Taiwan Epidemiology Bulletin

October 4 , 2011/ Vol.27 / No.19

Outbreak Investigation Express

Report of a Child Botulism Incident in Pingtung County, June 2011

Ching-Li Lin, Shu-Hua Huang, Chi-Chuan Huang, Sheng-Tang Wei, Li-Jen Lin

Fifth Branch, Centers for Disease Control, Taiwan

Abstract

On June 22, the Fifth Branch of Centers for Disease Control received a case report of botulism from a Kaohsiung medical center. The case was a four-year-old boy who was sent to a local hospital in Pintung County in the earlier hours of June 22nd for emergency resuscitation and then transferred to this medical center. The attending physician reported a suspected botulism case due to the symptoms shown and applied for the use of botulism antitoxin. After receiving two bottles of antitoxin treatment and careful medical care, the case's situation quickly improved and was transferred from the ICU to the general ward on July 21st. The serum samples taken from the case were tested positive for *Clostridium botulinum* toxin type A and negative for both toxin and microbiological tests for food samples, with no certain contamination source. Experience gained from this case is as following: although the case was only four years of age, the amount of serum samples taken and the antitoxins administered were no different from those for an adult. During this investigation, appropriately expressed support for the anxious family members and good interaction are necessary for receiving effective information. In addition, the cooperation between involved facilities and counties is essential in the completion of swift and preventive measures.

Keywords: botulism, botulism antitoxin

The Taiwan Epidemiology Bulletin series of publications is published by Centers for Disease Control, Department of Health, Taiwan (R.O.C.) since Dec 15, 1984.

Publisher : Feng-Yee Chang

Editor-in-Chief : Yi-Chun Wu

Executive Editor : Li-Gin Wu, Hsiu-Lan Liu

Telephone No : (02) 2395-9825

Address : No.6, Linshen S. Road, Taipei, Taiwan 100 (R.O.C.)

Website : http://teb.cdc.gov.tw/

Suggested Citation :
[Author].[Article title].Taiwan Epidemiol Bull 2011;27:[inclusive page numbers].

INSIDE

Original Article

Survey of Dengue Fever Vector Density before and after Insecticide Spraying

Chih-Yuan Wang[1], Chao Lin[1], Ying-Feng Ge[2], Hsin-Fan Chien[2], Chein-Sheng Lin[3],Chin-Shyan Wang[3], Shu-Wan Jian[1], Yu-Hsin Chen[2], Yu-Zhu Wang[2], Hwa-Jen Teng[1]

1. Research and Diagnostic Center, Centers for Disease Control, Taiwan
2. Fifth Branch, Centers for Diseases Control, Taiwan
3. Fourth Branch, Centers for Diseases Control, Taiwan

Abstract

Because of the emergency dengue fever mosquito control and the low temperature and dry condition in winter, dengue viruses usually can not overwinter in Taiwan. In 2010, Kaohsiung City Government launched a control program to prevent these circulating dengue virus strains sustaining through the winter in Kaohsiung City. This study conducted the adult vector density surveys before and after insecticide application within a 100-meter (in radius) area around the house of a confirmed dengue fever patient in Kaohsiung City. A comparative study was also conducted in Fengshan City, Kaohsiung County. Before insecticide spraying, the adult mosquito index was 0.77 in Cianjhen District, Kaohsiung City, and was reduced to 0.23 after insecticide spraying (reduction rate=69.6%). The adult vector index in Fengshan City, Kaohsiung County, was 0.33 and 0.10 before and after the insecticide spraying, respectively (reduction rate=70%). Forty-three female and 12 male *Aedes aegypti* were collected in these 2 study areas. The main resting sites of these female mosquitoes included kitchen (35.5%), bedroom (32.3%), living room (19.4%), bathroom (6.5%), basement (3.2%), and storage room (3.2%). This result indicated that single insecticide spraying might reach 70% reduction rate and, thus, should combine with the second insecticide application, environmental factors, or other control measures to interrupt the transmission cycle.

Keyword: Dengue fever, Kaoshiung City, effacicy of insecticide spray, resting site, *Aedes aegypti*

Introduction

Dengue fever is one of the global important vector-borne diseases. The prevalence range of this disease is expanding and the prevalence frequency is also increasing. At present, dengue fever is endemic in 100 countries, especially in southeastern Asia and western Pacific areas. WHO estimated that over 50 million people were affected by dengue fever yearly. In which, about 500,000 patients were dengue hemorrhagic fever cases and caused 12,500 casualties [1]. In Taiwan, after the dengue fever was silent for 40 years, a type 2 dengue outbreak was recorded in Liuchiu in 1981. Later, a dengue outbreak was found in southern Taiwan in 1987, and in the next year, another outbreak with 4,389 confirmed cases was recorded. Since then, small to medium outbreaks were then found every 3-5 years in different areas. However, since the serious

outbreak in 2002 (5,366 confirmed cases), the frequency of endemic event with the case number between 34 to 2,000 was increased to once per year [2-3]. Moreover, due to frequent travelling and traffic convenience, circulating of multiple viral serotypes was found each year and dengue endemic was also repeatedly occurred in Cianjhen District and Lingya District, Kaohsiung City.

