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Epidemiological Investigation of Visceral Leishmaniasis Caused by *Leishmania martiniquensis* in a Non-endemic Area of Thailand

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Abstract

On 5 Sep 2013, a patient suspected to have leishmaniasis from Lamphun, a northern province of Thailand, was reported to the Bureau of Epidemiology. An investigation was carried out to identify reservoirs and vectors. Active case finding was carried out among those who lived within 200-meter radius from the patient's residence. Blood samples were collected from humans, domestic mammals and rodents, and adult sandfly trapping was conducted by light traps. The patient was a 38-year-old man infected with human immunodeficiency virus (HIV) who had worked as a lumberjack and a woodcraftsman. In addition to many kinetoplasts in the bone marrow and amastigote form of *Leishmania*, *Leishmania martiniquensis* was identified by polymerase chain reaction (PCR). A total of 12 suspected cases out of 123 surveyed villagers were identified. However, all human specimens were tested negative by direct agglutination test and PCR. Female *Sergentomyia* sandflies were found. Deltamethrin fogging was done to control the vectors. Visceral leishmaniasis was confirmed in an HIV positive male from northern Thailand. Awareness of leishmaniasis among immunocompromised people should be raised for timely appropriate medical attention and specific vector control for sandflies in the area should be implemented.

Keywords: leishmaniasis, kala azar, *L. martiniquensis*, northern Thailand

Introduction

Leishmaniasis is a vector-borne zoonosis which is caused by intracellular protozoa, *Leishmania* species. There are two main forms of leishmaniasis, namely cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL or kala azar). CL often involves only skin with painless papules. Common symptoms of VL are prolonged fever, weight loss, signs of bone marrow invasion (anemia, thrombocytopenia and leucopenia), abdominal distension with hepatosplenomegaly, and lymphadenopathy.[1] In Thailand, VL was first reported in 1960 among south Asians who were probably infected from abroad. During 1998-2010, there were 43 CL cases and 13 VL cases reported in Thailand, including autochthonous and imported cases. At least six of the 13 VL cases were autochthonous and most were reported from the southern Thailand.[2]

Both humans and animals could be reservoirs, depending on type of *Leishmania* species. The parasite is transmitted by sandflies. Among over 1,000 known species of sandflies, about 70 female sandfly species can transmit *Leishmania* spp.[3], especially *Plebotomus* and *Lutzomyia* genera. Sandflies tend to bite humans and animals in the evenings within 200 meters from its breeding site.[4]

On 5 Sep 2013, the Bureau of Epidemiology, Ministry of Public Health, Thailand, received a notification from a local authority that a suspected leishmaniasis patient was admitted to a university hospital in Chiang Mai, a northern province of Thailand. We conducted an investigation in collaboration with the local authorities. The objectives of the investigation were to confirm the diagnosis of the reported patient; identify other possible cases through active case finding, reservoirs and vectors; institute control measures; and provide recommendations for prevention of future infection.

Methods

Epidemiological Investigation

We reviewed the medical records, and conducted interview and physical examination of the patient. Active case finding was performed among neighbors who lived within 200-meter radius from the patient's residences in two villages (Villages A and B) since onset of the illness. Information on gender, age, nationality, occupation, signs and symptoms of leishmaniasis, travel history, and behaviors related to insect bites was obtained by face-to-face interview. They were also physically examined and blood samples were collected for direct agglutination test (DAT) and polymerase chain reaction (PCR).

A suspected case was defined as a person who had at least one of the followings: wound or ulcer lasting more than one month, fever or feverish for more than six weeks, anemia, hepatomegaly or splenomegaly, or weight loss. A probable case was a suspected case with positive DAT for *Leishmania* species. A confirmed case was a suspected or probable case with positive result for *Leishmania* spp. by PCR, Giemsa stain of wound or ulcer biopsy, or amastigote form in bone marrow.

Reservoirs and Potential Vectors

An environmental study was performed by surveying the patient's residences in Villages A and B, including the presence of foreign workers in those villages. In addition, blood samples were collected from villagers and domestic mammals such as cow, dog, cat, rabbit living within 200-meter radius from the patient's residence. Serum and blood samples were tested by DAT and PCR respectively, while DAT of more than 1:100 for *Leishmania* spp. was considered as positive.

Furthermore, we set up 60 live rodent traps in Village A and 30 live rodent traps in Village B for two consecutive nights from 6 pm to 7 am. Traps were placed around 20 meters apart, starting from the patient's residences. Banana or fish was used as bait.

