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Surveys on Japanese Encephalitis Vectors in Taiwan Area during 2004-2008

Cheo Lin, Shu-Wan Jian, Hwa-Jen Teng

Research and Diagnostic Center, Centers for Disease Control, Taiwan

Abstract

The first Japanese encephalitis (JE) case in Taiwan in 2009 was detected in Pingtung County with the onset of the disease symptom in a patient on March 30, which was more than a month earlier than the first case in previous years, usually in May. Thus, it is necessary to obtain updated information on the temporal change of vector status. The purposes of this study are to understand the current status of JE vectors and the activity of JE virus in field mosquito population, and to understand the change of species composition of JE vectors over time in Taiwan. The surveys carried out by using light traps in different areas in Taiwan during 2004-2008, *Cx. tritaeniorhynchus* Giles was the dominant species in all areas, accounted for 64.1% to 100% of the total number of captured vectors. The species of *Cx. fuscocephala* Theobald accounted for 30.0% (the highest percentage among survey sites) of the mosquitoes caught in Kaohsiung County. *Culex annulus* Theobald was commonly captured in all the survey areas albeit in relatively small number. A single or double peak pattern in June, July, or September was seen in local population of *Cx. tritaeniorhynchus* in Pingtung County. The highest number of mosquitoes for single capture in each County/City ranged from 387 to 37,440 per trap-night, with the highest found in Puzi, Chiayi County. JE virus became active in field mosquito population mainly in May and June and 96.2% of total positive pools were from *Cx. tritaeniorhynchus*. In conclusion, *Cx. tritaeniorhynchu* is currently the major vector for JE transmission in Taiwan. In light of its relatively high population density and wide distribution as well as being the predominant species carrying JE virus every year, we suggest that in addition to continuing the current JE vaccination policy, an environmental management measure, as a part of integrated control, should be initiated to decrease the number of *Cx. tritaeniorhynchus* in JE high-risk counties and, thus, to prevent the occurrence of the related mosquito-borne diseases.

INSIDE

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Keywords: Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles, *Culex fuscocephala* Theobald, *Culex annulus* Theobald, population density

Introduction

In Taiwan, three species of mosquitoes are responsible for the transmission of Japanese encephalitis (JE). They are *Culex tritaeniorhynchus* Giles, *Cx. annulus* Theobald (*Cx. vishnui*), *and Cx. fuscocephala* Theobald. The relative importance of each species in JE transmission depends on the number of mosquitoes infected with virus in each species in a given area. For example, *Cx tritaeniorhynchus* and *Cx. annulus* played a major role in northern and central Taiwan [1-5], but in southern Taiwan, three vector species shared the same role [6]. No relevant report has been found for eastern Taiwan. In earlier studies, Japanese encephalitis virus (JEV) was isolated from larvae of *Cx. tritaeniorhynchus* collected in northern Taiwan [7] and the transovarial transmission rate of JEV in laboratory-infected field mosquitoes was 1/281 [8]. These evidences supported that *Cx. tritaeniorhynchus* were very likely to be the natural host of JEV in Taiwan area to maintain virus circulation. Other possible reservoirs included are bats and birds.

Surveys conducted in early years indicated that JEV became active among field mosquito population during May-December in northern Taiwan. However, there seemed to be seasonal and geographical variations of JE virus isolations from mosquitoes over time. Twelve, and one isolates of JEV were identified in Taipei area in July and August, respectively, in 1958 while in 1959 only nine isolates were collected in June [9]. In 1963, JEV were isolated from two and seven pools of mosquitoes collected in Taipei area in July and August, respectively [3]. During 1967-1968, three, two, and two isolates of JEV were identified in July, August, and September, respectively, in Kuanhsi, Hsinchu County, which was concurrence with the peak of vector density [1, 2]. In 1971, six isolates of JEV were found in Taoyuan in July [5]. In 1978, twelve (including one from *Cx. quinquefasciatus* Say) and three isolates were collected in Taipei in July and August, respectively [4]. During 1981-1983, JEV were isolated from sixteen, one hundred and twenty-three, five, one, three, fourteen, and two pools of mosquitoes collected in Taishan, Taipei County in May, June, July, August, September, October, and November, respectively [7]. Especially noteworthy is the findings from a survey conducted in southern Taiwan during 1974-1976 indicating that JEV isolates from mosquitoes were detected mainly (77.9%) between May and July while some detected between October and December [6]. In addition, a survey carried out in nature parks of Taipei City during 2002-2004 found that JEV were detected almost year round in population of *Cx. tritaeniorhynchus* [10].