Although several international organizations are devoted to develop dengue fever vaccine to reduce the dengue threat [4-5], the result is still unavailable for clinical application. Therefore, the control of this disease still depends on controlling the mosquito vectors to prevent the disease occurring and to interrupt the transmission. Up to date, there is no single effective method for vector control. A effective vector control program should be accommodated with local condition and long term promotion. In Vietnam, a long-term (7 years, 1998-2003) effective vector control was achieved by using *Mesocyclops* pp., mosquito fish and *Bacillus thuringiensis* to control larvae and to reduce the adult density [6-7]. Using the law to enforce the mosquito control was implemented in 1970 in Singapore, which reached a 15-year of disease prevention effectiveness [8]. However, during an outbreak, an emergency control of mosquitoes was needed to killing infectious female vectors to stop transmission cycle. Additionally, to decrease vector density in risk areas was also needed.

According to viral DNA sequence studies [9-10], epidemiologic analysis [11] and virus detection of vector mosquitoes [12] in Taiwan, the circulating viruses of most dengue outbreaks in Taiwan were imported from other countries. Later, the virus was transmitted to local vector mosquitoes and causing local endemic event. However, very few viruses may survive through warm winter and cause epidemic event in the next year, such as the case in 1998 and in 2002. During the 2010 outbreak in southern Taiwan, all 4 dengue virus serotypes were found simultaneously. Type 1 serotype was found in Tainan County, while serotype 2 and serotype 3 were found in Kaohsiung City and serotype 4 were found in Tainan City. In order to prevent viruses surviving through winter, Kaohsiung City government conducted an emergency insecticide application program. This study investigated adult vector density, combined with address mapping system before and after insecticide spraying using sweeping nets and backpack aspirators to evaluate the efficacy of the insecticide spraying.

Materials and Methods

Survey area

The survey site was a 100-meter (in radius) area around the house of the confirmed case in Ruihe, Cianjhen District, Kaohsiung City. Insecticide spraying was conducted in December 18, 2010. Department of Health, Kaohsiung City Government, was responsible for compelled indoor insecticide application by spray cans within a 20-meter area and outdoor spraying by fogging in a 50-meter area. Department of Environmental Protection, Kaohsiung City Government, was responsible for

outdoor insecticide spraying by foggers within a 100-meter area in fire alley, gutter, piles of unused objects, plants, and other mosquito resting sites. A comparative study was conducted in Shanmei, Fengshan City, Kaohsiung County, in December 22, 2010. Department of Health, Kaohsiung County Government, was responsible for compelled indoor insecticide spraying in a 100-meter area and Department of Environmental Protection was in charge of outdoor insecticide spraying in mosquito resting sites.

Kaohsiung City address mapping system (http://civil.kcg.gov.tw/address) and Kaohsiung County address mapping system (http://emap.kscg.gov.tw) were used to locate the house of the confirmed dengue case in the map (these 2 systems were merged to Kaohsiung City address mapping System after December 25, 2010). 20-meter, 50-meter and 100-meter area maps were marked out (Figure A and B). The number of houses was 28, 100 or 344 within 20-meter, between 20 and 50-meter, or between 50 and 100-meter blocks, respectively, in Kaohsiung City; while the house number in each block was 25, 75, 259 in Kaohsiung County, respectively.

Statistical analysis

Statistica version 5.0 software (StatSoft Holdings, Inc., Tulsa, Oklahoma, USA) was used for all statistical analyses. Initially, Fisher exact test was applied for 2x2 independent test due to the number of females smaller than 5 in some blocks (testing the independence of insecticide spraying and female vector number in insecticide spraying areas). After the independence of these variables was confirmed, a log-linear model was then used to compare the vector density before and after insecticide spraying and in blocks.