We also laid light traps at six locations in each village from 6 pm to 5 am, once during the rainy season and once in the cold season. Female sandflies caught were sent for PCR testing for *Leishmania* species. The density of trapped sandflies was calculated by the following formula.

$$\text{Trapping rate (TR)} = \frac{\text{Number of trapped sandfly}}{\text{Number of light trap} \times \text{Night}}$$

Results

Case Investigation

A 38-year-old Thai male with human immunodeficiency virus (HIV) infection had been under medication for antiretroviral therapy with zidovudine 250 mg, lamivudine 150 mg and nevirapine 200 mg. He developed itchy papules on both hands for the first time in 2010, and later slowly spread to elbows, feet, legs, knees, body and ears bilaterally. He had no fatigue or fever at that time. During 2011-2012, he developed sclerodactyly (Figure 1) and visited a community hospital where leprosy was ruled out by slit skin smear and he received symptomatic treatment.

Figure 1. Skin manifestations (a) hands with sclerodactyly, (b) elbow and (c) legs of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

On 2 Jul 2013, he was referred to a university hospital and his skin biopsy was tested positive for fungus, possibly histoplasmosis. Although he received 10% urea in triamcinolone acetonide cream, clobetasol cream and salicylic acid 40% ointment, his lesions showed no improvement. He was admitted to the same university hospital on 23 Aug 2013 as the dermatologist suspected that his chronic skin lesions were caused by leishmaniasis. Physical examination on admission to the hospital revealed multiple non-tender hyperpigmented nodules on legs, forearms and hands, with deformity of fingers (sclerodactyly). In addition, bone marrow biopsy on 27 Aug 2013 showed many kinetoplasts and amastigote form of *Leishmania* spp. (Figure 2), which were later identified as *Leishmania martiniquensis* by PCR.[5] After receiving intravenous amphotericin B 60 mg daily for 15 days, follow-up bone marrow biopsy found no *Leishmania* species.

Regarding the patient's exposure history, he lived in Village A and worked as a lumberjack in a nearby forest for 10 years until 2010 and later worked as a woodcraftsman for two years. While working in the forest which was about three kilometers far from his house, he used to wear long sleeves and long pants. Then, he moved to Village B where he worked as a woodcraftsman for a year. Village B is located about 30 kilometers from Village A. In March 2013, he moved back to Village A where he was currently living with his 82-year-old mother and a 10-year-old daughter. Village A is situated in Pong Mae Lob Sub-district while Village B is in Tha Tung Luang Sub-district of Mae Tha District, Lamphun Province.

He had no history of injection drug use (IDU) or blood transfusion. He and his family had never traveled to the southern Thailand or abroad.

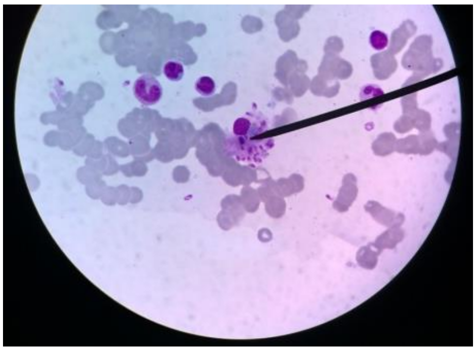


Figure 2. Amastigote form of *Leishmania* spp. in white blood cell from bone marrow biopsy of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Active Case Finding and Behavior Risk Assessment

In Village A with the patient's current residence, there were 106 villagers living within 200-meter radius. Among them, 88 (83.0%) from 36 households were interviewed and examined, including 47 (53.4%) males and 41 (46.6%) females. The median age was 49 years, with the range of 10-91 years. All of them were Thai, with the main occupation as farming (51.1%). Of 11 participants who met the suspected case definition, all were tested negative for *Leishmania* by both DAT and PCR. Behavior risk assessment showed that 51 (58.0%) villagers always protected themselves from insect bites using mosquito nets while sleeping, 44 (50.0%) villagers sometimes used insect repellant when going outside, and 50 (56.8%) villagers used indoor insecticide spraying.

In Village B where the patient lived previously, 46 villagers lived within 200-meter radius from the patient's house and 35 (76.1%) from 17 households were enrolled in the investigation. There were 21 (60.0%) females and 14 (40.0%) males. The median age was 53 years (range 12-78 years). They were Thai who were mainly working as woodcrafts (48.6%). Only one suspected case was identified and *Leishmania* spp. was not found in her blood samples by both DAT and PCR. Moreover, 17 (48.6%) participants never used mosquito nets while sleeping, 18 (51.4%) never used insect repellant when going outside, and 21 (60.0%) used indoor insecticide spraying (Table 1).

In both villages, none of them had traveled to the southern Thailand or abroad in the past two years. There was no history of IDU or blood transfusion in any participants.