In Taiwan, JE vectors maintain in a very low population density in winter and keep reproducing in whole year without overwinter processes [3, 8, 11]. The major breeding sites for *Cx. tritaeniorhynchus* and *Cx. annulus* were paddy fields (46.0-63.1%), water-holding holes in the ground (16.6-26.0%), irrigation ditches (10.2-13.6%) and others, including buffalo footprints, fish ponds, wetland, cisterns, concrete water tanks, creeks, and natural ponds [12]. Earlier investigation showed that the monthly fluctuation of adult mosquito population was a single-peaked or double-peaked pattern depending on collection areas. The major peak occurred during the July-August period and the minor peak was found during April-May [8, 13, 14]. A survey conducted during 1990-1992 revealed that the highest density of *Cx. tritaeniorhynchus* was recorded in Changhua, central Taiwan in August (an average of 13,022-17,839 adult female mosquitoes captured per trap-night), followed by Taishan, Taipei County in northern Taiwan, peaked in July or September (an average of 426-444 female mosquitoes captured per trap-night), and Chaochou, Pingtung County in southern Taiwan, peaked in April or July (an average of 211-1,955 female mosquitoes captured per trap-night) [15].

All JE vectors prefer to feed on animal blood and occasionally on human blood. The analysis of blood-feeding source of JE vectors during 1970-1974 showed that the majority of *Cx. annulus* fed on pigs, followed by bovines, dogs, human, and others [16]. *Culex tritaeniorhynchus* and *Cx. fuscocephala* fed mainly on bovines and, then, on pigs. Another survey conducted during 1973-1974 indicated that *Cx. annulus* preferred to feed on pigs, and then on bovines, dogs, human, and others [17], while most *Cx. tritaeniorhynchus* fed on pigs, followed by bovines and visa versa for *Cx. fuscocephala*. These results described from above surveys referred only to the relative frequency of blood source available. In a recent survey on blood feeding sources of mosquitoes found that *Cx. tritaeniorhynchus* fed on pigs, bovines, dogs, human, horses, and non-chicken avian species, *Cx. annulus* fed on human, dogs, and bovines, and *Cx. fuscocephala* fed on bovines [18]. However, due to the small samples of blood-fed mosquitoes analyzed in this study, no preference could be implied. In other experiment providing choice on blood sources with three animals, all three vector species displayed high preference to feed on bovine (with the percentage of 98.5%, 73.0%, 69.1% for *Cx. fuscocephala*, *Cx. annulus*, and *Cx. tritaeniorhynchus*, respectively), followed by pigs (with the percentage of 1.5%, 20.7%, 26.9%, respectively), and human (with the percentage of 0.03%, 6.3%, 4.0%, respectively) [8]. The first JE case in 2009 was detected in Pingtung County in April (date of onset was March 30), more than one month earlier than previous years. To update the vector status through time was need. Therefore, the purposes of this study are to update the current status of JE vectors and the activity of JE virus in field mosquito vectors, and to understand the species distribution of JE vectors in Taiwan.

Materials and methods

This study was part of a field survey on *Anopheles minimus* Theobald conducted by local public health personnel in Tainan, Pingtung, Taitung, Hualien, and Kaohsiung Counties during June 2004-December 2008. The Pest-O-Lite light traps were hanged for one night (from 6:00 pm to 8:00 am in the following morning) in households with animals and streams around under mountain foothills. In 2008, Chiayi, Nantou, Ilan, and Miaoli Counties were added to the survey. All the mosquitoes caught in the survey were sent to the laboratory of Taiwan Centers for Disease Control (Taiwan CDC) for species and sex identification. Because the characteristics of survey sites (household with animals) and the collection method (hanging light trap at night) in this survey were the same as those for *Cx tritaeniorhynchus* survey, the data were also valid for the analysis for JE vectors. Additionally, JE vectors were collected in Tainan, Kaohsiung, Pingtung, Haulien, Taitung, Taichung, and Ilan Counties and Taipei City during 2005-2008 at high-risk areas, including pig farms, rural-type villages, and natural parks by light traps, sweep nets, and gravid mosquito traps. All mosquitoes captured were kept in dry ice and were sent to the laboratory of Taiwan CDC for species and sex identification. These mosquitoes, pooled by species and sex with a maximum of 50 per pool, were tested by real-time RT-PCR methods for detection of JEV.

Results

1. Species composition of JE vectors

The composition analysis of JE vectors caught from light trap in Taiwan showed that *Cx. tritaeniorhynchus* was the dominant species in all the survey sites, accounting for 64.1% (Kaohsiung County) to 100% (Chiayi County) of the total captured vector mosquitoes (Figure 1). The species of *Cx. fuscocephala* were collected in southern and eastern areas of Taiwan as well as Taipei City, accounting for 0.0008% (Chiayi County) to 30.0% (Kaohsiung County). No *Cx. fuscocepthala* adults were caught in Ilan, Miaoli, and Nantou Counties. *Culex annulus* was commonly captured in survey areas with a relatively small number, accounting for only 0.02% (Chiayi County) to 5.9% (Kaohsiung County).

