Outbreak, Surveillance, Investigation & Response (OSIR) Journal

Volume 11, Issue 3
September 2018

www.osirjournal.net

ISSN: 2651-1061

The Outbreak, Surveillance and Investigation Reports (OSIR) Journal was established in 2008 as a free online publication in order to encourage and facilitate communication of health information and disease reporting across Asia and the Pacific. In September 2018, the journal is retitled as the "Outbreak, Surveillance, Investigation and Response" while maintaining the same abbreviation as OSIR.

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Volume 11, Issue 3, September 2018

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Editorial

Asking the Right Questions

Alden Henderson, Chief Editor

One basic element of epidemiology is asking the right question. When you do, you are more likely to identify the risk factors for disease transmission, the source of the outbreak, and take the proper actions to control the outbreak. The authors of the article on Dengue Fever Outbreak in Indonesia in this issue of OSIR asked the right questions. They identified "not eliminating mosquito breeding sites routinely (aOR 3.7, 95% CI 1.48-9.26)"and "the habit of hanging worn clothes (aOR 2.9; 95% CI 1.21-6.96)" as the primary risk factors for the outbreak. The first risk factor, presence of mosquito breeding sites, is intuitive and commonly reported in the literature as a risk factor along with age, education, income, race, rainy season, screens, safe water, air conditioning and density of community. However, the habit of hanging worn clothes has not been reported in the literature. The authors recommended against hanging clothes that were previously worn.

As epidemiologists develop their questionnaire and decide on which questions to ask during their investigation to identify risk factors, there are three types of questions to use. The first is to include known factors to ensure that your questionnaire, interviewers, respondent and analysis will identify these as risk factors. The second is to ask about a few risk factors that are not associated with transmission of dengue fever and is included for the same reasons as known risk factors. Both types of questions provide an internal positive and negative quality control. However, epidemiologists should not stop there and ask the third type of question as the authors of the dengue article asked about the "habit of hanging worn clothes". This question involves a risk factor that has not been reported in the literature and if the authors limited their questions to what was reported in the literature, they would just repeat what is already known and perhaps not identify a unique risk factor involved in this dengue outbreak.

How does one identify these new questions? Through observation during a field investigation, you may, as the authors did, see mosquitoes resting on clothes. Focus groups contain the collective wisdom and experience of the crowds, and can identify potential unusual risk factors and the reasoning behind the behavior. In this case, the authors found out that people felt the clothes worn during the day were clean enough to wear another day. Finally, interviews with the cases, their physicians and locals may provide insight to transmission routes that are unique to the site and conditions of the outbreak.

Consequently, asking the right question can lead to understanding and discovery and is part of the larger sphere of critical thinking. These topics are encouraged in our FETPs but not taught directly. Hopefully, one day it will be part of the core curriculum for all FETPs.

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Avian Influenza Outbreaks and Surveillance in Live Bird Markets, Quang Ninh Province, Vietnam, 2015-2017

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Abstract

Over 5,000 outbreaks of avian influenza (AI) have occurred in Vietnam, with more than 60 million birds infected and destroyed from 2003 to 2015. This study aimed to describe the AI situation and associated risk factors after 2015. Outbreaks, surveillance and molecular characteristics data in Quang Ninh Province from 2015 to 2017 were gathered from Regional Animal Health Office Number 2. Risk factors for AI virus found in live bird markets (LBMs) were identified using odds ratios (OR) with 95% level of confidence. Ten outbreaks of AI were reported in the border area between Quang Ninh Province and China. The AI active surveillance detected viruses in LBMs from 37.3% (227/608) of the samples. Of these, 7.0% (16/227) were H5N6, and all 608 samples were negative for H5N1 and H7 subtypes. Poultry at LBMs in Quang Ninh imported from Bac Giang Province was slightly more likely to be infected with AI (OR = 1.4, 95% CI = 1.01-2.04). Provincial and national animal health authorities should continue to conduct active surveillance and strictly enforce poultry movement control from China as well as stop transportation of infected poultry across provinces in Viet Nam.

Keywords: avian influenza, H5N6, live bird market, Quang Ninh, Vietnam

Introduction

Avian influenza (AI) is a zoonotic disease, and can cause severe illness and death in humans and poultry. Many subtypes of AI are spreading globally. The identified subtypes of influenza H5 include H5N1, H5N2, H5N6, H5N8 and H5N9.[1] Since the beginning of influenza A(H5N1) epidemic in 2003 till March 2017, hundreds of millions of birds have died[1] and 453 human fatalities in 16 countries were attributable to H5N1 virus[2]. Influenza A(H7N9) was firstly reported in Shanghai, China, during 2013, and associated human cases were found in other territories such as Hong Kong, Taiwan, Canada and Malaysia.[3] From March 2013 to February 2018, a total of 1,624 people were infected with influenza A(H7N9) virus in China, resulting in 621 deaths.[3]

Highly pathogenic avian influenza (HPAI) subtype H5N1 first appeared in Vietnam during late December 2003. By 2015, there were over 5,000 outbreaks in poultry, with more than 60 million birds infected and

destroyed. Consequentially, 127 people were infected with influenza A(H5N1), including 64 deaths.[4] Vietnamese consumers prefer to buy live poultry from live bird markets (LBMs) and slaughter them for consumption. Several poultry species are sold at LBMs, including ducks, chickens, geese and quails. LBM is a location where AI viruses can accumulate, multiply and spread the infection. In addition, the majority of LBMs are lack of appropriate waste management system. Poultry vendors usually work without personal protection such as masks and gloves. The remaining poultry are kept overnight and mixed with new batch of poultry arriving each day.

Quang Ninh Province is a tourist attraction that shares a border with China, and high demand for live poultry exists in the province while the supply is limited. High capacity of poultry imported from other provinces and China poses a risk of AI outbreaks in the province. Both national and provincial animal health authorities required better understanding on AI situation for better control and prevention. Hence, this study aimed to describe the occurrence of AI from outbreak reports and LBM surveillance, and assess risk factors for AI in Quang Ninh Province from 2015 to 2017.

Methods

Information from the AI surveillance at LBMs and the outbreak reports from the Regional Animal Health Office 2 (RAHO2) were compiled into a single database for analysis, including details of outbreaks reported by provincial animal health office in Quang Ninh, and laboratory and sequencing results tested by the laboratory section of RAHO2.

In Quang Ninh Province, there were 26 registered LBMs that each had a volume of at least 100 birds sold per day. Of 26 LBMs, 10 were randomly selected and samples were collected once a month during the study period (Figure 1). In each market, the sample collectors selected six vendors. Five poultry swabs from chickens and ducks, and other environmental samples such as fresh feces, waste in the cage, waste water outside the cage and drinking water from each vendor were collected. The samples were obtained for four rounds (A to D) during 2015-2017 (Table 1). At the time of sample collection, the sample collectors used a standard questionnaire to gather information from the vendor on risk factors as well.

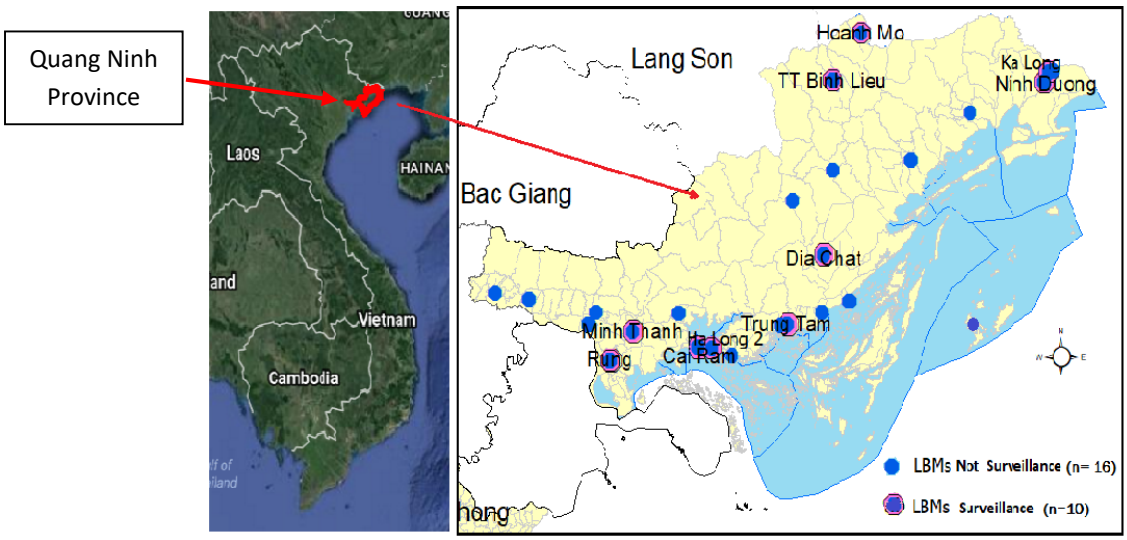

Figure 1. Location of over 100 birds sold and randomly selected live bird markets for avian influenza surveillance in Quang Ninh Province, Vietnam, 2015-2017

Table 1. Samples collected under the avian influenza surveillance at live bird markets (LBMs) in Quang Ninh Province, Vietnam, 2015-2017 (n=608)

Round	Period	LBM	Month	Pooled samples by type (per LBM/time)			Total pool sample
				Throat swab		Environmental swab	
				Chicken	Duck		
A	Dec 2015 - Feb 2016	4	3	6	6	6	216
B	Jun - Aug 2016	4	3	6	6	6	216
C	Mar - May 2017	2	3	-	-	8	128
		2	2	-	-	8	
		2	2	12	-	-	
D	Jun - Aug 2017	2	3	-	-	8	48

Five cotton swabs from the similar type of sample or similar species were pooled into one tube and tested for AI. If the sample was positive to influenza A, it was then tested for H5, N1, N6, H7 and H9 by reverse transcriptase polymerase chain reaction (RT-PCR). The tests were performed in the laboratory of RAHO2 using a standard protocol for AI surveillance[5]. Furthermore, two H5N6 viruses from outbreaks were selected for molecular sequencing and the HA gene was amplified by PCR[6]. The amplified products were later sent to the Macrogens Company in the Republic of Korea for sequencing. Phylogenetic analysis on HA sequences, including sequences downloaded from Genbank, was conducted using MEGA 6.0 with neighbor-joining, bootstrap 1000 replications[7].

The results were described using descriptive statistics. ArcGIS 9.3 program[8] was used to create a distribution map. Epicals 2000 program[9] was also used to calculate odds ratios (OR) with 95% confidence level (CI) to determine the association between AI and potential risk factors, including poultry source (province), poultry species and types of sample.

Results

HPAI Outbreaks

From 2015 to 2017, 10 HPAI outbreaks were reported in five out of 14 districts of Quang Ninh Province. These outbreaks could be grouped into three waves: wave 1 from October to December 2015 caused by H5N6 virus, wave 2 from October 2016 to January 2017 caused by H5N6 virus, and wave 3 in April 2017 caused by H5N1 virus (Figure 2). All outbreaks occurred in the areas with moderate to high density of poultry population (Figure 3).