Environmental Study

The patient's house in Village A was a one-storey wooden house which was built eight years ago, located one meter above the ground and surrounded by fig trees, with plenty of longan plant sprouts in plastic bag pots underneath the house. There was also a cottage situated nearby to store sculptures. There was a neighbor's cattle pen 30 meters away from his house as well. Inside his bedroom, there was a long-lasting insecticide impregnated net that he received two years ago. His sister's house, a one-storey cement house with a grocery store, was approximately 500 meters away. Before he developed symptoms, the patient visited his sister's shop every evening after logging trees in the forest and stayed for 1-1.5 hours.

In Village B, the house that he had stayed was a renewed one-storey cement house with two bedrooms, which was adapted from a wooden house. The mosquito-screened windows in the house were torn and three windows were always left opened.

No foreign worker or people from the southern Thailand had visited, lived or worked in these two villages during the past two years.

Table 1. Characteristics of villagers who lived within 200-meter radius from a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Variable	Village A (n = 88)		Village B (n = 35)	
	Number	Percent	Number	Percent
Gender				
Male	47	53.4	14	40.0
Median age in year (range)	49 (10-91)		53 (12-78)	
Thai nationality	88	100.0	35	100.0
Occupation				
Farming	45	51.1	5	14.3
Housekeeper	11	12.5	5	14.3
Woodcraftsman	1	1.1	17	48.6
General laborer	12	13.6	1	2.9
Student	7	8.0	4	11.4
Others	12	13.6	3	8.6
Protection behavior				
Using mosquito net				
Never	28	31.8	17	48.6
Sometimes	7	8.0	3	8.6
Always	51	58.0	15	42.9
Unknown	2	2.3	0	0
Using insect repellent				
Never	24	27.3	18	51.4
Sometimes	44	50.0	11	31.4
Always	18	20.5	6	17.1
Missing	2	2.3	0	0
Insecticide spraying				
No	36	40.9	14	40.0
Yes	50	56.8	21	60.0
Unknown	2	2.3	0	0

Table 2. Potential reservoirs and laboratory results of villagers who lived within 200-meter radius from a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Pontential reservoir	Village A				Village B			
	Total	Sample collected	DAT*	PCR†	Total	Sample collected	DAT*	PCR†
Human	106	77	0	0	46	34	0	0
Domestic animal								
Dog	20	18	18	0	0	0	-	-
Cat	3	0	-	-	0	0	-	-
Cow	2	1	1	0	0	0	-	-
Rabbit	2	1	1	0	0	0	-	-
Rodent	NA	2	-	0	NA	0	-	-

*DAT: Positive result by direct agglutination test

†PCR: Positive result by polymerase chain reaction

Reservoirs and Potential Vectors

Total 77 (87.5%) people in Village A and 34 (97.1%) people in Village B without symptoms were all tested negative by DAT and PCR.

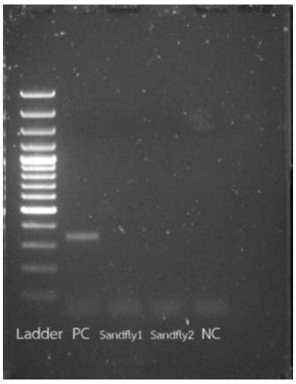
In Village A, all 20 blood samples of 18 dogs, one cow and one rabbit revealed positive DAT (Antibody titer >1:100). However, all PCR results from 20 samples were negative for *Leishmania* species. PCR results of two rodents trapped showed negative as well (Table 2). No domestic animal, rodents or sandflies were trapped in Village B.

To identify the potential vectors, the first trapping was performed just after the case investigation which was during the rainy season. In Village A, sandflies were captured in three traps, including one male sandfly (TR = 0.2 per trap-night) from a trap placed at the patient's workplace and two female sandflies (TR = 0.3 per trap-night) from a trap under the

(a) (b) (c)

Figure 3. Locations of the light traps (a) under patient's house, (b) patient's workplace, (c) cattle pen of neighbor's house that captured sandflies in the village of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

patient's house and a trap in neighbor's cattle pen (Figure 3). All sandflies were identified as *Sergentomyia* species. Thoraxes of female sandflies were tested by PCR and negative for *Leishmania* (Figure 4). Trapping was repeated for Village A during the cold season and two more male *Sergentomyia punjabensis* (TR = 0.5 per trap-night) were identified.



