were kept in the health department's breeding room until they developed into adult mosquitoes.

(3) Mosquito breeding: Susceptible and wild-type larvae were raised in separate plastic tubs and fed on yeast and pork liver powder. After pupation, they were placed into another paper cup until the emergence of adult mosquitoes, after which they continued to breed in 10% sugar water. The mosquito breeding room was maintained at 25-28℃ with relative humidity of 70±5% and illuminated for 12 hours [5].

2. Susceptibility Testing

On the day of spraying (August 9th and October 11th, 2006), susceptible and wild-type female mosquitoes were aspirated separately into foldable cages (25*11*11cm) covered with netting (16 mesh). Each cage contained 20 mosquitoes (1-3 days old without feeding) [6] that were placed on cotton soaked in 10% sugar water. One hour before spraying, we went to the 5 households selected the day before (experimental group). Each set of cages (containing one cage each of susceptible and wild-type strains) was placed either in the interior (living room, kitchen, bedroom, and bathroom) or the exterior (fire alley, yard and deck) of every house. Each household received 5 sets of cages, with 2 sets placed outside and 3 sets placed inside. If the house covered more than one floor, then each floor had at least one set of cages. The comparison and control groups each consisted of one household located at least 10 meters away from the spraying area. In addition, a laser gauge (Trimble HD150) measured each selected house's length, width and height to calculate the spraying area. The results for the Chiencheng district's 5 households were 750, 350, 250, 1100, and 550 cubic meters; for the 5 households in the Linya district, they were 880, 850, 700, 800, and 800

cubic meters.

A timer was used to record the spraying time that started from when the worker entered the house and initiated the thermal fogger to when the worker left the house. The time for the 5 households in the Chiencheng district were 14' 30", 4' 52 ", 1' 5 ", 7' 26", and 6' 10", respectively. The households in the Linya district recorded 5' 9", 8' 35", 8' 27", 10' 32", and 9' 20", respectively. For the 5 households in the experimental group, control workers (professionally-licensed vector experts in Kaohsiung City) wore Level-D safety equipment and entered each house 30 minutes after spraying to collect all cages previously placed. The cages for the comparison and control groups were sprayed with similar volumes of glycol and water, respectively, using the same thermal fogger. The knockdown rate for the adult mosquitoes in each cage was immediately observed and recorded. Then, all of the female mosquitoes were aspirated onto cotton soaked in 10% sugar water to observe their breeding inside paper cups. After 24 hours, the number of deaths was observed and recorded. The laboratory was maintained at 25$\pm$2℃ with a relative humidity of 70$\pm$5% and illuminated for 12 hours [7].

The thermal fogger used was pulsFog K10 (Dr. Stahl+Sohn GmbH & Co. KG) with a nozzle opening of 0.8μm and flow rate of 196.7$\pm$5.8ml/min, as determined using the consumption method. According to the type of insecticide used, the actual dilution factor and each house's interior spraying area, we calculated the household's actual active ingredient dosage (spraying time * fogger flow rate * active ingredient concentration ÷ spraying area ÷ actual dilution factor), the recommended active ingredient dosage (1ml/m^3 * active ingredient concentration ÷ recommended dilution factor), and the

ratio (actual dosage/recommended dosage). This time, the Chiencheng district used the insecticide Perdelta E.C. (7% Permethrin and 0.5% Deltamethrin with recommended 25x dilution). The Linya district used Deltamethrin 2.8% E.C. with recommended 100x dilution.

3. Evaluation of Control Efficacy

From 1-2 days before spraying to 1-2 days afterwards, we made on-site visits and randomly selected 50 households (as required by dengue fever control guidelines) during the peak time of mosquito activity (7-9AM) to measure the adult index, with different households selected each day if possible. The worker waved a mosquito net in the shape of a figure 8 and proceeded from the top floor to the basement while disturbing items such as clothing, kitchen shelves, tables, chairs, curtains, and bed nets. All adult mosquitoes were collected in the interior and exterior of the house in order to identify their species and sex. The adult index (number of female adult mosquitoes/number of investigated households) and the relative control efficacy [(adult index before spraying – adult index after spraying) ÷ adult index before spraying * 100] were calculated to evaluate the results.

