Outbreak, Surveillance and Investigation Reports

Volume 8, Issue 1
March 2015

Outbreak, Surveillance and Investigation Reports

eISSN: 2286-8933

Outbreak, Surveillance and Investigation Reports (OSIR) Journal

Field Epidemiology Training Program

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Contents

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Outbreak of Newcastle Disease among Recipients of the Poultry Dispersal Program in Bohol, Philippines, February 2013

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Abstract

Newcastle disease (ND) is a disease endemic among poultry that causes huge economic losses in the Philippines, including the Province of Bohol. On 5 Mar 2013, the Office of the Provincial Veterinarian (OPV) received a report on high mortality of native chickens in four villages of Valencia, Bohol. A team from OPV went to the affected villages to conduct a disease investigation. Objectives of the investigation were to determine the cause and source of the outbreak, and recommend control measures to prevent future outbreaks. Initial information revealed that all affected farms received native chickens distributed by a government agency. The investigation was, hence, conducted in all 47 recipient farms using a structured questionnaire. Blood samples from ill chickens without history of vaccination were collected for hemagglutination inhibition (HI) test to determine antibodies against ND virus. Clinical signs and serological test suggested that the outbreak was caused by ND virus. Univariate analysis revealed that parent stocks which were introduced to farmers on 28 Feb 2013 were the risk factor (OR=19.2, 95% CI=3.5-105.9) for the outbreak in the four villages. Traceback investigation identified possible source of infection as the introduction of infected poultry from a dispersal program of the government. This outbreak underscored the need to strengthen movement control in all ports of entry in the province. Likewise, chickens under the dispersal programs must be vaccinated and quarantined before distribution to recipient farmers.

Key words: newcastle disease, poultry, Bohol, Philippines

Introduction

Newcastle disease (ND) is a poultry disease caused by paramyxovirus type 1 (APMV-1), genus Avulavirus, belonging to the subfamily Paramyxovirinae, family Paramyxoviridae.[1,2] It is characterized by gastrointestinal, respiratory and neurological signs.[1-3] Mode of transmission is through direct contact with infected birds or indirect contact through inanimate objects.[1-4] Newcastle disease virus (NDV) is also a human pathogen and the most common sign of infection in humans is conjunctivitis that develops within 24 hours of NDV exposure to the eye.[3,4] Reported infections in human have been non-life-threatening and there is no evidence of human-to-human spread.[3-6]

ND affects about 200 birds species, including chickens around the world.[5] ND has been regarded as endemic since its recognition in Indonesia and England during 1926.[7] In the Philippines, the disease occurs all year round in all 81 provinces, including the Province of Bohol wherein poultry raising is an important mean of livelihood for smallhold farmers. Poultry population in the province comprised largely of native chickens produced by smallholders for about 90%. Thus, ND has been a serious problem as it causes major losses to these farmers. To prevent these losses, the Philippine Bureau of Animal Industry (BAI) has developed the Newcastle Disease Control Program (NDCP) in 2003. The program included disease surveillance, vaccination, quarantine, information, education and communication campaign, and seminars for livestock technicians, which aimed for early detection and recognition of suspected ND cases. In vaccinating poultry, live viruses of low virulence (lentogenic) or moderate virulence (mesogenic) and inactivated vaccines are used depending on disease situation and requirements of BAI.[8] ND is diagnosed through virus isolation and subsequent characterization as well as hemagglutination inhibition (HI) test.[2-7] Although HI test is used widely in ND serology, its usefulness in diagnosis depends on immune status of the birds to be tested and prevailing disease conditions.[2]

Although the disease is considered as endemic in Bohol, there has been no record of ND outbreaks since 2011, based on available data from the Philippine Animal Health Information System and records of the Office of the Provincial Veterinarian (OPV). Surprisingly, on 5 Mar 2013, the OPV received a report from the Municipal Agriculture Office (MAO) of Valencia, Bohol about massive deaths of native chickens in four villages. Furthermore, MAO and village officials confirmed that there had been no unusual high mortality among chickens in smallhold farms of the area for many years prior to this event.

On 7-8 Mar 2013, a team with veterinarians and livestock technicians was dispatched by OPV to the affected villages to determine the cause, possible risk factors and source of the outbreak, and provide recommendations to prevent future outbreaks.

Methods

The outbreak investigation was conducted on 7-8 Mar 2013 in four villages (barangays) of Valencia, Bohol, Philippines, which included Adlawan, Canduao Occidental, Canduao Oriental and Canlusong (Figure 1). All 47 farms receiving parent stocks of native chicken in Bohol were studied.

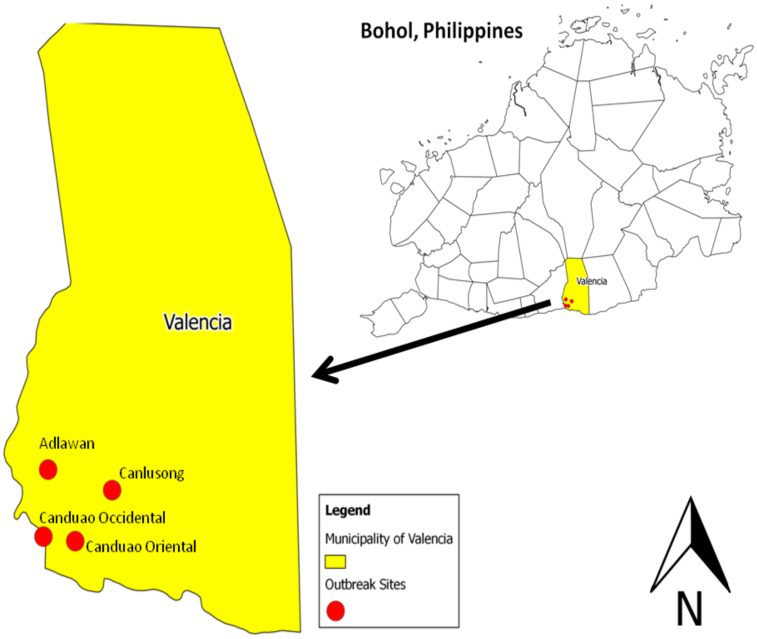

Figure 1. Map of villages affected by newcastle disease outbreak in Valencia, Bohol, Philippines, February 2013

A case was defined as a poultry farm in one of the four villages with a mortality rate 10% or more and reported any of the following signs: respiratory signs (sneezing, gasping for air, nasal discharge, coughing), nervous signs (depression, muscular tremors, drooping wings, twisting of head and neck, circling, complete paralysis), digestive signs (watery diarrhea),[1,3-6] swelling of eyelids or neck, and sudden death[3] during the period from 1 Feb to 7 Mar 2013.

Structured questionnaires were used to interview the affected farm owners and captured possible risk factors, including type of raising system, practice of vaccination, source of water, feeds used, distance to the nearest poultry farm and source of newly introduced parent stocks. Total 23 blood samples were taken from ill chickens without history of vaccination for HI test[9] to determine presence of antibodies against NDV. The test was conducted at the Regional Animal Disease Diagnostic Laboratory (RADDL) of the Department of Agriculture - Regional Field Office (DA - RFO) in Cebu Province.

Descriptive statistics was calculated using Microsoft Excel® and map of the outbreak location was plotted using Quantum Geographic Information System (QGIS) software. Level of association and significance between possible risk factors and disease outcome were computed using Epi Info version 3.5.4.[10]

Results

Of total 36 farms affected (76.6%), all were smallholders with less than 60 birds. Adlawan and Canduao Oriental villages had the highest attack rate (Table 1). Two waves of the outbreak were observed, and the number of affected farms during the second wave, which took place from 28 Feb to 1 Mar 2013, was much higher (Figure 2). The second wave started on 28 Feb 2013, the same day that parent stocks of native chicken were introduced into the farms.

Almost half of farmers in Adlawan (46.8%) had less than 20 heads of native chickens. Majority of the affected farmers had more than 30 years experience in poultry raising (97.8%).

HI test revealed that 91.3% (21/23) of bird samples tested positive for antibodies against NDV. Although the affected farms reported several respiratory, nervous and digestive signs of poultry disease within the period from 1 Feb to 8 Mar 2013, the most frequent signs reported were serous nasal or oral discharge and sudden death among birds (Figure 3).

All affected farms received the parent stocks of native chicken from a national government agency under the livestock and poultry dispersal program for smallholders. The parent stocks were introduced into the affected farms for two times on 13 and 28 Feb 2013, which were provided by a supplier in Bohol and a supplier from a live-bird market in nearby island province of Cebu respectively (Figure 4).