Remark:
PC = positive control
Sandfly1 = sandfly captured under the patient's house
Sandfly2 = captured from cattle pen
NC = negative control

Figure 4. Result of polymerase chain reaction amplification of a visceral leishmaniasis case in Mae Tha District, Lamphun Province, Thailand, 2013

Public Health Actions

Health education on clinical symptoms, reservoirs and prevention measures for leishmaniasis were provided to headmen, health volunteers and villagers in the villages to reduce panic and stigmatization to the patient and his family. We encouraged the communities to improve environmental sanitation in the area and cooperate with the local authorities to control adult vector by deltamethrin fogging.

Discussion

Visceral leishmanisis was confirmed in a man with HIV from Lamphun, a northern province of Thailand. No additional cases were found through active case finding. There were no human or animal reservoirs found in this study. Five *Sergentomyia* sandflies were identified.

This was an unusual event since leishmaniasis was usually found in the southern part of Thailand[2]. Only three cases were reported from the northern part during 1996-2012, with the latest case reported in Chiang Rai Province during 2012[2,6].

The case identified in this study was similar to the second leishmaniasis case reported from Chiang Rai Province in 2012 who also had underlying HIV infection and history of working in the forest and bamboo jungle for 4-5 times per month[6]. Immunocompromised hosts with HIV/AIDS are at higher risk of developing leishmaniasis when they contract the infection.[3,7] Source of the outbreak might not be in the community because we found no evidence of leishmaniasis from reservoirs and vectors. However, the evidence of sandflies in the village should be known as potential risk area. Though history of lumberjack working was likely to be associated with exposure to the parasite in the forest, other risk factors should be considered as well since the incubation period of leishmaniasis ranges from weeks to months[8]. The case might be using the mosquito net at home. However, as sandflies are 1.3-3.5 mm in size which is about one third of mosquitoes[2,9] (Figure 5), pores of the mosquito net cannot protect from sandflies.[4,8]

Figure 5. Photo of *Culex* mosquito (top) compared with a sandfly (bottom)

The diagnosis of VL was obtained by amastigote form of *Leishmania* spp. in bone marrow. Despite the fact that the result of skin biopsy revealed negative, CL could not be ruled out as *Leishmania* spp. could escape from necrotic tissue.[10]

We found female sandflies of genus *Sergentomyia* which can suck blood from humans and animals. However, there was no strong evidence that it could act as a vector for this disease while other genera such as *Phlebotomus* and *Lutzomyia* are known effective vectors in transmitting *Leishmania* parasites.[1] In Thailand, *Leishmania martiniquensis* was recently found.[11] Nevertheless, there was no evidence on the relationship between *Leishmania martiniquensis* and potential vector, which was *Sergentomyia* spp. in our study. Thus, we could not conclude that *Sergentomyia* spp. had a role in transmitting leishmaniasis.

Only few sandflies could be trapped as there was heavy rain during the first trapping and the thin wings of sandfly cannot tolerate flying through the rain. Despite the second attempt to trap in late cold season, the climate was drier than last year and this might lead to less number of sandflies than we expected. The second trapping was carried out only in Village A due to limited light traps available.

The antibody titer of all animal samples was more than 1:100 by DAT although PCR testing revealed negative. This might be caused by cross-reaction to other parasites such as *Trypanosome* spp. which belongs to the same *Trypanosomatidae* family as *Leishmania* species.[7]

In this investigation, a total of 90 live rodent traps were used. Despite that, only two rodents could be trapped in two villages after two nights. According to a study by Weihong published in 1999, rodents capture rate was the highest on the third day for grid trapping method.[12] Blood samples from rodent were sent only for PCR testing because of insufficient volume.

Our control measure by deltamethrin fogging could be appropriate to reduce number of sandflies.[13] Some studies have found that only 0.1% female phlebotomine sandflies are resistance to deltamethrin.[14,15]

The study was conducted on the weekday and it might be difficult for some villagers to participate in the study. In addition, due to limited field workers, survey on types of mosquito net that could protect from sandfly was not performed in these villages.

Conclusion

The results revealed that VL was confirmed in a HIV-positive patient by identifying *Leishmania* spp. in bone marrow biopsy in Lamphun, a northern province of Thailand. DAT and PCR testing of all suspected cases were negative. False positive of DAT was found in domestic animals. Only *Sergentomyia* sandflies were captured from the trapping. We recommended that people with symptoms such as chronic wound or ulcer, chronic fever, fatigue, abdominal discomfort and continuous weight loss should be made aware of leishmaniasis to receive appropriate investigation and medical attention, especially in immunocompromized people. Specific and effective vector control for sandflies in the area should be considered and implemented.

Acknowledgement

We would like to thank all field workers and staff from Lamphun Provincial Health Office, Office of Diseases Prevention and Control (ODPC) for Region 10, and Center of Vector Borne Disease Control 10.5 in Phrae Province for their contribution. We also appreciate all the people in the studied communities for their collaboration.