4. Statistical Analysis

(1) Adjusted mortality rate: the rate for each cage was calculated by the Abbott formula. The Abbott adjusted mortality rate = (experimental group's mortality rate – comparison group's mortality rate)/(100 – comparison group's mortality rate) [8].

(2) T-test: comparison of the differences in knockdown and mortality rates between wild-type and susceptible strains in each household.

(3) ANOVA: comparison of the knockdown and mortality rates for both mosquito strains among all the households.

Results

1. Chiencheng District

(1) Susceptibility testing

As seen in Table 1, the Chiencheng wild-type strain had a total average knockdown rate of 88.2% over all 5 households; the Tainan susceptible strain had an average of 98.2%; while both the comparison and control groups averaged 0%. When the T-test was used to compare knockdown rates between the 2 strains, we found (with the exception of the 1st household) a significant difference in each household ($p<0.05$), indicating that Perdelta had different effects on the 2 strains after 30 minutes. For the outside cages, the Chiencheng strain had an average knockdown rate of 77.8%; the Tainan strain had an average of 95.8%. After T-test analysis, the 2 strains showed a significant difference in the 5th household and in the total average for all 5 households ($p<0.05$). The other households did not show a significant difference. For the inside cages, the 2 strains were significantly different only for the 5th household. The ANOVA analysis showed a significance difference between the 5th household and the other 4 households when comparing interior rates for the Chiencheng strain ($p<0.05$). There were no significant differences when comparing the total average rate for all cages with the exterior rates of all the households.

When mosquitoes from all the cages were aspirated into paper cups, we found that the mortality rate was always higher than the knockdown rate after 24 hours of breeding, regardless of the strain. The average mortality rate for the Chiencheng strain for all the cages was 97.8%; the average rate for the Tainan strain was 99.4%. The Chiencheng strain in

the outside cages alone averaged a 94.6% mortality rate; the Tainan strain averaged 98.6%. The mortality rates for the Chiencheng and Tainan strains in the inside cages were both 100%. Meanwhile, all of the average mortality rates did not show a significant difference between the 2 strains according to the T-test ($p>0.05$).

We measured the interior space for 14 households and determined the actual spraying time. In addition, we found the ratio of each household's actual dosage and recommended dosage, as listed in Table 2. As the table shows, since the spraying area varied for each house, the spraying time also differed. When comparing the actual and recommended dosages, we found that every household had a ratio greater than 1 (from 1.22 to 8) with an average of 4.33.

(2) Evaluation of Results

As seen in Table 3, 2 days before spraying, 7 adult mosquitoes were captured, including 3 males and 1 female inside plus 1 male and 2 females outside. The adult index was 0.06. The day before spraying, the number of mosquitoes increased to 11, including 6 males and 1 female inside plus 2 males and 2 females outside. The adult index remained 0.06. An investigation 30 minutes after spraying showed that the number of mosquitoes decreased to 7, with all of them males. The adult index declined to 0. The day after spraying, 1 female mosquito was captured outside, and the adult index increased slightly to 0.02 for a control efficacy of 90.9%. Two days after spraying, no mosquitoes were captured. The adult index declined again to 0, and the control efficacy reached 100%.

2. Linya District

(1) Susceptibility testing

As seen in Table 4, the Linya wild-type strain had a total average knockdown rate of 89% over all 5 households; the Tainan susceptible strain had an average of 98.5%; while both the comparison and control groups averaged 5%. When the T-test was used to compare knockdown rates between the 2 strains, we found a significant difference only in the 1st household ($p<0.05$) 30 minutes after the use of Deltamethrin. For the outside cages alone, the Linya strain had an average knockdown rate of 84%; the Tainan strain had an average of 96.5%. For the inside cages, the Linya strain averaged 92.3%, while the Tainan strain averaged 100%. After T-test analysis, the 2 strains did not show a significant difference in either the interior or exterior locations ($p>0.05$). The ANOVA analysis showed that only the exterior rate from the 1st household differed significantly from the rates for all cages from the other 4 households for the Linya strain ($p<0.05$).