2. Density of JE vectors

A single-peaked pattern for *Cx. tritaeniorhynchus* population was detected in Pingtung County in July and in Tainan, Kaohsiung, and Hualien Counties in June but a double-peaked pattern was found in Taitung County with the major peak in September and a minor peak in June (Figure 2). Among the ten surveyed counties/cities in 2008, Chiayi County recorded the highest mean number of mosquitoes (3,329 mosquitoes per trap-night) (Table 1) with the highest number of mosquitoes for single capture occurred in Puzi City (37,440 mosquitoes per trap-night), followed by Ilan County (971 per trap-night) with the highest number of mosquitoes for single capture occurred in Zhuangwei Township (8,554 mosquitoes per trap-night). The average number of mosquitoes captured per trap-night per year for recent five years ranged from 1 to 613 in Hualien County, with the highest number of mosquitoes for single capture occurred in Xincheng Township

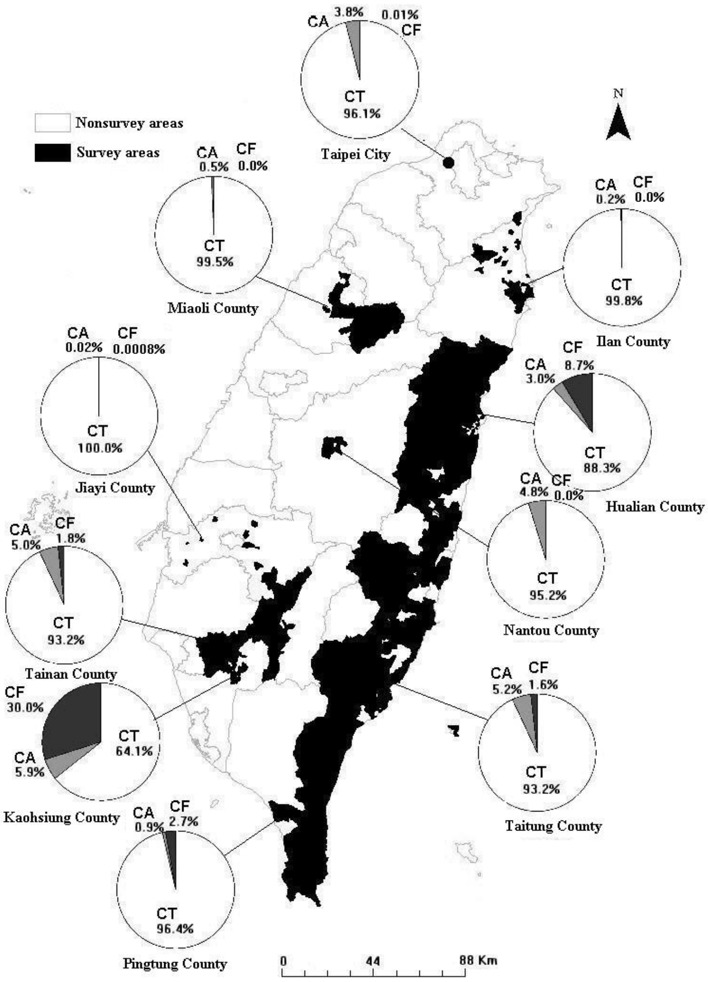

Figure 1. Species composition of three JE vector (*Cx. tritaeniorhynchus* Giles (CT), *Cx. annulus* Theobald (CA), and *Cx. fuscocephala* Theobald (CF)) captured by Pest-O-Lite light traps in each survey County, Taiwan, 2004-2008

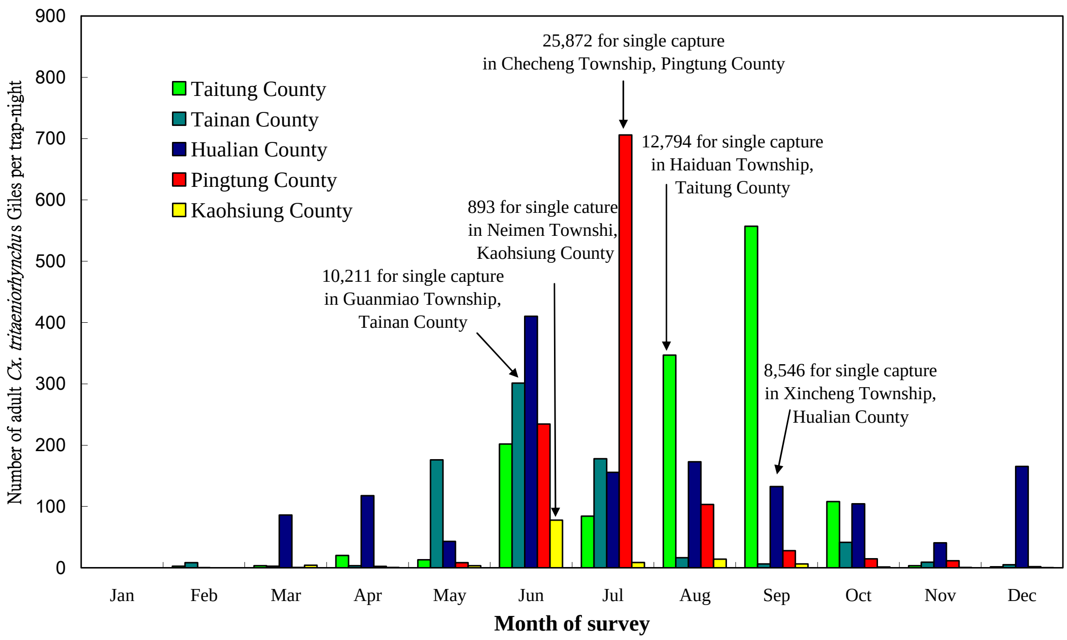

Figure 2. Monthly fluctuation of adult *Cx. tritaeniorhynchus* Giles captured by Pest-O-Lite light traps in Taitung, Tainan, Hualian, Pingtung, and Kaohsiung Counties during 2004-2008