Adult mosquito survey and virus detection in mosquitoes

Adult mosquito surveys were conducted 1 day before and after insecticide spraying. Each location was divided into 3 blocks (0-20 meter, 20-50 meter, 50-100 meter) and each block was then split into 2 parts (randomly selected as survey site before or after insecticide spraying). Ten houses were selected in each block which the 1st house was selected by random. Among 3 survey team members, 1 team member entered the house with the permission of the resident to collect mosquitoes by a sweeping net. Then another

(A) (B)

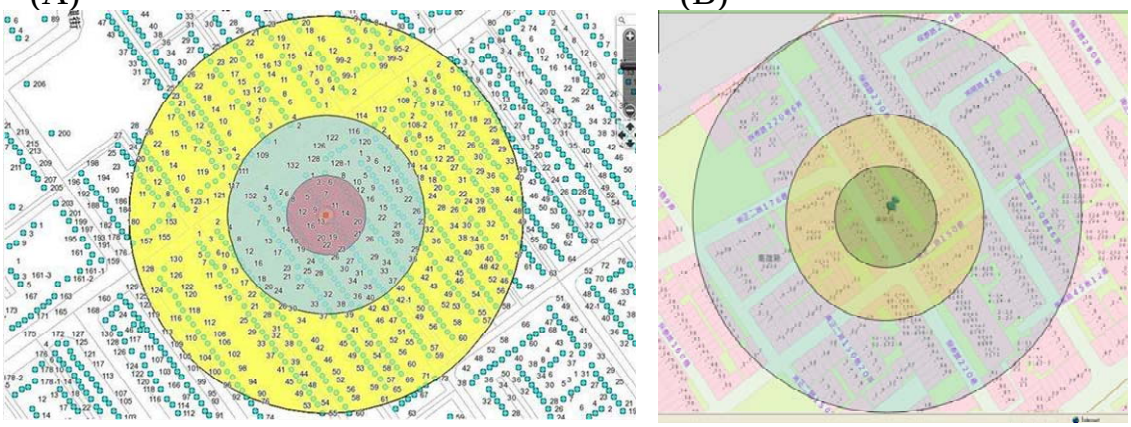

Figure The maps with the house distribution in Ruihe, Kaohsiung City (A) and Shanmei, Kaohsiung County (B)

member collected mosquitoes by a backpack aspirator (Model 1412, John W. Hock Company, Gainesville, Florida) and the third member recorded mosquito number and the collecting site (outdoor, living room, kitchen, bedroom, bathroom, storage room and basement). The species and gender of mosquitoes were identified on site, and adult mosquito index (average number of collected *Aedes* female mosquitoes per house) and reduction rate (*Aedes* female number before insecticide spraying – *Aedes* female number after insecticide spraying/*Aedes* female number before insecticide spraying) were also calculated. Investigation was focused on the first floor and the basement and each house was visited one time only. The number of house for each team in every block was the same for removing the difference of the collectors to obtaining the accuracy. Furthermore, all collected mosquitoes were frozen in dry ice and brought back to laboratory for virus detection by real time RT-PCR method [12].

Results

The numbers of *Ae. aegypti* females collected in Cianjhen District, Kaohsiung City were significantly different before and after insecticide spraying (χ^2=8.09, df=1, P<0.01), however, there was no significant difference in blocks (χ^2=2.97，df=2，P>0.05). One day before insecticide application, 23 females and 6 males of *Ae. aegypti* were collected in 30 houses. The adult mosquito index was 0.77 (=23 *Aedes* females/30 houses). Among blocks, the highest number of *Aedes* females collected were recorded in the 20-50 meter block (11 females) and followed by the 50-100 meter block (8 females) and the 0-20 meter block (4 females). Percentage of the *Aedes* female resting indoors was between 18.2-100.0%. One day after the insecticide spraying, 7 female sand 1 male of *Ae. aegypti* were collected (Table 1). Adult mosquito index was reduced to 0.23 (7 *Aedes* females/30 houses). The total reduction (0-100 meter area) was 69.6%, while the outdoor reduction was 90.0% and indoor

Table 1. The number of *Aedes* mosquitoes collected and the efficacy of insecticide application before and after insecticide spraying in Ruihe, Cianjhen District, Kaohsiung City, December 17-19 2010