Suggested Citation

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A Large Outbreak of Japanese Encephalitis in Rakhine State, Myanmar: Implication for Vaccine Policy

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Abstract

Japanese Encephalitis is endemic in Rakhine State, western Myanmar. In July 2014, 27 patients were admitted to Sittwe General Hospital, Rakhine State. Few patients died and some patients were confirmed as having Japanese Encephalitis (JE) infection. An outbreak investigation was conducted in forty six villages of nine JE affected townships in Rakhine state. The objectives were to describe the outbreak and to identify potential risk factors, reservoir and vector of JE virus in Rakhine State. Active case findings, environmental and entomological study were performed. We found 49 JE suspected cases with 10 deaths. Among them, 21 cases were confirmed as Japanese encephalitis infection by positive immunoglobulin M (IgM) ELISA. *Culex tritaeniorhynchus* was found in most JE affected townships. Almost all villagers had no awareness about JE transmission and prevention. Our investigation underscored the importance of collaboration between human and animal doctors, the raising of knowledge and awareness about JE transmission and prevention before the rainy season in Rakhine State, and the need for more studies on usefulness of JE vaccination among high risk population.

Keywords: Japanese encephalitis, outbreak investigation, Rakhine State, Myanmar

Introduction

Japanese Encephalitis (JE) is endemic in Rakhine State of Myanmar due to virus circulation among domestic animals through *Culex* mosquito.[1] From 2009 to 2012, an average of 25 JE cases was reported every year in Myanmar. The highest reported areas were Rakhine State and Yangon Region.[2] However, in 2013, there was only one JE case reported in Rakhine State.[1]

Most of JE virus infection in human is asymptomatic as less than 1% of infected people develop symptoms. The incubation period is 5-15 days.[3] Illness usually begins with acute fever, followed by mental change, focal neurologic deficits and movement disorders. The case fatality rate is approximately 20-30%. Among survivors, 30-50% have serious neurologic or psychiatric sequelaes.[4] JE is one of the vaccine preventable diseases. Although one dose could be protective, vaccination with two doses is more likely to achieve 100% seroconversion.[5]

JE virus is transmitted by mosquitoes. In Myanmar, *Cx. tritaeniorhynchus* is the important vector in spreading this virus to humans and domesticated animals such as horses, donkeys, and pigs.[6] *Cx. tritaeniorhynchus* breeds in rice paddy field and is active at twilight. Sporadic human and animal JE cases are usually reported during the monsoon rains.[4]

In July 2014, 27 patients were admitted to Sittwe General Hospital in Rakhine State. Among them, eight died with neurological symptoms. Serum samples of all patients were sent to National Health Laboratory for enzyme-linked immunosorbent assay (ELISA) testing and seven were tested positive for JE. After receiving notification from the vector borne disease control (VBDC) team in Rakhine State, a central VBDC team was sent to investigate the outbreak. The objectives of this study were to conduct an outbreak investigation and describe magnitude of the outbreak. Potential risk factors, reservoirs and vectors of JE virus were explored as well.

Methods

A descriptive study was conducted in 46 villages of nine JE affected townships in Rakhine State from 3 to 18 Aug 2014. To confirm the diagnosis, data on JE cases from the national VBDC program were reviewed. Information on demographic, clinical manifestations and laboratory results were reported to the national VBDC surveillance system by VBDC team leader. Medical records in Sittwe General Hospital (Reference hospital), including death certificates, were assessed as well (Figure 1).

A suspected JE case was a person who developed acute encephalitis syndrome with three or more of the following symptoms: acute fever, headache, convulsion, muscle spasticity, and alteration in mental and neurological signs[4] between 7 Jun and 30 Sep 2014. A confirmed JE case was a suspected patient with positive immunoglobulin M (IgM) ELISA for JE virus.

To confirm the existence of an outbreak, we compared JE report from VBDC program in the previous five years, 2009-2013. In addition, we also reviewed the clinical and demographic data from medical records in Sittwe General Hospital to describe magnitude of JE outbreak, and active case finding that was conducted in three villages with high morbidity rate from three JE affected townships (Sittwe, Minbya and Kyauktaw Townships) to find out new JE cases. Parents of children aged 5-15 years old who lived within one mile (1.6 km) around the patient house, either with or without fever, were approached. Moreover, we collected blood samples and sent to National Health Laboratory (NHL) in Yangon for laboratory confirmation.

An entomological survey was conducted in six villages with the highest attack rate of five JE affected townships to identify vector of JE virus. We used indoor collection in the morning, light trap, outdoor human landing catch or spray sheet collection[7]. This was conducted by the entomology team of central VBDC.