After the mosquitoes had come in contact with the insecticide for 30 minutes and after 24 hours of breeding, we found that the mortality rate was always higher than the knockdown rate, regardless of the strain. The average mortality rate for the Linya strain over all the cages was 92%; the outside cages averaged 85%; while the inside cages averaged 96.6%. Aside from the 83% mortality rate in the 1st household, the rates for all the other households were 100%. The mortality rate was 100% in every household for the Tainan strain. All the average mortality rates between the 2 strains were compared by the T-test, and the results found a significant difference only for the outside cages from the 1st household ($p<0.05$). The ANOVA analysis compared differences among

households and locations. Results showed that only the rate for all cages and for outside cages from the 1st household differed significantly from the rates of the other 4 households ($p<0.05$).

We measured the interior space for 14 households and determined the actual spraying time. In addition, we found the ratio of each household's actual dosage and recommended dosage, as listed in Table 5. As the table shows, except for the 1st household, the other households used the same dosage. However, the ratio for every household was greater than 1 (from 2.86 to 6) with an average of 4.86.

(2) Evaluation of Results

As seen in Table 6, 2 days before spraying 13 adult mosquitoes were captured, including 4 males and 8 females inside plus 1 male outside. The adult index was 0.16. The day before spraying, the number of mosquitoes increased to 23, including 7 males and 8 females inside plus 4 males and 4 females outside. The adult index increased to 0.24. An investigation 30 minutes after spraying showed that only 1 male mosquito was captured. The adult index declined to 0.02. The day after spraying, no mosquitoes were captured, and the adult index decreased to 0 for a control efficacy of 100%. Two days after spraying, the number of mosquitoes increased to 8, with 4 males and 3 females inside plus 1 male outside. The adult index increased to 0.06, and the control efficacy declined to 65.2%.

Discussion

1. Insecticide Resistance of *Aedes Aegypti* Mosquitoes in Kaohsiung

A sample of 5 households in the Chiencheng district was chosen for

investigation, and the results showed that both the knockdown and mortality rates were greater than 80%, indicating that the insecticide was not ideal. The knockdown rate differed significantly between the 2 species. Perhaps the reason lies in the insecticide used, as the active ingredients in Perdelta are Permethrin and Deltamethrin. These two ingredients kill on contact, and the knockdown effect is not known to be good. After 24 hours of breeding inside a paper cup, the Chiencheng strain mosquitoes had been confined within a certain space and forced to come into contact with a sufficient amount of Deltamethrin [9]. As a result, the mortality rate increased and was not significantly different from the rate in the Tainan strain.

Results from the Linya district showed that exterior rates for the 1st household and the other households were significantly different. This finding could be explained by one worker's observation that the cage was hidden on the top floor and that the spraying did not reach its target. Thus, the insecticide could not have an immediate impact on the mosquitoes. The Tainan susceptible strain, combined with the fact that small droplets from the thermal fogger (12-16 μm) could drift to hidden areas [10-11], had a knockdown rate of 82.5%. Meanwhile, after trapping the mosquitoes for 24 hours, the mortality rate increased. In general, the knockdown and mortality rates for the Linya strain were not significantly different from the rates of the Tainan strain.