Survey area	No. of houses	Cianjhen District, Kaohsiung City (experimental study)			Fengshan City, Kaohsiung County (comparative study)		
		No. of *Aedes aegypti* female (male)		Percent reduction	No. of *Aedes aegypti* females (male)		Percent reduction
		Before	After		Before	After	
0-20m		4(0)	2(1)	50.0%	1(2)	1(0)	0.0%
outdoor	10	1(0)	1(1)	0.0%	0(1)	0	N/A
indoor		3(0)	1(0)	66.7%	1(1)	1(0)	0.0%
20-50m		11(5)	3(0)	72.7%	4(0)	0	100.0%
outdoor	10	9(3)	0	100.0%	0	0	N/A
indoor		2(2)	3(0)	-50.0%	4(0)	0	100.0%
50-100m		8(1)	2(0)	75.0%	5(3)	2(0)	60.0%
outdoor	10	0	0	N/A	1(0)	0	100.0%
indoor		8(1)	2(0)	75.0%	4(3)	2(0)	50.0%
Total		23(6)	7(1)	69.6%	10(5)	3(0)	70.0%
outdoor	30	10(3)	1(1)	90.0%	1(1)	0	100.0%
indoor		13(3)	6(0)	53.8%	9(4)	3(0)	66.7%

reduction was 53.8%. As for blocks, average reductions were 50% in the 0-20 meter block (mainly came from indoor reduction rate 66.7%), 72.7% in the 20-50 meter block (mainly came from outdoor reduction rate 100%) and 75.0% in the 50-100 meter block (mainly came from indoor reduction rate 75%). Percentage of the *Aedes* female resting indoors was between 50-100%. As for the comparative study, there was no significant difference recorded in female vector density before and after insecticide spraying in Fengshan City, Kaohsiung County (χ^2=3.17, df=1, *P*=0.07), and in insecticide spraying blocks (χ^2=2.39, df=2, *P*>0.05). One day before insecticide spraying, 10 females and 5 males of *Ae. aegypti* were collected in 30 houses. The adult mosquito index was 0.33 (=10 *Aedes* females/30 houses). The highest number of females was recorded in the 50-100 meter block (5 females) and followed by the 20-50 meter block (4 females) and the 0-20 meter block (1 female). Percentages of *Aedes aegypti* females resting indoors were between 80.0-100.0%. One day after insecticide spraying, 3 female *Ae. aegypti* were collected (Table 1). Adult mosquito index was reduced to 0.1 (=3 *Aedes* females/30 houses) and. The total reduction was 70.0%, while outdoor reduction was 100.0% and indoor reduction was 66.7%. As for blocks, average reductions were 0.0% in the 0-20 meter block, 100.0% in the 20-50 meter block (mainly came from indoor reduction,100%) and 60.0% in the 50-100 meter block (indoor=50%, outdoor=100%).

In this study, 72.1% of *Ae aegypti* females were collected indoor (27.9% in outdoors). Among them, the most common resting site was kitchen (35.5%) and followed by bedroom (32.3%), living room (19.4%), bathroom (6.5%), storage room (3.2%) and basement (3.2%) (Table 2). In spraying time analysis, before the insecticide spraying, 66.7% of females were collected in indoors (33.3% in outdoor), including kitchen (40.9%), bedroom (36.4%) and living room (22.7%). After insecticide spraying, 90% of female vectors were collected indoors, which scattered evenly. In district analysis, 56.5% and 43.5% of *Ae. aegypti* females rested indoors and outdoors In Cianjhen District, respectively before insecticide spraying. The percentage became 85.7% (indoor) and 14.3% (outdoor) after insecticide spraying. As for Fengshan City, 90.0% of *Ae. aegypti* females

Table 2. Resting sites of adult *Aedes aegypti* before and after insecticide spraying in Cianjhen District and Fengshan City

Resting sites	Cianjhen District		Fengshan City		Total			
	No. of *Ae. aegypti* females (No. of Male)		No. of *Ae. aegypti* females (No. of Male)		No. of *Ae. aegypti* females (No. of Male)			Reduction
	Before	After	Before	After	Before	After	Total	%
Outdoor	10(3)	1(1)	1(1)	0	11(4)	1(1)	12(5)	27.9%
Indoor	13(3)	6(0)	9(4)	3(0)	22(7)	9(0)	31(7)	72.1%
Living room	3(2)	0	2(1)	1(0)	5(3)	1(0)	6(3)	19.4%
Kitchen	3(0)	2(0)	6(3)	0	9(3)	2(0)	11(3)	35.5%
Bedroom	7(1)	2(0)	1(0)	0	8(1)	2(0)	10(1)	32.3%
Bathroom	0	1(0)	0	1(0)	0	2(0)	2(0)	6.5%
Storage room	0	1(0)	0	0	0	1(0)	1(0)	3.2%
Basement	0	0	0	1(0)	0	1(0)	1(0)	3.2%
Total	23(6)	7(1)	10(5)	3(0)	33(11)	10(1)	43(12)	100.0%

were collected indoors (10.0% in outdoors) before insecticide spraying, and all females were collected indoors after insecticide spraying. All collected mosquitoes were tested for dengue fever virus. 75 samples collected before insecticide spraying (including 33 female and 11 male *Ae. aegypti*, 18 female and 13 male *Culex quinquefasciatus*) and 19 samples after insecticide spraying (including 10 female and 1 male *Ae. aegypti*, 7 female and 1 male *Cx. quinquefasciatus*) were tested and all were negative for dengue virus.