In order to search for high risk population, potential risk factors and reservoirs for JE, an environmental study was conducted by observing around 11 villages out of seven townships. In each village, one staff in the local authority, one basic health staff and one family member of JE patients were interviewed using unstructured questionnaires about knowledge and prevention behaviors.

Descriptive statistics were employed to display the distribution of suspected JE cases by time of onset, townships, and signs and symptoms. Attack rates and case fatality rates were explored by townships, including entomological and laboratory results. Association between potential risk factors and survival was examined by odds ratio and 95% confidence intervals. All analyses were performed using Epi Info 7[8].

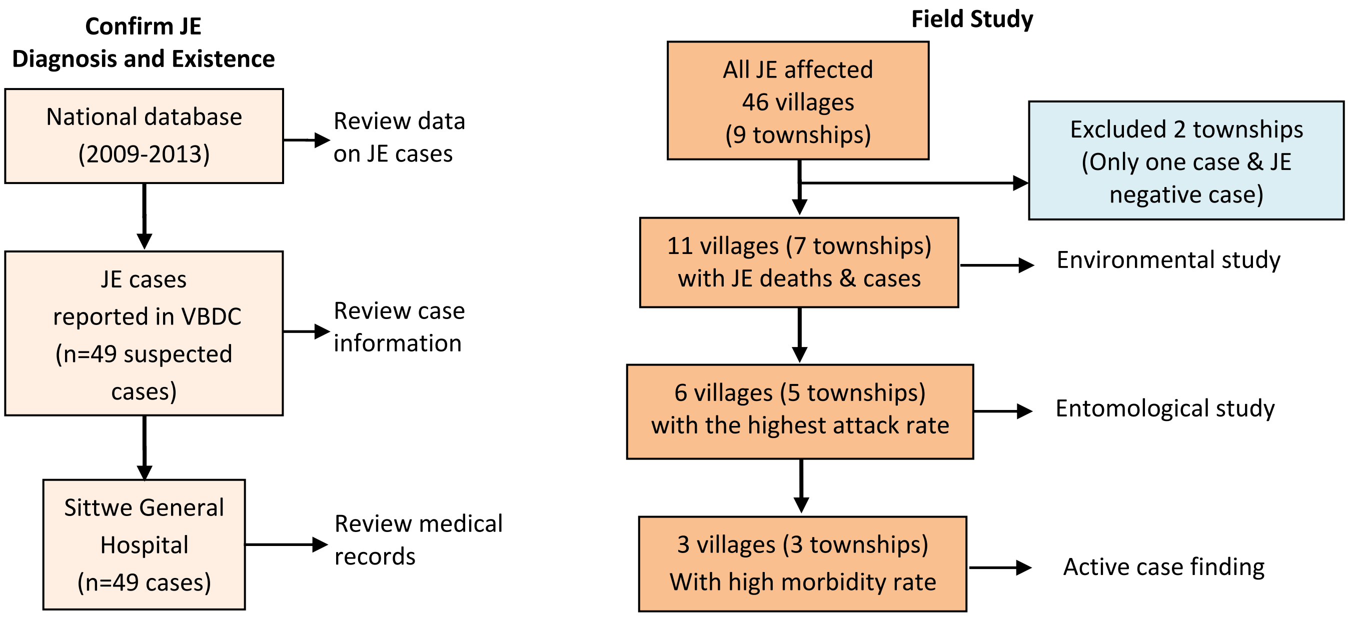


Figure 1. Method of data collection and field investigation in a Japanese encephalitis (JE) outbreak, Rakhine State, Myanmar, 2014

Laboratory Investigation

Blood specimens of suspected JE cases were confirmed for JE infection at NHL using JE IgM ELISA kit. The IgM ELISA consists of one enzymatically amplified 'two-step' sandwich-type immunoassay. In this assay, JE detect negative control (represents non-reactive serum), JE detect IgM positive control (represents reactive serum), and unknown serum samples were diluted with 1/100. The process was performed following the manufacturer's instruction. Sample dilution buffer for JE detect IgM was then incubated in microtitration wells coated with anti-human IgM antibodies. This was followed by incubating with both JE virus (JEV) derived recombinant antigen (JERA) and normal cell antigen (NCA) separately. After incubation and washing, the wells were treated with a JERA-specific antibody labeled with the enzyme horseradish peroxidase. After the third incubation and washing step, the wells were incubated with the tetramethylbenzidine substrate. An acidic stopping solution was added and the degree of enzymatic turnover of the substrate was determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbencies of the JERA and the control wells was accurately determined for presence of JEV antibodies.[9]