Table 1. Descriptive analysis of native chicken farms in Bohol, Philippines, February 2013 (n=47)

Variable	Category	Total number of farm	Attack rate (%)
Village	Adlawan	20	42.6
	Canduao Occidental	5	10.6
	Canduao Oriental	16	34.0
	Canlusong	6	12.8
Farm size (bird)	(Mean=24.7, median=20, mode=20)		
	<20	22	46.8
	20-40	15	31.9
	41-60	8	17.0
	>60	2	4.3
Age of owner (year)	(n=46, mean=53.7, median=54, mode=54)		
	<30	1	2.2
	30-40	6	13.0
	41-50	12	26.1
	51-60	17	37.0
	>60	10	21.7
Distance from the nearest farm (meter)	(n=45, mean=55.4, median=20, mode=20)		
	<20	12	26.7
	20-40	10	22.2
	>41	14	31.1

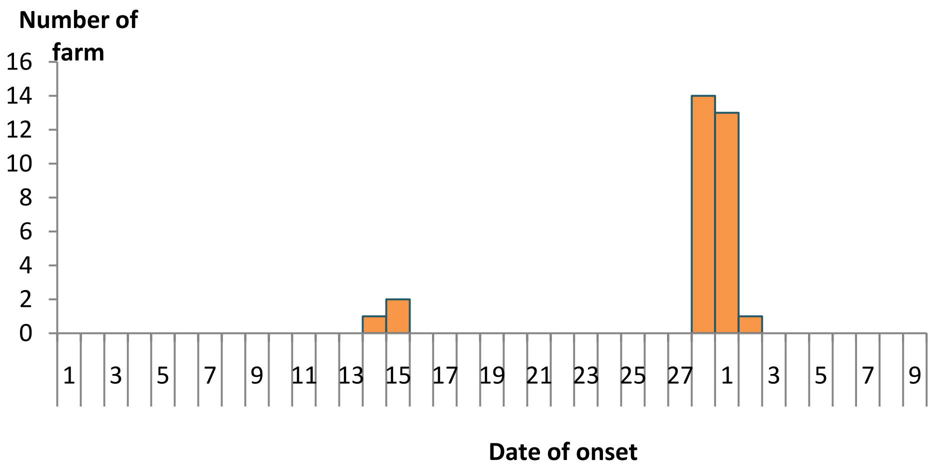

Figure 2. Date of onset of affected native chicken farms in Bohol, Philippines, February 2013 (n=36)

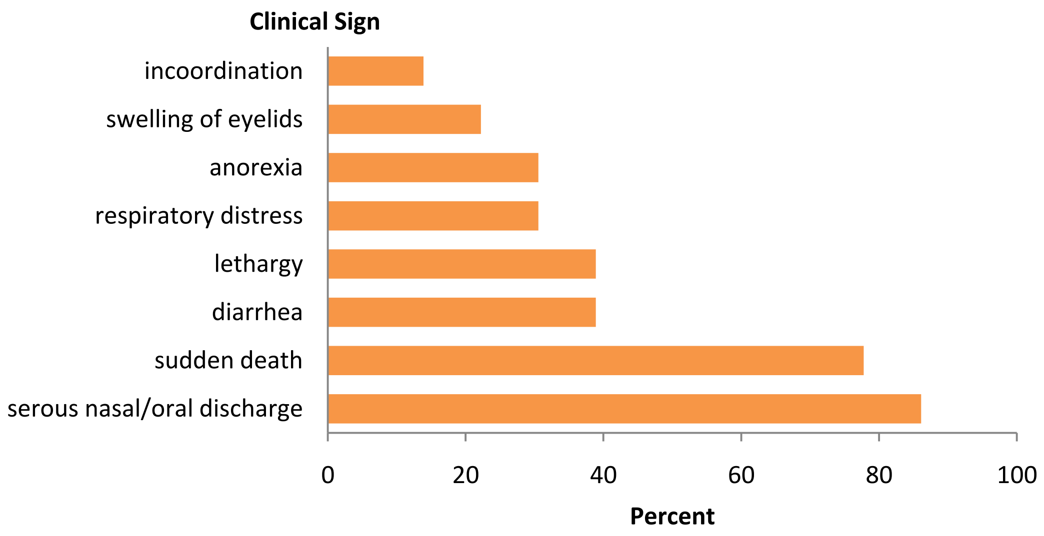

Figure 3. Most frequently reported clinical signs in affected native chicken farms in Bohol, Philippines, February 2013

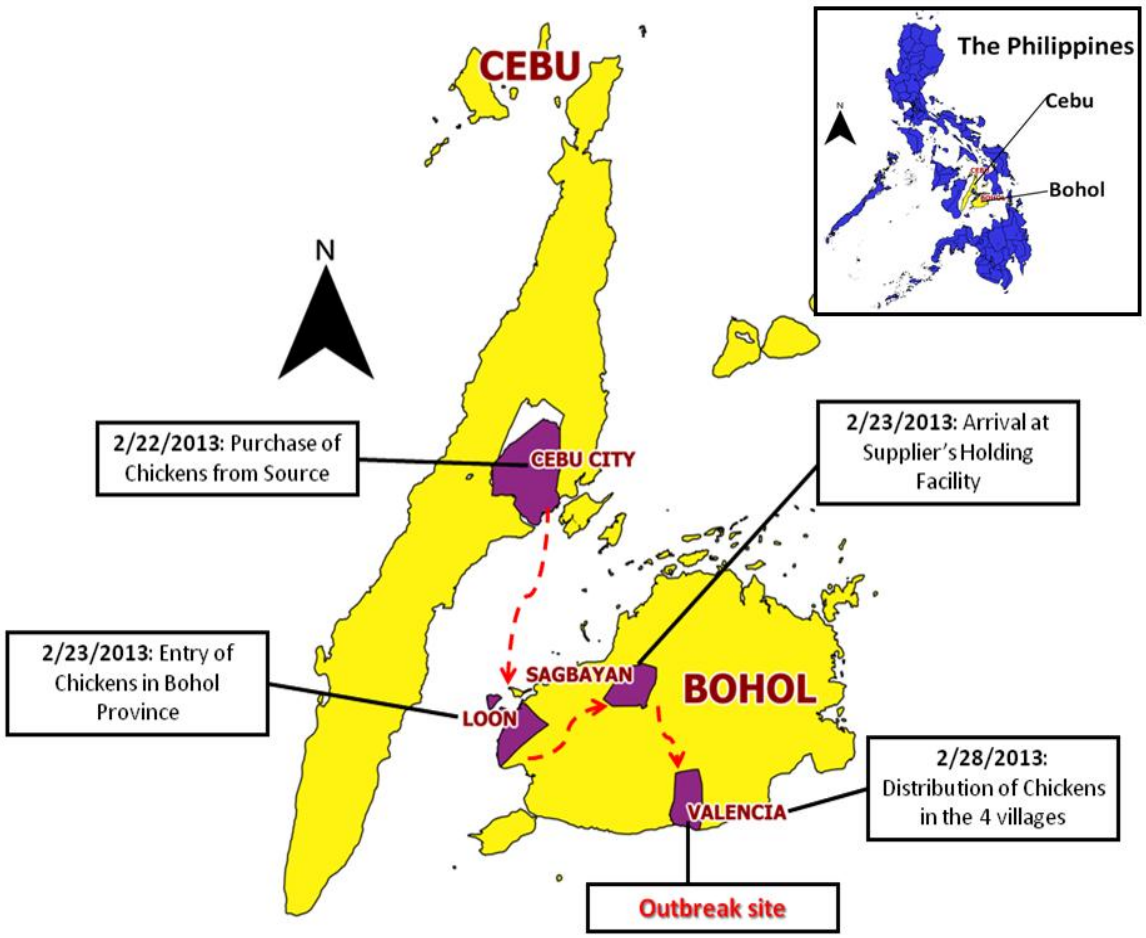

Figure 4. Transport route of parent stocks of native chickens introduced to farms in Valencia, Bohol, Philippines on 28 Feb 2013

Introducing parent stocks to farms on 28 Feb 2013 was a significant risk factor (OR=19.2, 95% CI = 3.50-105.87) for ND outbreak among native chickens in four villages (Table 2). Massive ND vaccination in all chicken population in the 4 affected and adjacent villages, education and communication campaign among villagers and local officials about backyard biosecurity and movement restriction were done by OPV to control the outbreak. The disease outbreak lasted for two and half months from 28 Feb until 25 May 2013 when the livestock technicians no longer reported additional cases in four villages.

Table 2. Univariate analysis of farm characteristics and disease outcome in Bohol, Philippines, February 2013 (n=47)

Variable	Number of farm (%)	Odds ratio (95% CI)
Farmer's age <60 years (n=46)		
Yes	33 (71.7)	1.7 (0.39-6.99)
No	13 (28.3)	Reference
Years in poultry raising (n=46)		
< 10	10 (21.7)	1.3 (0.24-7.47)
≥ 10	36 (78.3)	Reference
Farmer's education (n=45)		
Elementary and below	14 (31.1)	0.6 (0.14-2.59)
High School and higher	31 (68.9)	Reference
Type of raising system		
Free-raising	36 (76.6)	1.3 (0.28-6.14)
Strictly confined	11 (23.4)	Reference
Vaccination		
No	42 (89.4)	0.8 (0.07-8.01)
Yes	5 (10.6)	Reference
Source of water		
Rain water, canal, stream	30 (63.8)	0.6 (0.14-2.83)
Municipal water	16 (34.0)	Reference
Feeds used		
Own feed formulation	34 (72.3)	0.2 (0.02-1.75)
Commercial feeds	13 (27.7)	Reference
Distance to the nearest poultry farm (meter)		
≤ 20	24 (51.1)	0.8 (0.22-3.23)
≥ 21	23 (48.9)	Reference
New parent stocks introduced on 28 Feb 2013		
Yes	37 (78.7)	19.2 (3.50-105.87)
No	10 (21.3)	Reference
New parent stocks introduced on 13 Feb 2013		
Yes	17 (36.2)	0.1 (0.03-0.58)
No	30 (63.8)	Reference

Discussion

Clinical signs and serological test suggested that the outbreak was caused by NDV. Although the majority of clinical signs in affected poultry farms began on 28 Feb and 1 Mar 2013, the epidemic curve showed that there was another outbreak that started on 14 Feb 2013. This information coincided with the introduction of new stocks through the poultry distribution program of the government, which was a significant risk factor as well. The infection might occur from their origin or during transportation. According to a Provincial Quarantine Officer, under the Philippine quarantine regulations, livestock and poultry traders must be able to secure a livestock handler's license and shipping permit issued by provincial or regional quarantine officers and a veterinary health certificate issued by a licensed veterinarian before their shipments were allowed to enter any island province like Bohol. However in this case, the supplier admitted that he did not possess any of the above-mentioned documents. Furthermore, the shipment had not been inspected by quarantine officers at the port of Bohol since the trader disembarked at a small port outside the capital city, where there was no designated quarantine officer.