Discussion

This study used mapping system of Kaohsiung City and Kaohsiung County and collected mosquitoes by backpack aspirators and sweeping nets to evaluate the insecticide spraying efficacy. Furthermore, this method plus the evaluation during insecticide spraying period could provide promptly information about insecticide spraying deficiency to improve disease control. However, this report only provided an one-time survey, no repeats were available because of the time constrain. Our result revealed that single insecticide application usually reached 70% of mosquito reduction, which mainly came from outdoors than indoors. Insecticide spraying might also cause vector dispersing. Hence, the emergency dengue control should combine with other alternatives, such as environmental factors (low temperature, dry condition in winter), other vector control measures (source reduction, larvicide application, prevention of mosquito biting) or the second insecticide spraying, to stop transmission.

In our study, the reduction of adult mosquito index was reduced from 0.77 to 0.23 after insecticide spray in Cianjhen District, Kaohsiung City; however, the risk of disease transmission was still existed (index$\geq$0.2). In the comparative study (Fengshan City, Kaohsiung County), adult mosquito index was decreased from 0.33 to 0.10 and there was no immediate risk of transmission. As for the efficacy of insecticide application for blocks, 66.7% reduction of indoors in Kaohsiung City was found in the 0-20 meter block, 100.0% reduction of outdoors in the 20-50 meter block (0% for indoor) and 75% reduction of indoors in the 50-100 meter block. The indoor control in 20-100 meters might be due to the insecticide droplets spreading into indoors through the opened doors or windows [13]. In this survey, the number of females in the 0-20 meter block was the lowest comparing with other blocks and viral detection was negative in all tested samples. Therefore, it was unable to know the distribution of infectious females and efficiency of killing these target mosquitoes. Therefore, the infection site (for example 2 confirmed cases) should be selected in the future study than one confirmed case in our study in order to obtain more reliable result.

The indoor resting proportion for *Ae. aegypti* females was 72.1% in our study, which was similar to our previous study in 3 counties (79.3%) in southern Taiwan in 2003 [14] and in other countries (75.6%) [13]. Before insecticide spraying, 66.7% of *Ae. aegypti* females were collected indoors (40.9% in kitchen, 36.4% in bedroom and 22.7% in living room), while 90.0% were

collected indoors (scattered in all indoor areas) after insecticide spraying. This implied that insecticide spraying of using fogging might affect the distribution of females. After the dengue outbreak in 1987-1988, ultra-low volume (ULV) spray was used for emergency dengue control. Since 2004, ULV method was gradually replaced by fogging due to its long spraying distance to save spraying time and visible spraying effect. However, organic solvent or oil used for these foggers may remain on the furniture and wall after spray and the residents need to clean later, which affect the public's acceptance. In this study, the reduction rates reached only 70% whether in the experimental study (Cianjhen District) or the comparative study (Fengshan City). The other 30% failure might be due to techniques of insecticide spraying, small insecticide droplets by fogging, and mosquitoes moving from the borders of the study site, and/or emerging from the breeding containers in the study site. Thus, single insecticide spraying is unable to stop disease transmission and needs other control measures or multiple insecticide sprayings [15-16]. Therefore, the insecticide spraying method, technique and frequency are worth to be evaluated further. In early stage for preventing dengue virus established in Taiwan, insecticide spraying was applied to the house of the suspected dengue case and their surroundings and the activity areas of the viremic patients. However, because of the dramatically increased imported dengue cases, increased travelling frequency and public's life quality requirement, dengue control policies were modified to working on source reduction and limited insecticide application. Furthermore, dengue control policies could be changed to prevention policies to prevent the occurring of the disease and magnitude of the outbreak.

Acknowledgements

We highly appreciate the people who worked in the first-line of Dengue control. Furthermore, address mapping system developed by Kaohsiung City Government and Kaohsiung County Government helped this survey more successfully proceeded within space work.

References

1. WHO. Dengue. Available at: http://www.who.int/mediacentre/factsheets/fs117/en/index.html
2. King CC, Wu YC, Chao DY, et al. Major epidemics of dengue in Taiwan in 1981-2000: related to intensive virus activities in Asia. Dengue Bull 2000, 24: 1-10.
3. Taiwan CDC. Dengue-Notifiable infectious diseases statistics system. Available at: http://nidss.cdc.gov.tw/SingleDisease.aspx?Pt=s&Dc=1&Dt=2&disease=061
4. Durbin AP, Whitehead SS. Dengue vaccine candidates in development. Curr Top Microbiol Immunol 2010;338: 129-43.
5. Guy B, Almond J, Lang J. Dengue vaccine prospects: a step forward. The Lancet 2011;377:381-2.
6. Kay BH, Nam VS. New strategy against *Aedes aegypti* in Vietnam. The Lancet 2005;365:613-7.