Results

Rakhine State is situated on the western coast of Myanmar. The main economy of the state is agriculture-based and others are forest-related works, animal husbandry, fishery, prawn breeding and some domestic small industries. Majority (75%) of the population is living in the rural area. There are total 17 townships, three sub-townships, 120 wards, 1,040 village tracts and 3,862 villages.[1]

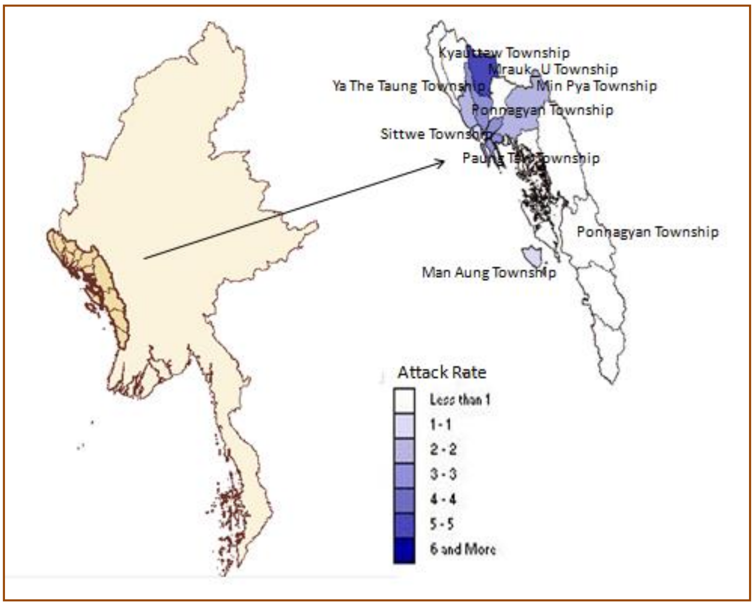
Diagnosis and Magnitude of the Outbreak

During the study period, active case finding and reports from VBDC identified a total of 49 suspected cases, with attack rate of 3.1 per 100,000 population, in 46 villages of nine townships in Rakhine State, including Kyauktaw, Minbya, Pauktaw, Ponnagyun, Sittwe, Rathedaung, Mrauk-U, Munaung and Toungup Townships (Figure 2).

Kyauktaw Township had the highest attack rate (5.4 per 100,000 population) among all the affected townships. The highest mortality rate (2.2 per 100,000 population) was recorded in Ponnagyun Township while the highest case fatality rate (50.0%) was in Sittwe Township (Table 1). From active case finding, 30 blood samples were collected. Nevertheless, all of them were tested negative for JE IgM by ELISA.

Among 49 suspected cases, there were 29 males (59%) and 20 females (41%). Most of cases were school-children and aged range from three months to 36 years, with median age as eight years. The highest attack rate, 5.4 per 100,000 population, was observed among 5-9 years old.

Samples of 35 JE suspected cases were sent to NHL and 21 cases (60%) were confirmed for JE by IgM ELISA. Two cases were with equivocal (uncertain) results. Among the 21 laboratory confirmed cases, four cases died and 10 survived with sequelae such as mental changes, focal neurological deficits while the others recovered completely.



Source: Data from Sittwe and Kyauktaw Hospitals in Rakhine State

Figure 2. Attack rate of suspected Japanese encephalitis cases by townships in Rakhine State, Myanmar, 3-18 Aug 2014 (n = 49)

Table 1. Attack rate and case fatality rate of suspected Japanese encephalitis cases by townships in Rakhine State, Myanmar, 3-18 Aug 2014

Township	Number of population[1]	Total number of cases*	Number of death*	Attack rate per 100,000	Mortality rate per 100,000	Case fatality rate (%)	Distance to	
							Rural health center (min)	Hospital (hour)
Sittwe§, ‡	269,205	8	4	3.0	1.5	50.0	-†	0.5 (car)
Kyauktaw§, ‡	221,644	12	1	5.4	0.5	8.3	20-25 (foot)	1 (car/boat)
Mrauk-U	214,307	2	0	0.9	0	0	20-25 (car/ boat)	1 (boat)
Minbya§, ‡	195,150	4	0	2.0	0	0	20-25 (boat)	1 (boat)
Pauktaw	191,426	8	1	4.2	0.5	12.5	30 (boat)	3 (boat)
Rathedaung‡	169,277	5	1	3.0	0.6	20.0	30 (boat)	2 (boat)
Toungup	148,417	1	0	0.7	0	0	45 (boat)	1 (boat)
Ponnagyun‡	135,942	8	3	5.9	2.2	37.5	30-35 (boat)	2 (boat)
Munaung	61,360	1	0	1.6	0	0	45 (boat)	1 (boat)

* Data sources: Sittwe and Kyauktaw Hospitals

‡ Entomological study was performed in this area.