Table1. Numbers of JE vectors captured by Pest-O-Lite light traps in counties of Pingtung, Taitung, Hualian, Tainan, Kaohsiung, Ilan, Miaoli, and Chiayi during June 2004-December 2008 (CT: *Cx. tritaeniorhynch*us, CA: *Cx. annulus*, and CF: *Cx. fuscocephala*)

Year	Counties	Pingtung	Taitung	Hualian	Tainan	Kaohsiung	Ilan	Miaoli	Chiayi	Nantou
2004	No. of traps	292	233	346	411	72	—	—	—	—
	Total no. of CT	2,955	678	506	3,558	43	—	—	—	—
	(mean*)	(10)	(3)	(1)	(9)	(1)				
	Total no. of CA	3	183	1,288	1,945	28	—	—	—	—
	Total no. of CF	244	3	8	122	0	—	—	—	—
2005	No. of traps	164	93	179	118	40	—	—	—	—
	Total no. of CT	2,835	301	2,162	2,512	17	—	—	—	—
	(mean)	(17)	(3)	(12)	(21)	(0)				
	Total no. of CA	642	26	1,121	774	8	—	—	—	—
	Total No. of CF	181	2	51	139	0	—	—	—	—
2006	No. of traps	200	170	140	120	40	—	—	—	—
	Total no. of CT	68,848	3,148	9,155	31,707	96	—	—	—	—
	(mean)	(344)	(19)	(65)	(264)	(2)				
	Total no. of CA	94	295	171	294	47	—	—	—	—
	Total no. of CF	1,323	26	1,017	232	0	—	—	—	—
2007	No. of traps	188	174	164	217	38	—	—	—	—
	Total no. of CT	2,373	26,341	100,507	17,969	466	—	—	—	—
	(mean)	(13)	(151)	(613)	(83)	(12)				
	Total no. of CA	29	363	1,208	178	185	—	—	—	—
	Total No. of CF	192	969	11,874	314	9	—	—	—	—
2008	No. of traps	122	214	167	175	108	36	27	36	36
	Total no. of CT	9,105	95,451	24,102	4,550	2,978	34,965	5,440	119,852	2,080
	(mean)	(75)	(446)	(144)	(26)	(28)	(971)	(201)	(3,329)	(58)
	Total no. of CA	33	6157	778	62	61	73	26	18	36
	Total no. of CF	463	1173	523	356	1,678	0	0	1	0

*Mean indicated the number of mosquitoes captured per trap-night.

(8,546 mosquitoes per trap-night). The mean numbers of mosquitoes per year collected in Taitung County were 3 to 446 mosquitoes per trap-night, with the highest number of mosquitoes for single capture occurred in Haiduan Township (12,794 mosquitoes per trap-night). Pingtung County and Tainan County collected 10 to 334 and 9 to 264 mosquitoes per trap-night in average with the highest single capture in Checheng Township (25,872 mosquitoes per trap-night) and Guanmiao Township (10,211 mosquitoes per trap-night). The average number of mosquitoes captured per trap-night in Miaoli County in

2008 was 201 and the highest single capture (2,559 mosquitoes per trap-night) was found in Nanzhuang Township. In Kaohsiung County, the average number of mosquitoes captured per trap-night per year for recent five years ranged from 0 to 28, with the highest number of mosquitoes for single capture occurred in Neimen Township (893 mosquitoes per trap-night). An average of 58 mosquitoes per trap-night was captured in Nantou County in 2008 with the highest single capture (387 mosquitoes per trap-night) in Yuchi Township.

3. JEV infection on local vector mosquitoes

JE virus became active among field mosquito population mainly in May and June but detected occasionally in other months (Figure 3). Six pools of *Cx. tritaeniorhynchus* (four from Haulien County, one from Ilan County, and one from Kaohsiung County) tested were positive for JE virus collected in May, seventeen pools (twelve from Taipei City and five from Ilan County) were positive in mosquitoes collected in June, one (from Taipei County) was positive in July, and one (from Haulien County) was positive in September. One pool of *Cx. fuscocephala* collected from Kaohsiung County in May was positive for JE virus.