7. Kay BH, Tuyet Hanh TT, Le NH Quy TM, et al. Sustainability and Cost of a Community-Based Strategy Against *Aedes aegypti* in Northern and Central Vietnam. Am J Trop Med Hyg 2010; 82:822-30
8. Ooi EE, Goh KT, Gubler DJ. Dengue prevention and 35 years of vector control in Singapore. Emer Infect Dis 2006; 12:887 -93.
9. Shu PY, Su CL, Liao TL, et al. Molecular characterization of dengue viruses imported into Taiwan during 2003-2007: geographic distribution and genotype shift. Amer J Trop Med Hyg 2009; 80:1039-46.
10. Huang JH, Liao TL, Chang SF, et al. Laboratory-based dengue surveillance in Taiwan, 2005: a molecular epidemiologic study. Amer J Trop Med Hyg 2007;77: 903-9.
11. Lin CC, Huang YH, Shu PY, et al. Characteristic of Dengue Disease in Taiwan: 2002–2007. Amer J Trop Med Hyg 2010;82:731-9.
12. Chen CF, Shu PY, Teng HJ, et al. 2010. Screening of dengue virus in field-caught *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) by one-step SYBR green-based RT-PCR Assay during 2004-2007 in Southern Taiwan. Vec Zoon Dis 2010;10:1017 -25.
13. Perich MJ, Davila G, Turner A, et al. Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. J Med Entomol 2000;37:541-6.
14. Wu JW, Teng HJ, Lin C, et al. Recent distribution of vector mosquitoes and epidemiology of the diseases they transmitted in Taiwan. Med Entomol Zoo 2009;60:241-52.
15. Teng HJ, Chen TJ, Tsai SF, et al. Chang NT. Emergency vector control in a DENV-2 outbreak in 2002 in Pingtung City, Pingtung County, Taiwan. Jap J Infect Dis 2007;60:271-9.
16. Esu1 E, Lenhart A, Smith L, et al. Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. Trop Med Inter Health 2010,15:619–31.

Biosafety and Biosecurity

Practices in Risk Assessment of Laboratory Biosafety

Yu-Yen Shih, Wen-Chao Wu, Jer-Jea Yan

Fifth Division, Centers for Disease Control, Taiwan

Risk assessment can be divided into three groups by the characteristics: qualitative, quantitative and semi-quantitative risk assessment [1]. Qualitative risk assessment is to literally classify the risk levels by describing the incidence and impact [1]. For example, in Taiwan CDC's, the epidemic signals of red, yellow, and green indicate high, moderate, and low risks, respectively. Quantitative risk assessment is to use the computational data to demonstrate the risk

probability and impact that provides statistical risk prediction [1]. Semi-quantitative risk assessment is to represent the qualitative levels by using actual values that can facilitate the calculation to determine the priority more accurately than qualitative analysis, such as the risk matrix [1].

Risk assessment is not limited to use one method. One may choose the appropriate methods based on industry characteristics and the assessing subjects. Risk matrix and Structured What-If Technique (SWIFT) can be used for general risk assessment. Instead, hazard and operability study (HAZOP), event tree, fault tree analysis (FTA), and failure modes and effects analysis (FMEA) can be used for risk assessment for the facilities and equipments [2]. Threat assessment and weakness assessment are usually used in security assessment.

In this article, we mainly introduce the risk assessment tools for laboratories including: what if brainstorming, HAZOP and SWIFT.

“What If Brainstorming” can be applied to task biorisk assessment in laboratories. Through brainstorming in each testing step or the overall procedure, hazards can be identified by asking potential problems or errors. The problems in this brainstorming stage don’t matter in order, but to the problems should comprise all the possible reasons of the hazards. Consequences could be caused by multiple factors, and existing control methods and effectiveness should be taken into account when assess the risk [3]. This assessment should contain the causes, consequences, current control methods, risk matrix, actions and recommendations [3]. An example of applying “what if brainstorming” to task biorisk assessment in laboratories is shown in Table 1.