Limitations

Although all 48 recipients of the native chicken dispersal program in the province were included in the study, the small sample size was the most likely reason for very wide confidence intervals in the inferential analysis of risk factors and disease outcome.

Conclusion

The outbreak was caused by ND brought about by parent stocks of native chickens introduced into the farms on 28 Feb 2013 under a government-initiated native chicken dispersal program. Chickens without history of vaccination were provided by the commissioned supplier from different live-bird markets in the neighboring island province and introduced into the four villages in Bohol where there was no vaccination. Failure to intercept the entry of poultry shipment at the port of disembarkation in Bohol also contributed to the disease outbreak.

Recommendations

This outbreak underscored the need to strengthen veterinary quarantine regulations in all ports of entry in the Province of Bohol, including sub-ports outside the capital city. Chickens under the dispersal programs must be vaccinated against ND and quarantined before distribution to farmers. The results of this investigation, including the recommendations, were presented to the concerned government authorities such as the Provincial Quarantine Office, Department of Agriculture, poultry farmers and other relevant stakeholders for their information and corresponding actions.

Acknowledgment

The authors would like to thank the following institutions that made this study possible: Field Epidemiology Training Program for Veterinarians (FETPV) Thailand, Field Epidemiology Training Program (FETP) Thailand, Food and Agriculture Organization of the United Nations (FAO), Provincial Government of Bohol, Philippines through the Office of the Provincial Veterinarian (OPV) and Local Government Unit of Valencia, Bohol, Philippines.

Suggested Citation

Tapdasan EP, Wongsathapornchai K, Chanachai K, Benigno CC, Gundran RS, Daguro LI, et al. Outbreak of newcastle disease among recipients of the poultry dispersal program in Bohol, Philippines, February 2013. OSIR. 2015 Mar;8(1):1-6. <http://www.osirjournal.net/issue.php?id=75&year=2015>.

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OSIR, March 2015, Volume 8, Issue 1, p. 7-12

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Environmental and Social Conditions Prevailing Dengue Fever Outbreak in Pasir Mas District, Kelantan State, Malaysia, 2010

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Abstract

A dengue outbreak occurred in Pasir Mas District, Kelantan, Malaysia during 2010. We conducted an investigation to describe the clinical and epidemiological characteristics of cases, determine risk factors for transmission and recommend appropriate control measures. A dengue case was defined as an individual with acute febrile illness and two or more of the following symptoms: rash, arthralgia, headache, myalgia, retro-orbital pain, hemorrhagic manifestation or leucopenia. Environmental surveys were done to search for *Aedes* species. A matched case-control study was done. From 5 May to 12 Aug 2010, total 465 cases were identified, with male to female ratio as 1.2:1. Most cases (34%) belonged to 11-20 years old group, followed by those aged 21-30 years (14%). Cases were mostly students (40%) or rubber tappers (15%). *Aedes aegypti* and *Aedes albopictus* species were identified in the district while many cases resided in areas with *Aedes* index of more than 1% and breteau index of more than 5%. Risk factors for getting dengue infection were presence of discarded containers within household premises (adjusted OR = 15.1, 95% CI = 5.41-41.97) and not using protective measures (adjusted OR = 3.9, 95% CI = 1.21-12.55). Control activities focused on mass clean-up and health education campaigns in affected communities. As a conclusion, this outbreak involved active individuals, and contributed by presence of breeding containers and not using personal protective measures.

Key words: dengue, *Aedes*, disease outbreak, case-control study

Introduction

Dengue is found in tropical and sub-tropical regions, predominantly in urban and semi-urban areas. There are four distinct serotypes of dengue virus (DEN 1, 2, 3 and 4), which are transmitted by *Aedes* mosquitoes. Although infection with one dengue serotype provides lifelong immunity to that serotype, there is no cross-protective immunity to other serotypes.[1] Symptoms usually appear after incubation period of 4-10 days and last for 2-7 days. Dengue virus infections can be asymptomatic or lead to undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[2]

In Malaysia, dengue is one of the notifiable diseases. All medical practitioners who diagnose dengue cases must report them to the nearest district health office.[3] Verification, investigation and control activities are carried out within 24 hours of notification by district health staff (Figure 1). Criteria for a dengue outbreak are occurrence of at least two cases in 14 days within an area of 400 km or at least two cases in 14 days with the epidemiological linkage.[4]

In May 2010, there were increased reported dengue cases from Pasir Mas District of Kelantan Province. Pasir Mas District has an area of 577.52 km^2, with a population of 206,400. It is bordered by Thailand to the west (Figure 2). Pasir Mas has a tropical climate, with 21-32°C temperature and intermittent rain throughout the year. There are many small rubber plantations in the area.[5] Pasir Mas was not a dengue problematic district. The incidence rate in 2009 was 56 per 100,000 population. The incidence was low compared to the incidence rate of Kelantan State (63.1 per 100,000 population) and the weekly number of cases for 5-year median was less than 10 cases. This district never experienced more than two localities of dengue outbreak at one time.

Since the number of reported dengue cases was much more than the median number reported during the past five years, we conducted an investigation to describe the clinical and epidemiological characteristics of cases, determine the risk factors for transmission and recommend appropriate control measures.

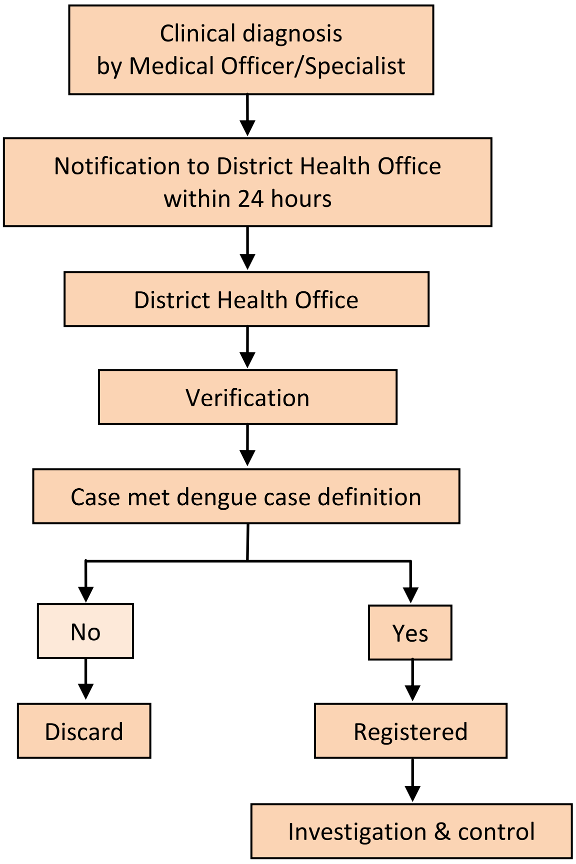

Figure 1. Dengue surveillance and response system in Malaysia, 2010

Methods

Descriptive Study

We reviewed case report forms of dengue cases that were reported from Pasir Mas District during 5 May to 12 Aug 2010. A dengue case was defined as an individual with acute febrile illness and two or more of the followings: rash, arthralgia, headache, myalgia, retro-orbital pain, hemorrhagic manifestation or leucopenia. Definitions were set up for all variables under interest as well (Table 1).

Blood samples were collected from some hospitalized cases for dengue IgM antibody detection by enzyme-linked immunosorbent assay (ELISA).[6]

Environmental Investigation

Households of the reported cases were visited and premises were searched for possible *Aedes* breeding sites within 50 meter radius from the house. Possible breeding containers were inspected for presence of *Aedes* larvae. If no larvae were detected, the search was widened up to 200 meter radius. *Aedes* index (AI) more than 1% and/or breteau index (BI) more than 5% indicated dengue sensitive areas. The following indices were computed for area around each house:[4]

$$\textit{Aedes}\text{ Index (AI)} = \frac{\text{Number of premises positive for } \textit{Aedes} \text{ larvae}}{\text{Number of premises inspected around the case's household}} \times 100$$

$$\text{Breteau Index (BI)} = \frac{\text{Number of containers positive for } \textit{Aedes} \text{ larvae}}{\text{Number of premises inspected}} \times 100$$

Case-control Study

We conducted a 1:1 matched case-control study with 80 subjects in each group. Cases who met the surveillance dengue case definition were selected among those reported by the surveillance system from 25 Jul to 14 Aug 2010 with systematic sampling until