Discussion

Culex tritaeniorhynchus was the dominant species among JE vectors in Taiwan, accounted for 64.1% to 100.0% of total number of JE vectors. Its population reached the peak during June-September. JE virus became active among field mosquito population during May-June, which occurred earlier (in May) in southern Taiwan and later (in June) in Taipei. JE virus activity was

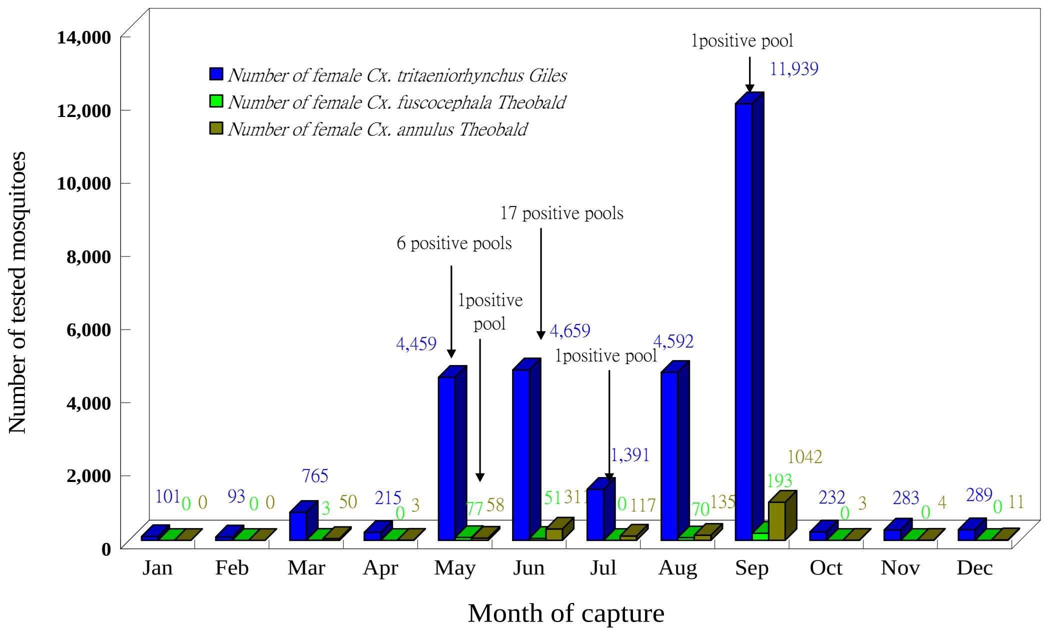

Figure 3. JE virus infection on JE vector mosquitoes by month in Taiwan, 2004-2008

Note: The numbers above the top of each bar mean the number of the tested mosquitoes in a specified species.

coincidence with the minor peak of JE vector density, not the major peak. Previous study indicated that the duration of viremia of JE virus in pigs or birds was 2-5 days. The infected JE vectors become infectious 5-15 days after blood-feeding of virus and maintain the transmissibility during their lifetime. The average survival time for female *Cx. tritaeniorhynchus* was 19.2 days [19]. During April-June, the JE mosquito density displayed in an increasing trend and the JE virus largely amplified inside pigs in Taiwan. Subsequently, Pigs develop antibody to JE virus and, thus, mosquitoes will no longer contract infection from these pigs. The scenario regarding JE virus activities indeed corresponds to the occurrence of JE human cases between 2004 and 2008, during which the cases began in May (accounted for 9.3% of total cases), reached its peak in June (41.3%), and then presented a decreasing trend during the later period (34.7% in July, 3.3% in August, 2.7% in September, 6.0% in October, and 2.7% in November) [20]. The first JE cases in 2009 occurred more than one month earlier than that in 2008, which probably resulted from warm winter weather. According to the data provided by Taiwan Central Weather Bureau, the average temperature in Hengchun area in January, February, March, and April 2009 is 21.3 ^{o}C, 23.4 ^{o}C, 23.6 ^{o}C, and 24.1^{o}C, respectively, in contrast to 21.9 ^{o}C, 20.3 ^{o}C, 22.9 ^{o}C, and 25.9^{o}C in 2008.

Culex annulus used to be the dominant species of JE vectors in Taiwan but it was replaced by *Cx. tritaeniorhynchus* in recent years. This replacement attributed probably to the fact that a large amount of cheap organophosphate insecticides were applied frequently in the paddy fields. This has resulted in a differential mortality of *Cx. annulus* and *Cx. tritaeniorhynchus*, which mainly breed in paddy fields and their surrounding irrigation ditches. Although this explanation lacks direct evidence, a previous study in Taichung County found that temephos, an organophosphate insecticide, had significantly increased the number of *Cx. tritaeniorhynchus* larvae [21] and a Japan study indicated that severe resistances to some organophosphate insecticides, such as temephos, malathion, Fenthion, and Fenitrothion, were commonly found among the field populations of *Cx. tritaeniorhynchus* [22]. Moreover, *Cx. tritaeniorhynchus* in Taiwan exhibited a r-type species of reproduction strategy [19] that made the species had a strong ecological adaptability and be able to survive in dramatically changing environment. The population density of *Cx. tritaeniorhynchus* was less than 250 mosquitoes per trap-night in the investigation conducted in sentinel sites during 1959-1970 [13]. Afterwards, the density increased substantially and reached an average of 211-17,839 female mosquitoes per trap-night in sentinel site investigation during 1990-1992 [15]. In this study, an average of 0-3,329 mosquitoes was collected in a larger area, not sentinel sites. Therefore, the highest number of mosquitoes for single capture ranged from 387 to 37,400, which indicated the overall high population density of *Cx. tritaeniorhynchus* in Taiwan. Additionally, the major peak of the population density of *Cx. tritaeniorhynchus* in these two surveys, for example Pingtung County, both occurred in July.