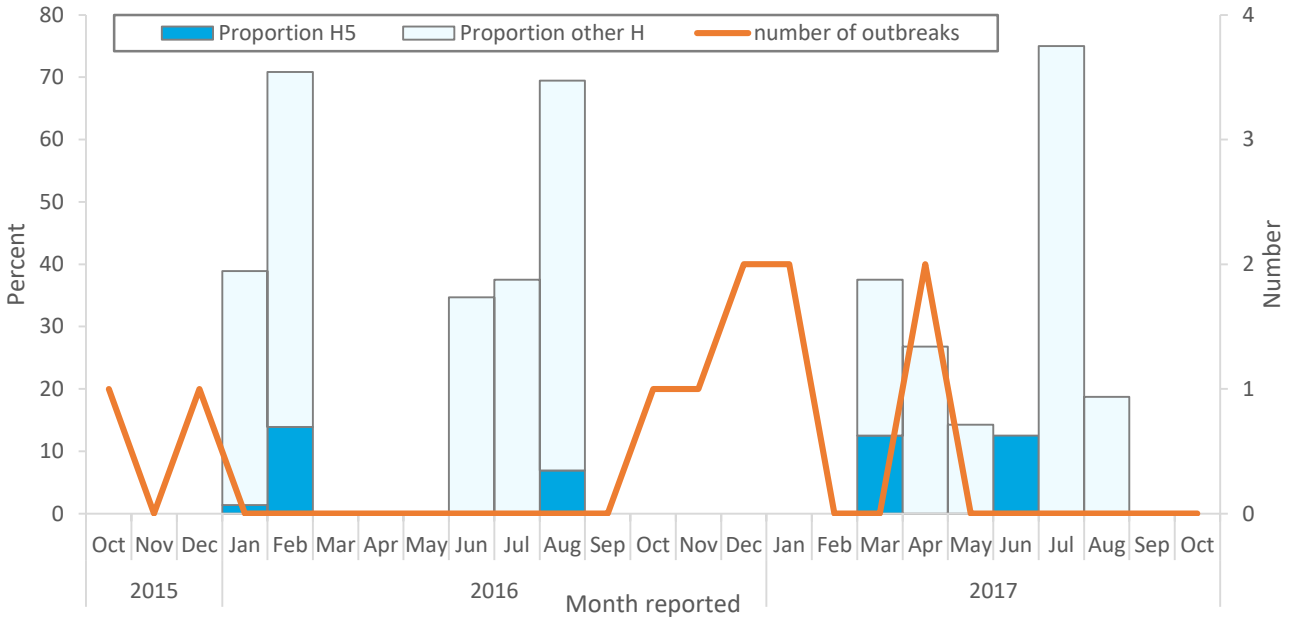

Figure 2. Avian influenza subtypes detected by surveillance and causing outbreaks in Quang Ninh Province, Vietnam, October 2015 to August 2017

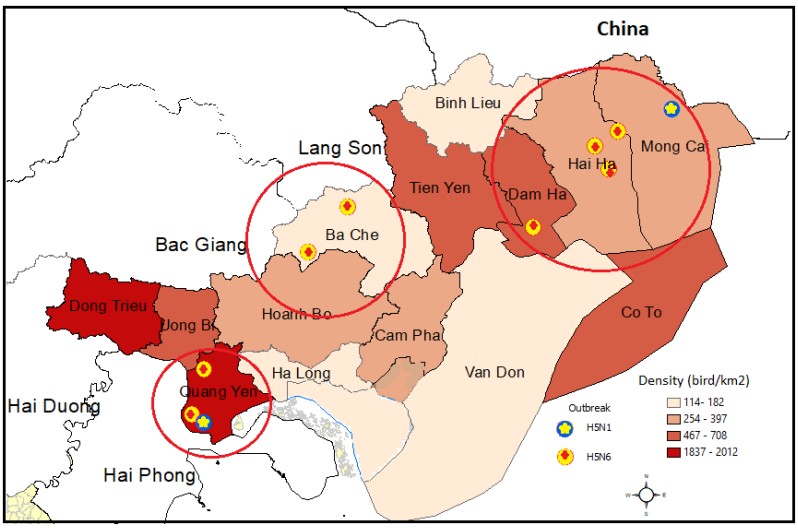

Figure 3. Location of highly pathogenic avian influenza outbreaks in Quang Ninh Province, Vietnam, October 2015 - August 2017 (n=10)

AI Surveillance at LBMs

A total of 608 pool samples were collected from LBMs from 2015 to 2017. Of which, the majority (66.0%) of poultry swab samples were collected from poultry produced in Quang Ninh Province while others were collected from poultry produced in Bac Giang (31.7%), Nam Dinh (1.3%) and Hai Duong (1.0%) Provinces. Throat swabs made up to 55.3% (336/608) of the samples while 44.7% (272/608) of all samples were collected from the environment. The proportion of AI virus was found to be 37.3% (227/608) in LBMs during 2015-2017 (Table 2).

A high proportion of samples were positive for AI in round B (89.5%, 102/114), followed by round A (57.7%, 79/137), and round D (54.8%, 17/31), with the lowest in round C (29.3%, 29/99). About 34.9% (95/272) of environmental samples and 39.3% (132/336) of throat swabs samples were found to have AI infection. Further characterization showed that 7.0% (16/227) of the samples positive for influenza A were H5N6. Nine out of AI positive samples collected during April-May 2017 were selected and four (44.4%) out of nine samples were found to have H9. All 608 samples were negative for H5N1 and H7 (Table 2). HPAI virus occurrence in LBM was significantly associated with poultry coming from Bac Giang Province (OR = 1.4, 95% CI = 1.01-2.04) (Table 3).

The surveillance system in LBMs detected AI in every month during the sampling period. HPAI H5N1 outbreaks were reported during the period when no AI was detected in the surveillance system (Figure 2).

Molecular Analysis

Two AI subtypes of H5N6 collected from outbreaks belonged to clade 2.3.4.4B. The virus circulating in Quang Ninh Province were closely related to the virus found in neighboring Lang Son Province. They were also closely related to AI viruses found in Dong Quan (H5N6), Sichuan (H5N1) and Shantou (H5N6) isolated in China during 2013 and 2014 (Figure 4).

Table 2. Laboratory results of avian influenza surveillance at live bird markets in Quang Ninh Province, Vietnam, 2015-2017 (n=608)

Type of sample		Number tested	Type A (%)	H5N6 (%)	H5N1 (%)	H7 (%)	H9 (%) (n=9)
Environmental swab	Drinking water	24	10 (41.7)	0	0	0	-
	Fresh feces	24	9 (37.5)	1 (11.1)	0	0	-
	Cage	176	59 (33.5)	4 (6.8)	0	0	-
	Waste water	48	17 (35.4)	1 (5.9)	0	0	-
	Total	272	95 (34.9)	6 (6.3)	0	0	-
Throat swab	Chicken	192	72 (37.5)	2 (2.8)	0	0	4 (44.4)
	Duck	144	60 (41.7)	8 (13.3)	0	0	-
	Total	336	132 (39.3)	10 (7.6)	0	0	-
Total		608	227 (37.3)	16 (7.0)	0	0	-

Table 3. Association between poultry source, species, type of sample and highly pathogenic avian influenza virus in Quang Ninh Province, Vietnam, 2015-2017

Variable	Total tested	Number positive	Percent	Odds ratio	95% CI	P-value
Poultry source (province)						
Bac Giang	193	84	43.5	1.4	1.01-2.04	0.05
Quang Ninh	401	140	34.9		Ref	
Poultry species						
Duck	144	60	41.7	0.9	0.60-1.35	0.68
Chicken	192	72	37.5		Ref	
Type of sample						
Throat swab	336	132	39.3	1.21	0.87-1.68	0.31
Environmental swab	272	95	34.9		Ref	

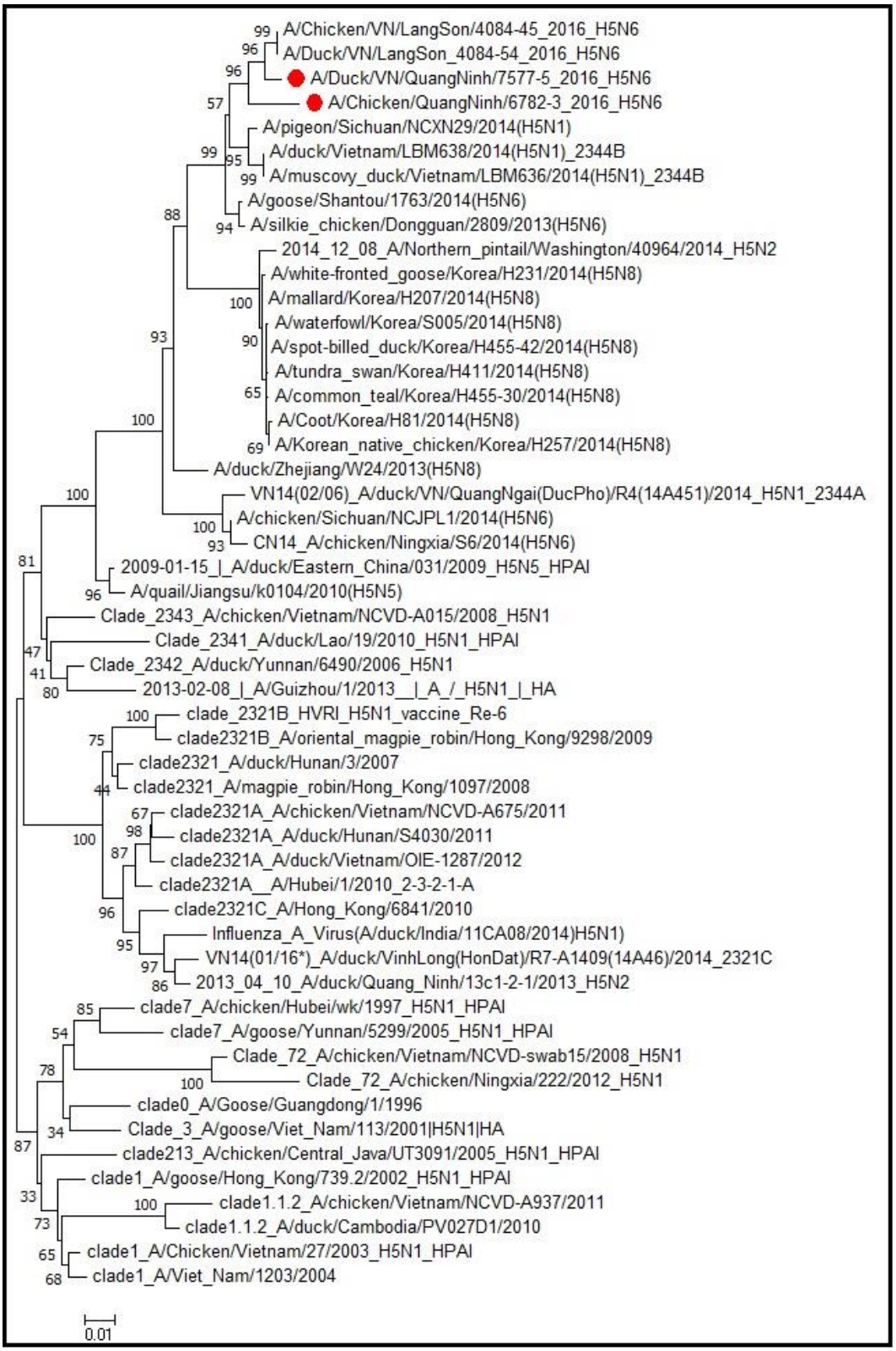

Figure 4. Phylogenetic analysis of HA genes from highly pathogenic avian influenza H5N6 detected in Quang Ninh Province, Vietnam, 2016

Discussion

Ten AI outbreaks among poultry were reported in Quang Ninh Province from 2015 to 2017, including eight outbreaks caused by H5N6 virus subtype and two by H5N1.

AI outbreaks reported during 2015-2017 occurred in the districts close to China, and Bac Giang Province which is connected to the western part of Quang Ninh Province and had higher poultry density (4,242 birds/km^2) than average in Vietnam (1,096 birds/km^2)[10]. A large amount of live poultry from China (80,000 tons per year) were imported to Vietnam for consumption due to the competitive price of live poultry.[11] Quang Ninh Province is the main entry of live poultry. Our molecular analysis showed that HPAI H5N6 virus was

shared in this region, regardless of country and provincial boundaries.

The proportion of AI virus found in LBMs from 2015 to 2017 (37.3%) was higher than the national average from 2011 to 2013 (22.1%, 2,162/9,790)[12]. Poultry sold at LBMs in Quang Ninh were imported from various areas, including nearby provinces and China, where poultry density is high. Poultry from Bac Giang Province were 1.4 times more likely to be infected with AI than poultry produced in Quang Ninh.

In LBMs, there were H5N6 subtype among influenza A positive samples and H9 in some selected samples while H7 and H5N1 subtypes were not detected. It had been shown earlier that H9 virus was a common donor of internal gene for other HPAI viruses.[13-16] Therefore, LBM could act as a mixing site for recombination among various influenza viruses which might result into a new HPAI subtype.

The fact that there was no HPAI H5N6 outbreaks during the study periods might reflect the under-reporting of HPAI outbreak from the local parties. AI surveillance in LBMs indicated that the AI viruses were circulated in the markets of Quang Ninh Province, and revealed a higher chance for genetic mutation and reassortment, including the risk of virus transmission to humans.

In conclusion, Quang Ninh Province was at risk for HPAI and HPAI viruses circulating in LBMs. The results of this study would be useful to improve short and long-term strategies for targeted surveillance at LBMs, relating to cross-border trade with neighboring provinces and China.

Limitations

The AI surveillance was not conducted at all LBMs in the province and the proportion might not represent the situation of AI in the whole province. In addition, the risk associated with the source of poultry might be due to sampling bias where the number of samples (n=193) collected from Bac Giang Province was much greater than Nam Dinh (n=8) and Hai Duong (n=6) Provinces.

The selected LBMs were those with more than 100 birds sold per day, in the area with crowded population, or located near the main road. Therefore, the proportion of positive AI results that we found in this study might be higher than the prevalence of all LBMs in the province.