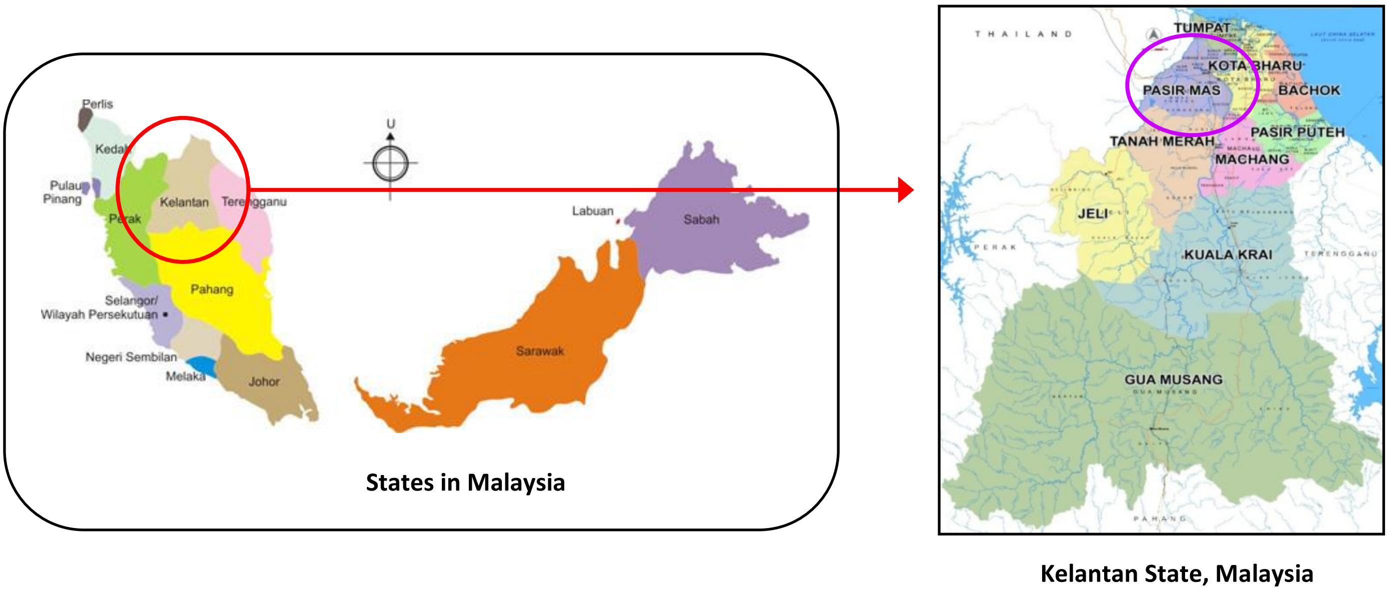

Figure 2. Location of Pasir Mas District, Kelantan, Malaysia

Table 1. Definitions of the variables under interest

No.	Variable	Definition
1	House in the estate	The house was located inside the estate, regardless the type of estate.
2	Often outside house in the morning and evening	Spent at least 30 minutes outside the house in the morning or evening
3	Protective measures	long-sleeved shirt and pant, using of mosquito net, spray, coil or repellent
4	Water container inside the house	Presence of any container to collect the water inside the house
5	Ant trap	Presence water container to trap the ant, usually put under the legs of table
6	Open well	Presence of open well inside or outside the house
7	Flower pot plate	Presence of water collector under the flower pot
8	Water container for bird drinking	Presence of water container which used as drinking water for birds
9	Bamboo stump	Presence of bamboo stump which can collect the rain water in the house compound
10	Water container outside the house	Presence of any container to collect the water outside the house, including rain water or tap water
11	Latex collector cup	Presence of any container used for latex collector, including bowl or coconut shell
12	Disposable container	Present of disposable container in the compound such as unused bottles, cans, plastic containers, polystyrene as there was no proper solid waste disposal
13	Tyre	Presence of unused tyre outside the house
14	Water container for animals	Presence of water container for drinking by cow, goat or other animals

the required sample size was reached. The cases were randomly and independently selected, and had equal variance. Controls were asymptomatic individuals from the nearest house without any ill family member and were matched for age. Face-to-face interviews of both cases and controls were conducted by five staff who were trained to use a standard questionnaire, and obtain information on demographic characteristics and possible behavioral risk factors such as using of protective measures against mosquitoes. In addition, inspection of the subjects' premises was done to identify the environmental variables such as location of houses, and presence of water containers and disposable containers. Data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago III) to calculate odds ratio (OR) and 95% confidence interval (CI). Logistic regression was also performed for all variables and those with p-value less than 0.05 were included in the multivariate model.

Results

Dengue Surveillance

From 5 May to 12 Aug 2010, there were total 465 dengue cases reported in Pasir Mas District. Blood samples were collected from 210 cases, and of these, 162 (77%) were positive for dengue IgM antibody.

All the cases presented with fever, and other associated common symptoms were myalgia (95%), headache (95%), vomiting (73%) and arthralgia (65%) (Figure 3). About 64% (298/465) of the cases were hospitalized while there were four fatalities, with case fatality rate of 0.9%. During 2010, the number of cases began to increase in May (the epi week 20), reached the peak in August and then, declined to normal level by the end of October (Figure 4). The cases were distributed throughout the district.

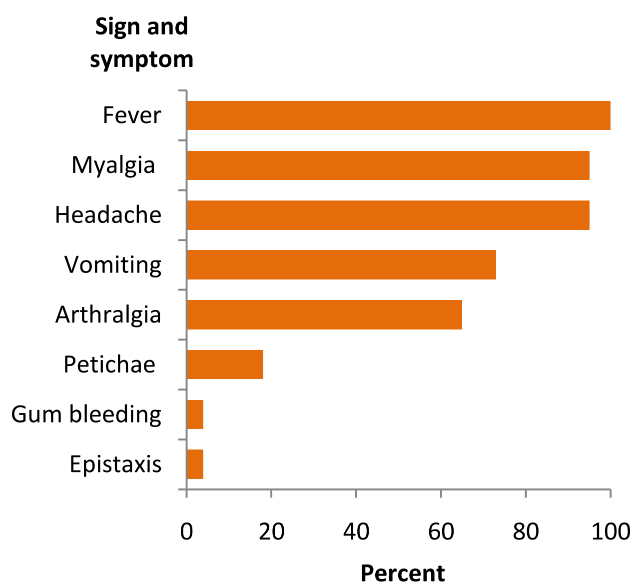

Figure 3. Signs and symptoms of dengue cases in Pasir Mas District, Kelantan, Malaysia, 5 May to 12 Aug 2010 (n=465)

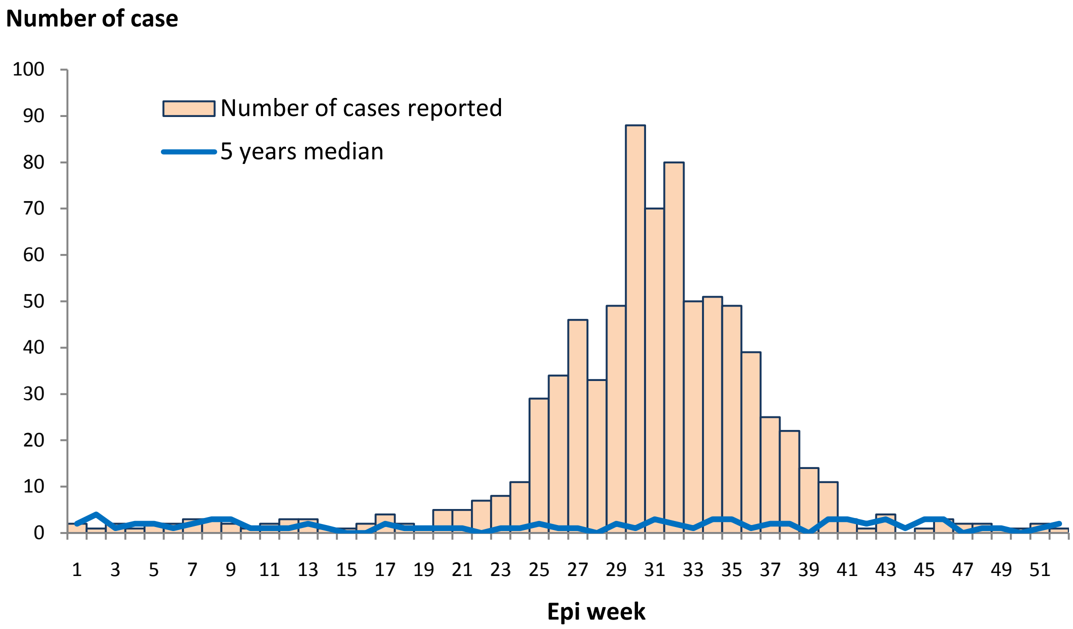

Figure 4. Number of reported dengue cases by week in Pasir Mas District, Kelantan, Malaysia, January to December 2010 (n=465)

The male to female ratio was 1.2:1. Most cases (34%) were 11-20 years of age, followed by those aged 21-30 years (14%). Cases were mostly students (40%) or rubber tappers (15%) (Table 2).

Table 2. Demographic characteristics of dengue cases in Pasir Mas District, Kelantan, Malaysia, 5 May to 12 Aug 2010 (n=465)

Characteristic	Number	Percent
Gender		
Male	251	54.0
Female	214	46.0
Age group (year)		
1-10	53	11.4
11-20	160	34.4
21-30	65	14.0
31-40	62	13.3
41-50	57	12.3
51-60	41	8.8
61-70	21	4.5
>70	6	1.3
Occupation (n=453)		
Student	182	40.2
Rubber Tapper	67	14.8
Self-employed	47	10.4
Housewife	40	8.8
Government worker	37	8.2
Private sector worker	36	7.9
Unemployed	37	8.2
Pre-school age children	7	1.5

Environmental Survey

Majority of houses in the district had no proper solid waste disposal. There were many discarded containers in the area such as plastic bottles, styrofoam food containers, broken glass containers and coconut shells (Figure 5). In rubber plantations, the rubber taps could also be breeding sites for *Aedes spp.*

Figure 5. Containers for mosquito breeding sites in Pasir Mas District, Kelantan, Malaysia, 2010

All houses where the reported cases lived, except one house, were visited by a district health team. The team inspected premises for larvae of *Aedes aegypti* and *Aedes albopictus* species. About 39% (182/464) of the reported dengue cases lived in areas with *Aedes* positive containers (within 200 meter radius from houses) while 99% (180/182) of the cases were with more than 1% AI and 63% (115/182) with more than 5% BI.

Case-control Study

There were total 160 respondents (80 cases and 80 controls). Demographic characteristics of the cases and controls were similar (Table 3).