Culex. tritaeniorhynchus was the dominant species of three JE vectors in Taiwan and it can also act as a secondary vector of West Nile fever, Rift Valley fever, and filariasis. Recently, integrated vector management (IVM), which utilize various control measures, was proposed to control vectors. The control measures recommended using in paddy fields include environmental management, biological control, mechanical control and others [22]. The elements of environmental management are intermittent irrigation, flushing of fields, use of water-resistant rice cultivars, and shifting of planting schedules to avoid mosquito breeding. Other effective mosquito control measures in the IVM program include applying larvivorous fish [23] and *Bacillus thuringiensis israelensis* to kill mosquito larvae for reducing adult mosquito density [18]. Because of the high population density of *Cx. tritaeniorhynchus* and JE virus isolation routinely from field mosquito population every year in Taiwan, we suggest continuing the current JE vaccination policy, and initiating an integrated control program in JE high-risk counties to decrease vector density and, thus, to prevent the occurrence of the related mosquito-borne diseases.

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Botulism in Taiwan, 2007-2009

Yu-Ling Chou, Ji-Jia Huang, Wei-Ju Su, Ding -Ping Liu

Second Division, Centers for Disease Control, Taiwan

Abstract

Clostridium botulinum exists as spores in the environment. Intoxication caused by its toxin is called botulism. Botulinum toxin block the release of acetylcholine and hence nerve transduction, leading to partial or generalized paralysis and other neurological symptoms. However, consciousness remains clear. Without proper medical intervention, cases could develop paralysis of the respiratory muscles; without mechanical ventilation, patients may die. Botulism has been a reportable disease since infectious disease surveillance began in Taiwan. During 1991—2006, an average of 3 cases was reported each year. In 2007, the Department of Health promulgated botulism as a category IV communicable disease. The peak of case numbers appeared in 2008, followed by a decline in 2009. A retrospective analysis was conducted to understand risk factors of botulism. Twenty confirmed cases were identified during 2007–2009, and type A was identified as the major cause. Ingestion of soy products was identified as a possible risk factor (p value < 0.0001, Fisher exact test). However, many different kinds of food may lead to botulism. Hence, for the purpose of health education everyone should be taught about the risk to intoxication. In case investigation, public health professionals should know that, all foods and their process, storage, transportation and seasoning should be considered as possible source of intoxication, in addition to canned food, which traditionally thought to be the cause. Records should be obtained regarding storage of food and leftovers, to identify source of botulinum toxin.

Keywords: *Clostridium botulinum*, botulism, food poisoning

Introduction

Clostridium botulinum is a gram-positive, spore-forming, obligate anaerobic bacillus that can secrete neurotoxins. It can exist as spores in soils of land and sea [1]. Its spores remain active after heating at 100 °C for 1 hour at 1 atmospheric pressure; spores can also germinate, proliferate and produce toxins at 3 °C [2]. Intoxication by its toxin is called botulism. Initially, cases may have focal neurological symptoms, including ptosis, paralysis of the oculomotor muscles, and diplopia. Severe cases may have progressive generalized paralysis including the respiratory muscles [3]. Without proper treatment, neurological symptoms of botulism could lead to death when respiratory muscles are involved. Botulinum toxins can be categorized into seven types (A-G) according to their antigenic properties. Among them, types A, B, E and F cause disease in humans [4].The toxins can be inactivated by heating above 85 °C for at least 5 minutes. Spores of *C. botulinum* are ubiquitous in the environment and can germinate under proper conditions. Hence, contamination by botulinum toxins should be considered in food processing, packaging and storage, unless it is maintained at >100°C, < 3°C, or is dried [2, 5].

The 1986 botulism outbreak in Changhua County was the first fully documented botulism outbreak in Taiwan. Nine cases had type A botulism after eating canned peanuts from an unlicensed factory. Two people died. Epidemiological investigation showed that the food was contaminated by spores that germinated in the jars [6]. In 1986, the Department of Health published “Low Acid Canned Food Manufacturing, Formulating and Processing Facility Hygiene Standards” according to the Food Sanitation Management Law, and established a system for examination and registration of domestic low acid canned foods. Furthermore, the Department of Health issued the commercial sterilization standards in 1989. It was stated that after sterilization, no microorganism should proliferate in food while store at room temperature and no pathogenic live microorganism or spores should exist. Since then, botulism caused by canned foods has been rare.