Surveillance design aimed to detect AI in each selected LBM and was not designed specifically to determine risk factors for AI. Thus, this study was simply able to measure general risk factors rather than the specific ones. However, the results could provide basis information to improve surveillance design, and control and prevention measures in the future.

Public Health and Policy Recommendations

As the HPAI H5N6 virus detected in LBMs was a potential risk for human infection, provincial health and market management authorities should enhance cleaning and disinfection at LBMs and encourage sellers to use face masks to protect themselves. While provincial public and animal health authorities should continue to conduct active surveillance and strictly enforce poultry movement control from China as well as prohibit the transportation of infected poultry across provinces in Vietnam.

Acknowledgements

We would like to thank colleagues in Regional Animal Health Office Number 2, Department of Animal Health, Quang Ninh Province, Vietnam. This study was part of the Field Epidemiology Training Program for Veterinarians (FETPV) hosted by Thailand Department of Livestock Development, and was achieved with the financial support from the United States Centers for Disease Control and Prevention, and the United States Agency for International Development (Grant no. GHA-G-00-06-00001 with FAO).

Suggested Citation

Tran DT, Hoang BT, Pham VT, Chanachai K, Prarakamawongsai T, Padungtod P, et al. Avian influenza outbreaks and surveillance in live bird markets, Quang Ninh Province, Vietnam, 2015-2017. OSIR. 2018 Sep;11(3):1-7.

References

1. World Organisation for Animal Health. Update on avian influenza in animals (types H5 and H7): 2016. 2017 Sep 18. [cited 2018 Jan 24]. <http://www.oie.int/en/animal-health-in-the-world/update-on-avian-influenza/2016/>.
2. World Health Organization. Influenza at the human-animal interface: summary and assessment, 14 Feb to 16 Mar 2017 [cited 2018 Jan 24]. <http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_03_16_2017.pdf>.
3. Food and Agriculture Organization of the United Nations. H7N9 situation update. 2018 February 14 [cited 2018 Jan 24]. <http://www.fao.org/ag/againfo/programmes/en/empres/h7n9/situation_update.html>.

4. Duong UT. What is H5N1 virus. 2017 Jul 18. Vietnamese [cited 2018 Jan 24]. <http://vietnamnet.vn/vn/suc-khoe/cac-loai-benh/cum-a-h5n1-la-gi-nhung-dac-diem-cua-cum-a-h5n1-382666.html>.

5. Heine HG, Foord AJ, Wang J, Vadeter S, Walker S, Morrissy C, et al. Detection of highly pathogenic zoonotic influenza virus H5N6 by reverse-transcriptase quantitative polymerase chain reaction. Virol J. 2015 Feb 8;12:18.

6. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A virus. Arch Virol. 2001 Dec;146(12):2275-89.

7. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013 Dec;30(12):2725-9. Epub 2013 Oct 16.

8. Environmental Systems Research Institute. ArcGIS. 2014 [cited 2018 Jan 24]. <http://resources.arcgis.com/en/help/main/10.2/index.html>.

9. R Project. Epicalc [cited 2018 Jan 24]. <https://cran.r-project.org/src/contrib/Archive/epicalc/>.

10. Vietnam. General Statistics Office of Vietnam. Ministry of Planning and Investment. Total poultry and cattle in Vietnam 2016. 2016 Oct 01. Vietnamese [cited 2018 Jan 24]. <http://www.gso.gov.vn/default.aspx?tabid=717>.

11. Vietnam Net. Consumption of 80 thousand tons of chicken from China. 2017 Feb 21. Vietnamese [cited 2018 Jan 24]. <http://www.vietnamnet.vn/vn/kinh-doanh/thi-truong/an-80-ngan-tan-ga-trung-quoc-thai-loai-am-anh-dich-benh-357386.html>.

12. Nguyen DT, Bryant JE, Davis CT, Nguyen LV, Pham LT, Loth L, et al. Prevalence and distribution of avian influenza a (H5N1) virus clade variants in live bird markets of Vietnam, 2011-2013. Avian Dis. 2014 Dec;58(4):599-608.

13. Liu J, Okazaki K, Ozaki H, Sakoda Y, Wu Q, Chen F, et al. H9N2 influenza viruses prevalent in poultry in China are phylogenetically distinct from A/quail/Hong Kong/G1/97 presumed to be the donor of the internal protein genes of the H5N1 Hong Kong/97 virus. Avian Pathol. 2003 Oct;32(5):551-60.

14. Bi Y, Chen Q, Wang Q, Chen J, Jin T, Wong G, et al. Genesis, evolution and prevalence of H5N6 avian influenza viruses in China. Cell Host Microbe. 2016 Dec 14;20(6):810-821. Epub 2016 Dec 1.

15. Chen H, Yuan H, Gao R, Zhang J, Wang D, Xiong Y, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. Lancet. 2014 Feb 22;383(9918):714-21. Epub 2014 Feb 5.

16. Yang L1, Zhu W1, Li X1, Bo H1, Zhang Y1, Zou S1, et al. Genesis and dissemination of highly pathogenic H5N6 avian influenza viruses. J Virol. 2017 Feb 14;91(5). pii: e02199-16. Print 2017 Mar 1.

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Epidemiological and Entomological Investigation of Dengue Fever Outbreak in South Nias District, North Sumatera Province, Indonesia, 2016

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Abstract

Dengue infection continues to present as a serious public health problem in North Sumatera, Indonesia. A dengue fever outbreak was reported in Teluk Dalam Subdistrict, South Nias District, North Sumatera during February 2016. An epidemiological investigation was conducted to identify the risk factors and recommend control measures. An observational study with a matched case-control design was conducted. A case was defined as a resident of Teluk Dalam District who had suffered two or more clinical symptoms of fever, headache, pain behind eyes, muscle and joint pain, and rash from 14 Feb to 16 Mar 2016. Blood samples were tested to confirm the diagnosis and serotype identification. Total 68 cases and 68 controls were included in the matched case-control study. The case fatality rate was 2.9%, age ranged from six months to 51 years (median 25 years). Three out of six cases were tested positive for DEN-3 serotype. In multivariate analyses, not eliminating mosquito breeding sites routinely (adjusted odds ratio = 3.7, 95% CI = 1.48-9.46) and having habit of hanging worn clothes (adjusted odds ratio = 2.9, 95% CI = 1.21-6.96) were risk factors. Elimination of mosquito breeding sites routinely, proper management of worn clothes, and conducting strict surveillance for dengue infection were recommended.

Keywords: dengue fever, outbreak, case-control study, Indonesia

Introduction

Dengue fever (DF) is a viral illness caused by four distinct serotypes of dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) and can transmit among people through the bite of *Aedes* mosquito.[1,2] Symptoms of infection usually begin 4-10 days after the mosquito bite and last for 2-7 days.[2] Infection with any one serotype confers lifelong immunity, yet there is no cross-protective immunity to other serotypes.[2,3]

In Indonesia, DF is an emerging vector-borne disease of high public health significance. North Sumatera is a province with DF as a public health problem as well.[4] The national dengue control program was initiated in 1974 and gradually expanded to be an integral part of general health services in the context of primary health care.[3] In the DF control program, surveillance data on endemicity of an area, season of transmission and disease progression are collected routinely.[4-6]

South Nias District was not an endemic area of DF in North Sumatera Province until the end of 2015.[4] On 26 Feb 2016, District Health Office in South Nias reported an outbreak of DF in Teluk Dalam Subdistrict. Surveillance officers from provincial health office and district health office jointly conducted an investigation from 27 Feb to 9 Mar 2016 to identify the risk factors and recommend the control measures.

Methods

Case-control Study

A case of DF was defined as a resident in Teluk Dalam Subdistrict who suffered from acute febrile illness for 2-7 days duration with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations or leucopenia[2], during 14 Feb to 16 Mar 2016, identified by active case finding in the affected area. A control was a neighbor of the cases, and did not have clinical signs and symptoms of DF. Cases and controls were matched for age and gender.

The house-to-house search was carried out to identify cases and controls in Teluk Dalam Subdistrict. The

standard outbreak investigation questionnaire from the Ministry of Health, including data on demographic profiles and sanitation practices, was administered to all cases and controls.

All data were analyzed by using the statistical software to calculate odds ratio (OR) and 95% confidence interval (CI). Logistic regression was also performed for all variables and those with p-value lower than 0.25 were included in the multivariate model. OR was used to determine the potential risk factors. Matched case-control was analyzed using McNemar's test.

Environmental Investigation

An environmental investigation was conducted through observing water and sanitation practices of all cases and controls. An entomologist of Puskesmas primary health center in Teluk Dalam inspected potential breeding sites of mosquitoes in and around houses of cases and controls in the subdistrict. The entomologist determined the species of mosquito larvae.

In Teluk Dalam and Nanowa Sub-villages, 100 households were randomly selected for calculation of the entomology indices as below: house index (HI), container index (CI) and Breteau index (BI). We compared the entomology indices with the goals of the Indonesia National Dengue Prevention and Control Program of lower than 5% for all three indices.[3]

$$HI = \frac{\text{Number of houses infested}}{\text{Number of houses inspected}} \times 100$$

$$CI = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

$$BI = \frac{\text{Number of positive containers}}{\text{Number of houses inspected}} \times 100$$

Laboratory Investigation

Laboratory investigation was conducted by collecting blood samples from the consented cases to confirm the diagnosis and detect the serotype. The samples were sent to the National Institute of Health Research and Development under the Ministry of Health of Indonesia. The real-time polymerase chain reaction was performed to detect the serotype of dengue virus.[3]

Results

Case Characteristics

The total number of DF cases identified in Teluk Dalam Subdistrict was 68. Age ranged from six months to 51 years (median 25 years) and 57% were female. All cases presented with fever and other associated common symptoms such as myalgia (90%), headache (87%), rash (80%), vomiting (63%) and arthralgia (55%). About 41% (28/68) of cases were hospitalized. Only 17.9% (5/28) were diagnosed with dengue hemorrhagic fever (DHF). Five cases experienced with thrombocytopenia (<100,000/mm^3) and hemoconcentration (≥20%)[2]. The total attack rate (AR) in this area was 12.7%.

Dengue cases were firstly reported on 14 Feb 2016, and peaked on 29 Feb 2016. Investigation was conducted on 27 Feb 2016. The outbreak continued for more than three weeks, with the last case identified on 9 Mar 2016 (Figure 1). Two deaths occurred, with the case fatality rate as 2.9%, including one female (age <1 year) and one male (age >44 year) (Table 1). The total number of respondents was 136 people (68 cases and 68 controls). There was no significant difference between cases and controls (Table 2).

Case-control Study

There were 68 cases and 68 controls included in the case-control study. In the bivariate analyses, not routinely eliminating mosquito breeding sites (OR = 3.0, 95% CI = 1.34-6.79), having habit of hanging worn clothes (OR = 2.9, 95% CI = 1.22-6.99) and without using personal protective measures against mosquitoes (OR = 2.5, 95% CI = 1.13-5.37) were statistically significant risk factors.

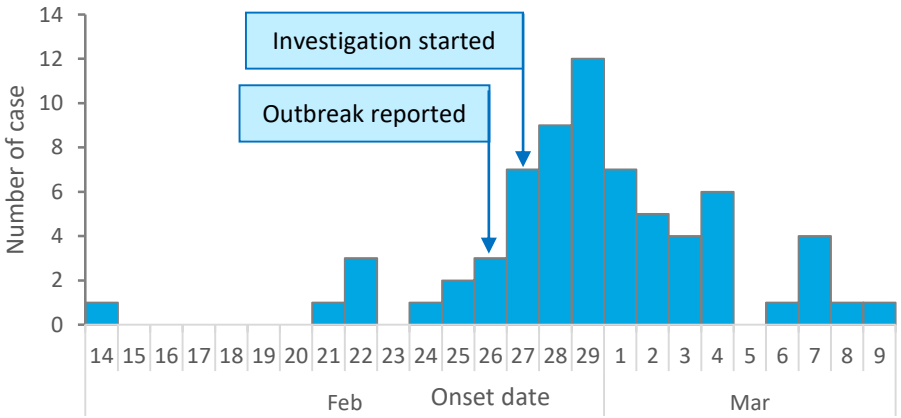

Figure 1. Dengue fever cases by date of onset in Teluk Dalam Subdistrict, South Nias District, North Sumatera Province, Indonesia, 14 Feb-9 Mar 2016 (n=68)

Table 1. Number of cases and deaths, attack rates, and case fatality rates of dengue fever outbreak in Teluk Dalam Subdistrict, South Nias District, North Sumatera Province, Indonesia, 14 Feb-9 Mar 2016 (n=68)

Variable	Population at risk (n=536)	Number of case (n=68)	Number of death	Attack rate (%)	Case fatality rate (%)
Age group (year)					
<1	15	2	1	13.3	50.0
1-4	68	4	-	5.9	-
5-14	83	8	-	9.6	-
15-44	150	35	1	23.3	2.9
>44	220	19	-	8.6	-
Gender					
Male	250	29	1	11.6	3.4
Female	286	39	1	13.6	2.6

Table 2. Characteristics of cases (n=68) and controls (n=68) of dengue fever outbreak in Teluk Dalam Subdistrict, South Nias District, North Sumatera Province, Indonesia, 14 Feb-9 Mar 2016

Characteristic	Number of case (%)	Number of control (%)
Level of education		
None	5 (7.4)	6 (8.8)
Primary	12 (17.6)	14 (20.6)
Secondary	18 (26.5)	17 (25.0)
Tertiary	33 (48.5)	31 (45.6)
Occupation		
None	15 (22.1)	16 (23.5)
Farmer	28 (41.2)	25 (36.8)
Businessman	10 (14.7)	12 (17.6)
Employee	5 (7.4)	8 (11.8)
Housewife	10 (14.7)	7 (10.3)

The findings in the multivariate analyses showed that without eliminating mosquito breeding sites routinely (adjusted OR = 3.7, 95% CI = 1.48-9.46) and having habit of hanging worn clothes (adjusted OR = 2.9, 95% CI = 1.21-6.96) were significantly associated as risk factors for DF (Table 3).