Since 2002, surveillance for drug resistance in *Aedes aegypti* mosquitoes has been ongoing in the southern region. Reports have shown that mosquitoes have developed resistance to the synthetic pyrethroid Permethrin for a long time. In addition, since 2006 Deltamethrin has

gradually had less effect on mosquitoes in Kaohsiung City's Chiencheng district [12-16]. The effect of Deltamethrin on Linya strain mosquitoes has also been declining since 2004. In order to exterminate mosquitoes, Kaohsiung City control workers have increased the insecticide concentration or even over-sprayed certain areas to get results. Although the Chiencheng and Linya strain mosquitoes have already developed resistance to Deltamethrin, we are not at a loss of what to do next. Local drug resistance surveillance results as well as the area's spraying history and biological examination results should be considered in deciding the appropriate concentration. Furthermore, switching insecticides or using one that does not contain pyrethroids can also achieve the same control effects [17]. These steps will prevent overdosage, use of the same insecticide for extended periods of time, environmental pollution, and drug resistance. This way, spraying is guaranteed to be effective and successful the first time around.

2. Evaluation of Emergent Spraying Results

Large-scale spraying reduces the ability of the outbreak to spread and allows more time for eliminating breeding sources. On the day of spraying, only male mosquitoes could be captured in both the interior and exterior of houses for the Chiencheng district, indicating that new breeding sources were being established and proving again that Deltamethrin kills on contact. Female mosquitoes were captured outside 1 day after spraying, which indicates that spraying was not sufficient to eliminate breeding sources. The control efficacy 2 days after spraying reached 100%. Thus, its residual effect was excellent. For the Linya district, the adult index had already increased gradually to 0.24 on the day before

spraying (0.2 is a critical level and indicates immediate transmission risks). Although 1 day after spraying the control efficacy reached 100%, mosquitoes could still be captured inside and outside the house on the 2nd day, causing the efficacy to drop to 65.1%. Since both male and female mosquitoes were still found inside, the breeding sources had not been completely eliminated. Only male mosquitoes were captured outside, indicating that breeding sources were still being created. Thus, teaching workers the correct spraying techniques and giving them the responsibility to increase outreach to every household are both necessary measures.

3. Spraying Quality

Although the Environmental Agents Control Act sees vector control as a professionally-licensed field, it does not require licenses for spray workers. In addition, the lack of professional training causes large gaps in the spraying quality. The spraying skills of control workers vary greatly, as was seen in the Chiencheng district, where overdosage (1-8x) was very common. Perhaps the public pressure on prompt initial control caused workers to seek a quick remedy while neglecting the correct procedures. In turn, mosquitoes developed drug resistance. Spraying began in the Linya district in the middle of the seasonal control efforts, after workers had attended several training sessions. As a result, their skills get improved. Although overdosage (2-6x) still occurred, the amount sprayed for each household gradually became more consistent. Of course, the quality is still on the rise. The key to eradicating dengue fever lies in the complete elimination of breeding sources. A priority of emergent control measures is spraying the interior of the house [18]. Last year, when Kaohsiung City implemented emergent spraying activities for

dengue fever, it faced the challenges of drug-resistant mosquitoes and citizens' refusal to have their homes sprayed. Each team member persevered and effectively controlled the native transmission. In the future, if we can use biological examinations early on, determine the accurate concentration, improve worker training, implement proper spraying methods, perform evaluations on susceptibility, and finally ensure the spraying quality, then we can achieve the ultimate goals of reducing mosquitoes and interrupting the transmission of dengue fever.

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Table 1. Susceptibility Testing in the Chiencheng District

Location	Household	30-min Knockdown Rate (%)		24-hr Mortality Rate (%)	
		Chiencheng	Tainan	Chiencheng	Tainan
Interior	1	100b	100	100	100
	2	100b	100	100	100
	3	100b	100	100	100
	4	100b	100	100	100
	5	84＊a	100	100	100
	Average	96.8	100	100	100
Exterior	1	100	100	100	100
	2	90	100	97	100
	3	77	98	100	100
	4	55	93	93	98
	5	67＊	88	83	95
	Average	77.8＊	95.8	94.6	98.6
All	1	100	100	100	100
	2	93＊	100	99	100
	3	91＊	99	100	100
	4	80＊	97	97	99
	5	77＊	95	93	98
	Average	88.2＊	98.2	97.8	99.4

1. * For the T-test, with 95% confidence intervals, the 2 strains are significantly different.
2. For ANOVA, rates with different letters indicate that in the same location for the same strain, the households are significantly different.