Recent literature review showed that, in the Americas and Europe, botulism has been caused by home-canned vegetables, e.g. asparagus, garlic in oil, home-made foods, e.g. potatoes, cream soups, sausages, chili sauce, salads in restaurants, and commercial carrot juice [5,7-11]. Botulism is also frequent in China, caused by fermented soy products such as fermented (stinky) tofu, fermented black beans, bean paste, and fermented bean curds, salted fish, bacon, and salted duck eggs [12-15]. This shows that all food could cause botulism. Other than commercially prepared canned food, eating any homemade food, home-canned food, and refrigerated food with low-acidity (pH 5.0-7.0) or low-salt concentration (0.1-5.0% w/w) may cause botulism if eaten without heating [2]. People in different geographic areas, with different cultures and eating habits might be affected by different toxin types. For example, type E botulism occurred in Alaska, where aborigines eat fermented fish and roe [5]. In 1987, 1990, 2006 and 2008, type B botulism caused by eating salted muntjac meat, salted goat meat and salted bird meat was found in hill tribes in Nantou County, Ilan County, and Miaoli County, respectively [16-18]. In recent years, the majority of botulism in Taiwan is caused by type A botulinum toxin, and only a few cases were caused by type B toxin.

There are four types of botulism. Food borne botulism is caused by ingestion of food contaminated by toxins. Intestinal botulism, traditionally called infant botulism, is caused by ingestion of spores by individuals without established intestinal normal flora. Wound botulism results from toxins produced by germinated spores colonizing wounds. Other (iatrogenic) botulism is caused by using unlicensed botulinum toxins or overdose during cosmetic procedures. In recent years, botulinum toxins have been considered a potential bioterrorism agent. Contaminated commercial products may cause panic, social disturbance and economic loss [19]. Therefore, causes of all botulism need to be clarified if possible. In October 2007, botulism became a category IV communicable infectious disease. Suspected cases need to be reported immediately, and preventive measures implemented to control potential outbreaks.

Botulism has been a reportable disease since infectious disease surveillance began in

Taiwan. Since 1991, about three cases were reported each year. However, during 2007–2009, there were 14, 17, and 1 case reported, and 8, 11, and 1 case confirmed, respectively, showing an increase (Figure). We reviewed the cases confirmed during 2007–2009, results of case investigation, and analyzed the relationship between cases and suspected foods, to find possible causes.

Cases included

All reported botulism cases reported during 2007–2009 in the Communicable Disease Reporting System of the Taiwan Centers for Disease Control (CDC).

Case investigations

Investigation was done by local health authorities for confirmed botulism cases during 2007–2009 and reports were transmitted to database of the CDC.

Case definition

A confirmed case is illness in a person with neurological symptoms (including diplopia, blurred vision, bulbar palsy, symmetric paralysis) with *Clostridum botulinum* isolated from stool or vomitus, or is serologically positive for botulinum toxins.

Results

During 2007-2009, CDC received 35 reported botulism suspected cases; 20 were confirmed. Among them, animal neutralization assays showed that 85% were caused by toxin type A and 10% by type B. Etiology of 5% of the cases could not be identified due to inadequate amount of sera.

During 2007–2008, there was clearly a peak, with 19 confirmed cases. Only one case was confirmed in 2009. In 2008, there were three clusters, including type A botulism in two sisters in Hsinchu City in February [20], two cases of type A botulism in Taichung City in the same month, and type A botulism in a family of four people in Kaohsiung County.

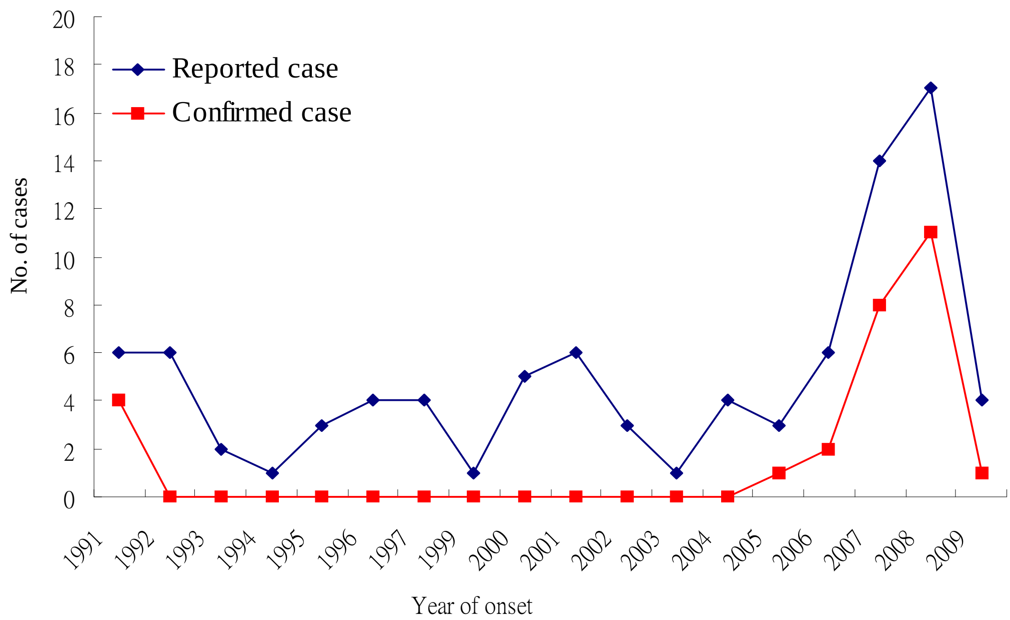

Fig. Numbers of reported and confirmed botulism cases in Taiwan during 1991—2009

There were seven men and 13 women (1:1.86); one men died. They were aged were 22-74 years (average: 47.5, median: 50.5). Most cases were aged 25-34, and 45-54 years. Most of the cases aged 25-34 were men, but cases aged 45-54 years were mostly women. Geographically, Kaohsiung County had the most cases (4), followed by Taichung City (3) and Hsinchu City (3). Sporadic cases appeared in western Taiwan and Ilan County. There were 5 (25%) farmers (Table).