Environmental Investigation

The team observed multiple breeding sites of *Aedes* in the backyard of cases' houses such as discarded tires, plastic bottles and other water containers. Majority of the cases had no proper waste disposal.

In addition, entomologist confirmed that of mosquito larvae that collected during the investigation were larvae of *Aedes*. The result of entomology indices were 30% for HI, 24% for CI and 32% for BI while all were above the national standard indices of less than 5%[2].

Table 3. Factors associated with dengue fever outbreak in Teluk Dalam Subdistrict, South Nias District, North Sumatera Province, Indonesia, 14 Feb-9 Mar2016

Variable	Crude odds ratio	95% CI	Adjusted odds ratio	95% CI
Activity around house in the morning and late afternoon	0.2	0.05-1.13	0.10	-
Without using personal protective measures against mosquitoes	1.0	1.13-5.37	0.36	-
Having habit of hanging worn clothes	2.9	1.22-6.99	2.90	1.21-6.96
Without routinely eliminating mosquito breeding sites	3.0	1.34-6.79	3.75	1.48-9.46

Laboratory Investigation

Blood samples were collected from six cases who were willing to be tested during the epidemiological investigation. All the cases presented with headache, rash, myalgia and arthralgia after three days of fever. Laboratory examination confirmed that three were positive for DEN-3 serotype and the rest were not dengue or other arbovirus infections.

Discussion

The epidemiological and entomological investigations revealed an outbreak of DF in Teluk Dalam Subdistrict. The main risk factors of the outbreak were not eliminating mosquito breeding sites routinely and having habit of hanging worn clothes. Mosquito breeding sites should be routinely managed as it can interrupt the lifecycle of mosquitoes by eliminating mosquito eggs and larvae. Without the breeding site or if the water containers are covered with a fine mesh, the mosquitoes have less opportunities to lay eggs and cannot develop through their aquatic life stages.[2,7] The large number of disposable containers (plastic, coconut shell, discarded bottles and tyres) were identified and *Aedes aegypti* were detected in the affected areas. The abundance of breeding habitats for *Aedes* signified that the area was sensitive and vulnerable to DF transmission.[4,8,9]

In addition, mosquito breeding sites can contribute to high entomological indices (HI, CI and BI) and poor sanitation as well.[2,7] The most effective way to control *Aedes aegypti* larvae is by removing or treating containers that can serve as larval habitats in the environment.[10] Prevention and control relies primarily on reducing the number of natural and artificial water-filled habitats that support the mosquitoes breeding. This requires persistent contribution from the affected communities.[10,11]

Habit of hanging clothes that have been worn was another risk factor of DF outbreak in South Nias District as well. *Aedes aegypti* prefers to rest indoor in the dark and humid houses/buildings or objects. Clothes that have been worn obtain human odors, amino acids, lactic acids, sweats and other substances that attracts *Aedes aegypti*[2,8]. Hanging worn clothes is a favorite resting place for *Aedes aegypti* after sucking human blood. After that, they will suck human blood again until the blood is enough for maturing their eggs.[12]

Personal Protective measure is the way to prevent *Aedes aegypti* bites as they are most active in the morning and late afternoon.[2]The protective measures included using of repellent creams, mosquito nets (plain or insecticide-treated), mosquito coils, repellents, electric rackets, mats and smokeless coils, and wearing long-sleeved shirts and long trousers.[2,13]

Strength and Limitation

This was the first epidemiological investigation of DF outbreak with serological testing in North Sumatera since the previous epidemiological investigations were performed merely with clinical symptoms.

Limited testing of clinical specimens was conducted in this study. Since not all cases were laboratory confirmed as DF infection, some of them might be affected by other illnesses with similar manifestations such as chikungunya.

Conclusion

An outbreak of DF was confirmed in Teluk Dalam Subdistrict, South Nias District, North Sumatera. Not eliminating mosquito breeding sites routinely and having habit of hanging worn clothes were the most potential risk factors associated with the outbreak.

Public Health Actions and Recommendations

During the outbreak, fogging was conducted on 29 Feb and 7 Mar 2016. Intensive information, education and communication (IEC) campaigns as well as promoting behaviors to remove, destroy or manage mosquito larva habitats were conducted in the affected communities, churches, mosques and schools.

In addition, people in the communities were recommended to conduct routine activities for eliminating mosquito breeding sites such as managing the natural and artificial water-filled containers, and to avoid hanging of clothes that have been worn. A strict surveillance of DF should be conducted and multi-sectoral collaboration should be enhanced to prevent and control DF in the future.

Funding

This outbreak investigation was conducted as part of routine public health work of provincial health office in North Sumatera and district health office in South Nias.

Acknowledgements

The authors would like to acknowledge all the surveillance staff in district health office of South Nias and Puskesmas Teluk Dalam for providing assistance in conducting the investigation, and Head of National Institute of Health Research and Development, Ministry of Health of Indonesia for valuable advice.

Suggested Citation

Sitepu FY, Nasution H, Supriyadi T, Depari E. Epidemiological and entomological investigation of dengue fever outbreak in South Nias District,

North Sumatera Province, Indonesia, 2016. OSIR. 2018 Sep;11(3):8-12.

References

1. Guzman MG, Harris E. Dengue. Lancet. 2015;385:453-65.
2. Regional Office for South-East Asia. World Health Organization. Comprehensive guideline: prevention and control of dengue and dengue hemorrhagic fever. New Delhi: WHO Reg Publ SEARO; 2009.
3. Indonesia. Ministry of Health. Guideline for prevention and control of dengue fever. Jakarta: Ministry of Health, Indonesia; 2017. Indonesian.
4. Indonesia. Provincial Health Office. North Sumatera. Ministry of Health. Report of arbovirosis control and prevention program in North Sumatera. Medan: Provincial Health Office, North Sumatera; 2016.
5. Sitepu FY, Suprayogi A, Pramono D. Evaluation and implementation of dengue hemorrhagic fever surveillance system in Singkawang City, West Kalimantan. BALABA. 2012;8(1):5-10. Indonesian [cited 2018 Feb 7]. <http://ejournal.litbang.depkes.go.id/index.php/blb/article/view/3259>.
6. Sitepu FY, Supriyadi T. Evaluation of dengue hemorrhagic fever control and prevention program in North Sumatra, 2010-2012. BALABA. 2013;9(1):1-6. Indonesian [cited 2018 Feb 7]. <http://ejournal.litbang.depkes.go.id/index.php/blb/article/view/3268>.
7. Dhimal M, Gautam I, Kreß A, Müller R, Kuch U. Spatio-temporal distribution of dengue and lymphatic filariasis vectors along an altitudinal transect in Central Nepal. PLoS Negl Trop Dis. 2014 Jul 31;8(7):e3035. eCollection 2014.
8. Lozano-Fuentes S, Hayden MH, Welsh-Rodriguez C, Ochoa-Martinez C, Tapia-Santos B, Kobylinski KC, et al. The dengue virus mosquito vector Aedes aegypti at high elevation in Mexico. Am J Trop Med Hyg. 2012 Nov;87(5):902-9. Epub 2012 Sep 17.
9. Hadisoemarto PF, Castro MC. Public acceptance and willingness-to-pay for a future dengue vaccine: a community-based survey in Bandung, Indonesia.PLoS Negl Trop Dis. 2013 Sep 19;7(9):e2427. eCollection 2013.
10. Buczak AL, Baugher B, Babin SM, Ramac-Thomas LC, Guven E, Elbert Y,et al. Prediction of high incidence of dengue in the Philippines.PLoS Negl Trop Dis. 2014 Apr 10;8(4):e2771. eCollection 2014 Apr.
11. Arima Y, Chiew M, Matusi T. Epidemiological update on the dengue situation in the Western Pacific Region, 2012. WPSAR. 2015;6(1):2-9.
12. Mubarok MA, Wahyuningsih NE, Riani DA, Putri R, Budiharjo A. The relationship between healthy hygiene behavior and dengue haemorrhagic fever (DHF) incidence in Semarang. J Phys Conf Ser. 2018:6-11.
13. Anand T, Kumar R, Saini V, Meena G, Ingle G. Knowledge and use of personal protective measures against mosquito borne diseases in a resettlement colony of Delhi. Ann Med Health Sci Res. 2014 Mar;4(2):227-32.

Outbreak, Surveillance, Investigation & Response (OSIR) Journal
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A Cluster of Suspected Cases of Zika Leading to Uncommon Dengue Serotypes with Possible Coexisting Zika Virus in Northern Thailand, 2016

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Abstract

Due to high morbidity and mortality since 1949, dengue is one of the notifiable diseases routinely reported to the Ministry of Public Health. On 10 Apr 2016, five people with fever and rash from a village in Phetchabun Province were notified. Descriptive, case-control, and environmental investigations were conducted to verify diagnosis, describe characteristics of the outbreak, and determine risk factors. A confirmed case was a person with dengue-related symptoms, and dengue Immunoglobulin IgM, or dengue nucleic acids tested by reverse transcription polymerase chain reaction (RT-PCR). Controls were those without any symptoms and were randomly selected in the village. Of 12 dengue confirmed cases, there were six with DEN-3 and two with DEN-4 viruses. Living near cases (odds ratio = 11.1, 95% CI = 1.2, 98.3) and using home for community services (odds ratio = 9.2, 95% CI = 1.1, 79.6) were associated with dengue infection. One *Aedes aegypti* mosquito was identified with Zika virus by RT-PCR. A dengue outbreak related to serotypes DEN-3 and DEN-4 was confirmed with potential coexisting Zika virus in the village. Intensive vector control, elimination of mosquito breeding sites and health education provided in align with community participation controlled the outbreak.

Keywords: Dengue fever, Zika virus, outbreak, investigation, Lomkao District, Phetchabun Province

Introduction

While half of the world population is at risk for dengue infection, the disease is commonly found in tropical and sub-tropical climates in Asian and Latin American countries.[1] Dengue virus is transmitted by *Aedes aegypti*, the same mosquito that spreads Zika virus. Both dengue and Zika virus are single-stranded RNA viruses and belong to genus *Flavivirus* in the family *Flaviviridae*.[2] Similar to other arbovirus infections, clinical manifestations of dengue and Zika virus infections include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache, which are usually mild and last for 2-7 days[2,3].

In Thailand, the first dengue infection was reported during 1949 and the first dengue hemorrhagic fever (DHF) outbreak occurred in Bangkok during 1958.[4] At present, dengue is one of the notifiable diseases in the country, and causes high morbidity and mortality every year. During 2015, morbidity rate was 63.3 per 100,000 population and mortality rate was 0.1 per 100,000 population. The highest morbidity rate was observed among children aged 10-14 years.[5,6]

The severity of dengue infection had been reported to specific serotypes while severity also depends on several factors such as immune response and underlying conditions.[7-9] Out of four dengue serotypes,

predominant serotypes in Thailand were DEN-1 in 2004 (56.4%), DEN-4 in 2007 (50.0%), DEN-1 in 2008 (57.4%) and DEN-3 in 2010 (38.7%).[10]

Regarding to Zika virus, the first human case was identified in Uganda during 1952 and outbreaks were reported in Africa and Asia in 1960-1980.[3] The disease re-emerged as a large outbreak of Zika virus and the associated microcephaly were reported in Brazil during 2015. In Thailand, Zika virus surveillance in humans was initiated since 2012, and an average of five confirmed cases of Zika virus were reported from the various parts of the country through the national surveillance system every year until 2015.[11] Two cases were reported in 2016 as of 18 Mar 2016.[12]

On 10 Apr 2016, a cluster of five people with fever and rash from village 4, Wang Ban Subdistrict, Lomkao District, Phetchabun Province (Village A) was reported to the Bureau of Epidemiology. In addition, outbreaks of dengue infection during 2010-2014 as well as an outbreak of Zika virus in humans during 2015 were reported to the provincial health office in Phetchabun. While clinical manifestations of fever and rash were consistent with dengue infection, the period of the outbreak was coincident with high transmission activity of Zika virus in the country as well. Hence, an investigation was conducted in the affected village on 12-18 Apr 2016 to verify the diagnosis, describe the characteristics of the outbreak, determine risk factors and recommend appropriate control measures.