Table 2. Spraying Status by Household in the Chiencheng District

Household	Spray Area (M3)	Time (Min. Sec.)	Actual Dosage (ml/M^3)	Ratio (Actual/Recommended Dosage)
1	750	14’30”	0.023	7.67
2	250	4’52”	0.024	8
3	350	1’5”	0.004	1.33
4	1100	7’26”	0.008	2.67
5	550	6’10”	0.014	4.67
6	550	5’35”	0.012	4
7	150	1’23”	0.011	3.67
8	150	2’38”	0.021	7
9	550	6’45”	0.015	5
10	650	7’13”	0.013	4.33
11	650	7’	0.013	4.33
12	350	3’15”	0.011	3.67
13	350	1’30”	0.005	1.67
14	350	1’15”	0.004	1.33
Mean	482.1	5’5”	0.013	4.33

Recommended Dosage (Active Ingredient Concentration/Recommended Dilution Factor)＝0.003ml/m3

Table 3. Control Efficacy in the Chiencheng District

Time	2 days before		1 day before		Spray date		1 day after		2 days after	
Location	Interior	Exterior	Interior	Exterior	Interior	Exterior	Interior	Exterior	Interior	Exterior
Sex	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
# Mosquito	3 1	1 2	6 1	2 2	5 0	2 0	0 0	0 1	0 0	0 0
Total	7		11		7		1		0	
Adult index	0.06		0.06		0		0.02		0	
Efficacy (%)	—		—		—		90.9		100	

Table 4. Susceptibility Testing in the Linya District

Location	Household	30-min Knockdown Rate（%）		24-hr Mortality Rate (%)	
		Linya	Tainan	Linya	Tainan
Interior	1	63.3	100	83	100
	2	100	100	100	100
	3	100	100	100	100
	4	98.3	100	100	100
	5	100	100	100	100
	Mean	92.3	100	96.6	100
Exterior	1	20a	82.5	25＊a	100
	2	100b	100	100b	100
	3	100b	100	100b	100
	4	100b	100	100b	100
	5	100b	100	100b	100
	Mean	84	96.5	85	100
All	1	46＊a	93	60a	100
	2	100b	100	100b	100
	3	100b	100	100b	100
	4	99b	100	100b	100
	5	100b	100	100b	100
	Mean	89	98.5	92	100

1. * For the T-test, with 95% confidence intervals, the 2 strains are significantly different.
2. For ANOVA, rates with different letters indicate that in the same location for the same strain, the households are significantly different.

Table 5. Spraying Status by Household in the Linya District

Household	Spray Area (M3)	Time (Min. sec.)	Actual Dosage (ml/M3)	Ratio （Actual/Recommended Dosage）
1	880	5’9”	0.00075	2.68
2	750	8’35”	0.00146	5.21
3	700	8’27”	0.00154	5.5
4	800	10’32”	0.00168	6
5	800	9’20”	0.00149	5.32
Mean	786	8’24”	0.00136	4.86

Recommended Dosage (Active Ingredient Concentration/Recommended Dilution Factor)＝0.00028ml/M3

Table 6. Control Efficacy in the Linya District

Time	2 days before				1 day before				Spray date				1 day after				2 days after			
Location	Interior		Exterior		Interior		Exterior		Interior		Exterior		Interior		Exterior		Interior		Exterior	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
# Mosquito	4	8	1	0	7	8	4	4	0	1	0	0	0	0	0	0	4	3	1	0
Total	13				23				1				0				8			
Adult index	0.16				0.24				0.02				0				0.06			
Efficacy (%)	—				—				—				100				65.2			