Table 3. Demographic characteristics of cases (n=80) and controls (n=80) in Pasir Mas District, Kelantan, Malaysia, 5 May to 12 Aug 2010

Characteristic	Number of case (%)	Number of control (%)	P-value
Gender			
Male	44 (55.0)	34 (42.5)	
Female	36 (45.0)	46 (57.5)	0.11
Ethnic			
Malay	79 (99.0)	77 (96.3)	
Non-Malay	1(1.3)	3 (4.0)	0.13
Mean age (year)	30.6	34.9	0.15
Educational status	(n=78)	(n=80)	
None	1 (1.3)	9 (11.3)	
Primary	21 (27.0)	22 (27.5)	
Secondary	46 (59.0)	42 (52.5)	
Tertiary	10 (12.8)	7 (9.0)	0.18

In univariate analysis, statically significant risk factors were presence of discarded containers and drinking water containers for animals in the premises, not using protective measures against mosquitoes, living in a rubber plantation, and staying outside the house in the mornings and evenings. These variables were included in multiple logistic regression model. Two factors that still showing statistically significant risks for contracting dengue were presence of discarded containers in the premises (adjusted OR = 15.1, 95% CI = 5.41-41.97) and not using protective measures such as mosquito repellent and long-sleeved clothing (adjusted OR = 3.9, 95% CI = 1.21-12.55) (Table 4).

Discussion

High AI and BI demonstrated that conditions in Pasir Mas District were favourable for breeding of *Aedes* mosquito and transmission of dengue virus. Lack of proper sanitary waste disposal in the area resulted in many disposable containers around the houses that served as mosquito breeding sites. The case-control study showed that those living in houses with disposable containers in the premises were more likely to get dengue infection.

Most of the cases were students or young adults working in rubber plantations. Rubber taps in plantations might collect rain water and serve as *Aedes* breeding sites. Workers in these sites might get infected while working in the plantations. Students could also expose while walking to school and passing through rubber plantations or premises with mosquitoes in the mornings and late afternoons. *Aedes* mosquitoes usually bite after sunrise and before sunset.[5]

The affected age group in this outbreak was similar to that of reported in Malaysia during 1991-2000, with most cases were in young and middle age groups.[7] More children were infected probably because adults in the endemic areas might have been infected and developed immunity to the circulating serotypes.

Non-use of protective measures while living in a dengue endemic area was a risk factor as well. A study conducted by Norli and Azmi in Johor during 2006 found that those living in houses with unscreened windows (OR = 4.2, 95% CI = 1.72-10.44) and those who did not wear long-sleeved clothes (OR = 5.4, 95% CI = 1.02-29.03) were at risk of acquiring dengue infection.[8] In our study, although we did not observe specific measures like having screened windows, the risk of getting infected without using any protective measure was significant (OR = 3.9, 95% CI = 1.21-12.55).

Table 4. Multivariate analysis of risk factors for dengue in Pasir Mas District, Kelantan, Malaysia, 25 May to 12 Aug 2010

Risk factor	P-value	Adjusted odds ratio (OR)	95% CI
Presence of disposable containers (e.g. bottles, styrofoam containers, coconut shells) in premise	<0.01	15.1	5.41-41.97
Not using protective measures (insect repellent, long-sleeved clothing, mosquito coil, mosquito net)	0.02	3.9	1.21-12.55
House located in a rubber plantation	0.20	1.9	0.71- 4.90
Often stay outside the house in the mornings and evenings	0.31	1.6	0.63-4.01
Presence of water container for animal in premise	0.90	0.9	0.15-5.51

Limitations

Since all cases were not laboratory confirmed, some of them might be other illnesses. In 2009, there was an outbreak of chikungunya in Malaysia, including Kelantan.[9] Dengue and chikungunya viruses can both be transmitted by *Aedes* species and clinical manifestations are similar.[10]

The questionnaire used in the case-control study did not obtain information about specific protective measures like wearing long-sleeved clothing, using insect repellents or having screened windows at home. Furthermore, during the environmental survey, the team did not note down the type of disposable containers found positive for *Aedes* larvae. Noting specific risk behaviors and breeding sites in the area could have been helpful for providing education campaign to the residents. In addition, as the existing surveillance system was passive surveillance, mild cases did not attend health care facilities might be missed.

Conclusion

There was a dengue outbreak in Pasir Mas District in 2010 which lasted for about five months. Environmental conditions and without utilizing the protective measures contributed to the outbreak.

Public Health Action and Recommendations

Although district health teams conducted fogging operations in response to detection of *Aedes* positive containers around the premises, the effects were only temporary since the underlying conditions favoring the breeding of mosquitoes continued to provide an opportunity for dengue transmission. Intensive community education and clean-up campaign could stop further spread of the disease. Therefore, local health staff conducted health education campaigns in schools and mosques to encourage residents to clean up their premises and use protective measures.

In addition, we recommended that local authorities should improve solid waste disposal system in the area, and a recycling program was designed and implemented in the communities in order to reduce the number of discarded containers in the premises.

Regular larval surveys should be done in order to identify dengue sensitive areas and preventive measures should be instituted regularly before outbreak starts. Multi-sectoral collaboration was necessary to prevent and control dengue outbreaks as well.

Acknowledgement

The authors thank all staff in Pasir Mas District Health Office and Kelantan State Health Department for their assistance while conducting this study. We are also grateful to Dr. Maria Concepcion Roces for her assistance in developing the manuscript.

Suggested Citation

Sulaiman S, Hamzah WM, Othman Y, Abdul Karim B, Kamaludin F. Environmental and social conditions prevailing dengue fever outbreak in Pasir Mas District, Kelantan State, Malaysia, 2010. OSIR. 2015 Mar;8(1):7-12.
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Epidemiological and Serological Study of Re-emerging Diphtheria in Dansai District, Loei Province, Thailand, June to October 2012

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Abstract

In July 2012, two fatal cases of diphtheria were reported from Dansai District of Loei Province, where had been free from diphtheria for a decade. An investigation was conducted to confirm the etiology, identify source of the outbreak and measure the prevalence of antibody to diphtheria toxin among the population. Medical records were reviewed. Active case finding and throat swab screening were done in all villages. Throat swab samples were sent for culture. *Corynebacterium diphtheriae* isolates were sent for toxin testing and a subset for multi-locus sequence typing (MLST). A serological survey of diphtheria antibody was done in 132 respondents in two villages. A total of 37 cases and 26 carriers were found. Total 18 (48.6%) cases were children. Two fatal cases (5.4%) were adults. All 16 (61.5%) children carriers had received the recommended doses of DTP or dT from the EPI program in the past. In the serological survey, 39.4% (52/132) had protective antibody, including 68.8% (11/16) of children and 35.3% (41/116) of adults. About 79% of people who had protective level received at least one dose of diphtheria toxoid. With MLST, three new subtypes were identified, including ST243, ST244 and ST245. Dansai outbreak was confirmed with the emergence of new pathogenic strains. A low proportion of sera (39.4%) had protective antibody level. A booster dose of diphtheria and tetanus toxoid should be considered.

Key words: diphtheria, outbreak, serological survey, Loei, Thailand, *C. diphtheriae*

Introduction

Diphtheria is a disease of upper respiratory tract caused by *Corynebacterium diphtheriae*. Toxins of the organism cause systemic effects and may lead to death. With an overall case fatality rate of 5-10% in the pre-vaccine era,[1] this disease is a major cause of illness and death among children worldwide.[1,2]

In the early 1970s, diphtheria was highly endemic in Thailand, resulting in child deaths.[2] After introduction of diphtheria vaccine into the expanded program for immunization (EPI) in Thailand during 1977, number of diphtheria cases declined.[2,3] The EPI program schedules five doses of diphtheria-tetanus-pertussis (DTP) vaccine at ages of 2, 4, 6 and 18 months, and 4-5 years.[3] Booster doses of diphtheria and tetanus toxoids (dT) are given to 6th grade students aged around 12 years.[3,4] In 2006, Thailand Ministry of Public Health (MOPH) began providing dT instead of tetanus toxoid (TT) to pregnant women in antenatal clinics and health promoting hospitals (HPH) so as to protect newborns from diphtheria as well as tetanus.[5]

In nationwide cross-sectional surveys conducted during 1999, 2003 and 2008, coverage for three doses of DTP vaccine (DTP3) was reported to be 97%, 98% and 99%, and for four doses (DTP4) was 90%, 93% and 97% respectively.[3,4] Coverage for five doses of DTP vaccine (DTP5) was 54% in 2003 and 79% in 2008 while coverage of dT vaccine among 6th grade students was 94% in 2008.[4]

With increasing vaccine coverage, Thailand aimed for diphtheria elimination.[2] Despite that, in 2010, an increase in locally acquired cases was reported in the far southern provinces of Thailand.[6] In addition, during July 2012, provincial health office in Loei, a northeastern province, notified Bureau of Epidemiology (BOE) of two laboratory-confirmed fatal diphtheria cases in Dansai District[7] although Loei

Province had been free of diphtheria for the past decade (Figure 1).[6] Thus, from July to October 2012, BOE, Office of Disease Prevention and Control for Region 6 and local surveillance and rapid response team (SRRT) conducted a joint investigation. Objectives were to confirm the diagnosis, identify source of the outbreak, measure the prevalence of antibody to diphtheria toxin among the population as an indicator for coverage of the vaccination program, and recommend prevention and control measures.

Method

This investigation involved case finding, active surveillance, serological survey for diphtheria antibody, and laboratory assays for toxin production and genetic analysis.