Methods

An investigation was jointly conducted by the Bureau of Epidemiology, the Office of Disease Control and Prevention 2 and local public hospitals using descriptive study methods to identify gap and potential risk factors. An analytic study was also performed to confirm the source of infection.

Descriptive Study

A descriptive study on fever and rash was initiated by reviewing medical records of patients with dengue symptoms, dengue diagnosis, or international classification of diseases (ICD) 10 code of A97, who visited Lomkao District Hospital and Wang Ban Subdistrict Hospital in 2011-2015.

The national guidelines for prevention and control of Zika virus recommended that surveillance should be carried out in four groups in order to monitor for infection and microcephaly, to gather information on high-risk groups and areas, and to develop guidelines in setting control and prevention measures. The four groups placed under surveillance were pregnant women, general patients, infants with abnormal head (head size much smaller compared with other babies of the same age and gender)[13], and those who developed Guillain-Barre syndrome or neurological diseases after being infected. For these four groups, the definition of patient under investigation (PUI) was recommended as per the national guideline. Investigation and disease control for Zika virus infection should be undertaken among all PUIs (Figure 1).[14]

Following the national guideline[14] on surveillance of Zika virus infection, active case finding for suspected cases was carried out among patients at the hospitals for previous five years and people in Village A during 12 Mar - 28 Apr 2016. A suspected case was a person with fever and at least two of the following symptoms, or rash and one of the followings: headache, muscle pain, joint pain, vomiting and conjunctivitis in Village A during 12 Mar - 18 Apr 2016. A confirmed case was a suspected case who was tested positive for dengue, Zika or *Flavivirus* by immunoglobulin M (IgM) or reverse transcription polymerase chain reaction (RT-PCR).

Laboratory Study

Following the national guideline[14] on active case finding and specimen collection for laboratory testing of Zika virus infection, urine and plasma specimens were collected from the suspected cases who were sick for less than five days and urine specimens from those with history of illness from five days to one month (Figure 2).

The specimens were tested for dengue and Zika virus by IgM or RT-PCR at the National Institute of Health and the Bamrasnaradura Infectious Diseases Institute. As a further step, urine and plasma specimens from all family members of the confirmed cases and all pregnant women in the village were sent to these two laboratories for testing.

Analytic Study

A case-control study was employed to identify risk factors. A case was either a suspected or confirmed case while a control was a person in the same village without any symptoms. The case and control ratio was 1:1. Controls were selected by simple random sampling from the list of village residents with no specified symptoms. Crude odds ratio were calculated for all potential risk factors. All those with p-value lower than 0.05 were included in the multiple logistic regression model. The Epi Info version 3.5[15] was utilized to calculate the odds ratios (OR).

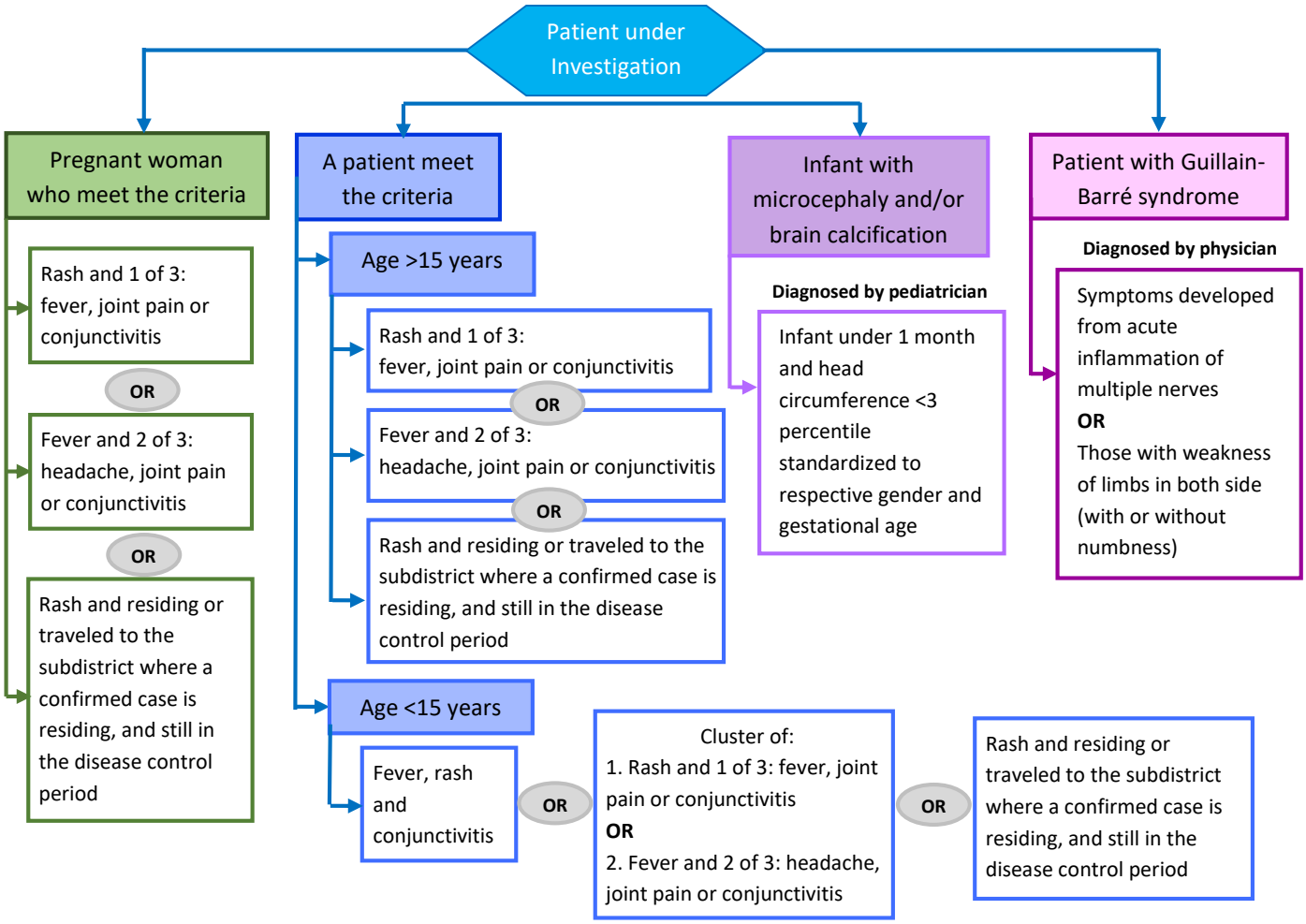
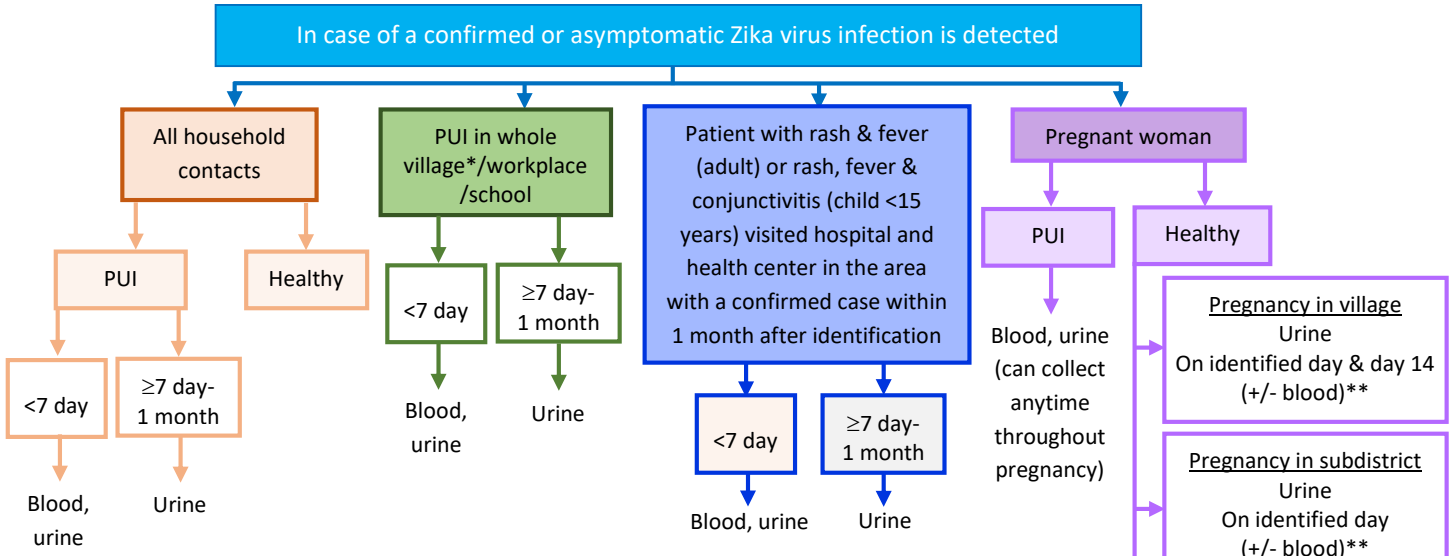


Figure 1. Criteria of patient under investigation for investigation of Zika virus infection in Thailand, specified by the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, 2016[10]



** In case of a patient residing in municipal area, look for patient under investigation (PUI) within 100 meter radius.*

*** If a widespread outbreak might occur for a certain period of time before identification of the index case, blood collection of pregnant women in the village or same subdistrict with all confirmed cases should be considered. The virus can be identified in blood but not in urine of some pregnant women as the virus can stay longer in blood and can still transmit the infection.*

Figure 2. Guideline on active case finding and specimen collection for confirmed cases and asymptomatic infection of Zika virus infection by the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, 2016[10]

Environmental Study

In order to find the source of infection, an environmental survey was carried out in the village by the investigation team. Using the Thai national guidelines issued in 2016[14], an environmental survey for mosquito larvae was performed in 100-meter radius around the houses of suspected and confirmed cases. House index (HI) and container index (CI) were calculated for days 0, 3, 5, 7, 14, 21 and 28, started from the day when a case was discovered. HI is the percentage of houses infested with larvae and/or pupae, and CI is the percentage of water-holding containers infested with larvae or pupae.[16] In addition, mosquitoes and larvae were trapped and tested for dengue and Zika virus by RT-PCR in the laboratory of Vector Borne Disease Control Center of Phetchabun Province.

Results

Descriptive Study

Reviewing the medical records in the hospitals for cases with fever and rash revealed that dengue clusters during 2011 and 2013, one rubella case during 2013 and two Zika virus infections during 2014 were reported in this area.

A total of 32 suspected cases were identified in Village A of Petchabun Province during 1-28 Apr 2016. The male-to-female ratio was 1:1.6 and the mean age was 24 years, with a range of 4-76 years. The index case was a student and 37.5% of the suspected cases were students as well. The most common clinical manifestation was rash (87.5%) (Figure 3). The majority (79.2%) of the rashes were maculopapular rash and 20.8% had erythema for 1-5 days, with average of two days. Rash was found mostly over the chest and back (51.6%). About 43.8% of cases had joint pain for 1-4 days (average 2 days). Almost all cases had fever (84.4%) and some had conjunctivitis (31.3%).

The index case was a 13-year-old girl and developed symptoms on 10 Mar 2016. Before the semester break, she travelled to the school in the subdistrict to receive her examination result eight days prior to disease onset. The school reported that no students got ill with fever and rash. She was completely recovered on 17 Mar 2016, without visiting a hospital or clinic. Later on, the district hospital identified four children with fever with rash in the same community.

Laboratory Study

Samples from 32 suspected cases were tested and 12 (37.5%) were tested positive for dengue by IgM or RT-PCR. Of these, four (33.3%) cases were found to have *Flavivirus* by IgM while six cases (50.0%) were identified with DEN-3 virus by RT-PCR and two cases (16.6%) had DEN-4 by RT-PCR. None of them were found to have Zika virus. There were two pregnant women in the village and both of them tested negative for dengue and Zika virus.