Table. Demographics of confirmed botulism cases in Taiwan During 2007–2009 (N = 20)

Characteristic	Cases	%
Ages		
15 - 24	1	5
25 - 34	5	25
35 - 44	2	10
45 - 54	5	25
55 - 64	3	15
65 - 74	4	20
Residence		
Kaohsiung County	4	20
Hsinchu City	3	15
Taichung City	3	15
Tainan County	2	10
Taipei County	2	10
Yunlin County	2	10
Taichung County	1	5
Miaoli County	1	5
Kaohsiung City	1	5
Ilan County	1	5
Occupation		
Farmer	5	25
None	3	15
Housewife	2	10
Public relations	2	10
Student	1	5
Security	1	5
Draftee	1	5
Nursery teacher	1	5
Tour bus driver	1	5
Chef	1	5
Recycle	1	5
Unknown	1	5

Most frequent clinical symptoms include difficulty breathing (55%), ptosis (55%), dysphagia (50%), paralysis of extraocular muscles (45%), diplopia (30%), vomiting (25%), and difficulty speaking (15%).

Food history was not available for two cases (one died and one was intubated). Most frequently suspected foods, in 18 of the cases with food history available, were: 1. soy products (19 items), including fermented soy products such as fermented bean curd, fermented (stinky) tofu, vacuum sealed bean curds, tofu, soy milk (65% of the cases); 2. canned foods (9 items), including vegetables, shredded bamboo shoots, conch (25% confirmed cases); 3. wheat products (8 items), including noodles, and steamed bread; 4. peanuts, alone or with pork (7 items); 5. mustard (6 items); and 6. sausages (6 items).

The majority of suspected foods was vegetable protein-related products (e.g. soy), not animal proteins, as traditionally believed. Among the 20 confirmed cases, 13 had ingested soy products, and the 15 cases without botulism did not consume soy products (*p value<0.0001*, Fisher exact test). All suspected foods were negative for the toxin.

Discussion and suggestion

Soy products are very likely risk factors of botulism. Although botulism cannot be transmitted from human to human in the community like other infectious diseases, it can threaten the health and lives of the cases. The toxin can block nerve transmission and paralyze respiratory muscles. Severe cases may require mechanical ventilation support. Its long recovery period may also become a financial burden to the family.

Although cases were confirmed, toxins were not found in food samples. In the past, health authorities usually suspected canned or pickled foods, and ignored other incompletely sterilized foods that may be contaminated by the spores. Sealing or vacuum–packing these food products create condition suitable for spore proliferation [5-9]. Restricting suspected foods to canned products may limit our ability to unify the findings in human specimen, case investigation and foods testing. In this retrospective study of suspected foods ingested by confirmed cases during 2007–2009, soy products including fermented bean curds, fermented tofu, vacuum sealed bean curds, were the most frequently associated foods. Statistic analysis showed that soy products are very likely risk factors for botulism. Protein contents of soy products are higher than other vegetable products. Commercial soy products are usually not sterilized by autoclaving, but vacuum sealed. They are also often stored at room temperature, not refrigerated. This creates the condition for soy products to become risk factors for botulism. Improving the quality and safety of soy products is a new challenge for the food industry. A cluster of botulism occurred in a family in Hsinchu City in early 2008 [20], and epidemiological study suspected that vacuum sealed soy products might be the cause. However, the suspected food cannot be confirmed by laboratory testing. TFDA reviewed recent sporadic botulism cases and found that soy products from a certain supplier were associated with two events and suspected that its soy products was the source of botulism. Local health authorities were asked to inspect the manufacturer to improve its environmental hygiene. A meeting for commercial vacuum sealed soy and wheat products was held on March 21, 2008 by the health bureaus, industry associations and other authorities. Discussions were also held in a meeting for food poisoning prevention on October 6, 2008, allowing manufacturers and local health authorities to understand more about botulism and improve food production and storage. In 2009, botulism confirmed cases had decreased, showing that strengthening management of food manufacturers can decrease risk of botulism significantly.

Factors contributing to botulism could be very complicated. In the future, health education should assume that everybody could be affected and explain in detail possible causes of botulism, especially for people consuming soy frequently. While conducting case investigation, public health professional should not only suspect canned foods. Any food, because of their manufacturing and transportation, could be the cause of botulism, including the seasoning used. Storage of leftover needs to be clearly documented as well [5, 7-11]. The communicable disease surveillance system of the CDC should add soy products as a suspected food to help find sources of the toxins and prevent further transmission.

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