Case and Carrier Finding

We reviewed medical records of hospitalized patients with diagnosis of exudative tonsillitis or oral candidiasis, as lesions of these diseases are similar to respiratory diphtheria, between April to August 2012 at Dansai Hospital. We also collected medical records of persons who visited Dansai Hospital or one of 15 HPH of Dansai District with symptom of white membrane in the upper respiratory tract, or was diagnosed as diphtheria or suspected diphtheria by a physician from August to October 2012. Case investigation reports and laboratory results from Dansai Hospital and provincial hospital were reviewed.

Active Surveillance for Diphtheria

In collaboration with health volunteers, active surveillance for diphtheria was done in all 97 villages of Dansai District through a weekly door-to-door survey which was done using a newly developed case screening protocol. A person who had sore throat, white membrane in the upper airway or history of direct contact with a known case or carrier was examined and a throat swab was collected by trained health care officers for culture.

Case Definitions

A suspected case of diphtheria in this investigation was defined as a person who lived in Dansai District and had fever, sore throat or white membrane in the upper respiratory airway between June to October 2012. A confirmed case was a suspected case tested positive for *C. diptheriae* by culture.

A carrier was a person who did not have any sign or symptom of diphtheria, but was positive for *C. diptheriae* and diphtheria toxin. However, in presence of a confirmed case or carrier in the same sub-district, a person tested positive only by culture was also regarded as a carrier, despite results of toxin testing.

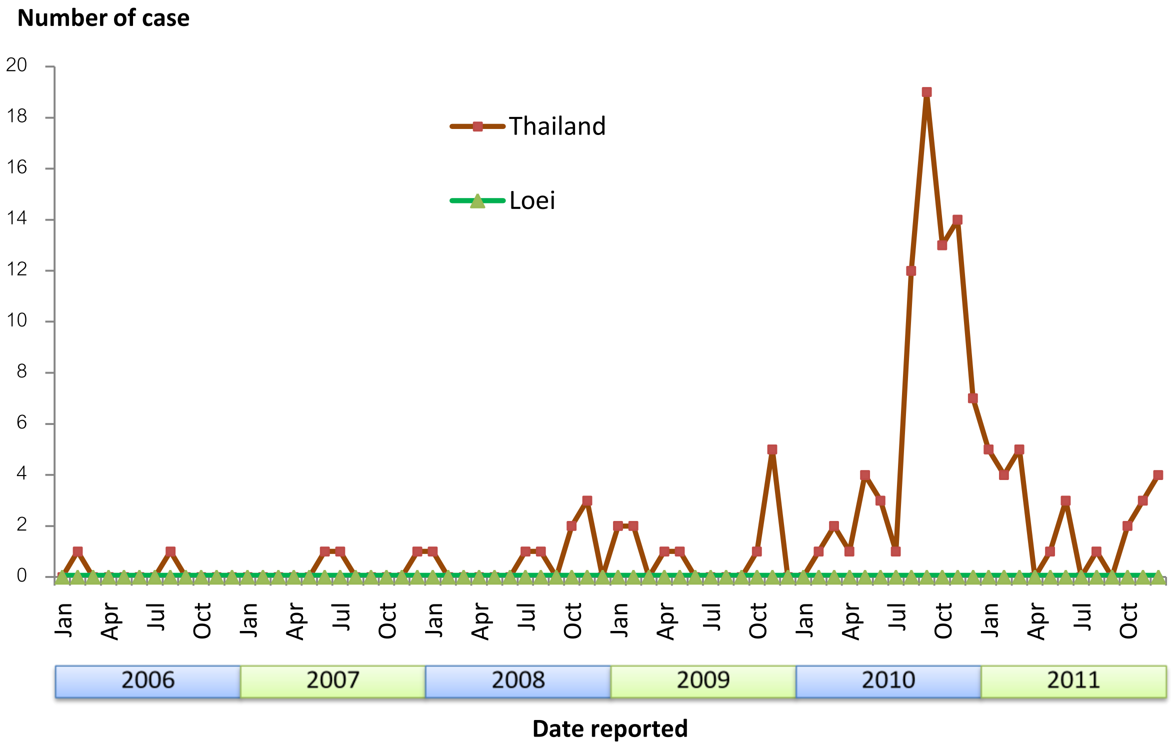

Figure 1. Number of diphtheria cases reported in the national passive surveillance in Dansai District, Loei Province and Thailand, 2006-2011[6]

A close contact was a person who had direct contact via sharing food, drinking glass or living in the same house with a suspected or confirmed case within 14 days before the case's onset of symptoms until recovery or death, or had direct contact with a carrier within 14 days before throat swab collection until last day of chemoprophylaxis of the carrier.

Laboratory Testing

Throat swab samples of villagers from 97 villages were sent for *C. diphtheriae* culture at the Dansai Hospital, Loei Provincial Hospital and National Institutes of Health. *C. diphtheriae* colonies growths from all positive swab samples were sent for toxin test by Elek method[7] which is used to detect in vitro toxin-producing organisms, including *C. diphtheriae*. Some of specimens with toxigenic *C. diphtheriae* were sent for identification using multi-locus sequence typing (MLST) and compared with known *C. diphtheriae* genetic sequences on the MLST website database.[8]

Serological Survey

A cross-sectional serological survey was done in two villages: Pakpong Village in Pakman Sub-district and Dandoo Village in Pong Sub-district, which were selected purposively because in Dandoo Village, there was one case with recent date of onset and thus, had not yet provided mass vaccination while in Pakpong Village, mass vaccination was administrated only a few days earlier, which was too soon to generate the antibodies. Mass vaccination was completed in the other villages more than two weeks before our serological survey and there was no confirmed case or carrier identified in there.

The target sample size of 145 persons was calculated based on 20% estimated prevalence on protective level of serum diphtheria antibody using 95% confidence interval (CI). Volunteers were selected by random sampling using a name list ordered by age. Excluded people were children less than five years old, disabled persons, pregnant women, people who did not stay in the villages during two weeks before the onset of confirmed case in that village, and those who were unwilling to participate. Finally, there were 132 total respondents, including 62 persons from Pakpong (total population 282 persons) and 70 persons from Dandoo (total population 129 persons).

A face-to-face interview was conducted using a structured questionnaire. The variables collected were demographic data, health status, immunization history, signs, symptoms and complications of diphtheria (fever, sore throat, cough, white membrane in throat, anorexia, swelling of neck, chest tightness, dyspnea and muscle weakness), and risk factors such as contact and travel history. Throat swabs and 5mL of blood samples were collected from all respondents.

Antibody Testing

Total 132 blood samples were sent to the Institute of Biological Products in MOPH to test for anti-toxin level by micro-cell culture technique.[9] Level less than 0.1 IU/mL indicated non-protection while level 0.1 IU/mL or more was considered as protective.

Ethical Clearance

As a rapid public health response to this outbreak, an ethical clearance for human research was not required.

Results

Study Site

Dansai District in Loei Province is located in the northeastern region of Thailand bordering Lao PDR. About 85% of the area is mountainous. According to records in Dansai Hospital, estimated population in this district during 2012 was 40,120. Approximately 2% were Hmong hill tribe and most people worked in agriculture.[10]

Case and Carrier Finding

Total 37 confirmed cases of diphtheria, with onset from 24 Jun to 17 Oct 2012, were found (Figure 2) in nine out of 10 sub-districts of Dansai District (Figure 3). Overall case incidence between June to October 2012 was 92 per 100,000 population. Among all confirmed cases, 18 cases were children less than 15 years while 19 cases were adults. Age ranged from 5-72 years (mean 22.1 years). Majority was in the age group of 5-14 years (45.9%), followed by 20-44 years (40.5%) and the lowest was those older than 44 years (13.5%). No cases were found among children less than five years and those aged 15-19 years. There were 15 males (40.5%) and 22 female (59.5%). Seven cases (18.9%) were fully vaccinated, according to the EPI program, while all were children (Table 1).

Most confirmed cases had mild symptoms although 24% needed hospitalization. Two adults died from cardiac complications (case-fatality proportion 5.4%). Both cases were never vaccinated and had history of heavy alcohol drinking. The first case, aged 40, was infected with human immunodeficiency virus (HIV) and the second case, aged 25, was a methamphetamine addict from the Hmong hill tribe.

Clinical manifestations of all cases were sore throat (94.6%), fever (89.2%), white membrane in throat (51.4%), cough (37.8%), poor appetite (35.1%) and neck swelling (16.2%). A toxigenic strain of *C. diphtheriae* was found in 78.4% of confirmed cases (Table 1).