Hence, 12 cases were confirmed to have dengue infection in this outbreak. The attack rate was 8.5% among 142 residents in the village. The outbreak started on 14 Mar 2016 and ended on 30 Apr 2016, with the peak in mid-April (Figure 3). After monitoring for 28 days from the onset of the last case, since there were no new case, the outbreak was declared an end.

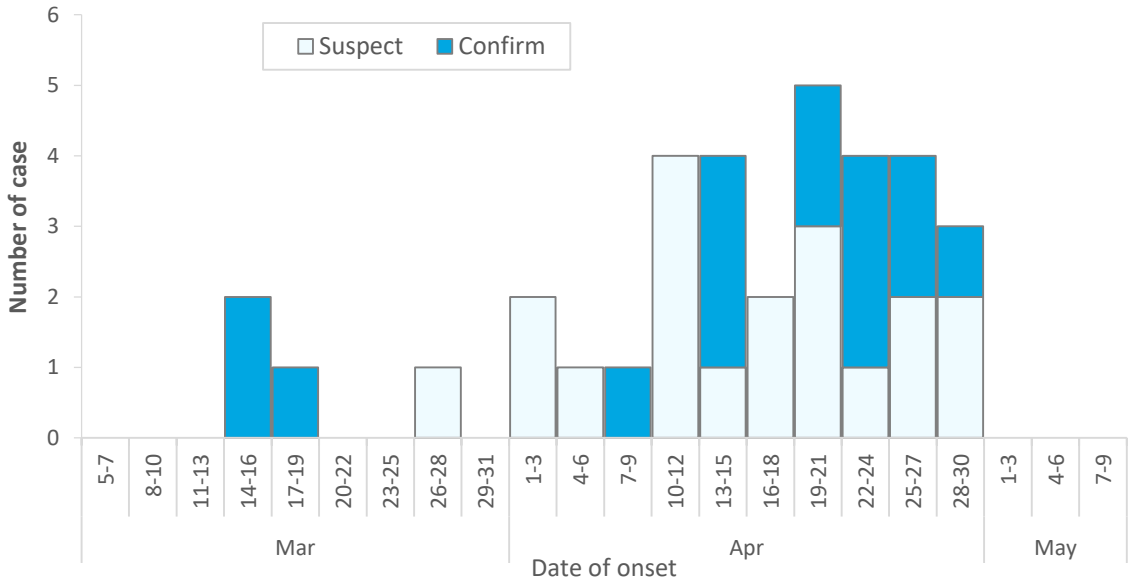


Figure 3. Number of dengue cases by date of onset in a village, Lomkao District, Phetchabun Province, Thailand, 3 Mar to 8 May 2016 (n = 32)

Analytic Study

In order to identify the potential risk factors for dengue virus infection, all 12 dengue confirmed cases and 20 suspected cases were included in the analysis. In univariate analyses, four statistically significant risk factors were: being a student (OR = 31.2, 95% CI = 1.7-563.2), having a suspected case in the family (OR = 23.0, 95% CI = 1.3-420.4), living within 100-meter radius of a suspected case's house (OR = 2.9, 95% CI = 1.0-8.7), and using home as a grocery shop or for community service (OR = 8.5, 95% CI = 2.3-32.2). A multivariate analysis revealed that living within 100-meter radius from a suspected case's house (Adjusted OR = 11.1, 95% CI = 1.2-98.3, p-value = 0.03) and using home as a grocery shop or for service (Adjusted OR = 9.2, 95% CI = 1.1-79.6, p-value = 0.04) were factors associated with dengue infection (Table 1).

Environmental Study

The time of the outbreak was at the end of summer and approaching the rainy season. There was a forest and a river running near the village. The village was far from schools. Containers with water were observed in an antique shop, and there was a home-made tamarind pickle shop. These pickle pots were used for one week and then left empty until the next batch of tamarind was available. Since there was no cover for the pots, water might collect in the vacant pots (Figure 4).

A total of 10 houses and a temple were surveyed for HI and CI in Village A. The highest HI (26.6%) was spotted within 100-meter radius of the suspected cases (average HI 13.1%) while the highest CI was discovered in another house with 11.5% (average CI 4.5%). No mosquito larvae or pupae were found either in the temple or containers around the temple.

Figure 4. Home-made tamarind pickle pots in a village, Lomkao District, Phetchabun Province, Thailand, 2016

Six *Aedes aegypti* and one *Culex* mosquitoes were trapped from seven areas near the suspected cases' houses. Among them, Zika virus was detected in the salivary gland of one *Aedes aegypti* by RT-PCR.

Actions Taken

In addition to strengthening of surveillance and active case finding, we coordinated with local authorities to identify more budget for the disease control team. Activities on vector control, health education, repellent distribution, and use of insecticides and ultra low volume fogging for adult mosquitos were enhanced. Unused water containers were destroyed and chemical control was implemented at 100-meter radius of the cases' houses. Health personnel from other districts and the Vector Borne Disease Control Center 2.2 in Phetchabun checked for HI and CI on 0, 3, 5 and 7 days after the outbreak alert. Concept of community participation was emphasized in parallel with enhancing control measures until 28 days after the onset of the last case.

Table 1. Univariate and multivariate analysis on risk factors for dengue infection in a village, Lomkao District, Phetchabun Province, Thailand, March - April 2016

Factor	Univariate		Multivariate	
	Crude Odds Ratio	95% CI	Adjusted Odds Ratio	95% CI
Being a student	31.2	1.7-563.2	8.0	0.6-99.2
Having a suspected case in the family	23.0	1.3-420.4	0.4	0.02-8.6
Living within 100-meter radius of a suspected case's house	2.9	1.0.-8.7	11.1	1.2-98.3
Using home as a grocery shop or for services in community	8.5	2.3-32.2	9.2	1.1-79.6

Discussion

Through identification of *Flavivirus* and dengue serotypes DEN-3 and DEN-4, an outbreak of dengue virus infection was confirmed in the Village A of Lomkao District, Phetchabun Province. The clinical manifestations of fever, maculopapular rash generally on large areas of body and joint pain were consistent with those of dengue and Zika virus infections[2,3].

The index case and 37.5% of the suspected cases were students. The period of outbreak from Mar to Apr 2016 corresponded with the semester break of the schools, February to May. This result was consistent with the previous knowledge showing that dengue virus infection most frequently occurs among those aged 5-14 and 15-24 years old, and 50% were students.[17] This could be explained by the fact that in general, children tend to have a lower rate of immunity to dengue infection.[18] Despite this, an increasing trend of dengue infection had also been observed in older age groups.[19]

Although dengue virus infection in Thailand generally revealed serotypes DEN-3 (30.9%) and DEN-4 (29.5%) in the previous decade,[6] a higher number of DEN-1 (79.6%) and DEN-2 (10.7%) serotypes were reported during 2014 (35.7%).[20] Especially in the northern region, DEN-2 and DEN-1 had been most frequently reported in 2013 (DEN-1 41.5%) and 2014 (DEN-1 79.5%). The fatality rate for DEN-4 was 50%. Many severe cases were reported in 2014 as people were not immune to the new serotype.[21,22] However, one study in 2014 reported that serotypes DEN-3 (46.6%) and DEN-4 (31.0%) were predominant in the northeastern Thailand.[18] This outbreak occurred in a northern village, and DEN-3 and DEN-4 serotypes were identified, which exhibited a discrepancy with the prior national data of dengue infection and yet, consistent with the findings in the northeastern area due to geographical proximity.

All the cases identified in this study, including DEN-3 and DEN-4 serotypes, presented as mild infections. A previous study in Thailand stated that DEN-2 and DEN-3 infections were two times more likely to result in severe infection compared with DEN-4.[7] While DEN-2 was noted to be more pathogenic in some studies[23,24], DEN-1 may also lead to severe disease, as shown in a study conducted by Balmaseda A, et al[25]. Despite that, DEN-4 was related to mild infections in some reports[26,27].

In addition, mosquito-infested places with high HI and CI were detected within 100-meter radius of the suspected cases' houses, which was higher than the standard criteria specified by the Bureau of Epidemiology (Table 2). High density of mosquito breeding sites was augmented by the analytic findings that living near the suspected cases' houses and using home to provide community services were associated with dengue infection. The houses attached to a shop or a service delivery place are liable to be crowded with visitors and tends to be a source for dengue outbreak, especially if the area is populated with high number of mosquitoes. Potential breeding sites in close vicinity were associated with outbreak spots and found to advance the outbreak as reported previously.[28,29]

Since *Flavivirus* was identified by IgM in four cases, antibody testing of dengue virus could be confounded by cross-reactivity with other *Flaviviruses* when a person was infected with or vaccinated against other *Flaviviruses* such as Zika virus, yellow fever or Japanese encephalitis, especially during the acute

Table 2. Environmental targets for larva survey of Zika virus infection, specified by the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, 2016[10]

No.	Target	General Areas	Municipal Areas	Bangkok and Pattaya City
1.	HI and CI 0% within 5 days	- Whole village - If a suspected or confirmed case's house is close to other villages, mosquito larva control must be done in half of each village located within 100 radius meter. - Within 100 meter radius of the case's regular route if the case go out of the home after onset of symptoms	- Within 100 meter radius of a case's house - Within 100 meter radius of the case's regular route if the case go out of the home after onset of symptoms	- Within 100 meter radius of a case's house - Within 100 meter radius of the case's regular route if the case go out of the home after onset of symptoms
2.	HI and CI <5% within 14 days	- Whole subdistrict where the case is residing	- Whole community or village where the case is residing	-
3.	HI and CI <5% within 28 days	- Whole district where the case is residing	- Whole subdistrict where the case is residing	- Whole community where the case is residing

phase of infection[30]. Furthermore, *Aedes aegypti* mosquito in the affected area was found to have Zika virus. Identification of two Zika infection in 2014 by reviewing of medical records and findings of Zika virus infection in Phetchabun Province from other studies[31,32] were in supportive as evidences of existing Zika virus in the area.

Due to the facts mentioned above, the findings suggested a dengue outbreak with potential coexisting of Zika virus in the area. Co-infection of dengue and Zika viruses was observed among two patients in New Caledonia during 2014[33] and a traveler returning from Haiti in 2016[34].

Conclusion

The identification of *Aedes aegypti* mosquitoes in conjunction with the epidemiological findings confirmed a dengue outbreak related to DEN-3 and DEN-4 serotypes, and potential coexiting Zika virus in Village A of Lomkao District, Phetchabun Province.

Recommendations

The remarkable fact was that the dengue outbreak occurred among students during the semester break although being a student was not a significant factor in the multivariate analysis. This highlighted the importance of implementing the vector control program in the communities during semester breaks in addition to the routine campaigns in schools.

Furthermore, results of dengue serotyping could pinpoint the potential source of endemic area as well as predict the severity of involved cases. Hence, the serotyping of the infected cases should be performed in the future investigations of dengue outbreaks.

While HI and CI are definite and simple indicators for entomological surveillance to predict the vector population, these should be carried out to prioritize areas for vector control as well as be vigilance with routine environmental surveys for potential outbreaks. Since HI and CI must be zero within five days in the affected village and less than 5% within 14 days in the subdistrict as per the national guideline[14], strict mosquito control measures were recommended to the related public health offices. Proper sanitation and elimination of mosquito breeding sites especially in houses with high HI and CI, and the service delivery places was educated to the local public.

Limitations

As the study was carried out among patients with clinical symptoms, asymptomatic cases were not included in the study and the findings might not represent the actual disease prevalence in the area. However, since the identified risk factors were related to the residence, recall bias was less likely to exist in this study.

As the *Flavivirus* was detected by IgM, the United States Centers for Disease Control and Prevention recommended that testing of acute specimens should be conducted by the molecular testing such as RT-PCR for confirmation of Zika virus infection in the future studies[36].

Acknowledgment

This study could not be achieved without assistance from the staff in Bureau of Epidemiology, Office of Disease Prevention and Control 2 in Phitsanulok Province, Phetchabun Provincial Health Office, Lomkao District Health Office, Lomkao District Hospital, and Wang Ban Subdistrict Hospital. Hence, we acknowledged and documented our deepest appreciation all of them in this manuscript.

Suggested Citation

Nurnchut A, Aye YM, Sookvech S, Sookkhum S, Suwannachairob A, Buathong R, et al. A cluster of suspected cases of Zika leading to uncommon dengue serotypes with possible coexisting Zika virus in northern Thailand, 2016. OSIR. 2018 Sep;11(3):13-21.