HAZOP and SWIFT can be used in risk assessment of laboratory facilities [1,6]. HAZOP is a structured and systematic analysis of existing procedures or operation to identify and assess the existing risks on facilities (including pipelines, pump valves or sub-systems), making effective operation on the existing operation and procedures [4]. To assess the laboratory facilities, for example, decontamination tank and the system, HVAC (heating, ventilation and air conditioning) are potential points to be checked for study, known as the study node. When analyze the study node, some guidewords will be used, such as no flow, backflow, flow reduction, higher pressure, less pressure, decontamination and cleaning, and maintenance. We should discuss once a guideword to ensure that all deviation of process or operating parameters were estimated, so that we can find out potential hazards, identify the possible causes and consequences, take safety precautions, and propose improvement measures, performing a more complete assessment technique [4]. An example of applying “HAZOP” to risk assessment in laboratory facilities is shown in Table 2.

SWIFT analysis, which is similar to HAZOP but more efficient, is also refers to discussion on possible abnormality in operation through group brainstorming. With system and sub-system assessment, group members view the failure in each category, and try to ask and answer questions that may occur (may use the checklist to quickly point

out additional problems), and record cause of the problems, consequences, existing control methods and recommendations [5-6]. For the protective equipments in BSL-3 laboratories, for example, assessing the protective barriers can be regarded as a system assessment while assessing the systemic integration, floors, ceilings, walls and penetration testing can be regarded as a sub-system assessment. Then we inspect the fault by each category, also try to ask and solve the problems that may occur (checklist may quickly point out additional problems) and record cause of the problems, consequences, existing control methods and recommendations. An example of applying "SWIFT" to laboratory risk assessment is shown in Table 3.

It can quickly determine the potential hazards, and then take correct and effective control measures to reduce the incidence and spread of accidents if we making good use of risk assessment methods for laboratories. Therefore, the concept of risk assessment and being proficient at application to risk assessment is an essential knowledge and ability for laboratory personnel.

Table 1. An example of applying "what if brainstorming" to task biorisk assessment in laboratories

Problem	Cause	Consequence	Existing control	Risk assessment			Action taken
				Consequence	Probability	Risk	
Virus spills on counter surface in biosafety cabinet	Technical defects	Aerosol generating	Operating in biosafety cabinets	Mild hazard	Inevitable	Moderate	Strengthening training on good microbiological practice

Table 2. An example of applying "HAZOP" to risk assessment in laboratory facilities

Study Node: Sewage system - the collection tank

Guidewords: No flow

Cause	Consequence	Control	Suggestion
Decontamination requirements (e.g. blocking)	1. Possible damage on the primary barriers (human error) 2. Possible damage on the primary barriers（failed equipment）	1. Establish standard operati procedures 2. Wear protective clothing 3. Work permit 4. All equipments can be isolated by technical devices	1.Ensure the disinfection and cleaning procedures/checks and tracking; prevent damage on the primary barrier; ensure effective decontamination and cleaning 2.Personnel education and training

Table 3. An example of applying "SWIFT" to laboratory risk assessment

System: The integrity of protective barriers

Sub-system: Windows

Problem	Cause	Consequence	Control	Suggestion
Leakage	Seal failure	Damage protection	Leak test	1. Identify materials and specifications of the window glass 2. Identify the size and location of all windows to prevent the damage caused during equipment delivery

References

1. Wang SH. Risk management overview and risk assessment methods. Green Energy and Environment Research Laboratories of Industrial Technology Research Institute, 2006.
2. Lin RU. Risk assessment practices in occupational safety and health. Industrial safety Technology, 2008;42-46. Available at: http://proj.moeaidb.gov.tw/cesh/data4/67-11.pdf
3. TECH 482/535 What-If analysis. Available at: http://www.ceet.niu.edu/depts/tech/asse/tech482/what_if_analysis.doc
4. "WHATIF" HAZARD ANALYSIS. Available at: http://risk.arizona.edu/healthandsafety/labchemicalsafety/What-IfHazardAnalysis.pdf
5. Rausand M. HAZOP Hazard and Operability. Department of Production and Quality Engineering. Norwegian University of Science and Technology. Available at：http://www.caia.co.za/files/Hazop_Technique_MarvinRausand.pdf
6. HAZOP and hazard identification services. Available at: http://www.lloydsregisterasia.com/services/pdfs/risk-management-services-factsheet2.pdf
7. Facility risk assessments. Available at: http://www.dnv.com/services/consulting/biorisk/facility_risk_assessment/

Common Deficiencies of Facilities and Equipments in Taiwan's High Containment Laboratories

Yi-Jhen Chen, Wen-Chao Wu, Jer-Jea Yan

Fifth Division, Centers for Disease Control, Taiwan

The purpose of laboratory biosafety is to avoid laboratory-acquired infections. The key is relied on biosafety management in organization (biosafety committee or designated person), as well as biosafety awareness and understanding of laboratory staff. Valid operation and use of laboratory safety facilities and equipments are also important [1].