During the investigation, 26 carriers of *C. diphtheriae* were found in Dansai District (64 per 100,000 population). Among them, 16 (61.5%) were children less than 15 years old and 10 were adults, with mean age of 13.5 years (range 4-41 years). As with the cases, most carriers aged between 5-14 years (57.7%), followed by 20-40 years (23.1%), 15-19 years (15.4%) and 4 years or less (3.8%). There were 15 males (57.7%) and 11 females (42.3%). All 16 child

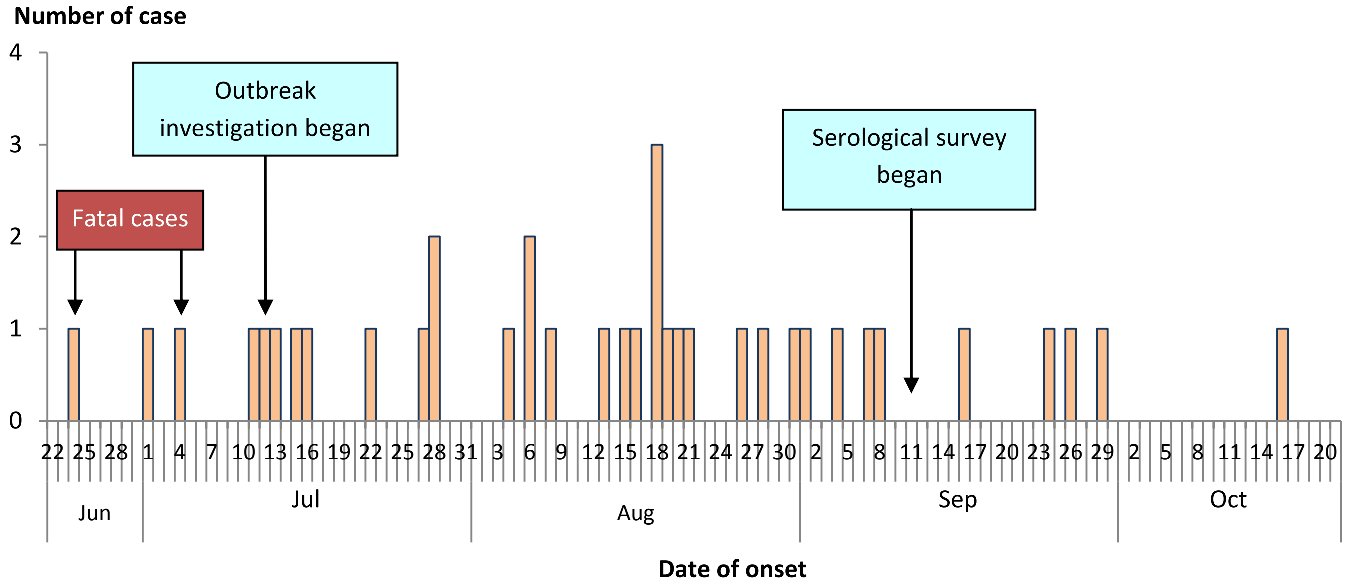

Figure 2. Confirmed diphtheria cases by date of onset in Dansai District, Loei Province, June to October 2012 (n=37)

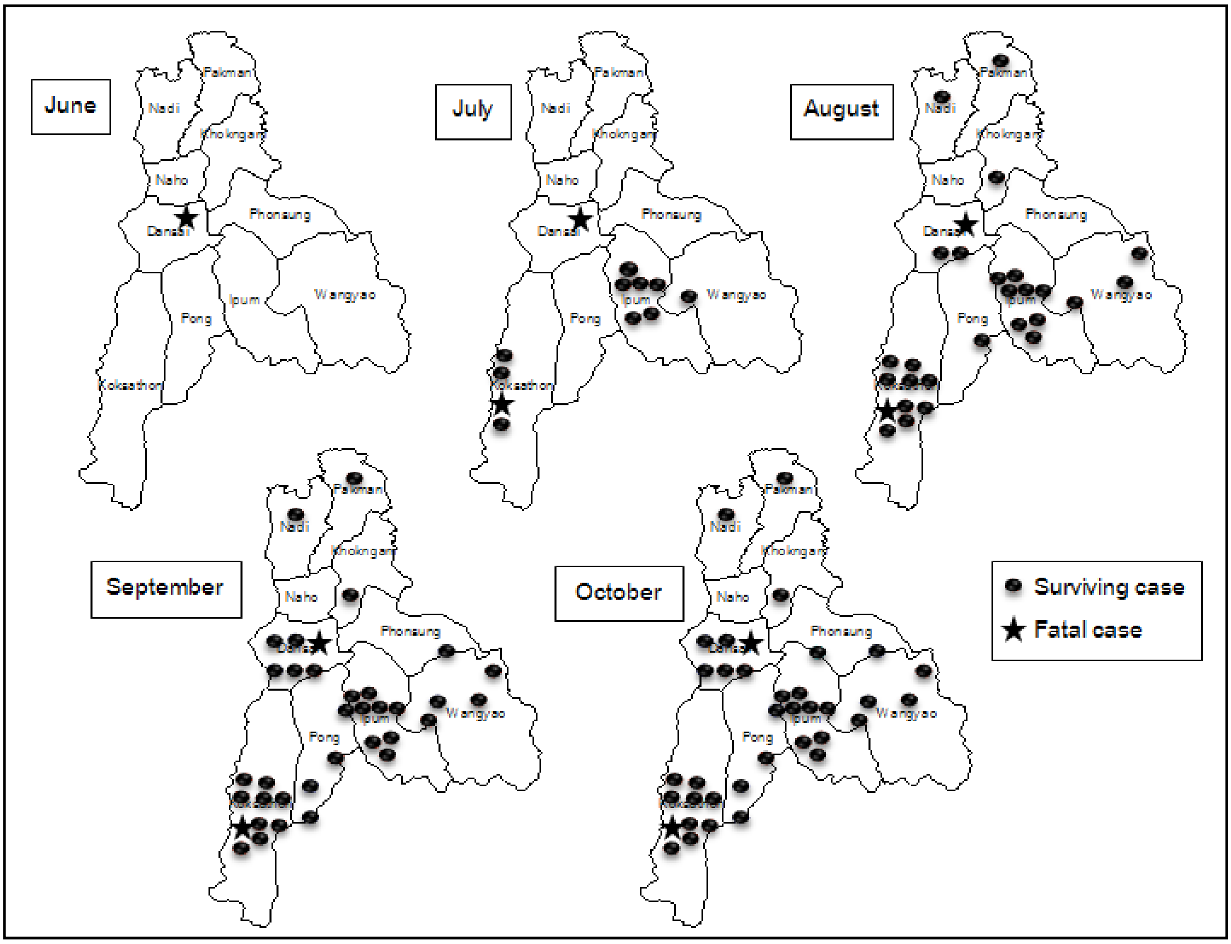

Figure 3. Distribution of confirmed diphtheria cases by month of onset and sub-district, and by case outcome, Dansai District, Loei Province, June to October 2012 (n=37)

Table 1. Characteristics of confirmed cases and carriers of diphtheria in Dansai District, Loei Province, June to October 2012

Variable	Case (n=37)		Carrier (n=26)	
	Number	Percent	Number	Percent
Gender				
Male	15	40.5	15	57.7
Female	22	59.5	11	42.3
Age (year)				
0-4	0	0	1	3.8
5-14	17	45.9	15	57.7
15-19	0	0	4	15.4
20-44	15	40.5	6	23.1
≥45	5	13.5	0	0
History of complete diphtheria vaccination				
0-14 years	7	18.9	16	61.5
≥ 15 years	0	0	4	15.4
Overall	7	18.9	20	76.9
Hospitalization				
Yes	9	24.3	0	0
No	28	75.7	0	0
Laboratory testing				
Positive to *C. diphtheriae*	37	100	26	100
Elek test				
Positive	29	78.4	17	65.4
Negative	5	13.5	8	30.8
Not done	3	8.1	1	3.8

carriers had received the recommended doses of DTP or dT from the EPI program and four (15.4%) adult carriers had completed immunization in the past. The Elek test showed that 65.4% of carriers were tested positive for toxin of *C. diphtheriae* strain while 30.8% were negative (Table 1).

Among 22 toxigenic *C. diphtheriae* specimens sent for MLST, three new clonal patterns of diphtheria genetic sequences were detected: ST243 (13 cases, two carriers), ST244 (one case, three carriers) and ST245 (one case, two carriers) (Figure 4,5). The first clonal emerged Dansai was ST243. All three clonal pattern were found at the center of Dansai, Ipum Sub-district.

Diphtheria Anti-toxin Serological Survey

Among a total of 132 persons included in the serological survey, 16 were children, including one confirmed diphtheria case, and 116 were adults. For gender, 54 persons were male and 78 were female.

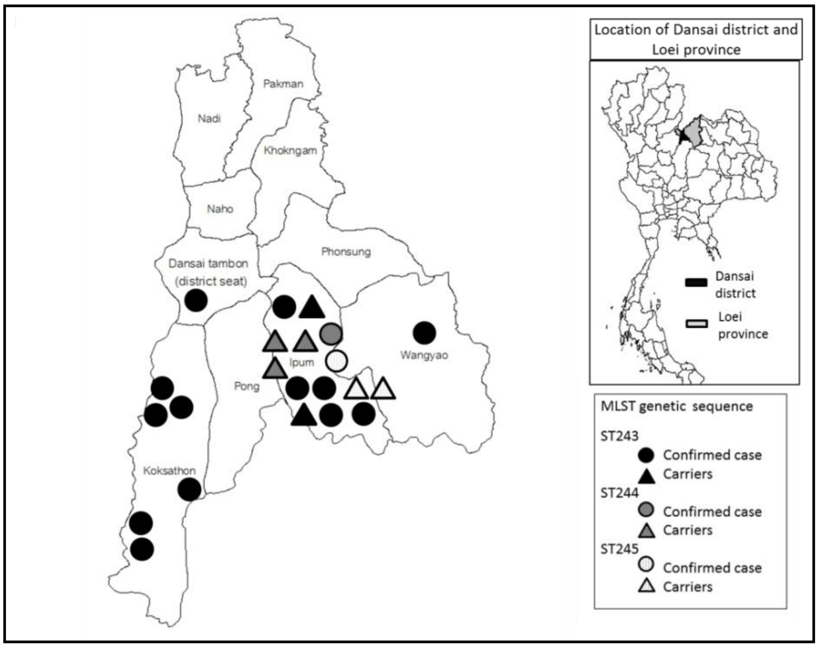

Figure 4. Distribution MLST of *C. diphtheriae* isolation from confirmed cases and carriers by sub-districts, Dansai District, Loei Province, June to October 2012 (n=22)