References

1. World Health Organization. Dengue and severe dengue. 2017 Apr 7 [cited 2017 May 10]. <http://www.who.int/mediacentre/factsheets/fs117/en/>.
2. Centers for Disease Control and Prevention. *Flaviviridae*. 2014 Apr 1 [cited 2017 May 10]. <https://www.cdc.gov/vhf/virus-families/flaviviridae.html>
3. World Health Organization. Zika virus. 2018 Jul 20 [cited 2018 Aug 20]. <http://www.who.int/mediacentre/factsheets/zika/en/>.
4. Limkittikul K, Brett J, L'Azou M. Epidemiological trends of dengue disease in Thailand (2000-2011): a systematic literature review. PLoS Negl Trop Dis. 2014 Nov 6;8(11):e3241. eCollection 2014.
5. Thailand. Bureau of Epidemiology. Department of Disease Control. Ministry of Public Health. National disease surveillance

(Report 506): communicable diseases [cited 2017 May 10]. <http://www.boe.moph.go.th/boedb/surdata/index.php>.

6. Thailand. Bureau of Vector Borne Diseases. Department of Disease Control. Ministry of Public Health. Dengue situation for week 27 in 2016. Thai [cited 2017 May 10]. <https://tinyurl.com/ybus6gfy>.
7. Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Yoon IK, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. PLoS Negl Trop Dis. 2010 Mar 2;4(3):e617.
8. Thein TL, Wong JG, Tan LK, Yung CF, Gan V, Ooi EE, et al. Dengue serotypes and disease severity in Singapore. 2012 Jun;16(1):e114.
9. Rocha BAM, Guilarde AO, Argolo AFLT, Tassara MP, da Silveira LA, Junqueira IC, et al. Dengue-specific serotype related to clinical severity during the 2012/2013 epidemic in centre of Brazil. Infect Dis Poverty. 2017 Aug 2;6(1):116.
10. Pongsiri P, Themboonlers A, Poovorawan Y. Changing pattern of dengue virus serotypes in Thailand between 2004 and 2010. J Health Popul Nutr. 2012 Sep;30(3):366-70.
11. National Trustworthy and Competent Authority in Epidemiological Surveillance and Investigation. Zika virus. 2016 Sep 6. Thai [cited 2017 May 10]. <https://tinyurl.com/y6ux5bbc>.
12. Thailand. Bureau of Emerging Infectious Diseases. Department of Disease Control. Ministry of Public Health. Zika virus disease. 2016 Mar 18. Thai [cited 2016 Jun 15]. <http://beid.ddc.moph.go.th/beid_2014/sites/default/files/situation_zika180359.pdf>.
13. World Health Organization. Microcephaly. 2016 Oct 14 [cited 2018 Aug 6]. <http://www.who.int/news-room/fact-sheets/detail/microcephaly>.
14. Luangon W, Mongklangkul N, editors. Manual on prevention and control of Zika virus infection for medical and public health personnel, 2016. Bangkok: Printing Office, The War Veterans Organization of Thailand Under Royal Patronage of His Majesty the King; 2016.
15. Centers for Disease Control and Prevention. Epi Info. 2016 Sep 15 [cited 2016 Jun 5]. <http://wwwn.cdc.gov/epiinfo/html/prevVersion.htm>.
16. World Health Organization. Dengue control: vector surveillance [cited 2018 Feb 5]. <http://www.who.int/denguecontrol/monitoring/vector_surveillance/en/>
17. Heymann DL. Control of communicable diseases manual. 19th ed. Washington DC: American Public Health Association, 2008.
18. Thailand. Department of Arbovirus. National Institute of Health. Department of Medical Sciences. Ministry of Public Health. Annual report 2015. Nonthaburi: Department of Medical Sciences; 2016. Thai.
19. Thailand. Bureau of Vector Borne Diseases. Department of Disease Control. Ministry of Public Health. Guideline for dengue and dengue fever for clinical and public health. Nonthaburi: Department of Disease Control; 2015. Thai.
20. Thara U, Pakdeenual P, Thawachasin A, Chomepoosri J, editors. The biology, serotypes and disease cycle of dengue virus in Thailand. Nonthaburi: National Institute of Health, Thailand; 2015. Thai.
21. Thailand. National Institute of Health of Thailand. Ministry of Public Health. Report of Dengue Virus Serotype in 2013. Thai [cited 2018 August 6]. <http://nih.dmsc.moph.go.th/dengue/10_11_56.pdf>.
22. Thailand. National Institute of Health of Thailand. Ministry of Public Health. Report of Dengue Virus Serotype by Region in 2014. Thai [cited 2018 August 6]. <http://nih.dmsc.moph.go.th/login/showimgpic2.php?id=703>.
23. Chen RF, Yang KD, Wang L, Liu JW, Chiu CC, Cheng JT. Different clinical and laboratory manifestations between dengue haemorrhagic fever and dengue fever with bleeding tendency. Trans R Soc Trop Med Hyg. 2007 Nov;101(11):1106-13. Epub 2007 Aug 30.
24. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with

disease severity. J Infect Dis. 2000 Jan;181(1):2-9.

25. Balmaseda A, Hammond SN, Pérez L, Tellez Y, Saborío SI, Mercado JC, et al. Serotype-specific differences in clinical manifestations of dengue. Am J Trop Med Hyg. 2006 Mar;74(3):449-56.
26. Nisalak A, Endy TP, Nimmannitya S, Kalayanarooj S, Thisayakorn U, Scott RM, et al. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. Am J Trop Med Hyg. 2003 Feb;68(2):191-202.
27. Klungthong C, Zhang C, Mammen MP Jr, Ubol S, Holmes EC. The molecular epidemiology of dengue virus serotype 4 in Bangkok, Thailand. Virology. 2004 Nov 10;329(1):168-79.
28. Wijayanti SP, Porphyre T, Chase-Topping M, Rainey SM, McFarlane M, Schnettler E, et al. The importance of socio-economic versus environmental risk factors for reported dengue cases in Java, Indonesia. PLoS Negl Trop Dis. 2016 Sep 7;10(9):e0004964. eCollection 2016 Sep.
29. Vincenti-Gonzalez MF, Grillet ME, Velasco-Salas ZI, Lizarazo EF, Amarista MA, Sierra GM, et al. Spatial analysis of dengue seroprevalence and modeling of transmission risk factors in a dengue hyperendemic city of Venezuela. PLoS Negl Trop Dis. 2017 Jan 23;11(1):e0005317. eCollection 2017 Jan.
30. World Health Organization and Special Programme for Research and Training in Tropical Diseases. Handbook for clinical management of dengue. Geneva: World Health Organization; 2012.
31. Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, et al. Detection of Zika virus infection in Thailand, 2012-2014. Am J Trop Med Hyg. 2015 Aug;93(2):380-3. Epub 2015 Jun 22.
32. European Centre for Disease Prevention and Control. Epidemiological update: outbreaks of Zika virus and complications potentially linked to the Zika virus infection, 1 September 2016. 2017 Jun 12 [cited 2018 Aug 6]. <https://tinyurl.com/y92ml2f4>.
33. World Health Organization. Dengue control [cited 2017 May 10]. <http://www.who.int/denguecontrol/human/en/>.
34. World Health Organization Regional Office for South-East Asia. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Geneva: World Health Organization; 2011.
35. Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daurès M, John M, Grangeon JP, et al. Co-infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014. Emerg Infect Dis. 2015 Feb;21(2):381-2.

Outbreak, Surveillance, Investigation & Response (OSIR) Journal
Field Epidemiology Training Program, Bureau of Epidemiology
Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

Grammar of Science: To Boost "Odds" to Reduce "Risks" and to Avoid "Hazards"

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A patient gets confused by listening to his doctors. Doctor A told him that "Knowing your risk factors for stroke is the first step in preventing a stroke. Risk factors that you can change or treat included, for examples, high blood pressure, smoking, diabetes, high cholesterol, physical inactivity and obesity, and sleep apnea. But the risk factors that you can't control are increasing age, gender, heredity and race."[1] Doctor B told him that "Nothing will help you prevent a stroke more than quitting smoking. Other important ways to lower your odds of having a stroke include lose weight, drink less alcohol, consume less sodium (salt), eat a healthy diet and spend less time in front of screens and more time walking."[2] Doctor C, warned him that "For people who were admitted to a hospital at the time of their index stroke and received the treatment in time, the chance of stroke recurrence was reduced by 16%. Based on their hazard ratios, factors associated with stroke recurrence include having comorbid conditions, both diabetes and urinary incontinence, and other cardiac conditions."[3]

So what are "Risk", "Odds" and "Hazard"?

By dictionary definition, "risk" is the possibility of loss or injury[4]. In epidemiology, however, "risk" is defined as "incidence". Statisticians further define "incidence" as "chance" or "probability" of developing outcome of interest no matter good or bad (e.g., disease, cure or die). Incidence means the occurrence of new outcomes in the study population over a specified period of time, and it also means the number of new cases per unit of population.[5,6] Thus, we can say that there are two types of incidence that are commonly used: incidence proportion and incidence rate. Incidence proportion or cumulative incidence is the proportion of a population that does not have the outcome (simply called disease-free population) and then some subsequently develop the outcome during a specified period of time. Basic statistical formula for Incidence proportion is number of new cases (numerator) divided by the total number of population (denominator); thus, a risk is a proportion. Incidence proportion does not take into consideration about time-at-risk (follow-up time from the starting point and still disease free, but the person is at risk of having the outcome).[7,8]

In a research study, if your question is about how many outcomes occur in the total population within a unit of time (e.g., per day, month, year), that means we want to know incidence rate or person-time rate. A person-time is an epidemiologic jargon and generally calculated from a total time of all people in a study contributed until they reach the "endpoints" (i.e., having the outcome of interest) or are "censored" (i.e., not having the outcome due to lost to follow-up or reaching the end of the study period). Basic statistical formula for Incidence rate is number of new cases (numerator) divided by the total time-at-risk of population (denominator). Thus, an incidence rate reflects how quickly disease occurs in a population.[6-8]

Odds can be defined as the risk (or probability) of an outcome occurring over the risk (or probability) of an outcome not occurring.[6] For example, if we follow 100 smoking people in a community for five years (each person contributes five years of follow-up time) and 10 of them eventually develop stroke at the end of 5 years. We now can say that among smokers the risk of having stoke is (10/100) 0.1 or 10%, the rate of having stroke is (10/500) 0.02 or 2% per year, and the odds of having stroke is 0.1/0.9 or 0.11:1.

By dictionary definition, a "hazard" is a source of harm or danger; where "danger" is exposure or liability to injury, pain, harm, or loss[9,10]. From this definition, hazard is danger, and risk is the probability of encountering the danger. However, in epidemiology, similar idea but not exactly the same as incident rate, the term "hazard" refers to the probability that a person has been followed and then develops an outcome or reaches the endpoint at time t[11,12]. We can say that hazard is the probability of an outcome occurrence of an individual, based on his/her

"time-to-event" (so-called "survival time"); thus, hazard represents the instantaneous event rate for an individual who has already survived to the time "t"[11]. For examples, we can calculate a hazard of a diabetes patient to develop second episode of stroke after he has been followed from his first stroke.