To construct high containment laboratories, the main safety facilities and equipments include ventilation system, high efficiency particulate air (HEPA) filter, biosafety cabinet and autoclave. During 2007 to 2010, Taiwan CDC checked domestic negative pressure laboratories for *Mycobacterium tuberculosis* and laboratories above biosafety level 3, and found common defects of facilities and equipments as described below.

Laboratory ventilation system consists of intake and exhaust. Its main function is to provide laboratory directional airflow to maintain negative pressure environment, effectively exhausting the pathogenic microorganisms in the laboratory. The common deficiencies include: (1) wrongly design, misplaced or narrowed space of laboratory air intake and exhaust vents,

resulting in turbulent flow or short air circulation; (2) no back-up exhaust fans or exhaust without HEPA-filter [1]; (3) dysfunction or non-function of exhaust portals in laboratory; (4) exhaust system without air control damper or dysfunction; (5) intake from indoor air or air from other contaminated areas instead of fresh air from outside.

HEPA filters should be installed in the intake and exhaust vents of laboratories. Foreign laboratories do not require filtering the air intake, but situation in Taiwan, taking environmental consideration, filter supply air will effectively prolong the lifespan of HEPA at exhaust vents. Exhaust HEPA filters capture contaminated aerosols or particulates produced from experimental operation or accidents, and then clean air is exhausted to the outside through the filter. Its common deficiencies include: (1) laboratories do not regularly test the efficiencies of the HEPA filters, or do not equip standard operating procedures with regard to inspecting, replacing, and disposal of the HEPA filters; (2) HEPA filter leak test failure without making any subsequent replacement.

Laboratory personnel should operate high risk pathogen at negative pressure settings to avoid incautiously dispersing the pathogens, and endangering the public safety in community. In previous years, we reviewed and found some laboratory staff misunderstood or ignorant to the negative pressure in laboratories so that were unaware of being exposed in high-risk environment. The common deficiencies include: (1) unaware of leakage inside the laboratory, resulting in insufficient or inconstant negative pressure; (2) not install manometers at the laboratory entrance or operating room, or not adjust regularly; (3) negative pressure value less than or exceeds the set range, and the laboratory did not have any subsequent correcting program [2].

Biosafety cabinets are local exhaust of the laboratory. Laboratory staff should handle possible infectious aerosols or splashing in a biosafety cabinet. Maintenance of negative pressure in biosafety cabinets can prevent the experimental operator from being infected by droplets. The common deficiencies include: (1) not regularly test functionality every year; (2) sharing same pipeline design for biosafety cabinets and laboratory exhaust system, and hence cause air flow interference; (3) inappropriate placement of biological indicators used for fumigation or inadequate test point that unable to prove fumigation is complete; (4) wrongly airtight connection of exhaust hood for Class II A2 type biosafety cabinets instead the correct canopy connection, or improper hood installation, resulting in positive pressure at the gap [3].

Autoclaves are mainly used for infectious waste sterilization. Most laboratory staff know they need to regularly test the biological validation of autoclaves, but don’t know the type of autoclaves they use (gravity type, vacuum type, or a small pressure vessel). The common deficiencies include: (1) autoclaves set outside the laboratories without proper transporting line for infectious waste and other related standards; (2) drainage and exhaust without HEPA filters for the vacuum type autoclaves; (3) seam leaks at the wall through site and around the equipments for through-wall type autoclaves.

Taiwan CDC implements safety check according to the law, and request organizations to accomplish improvement before the deadline on the basis of deficiency documents. It is necessary to have effective tools to do good work. Organizations should establish standard operating procedures and plans of management and maintenance for the laboratory safety facilities and equipments in laboratory management documents. Regularly perform testing in accordance with annual plan to ensure functioning well. In addition, in order to protecting the safety of laboratory staff, we should strengthen training for laboratory staff in safe operation with infectious materials, correct wear of personal protective equipments and use of laboratory facilities and equipments.

References

1. Wu WC, Tseng SH, Yan JJ. Laboratory biosafety certification of BSL-2 negative pressure laboratories of M. tuberculosis in Taiwan, 2009. Taiwan Epidemiol Bull 2010;26:350-7.
2. Taiwan CDC. Safety Guidelines for Biosafety Level 3 Laboratory. 2nd ed.,2011;11.
3. Wu WC, Lee LL, Wu HS. Audit report for laboratories of biosafety level 3 and higher in Taiwan, 2007. Taiwan Epidemiol Bull 2008;24:523-39.