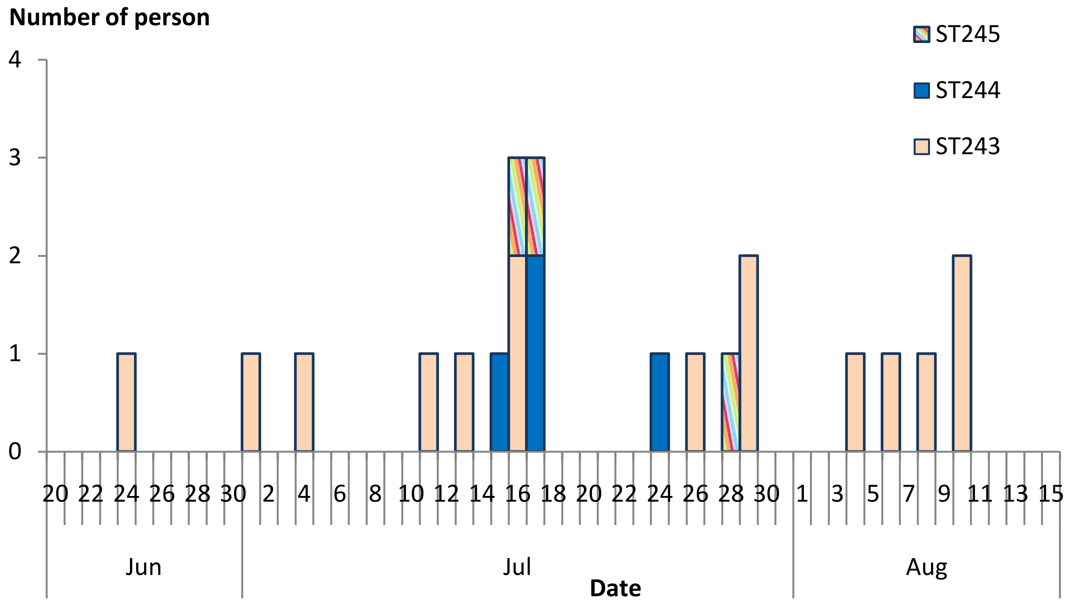

Figure 5. Confirmed diphtheria cases and carriers by MLST sequences by date of onset for cases or date of throat swab collection for carriers, Dansai District, Loei Province, June to October 2012 (n=22)

Mean age was 45.5 years (range 7-87 years) (Table 2). About 24% of the participants reported a sign or symptom of diphtheria in the questionnaire.

Protective level of diphtheria antibody were found in 52 out of 132 (39.4%), Of these, 68.8% (11/16) were children and 35.3% (41/116) were adults. Based on vaccination history, 78.8% (41/52) with a protective level received at least one dose of diphtheria toxoid while 50.0% (40/80) of volunteers with non-protective antibody level did not receive immunization or remember their vaccination history (Table 2).

Table 2. Immune status of respondents from serological survey in Dandoo and Pakpong Villages, Dansai District, Loei Province, September 2012

Variable	Total number (n=132)	Protective level (n=52)		Non-protective level (n=80)	
		Number	Percent	Number	Percent
Gender					
Male	54	23	42.6	31	57.4
Female	78	29	37.2	49	62.8
Age (year)					
≤ 15	16	11	68.8	5	31.2
> 15	116	41	35.3	75	64.7
Interval prior diphtheria toxoid vaccination					
Within 2 weeks	18	12	66.7	6	33.3
2 week - 1 month	6	4	66. 7	2	33.3
>1 month - 6 months	6	1	16. 7	5	83.3
>6 month - 10 years	34	20	58.8	14	41.2
>10 years	17	4	23.5	13	76.5
None or N/A	51	11	21.6	40	78.4
Culture and toxin assay					
Toxigenic *C. diphtheriae*	1	1	100	0	0
Non-toxigenic *C. diphtheriae*	2	0	0	2	100
Negative for *C. diphtheriae*	129	51	39.5	78	60.5
Case definition					
Suspected case	32	18	56.3	14	43.7
Confirmed case	1	1	100	0	0
Carrier	2	0	0	2	100
Not meeting any case definition	97	33	34.1	64	65.9

Out of 132 throat swabs collected for culture, the one sample collected from the confirmed case at Dansai Hospital found to have toxigenic *C. diphtheriae*.

Among 131 samples collected in Dandoo and Pakpong Communities, 2 carriers with non-toxigenic *C. diphtheriae* were found. The confirmed hospital case had a protective level against diphtheria toxin while the two carriers had none (Table 2). Nine adults (four males and five females) were found to have high levels of serum anti-toxin (>2.56 IU/mL). Although one of them had contacted with a confirmed case, that person did not meet our definition of a close-contact. All of them already had dT immunization and three of them (33.3%) had the most recent dose less than two weeks ago, followed by four (44.4%) at 2 weeks to 6 months and two (22.2%) at 6 months to 10 years. Throat swab results from all of them were negative for *C. diphtheriae*.

Discussion

This Dansai outbreak followed a pattern similar to re-emergence of diphtheria in the former Soviet Union in the 1990s. The epidemic was first recognized with reports of fatal adult cases. Then, additional child cases and carriers were detected.[12] The second similarity was a shift in age of diphtheria cases.[13,14] During diphtheria epidemics in Thailand around 1980s and 1990s, 98% and 91% of cases were children respectively.[2] However, in this outbreak, the adult cases nearly equaled the child cases. This situation might occur from lack of natural "wild" infection to boost immunity in adults. Another similarity was that adults tended to have more severe complications than children[13,15] while the last was a large number of carriers among well-vaccinated children.[13,16]

The diphtheria outbreak in Dansai District was widespread, with many cases and carriers detected. Considering the difficult geography and transportation, *C. diphtheriae* might silently circulate in remote sub-districts among clinical cases and carriers for a long period before a fatal case prompted the notification. This hypothesis was supported by the MLST results, which identified three genetic sequences in those areas. The variation of genetic sequences suggested bacterial reproduction of many generations.[8] The first likely genetic sequence was ST243 which was found in the first generation of Dansai cases and carriers. This subtype remained to be the major sequence found throughout the epidemic in Dansai. With the incubation period of diphtheria around 2-5 days,[1] the epidemic curve probably reflected many generations of spread of *C. diphtheria* (Figure 2). Despite that, it was not clear how ST243 entered Dansai as it did not match any genetic sequence found from previous outbreaks in the southern region of Thailand.[11]

In this investigation, vaccinated cases were likely to have milder symptoms and fewer complications than unvaccinated ones. Similar findings were reported from diphtheria outbreaks in the former Soviet Union, Finland and Sweden.[12,13,19,20] From the previous epidemics, a decreased in diphtheria cases was caused by a mass vaccination.[12,13] In order to rapidly control a diphtheria epidemic, emergency vaccination campaigns should target persons at high risk for severe complication or death such as immunocompromised hosts, those younger than five, those older than 40 years and people abusing drugs.[1,12,17,18,21]

Cardiac complications from diphtheria were causes of the two deaths. Contributing factors associated with mortality were non-vaccination, heavy alcohol drinking, severe underlying disease and delayed specific treatment for diphtheria. These findings were also found in the 1990s epidemics in the former Soviet Union[14] and the 2012 outbreaks in the southern provinces of Thailand.[17,18]

From a serological survey result, unlike a previous hospital-based seroprevalence study in Khon Kaen Province, a commerce center of northeastern region, which found 94.8% of the study population had protective antibody level,[21] we found only 39.4% had protective level in Dansai District. This difference was found in both children and adults, and was likely to cause by a variation in population since Dansai was a rural area.[21] Nearly half of the confirmed cases in children had received recommended vaccines from the EPI program. However, in our serological survey, 50% of people who claimed to have been vaccinated for diphtheria had low level of immunity. Thus, even for patients with a history of vaccination, the diphtheria diagnosis should not be ruled out. Misdiagnosis of diphtheria could lead to severe complications and even death,[17] as well as further spread of the disease in communities.[14,15] Our serological survey identified that most adults had non-protective level of antibody to diphtheria toxin, including those with a history of vaccination as children. This finding might have been caused by lack of natural "boosters" from circulating *C. diphtheria*.

Limitations

A major limitation of this investigation was inability to identify the primary source of the outbreak as the investigation was started late. Other limitations included incomplete medical records for vaccination history, especially in adults. In addition, many patients and respondents could not remember their contact and risk history.

Conclusion

The Dansai outbreak was possibly the first sign of a re-emergence of diphtheria in Thailand. A likely long period of occult propagation was suggested by the discovery of three new diphtheria genetic sequences. Nevertheless, mechanism of diphtheria spread in Dansai was still unknown. While most cases were found among children, incidence in adults also rose, with more severe complications. Vaccinated children tended to be carriers. Overall immunity levels remained low, especially in adults.

Recommendations

Better surveillance systems and control measures should be instituted, including an emergency vaccination campaign in the epidemic area. According to a poor immunity found in results of a study, we suggested scheduling dT booster vaccination to implement every 10 years in adults in this area. To prevent future epidemics, further studies on diphtheria serology and vaccine effectiveness were required. National vaccine strategy should also improved by increasing vaccine coverage of children and adding adult dT to the EPI.

Acknowledgement

We would like to express our thanks to the Thai National Institute of Health and the Institute of Biological Products, MOPH for laboratory testing; Dansai Hospital, Loei Provincial Health office and Office of Disease Prevention and Control for Region 6, Khon Kaen Province, for coordination and their hard work in the communities; Department of Communicable Disease, MOPH, for providing knowledge on diphtheria; and staff and trainees from Thailand EIP, Bureau of Epidemiology and Field Epidemiology Training Program for their support in this investigation.

Suggested Citation

Phupat P, Sittisak S, Pimrat K, Junti K, Jivapaisarnpong T, Paveenkittiporn W, Pittayawonganon C. Epidemiological and serological study of re-emerging diphtheria in Dansai District, Loei Province, Thailand, June to October 2012. OSIR. 2015 Mar;8(1):13-21.
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