Risk Comparisons – "Odds Ratio", "Risk Ratio" and "Rate Ratio"

Now we want to compare risks among those who have different exposures, which means that we want to assess a measure of association or relationship between exposure and outcomes among the two groups. Exposure is a generic epidemiologic term while it could be personal characteristics (e.g., gender, age, occupation, smoking), genetic/biologic characteristics (e.g., genotyping, immune status), acquired characteristics (e.g., disease status), or environmental characteristics (e.g., residential). Common measures of association include risk ratio (relative risk), rate ratio and odds ratio.[6,13]

A risk ratio or relative risk (RR) compares the risk of having the outcome of the two exposure groups. Basically, RR is calculated by dividing the risk (or incidence proportion) of one group against the risk in another group (baseline or reference group). A rate ratio (also abbreviated as RR) compares the incidence rates or person-time rates of the two groups. Odds ratio (OR) is another measure of association, comparing the odds of an outcome occurring in one group by the odds of the same outcome in another group.[6,8,12,13] As an example shown in figure 1, in a clinical trial, the AIDS patients with an initial episode of PCP (*Pneumocystis carinii* pneumonia) were randomly allocated to receive treatment A or B. Patients in each group were followed up, and some of them had PCP relapse. However, they were not all "relapsed" (reaching the endpoint) or "not relapsed" (being censored) at the same time. For example, patient obs#1 were followed and had relapse (pcp=1) at 11.9 months, while patient obs#2 were followed 11.6 months and not relapsed (pcp=0). The researchers then can compare the two treatments regarding the risk of having PCP relapse by calculating RR (risk/rate ratios) or OR as shown in figure 2.

	obs	trt	trtno	pcp	pdate
1.	1	B	0	1	11.9
2.	2	B	0	0	11.6
3.	3	A	1	0	12.8
4.	4	A	1	0	7.3
5.	5	B	0	1	4.5
6.	6	B	0	0	18.1
7.	7	A	1	0	14.7
8.	8	B	0	0	24
9.	9	A	1	0	16.2
10.	10	A	1	0	26.6

Figure 1. Example of raw data of a clinical trial to compare risk of relapse between two treatments

trtno	relapse (pcp: 1=yes, 0=no 0	1	Total	Total_time (months)
0	120 77.42	35 22.58	155 100.00	2073.7
1	140 90.91	14 9.09	154 100.00	2379.6
Total	260 84.14	49 15.86	309 100.00	4453.3

Odds ratio = Odds trtnoA / Odds trtnoB
= [(Probability Yes : Probability No) trtnoA] / [(Probability Yes : Probability No) trtno B]
= [(14/154)/(140/154)] / [(35/155)/(120/155)]
= 0.343

Risk ratio = Risk trtno A / Risk trtnoB
= Incidence Proportion trtnoA / Incidence Proportion trtnoB
= Probability Yes trtnoA / Probability Yes trtnoB
= (14/154) / (35/155)
= 0.403

Rate ratio = Rate trtnoA / Rate trtno B
= Incidence Rate trtnoA / Incidence Rate trtno B
= (14/2379.6) / (35/2073.7)
= 0.349

Figure 2. Basic statistics for comparing risk of relapse between two treatments

By statistical formula, we will find that OR approximates risk ratio when the outcomes are rarely happened. OR cannot be used to estimate rate ratio because the denominator of the rate is time-at-risk. When should we present odds ratio or risk ratio? If the outcome is incidence, we can present either risk ratio or odds ratio; if not, we have to present OR[6,7,8]. There is a recommendation that no matter we select to present risk ratios and OR, we should give information about the frequencies of the outcome and the exposure risk factor[7].

Risk Comparisons – "Hazard Ratio"

As previously mentioned, time-to-event or survival time is the expected duration of time until one or more events happen. Although it is called survival time, but the event or endpoint does not have to always be "dead"; the researchers may want to study time from date of drug initiation until date the patient is cured. Analysis of time-to-event takes into consideration for both cases that have complete time from the starting point to reaching the endpoint and cases that have time from the starting point until they are censored. Censoring that is random and non-informative is usually required in order to avoid bias in a time-to-event analysis; thus, the analysis will correctly incorporate information from both censored and uncensored observations[14,15].

Based on the time-to-event and the event status (endpoint or censored), we can estimate two functions that are dependent on time, the survival and hazard functions.[14] Both functions describe the distribution of event times. The survival function gives, for every time, the probability of surviving (or not reaching the outcome) up to that time. On the opposite, the hazard function gives the potential that the outcome event will occur, per time unit, given that an individual has survived (or not yet having the outcome) up to the specified time[14]. Based on the example of a clinical trial among PCP patients who were randomly allocated to treatment A or B, each patient had different follow-up "time" in the study (Figure 3). Some were "relapsed" (so-called "failure" cases) and some were "not relapsed" (so-called "censored" or "net loss" cases) at different follow-up times. For example, among 155 patients in treatment A (trtno=0) group at the beginning, there was one relapsed case and none loss (or censored) at the time of 0.2 month; thus, there were 154 patients at the beginning of next time period and another one relapsed and none censored at the next time period of 1.1 month, and so on. From those events throughout each time period, we can calculate survival function (probability of "not relapse" over time) and hazard function (probability of "relapse" over time) as shown in figure 3.

Time	Beg. Total	Relapse	Net Lost	Prob Relapse	Prob Not relapse	Survivor Function		Hazard Function		Cummulative Hazard
trtno=0										
0.2	155	1	0	0.00645	0.99355	0.99355	*(0.99355 x 1)*	0.00645	*(1 - 0.99355) / 1*	0.00645
1.1	154	1	0	0.00649	0.99351	0.98710	*(0.99351 x 0.99355)*	0.00649	*(0.99355 - 0.98710) / (0.99355)*	0.01294
1.2	153	1	0	0.00654	0.99346	0.98065	*(0.99346 x 0.98710)*	0.00654	*(0.98710 - 0.98065) / (0.98710)*	0.01948
1.3	152	0	1	0.00000	1.00000	0.98065	*(1.00000 x 0.98065)*	0.00000	*(0.98765 - 0.98765) / (0.98765)*	0.01948
1.4	151	2	0	0.01325	0.98675	0.96766	*(0.98675 x 0.98065)*	0.01325	*(0.98765 - 0.96766) / (0.98765)*	0.03272
1.5	149	0	1	0.00000	1.00000	0.96766	*(1.00000 x 0.96766)*	0.00000	*(0.96766 - 0.96766) / (0.06766)*	0.03272
:	:	:	:	:	:	:	:	:	:	:
trtno=1										
0.1	154	1	1	0.00649	0.99351	0.99351	*(0.99351 x 1)*	0.00649	*(1 - 0.99351) / 1*	0.00649
0.4	152	1	0	0.00658	0.99342	0.98697	*(0.99342 x 0.99351)*	0.00658	*(0.99351 - 0.98697) / (0.99351)*	0.01307
0.6	151	1	0	0.00662	0.99338	0.98044	*(0.99338 x 0.98697)*	0.00662	*(0.98697 - 0.98044) / (0.98697)*	0.01969
2.9	150	0	1	0.00000	1.00000	0.98044	*(1.00000 x 0.98044)*	0.00000	*(0.98044 - 0.98044) / (0.98044)*	0.01969
5.2	149	0	1	0.00000	1.00000	0.98044	*(1.00000 x 0.98044)*	0.00000	*(0.98044 - 0.98044) / (0.98044)*	0.01969
5.5	148	0	1	0.00000	1.00000	0.98044	*(1.00000 x 0.98044)*	0.00000	*(0.98044 - 0.98044) / (0.98044)*	0.01969
:	:	:	:	:	:	:	:	:	:	:

Beg Total = Number of cases at the beginning of time period
Relapse = Number of relapse cases at the time period
Net lost = Number of cases who lost to follow-up or exit the study at the time period

Prob relapse = Probability of relapse (failure) = Relapse / Beg_total
Prob Not relapse = Probability of not relapse (survival) = 1 – Prob Relapse

Survival Function (*St*) = Probability of not relapse at the end of that period
= Prob Not relapse at time t x Prob not relapse at the end of time t-1

Hazard Function (Hz_t) = Probability of relapse over time at time t ,
given that the person not relapse at time t-1
= *St* that changes at the end of time t from time t-1,
given that the person not relapse at time t-1
= *(St – St-1) / St-1*

Figure 3. Examples of survival function and hazard function of the two treatment groups

From the nonparametric estimators of the survival function (Figure 3), we usually present survival probabilities as a function over time using the Kaplan Meier graph as shown in figure 4. When we compare chance of reaching the outcome over time (hazard function) between two groups with different exposures (e.g., Treatment A-B, smoking Y-N), we will get "hazard ratio" (HR). Thus, we can say that HR is a measure of relative risk over time in circumstances where we are interested not only in the total number of events, but in their timing as well [8,14,15].

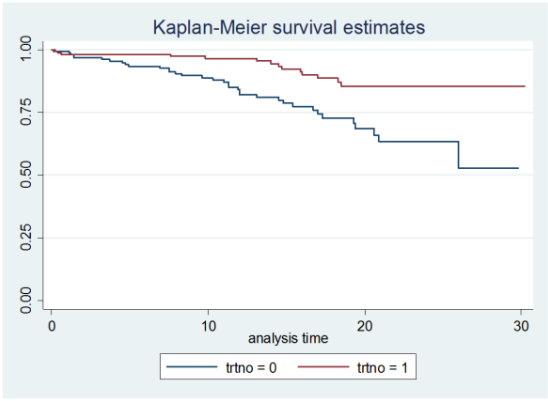

Figure 4. Kaplan-Meier survival function curve of two treatments

What can the Doctor Tell the Patient about "Odds", "Risks" or "Hazards"?

So we can calculate the OR/RR/HR from the study samples, but does it represent the "true" risk in population? Can you recommend your patient to boost or reduce odds (chance of having the outcome over not having the outcome), risk (chance of having the outcome), or hazard (chance of having the outcome over time)? Regression models that give you OR (logistic regression model), RR (Poisson regression model) and HR (Cox's proportional hazard model) usually provide hypothesis testing of the OR/RR/HR with p-value estimate.[6,7,14] In literature, sometimes they do not present p-value, but show OR/RR/HR with its 95% CI. Remember our definition of 95% CI in previous article: it represents the estimates of the true value in the population. For this statistics, if the 95% CI of the estimate does not include 1, we will say that such factor is statistically significant. For example, if OR of stroke between smoking versus not smoking groups is 4.5 (95% CI = 3.0-7.6), then we can say that the odds to have stroke were statistically significant different (increase) among smokers compared to non-smokers. If RR of stroke between male versus female is 2.8 (95% CI = 0.8-3.7), then we can say that the risk to have stroke were not statistically significant different between male and female. If HR of stroke between treatment A versus treatment B is 0.25 (95% CI = 0.2-0.5), then we can say that the risk to have stroke were statistically significant different (reduce) if the patients get treatment A compared to those who get treatment B. Note that when OR/RR/HR is 1, it means no statistically difference between comparison groups; when it is more than 1, that means one group has higher risk than its counterpart group (baseline/reference group); and when it is less than 1, that means one group has lower risk (protective) than its counterpart group. If the study is a clinical trial, we can also calculate "efficacy" of the treatment, technically called "prevented fraction among the exposed" from RR/HR; the formula is "Efficacy = 1-RR or 1-HR". For example, when HR of stroke between treatment A versus treatment B is 0.25 (95% CI = 0.15-0.45), then we can say that the efficacy of treatment A compared to treatment B is 75% (55-85%)[6,11].

Now the patient understands the terms "risks", "odds" and "hazards" that Doctor A, Doctor B and Doctor C are trying to tell him!

Suggested Citation

Kaewkungwal J. Grammar of science: to boost "odds" to reduce "risks" and to avoid "hazards". OSIR. 2018 Sep;11(3):22-6.

References

1. American Stroke Association. Let's talk about risk factors for stroke. 2017 [cited 2018 Sep 4]. <https://www.strokeassociation.org/idc/groups/stroke-public/@wcm/@hcm/documents/downloadable/ucm_309713.pdf>.
2. Harvard Health Publishing. How to lower your stroke risk. 2013 Aug [cited 2018 Sep 4]. <https://www.health.harvard.edu/heart-health/how-to-lower-your-stroke-risk>.
3. Lee AH, Somerford PJ, Yau KKW. Risk factors for ischaemic stroke recurrence after hospitalization. Med J Aust. 2004;181(5): 244-6.
4. Merriam-Webster. Risk [cited 2018 Sep 6], <http://www.merriam-webster.com/dictionary/risk>.
5. Cole SR, Hudgens MG, Brookhart MA, Westreich D. Risk. Am J Epidemiol. 2015;181(4):246-50.
6. Centers for Disease Control and Prevention. Principles of epidemiology in public health

practice: an introduction to applied epidemiology and biostatistics. 3rd ed. 2012 May [cited 2018 Sep 6]. <https://www.cdc.gov/ophss/csels/dsepd/ss1978/SS1978.pdf>.

7. Cummings P. The Relative merits of risk ratios and odds ratios. Arch Pediatr Adolesc Med. 2009 May;163(5):438-45.
8. Scott I. Interpreting risks and ratios in therapy trials. Aust Prescr. 2008;31:12-6.
9. Merriam-Webster. Hazard [cited 2018 Sep 6]. <http://www.merriam-webster.com/dictionary/hazard>.
10. Merriam-Webster. Danger [cited 2018 Sep 6]. <http://www.merriam-webster.com/dictionary/dange>.
11. Brody T. Clinical trials: study design, endpoints and biomarkers, drug safety, and FDA and ICH guidelines. 2nd ed. New York: Academic Press; 2016.
12. Stare J. Odds ratio, hazard ratio and relative risk. Metodoloˇski zvezki. 2016;13(1):59-67.
13. Jewell NP. Risk comparisons. Am J Ophthalmol. 2009;148(4):484-6.
14. Cornell Statistical Consultant Unit. Cornell University. What is Survival Analysis? [cited 2018 Sep 6]. <https://www.cscu.cornell.edu/news/statnews/stnews78.pdf>.
15. Hernán MA. The hazards of hazard ratios. Epidemiology. 2010;21(1):13-5.