§ Active case finding was performed in this area.

† As all 8 cases visited the hospital, no information was obtained from the interview.

All suspected JE patients first presented with fever, and followed by convulsion (63%), unconscious (53%), mental change (51%), nausea/vomiting (22%), headache (16%), or encephalitis (90%). Median duration from onset of illness to admission to hospital was four days, with inter-quartile range of 3-5 days. Ten patients died of acute encephalitis syndrome. No cases had history of JE vaccination.

All characteristics such as gender, age group, townships and symptoms of JE did not show any statistical difference when compared 10 deaths and 39 survivals of JE suspected cases (Table 2).

The JE outbreak started on 26 Jun 2014 and ended on 24 Sep 2014. The shape of the epidemic curve resulted gradual increase in nature (Figure 3). Compared with the previous five years data from VBDC program recorded in Rakhine State, less than five JE cases were recorded in each year during 2009-2013 and there was a clear increase in number of JE cases reported in 2014 (Figure 4).

Table 2. Analysis on characteristics and symptoms of suspected Japanese encephalitis cases in Rakhine State, Myanmar, 3-18 Aug 2014

Characteristic	Death (n=10)		Survival (n=39)	
	Number	Percent	Number	Percent
Gender				
Male	4	13.8	25	86.2
Female	6	30.0	14	70.0
Age group (year)				
0-10	8	22.2	28	77.8
>10	2	15.4	11	84.6
Townships				
Big*	6	27.3	16	72.7
Small**	4	14.8	23	85.2
Symptoms				
Fever	10	100.0	39	100.0
Headache	3	30.0	5	12.8
Convulsion	8	80.0	23	59.0
Mental change	6	60.0	19	48.7
Neck stiffness	0	0	8	20.5
Nausea/vomiting	1	10.0	9	23.1
Focal neurological sign	3	30.0	7	17.9

* Kyauktaw, Mrauk-U and Sittwe Townships

** Munaung, Minbya, Pauktaw, Ponnagyun, Toungup and Rathedaung Townships

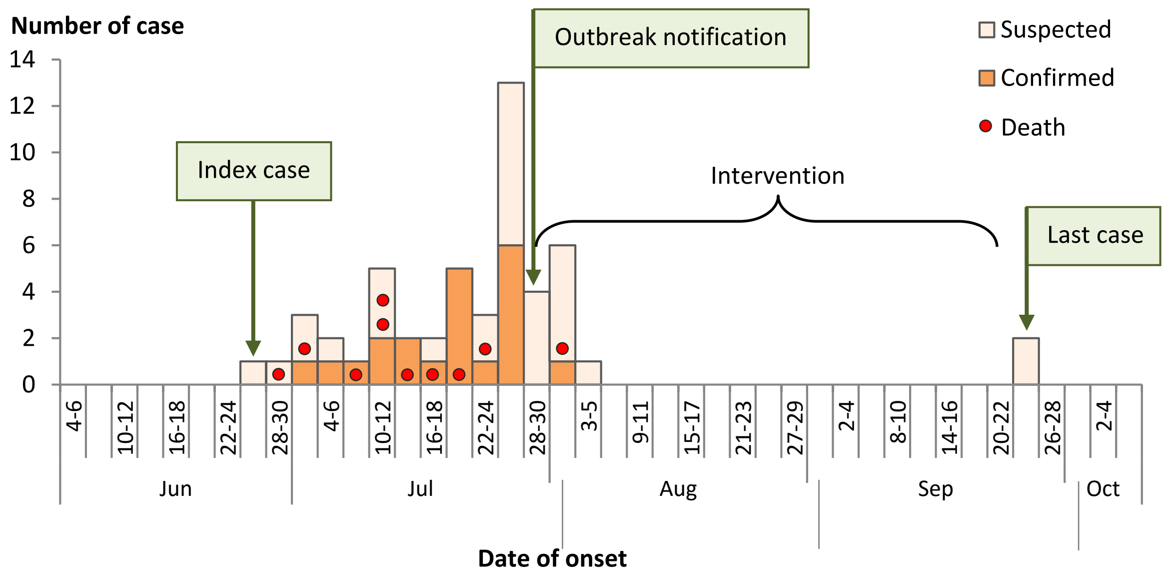


Figure 3. Distribution of Japanese encephalitis cases by date of onset in Rakhine State, Myanmar, 2014 (n=49)

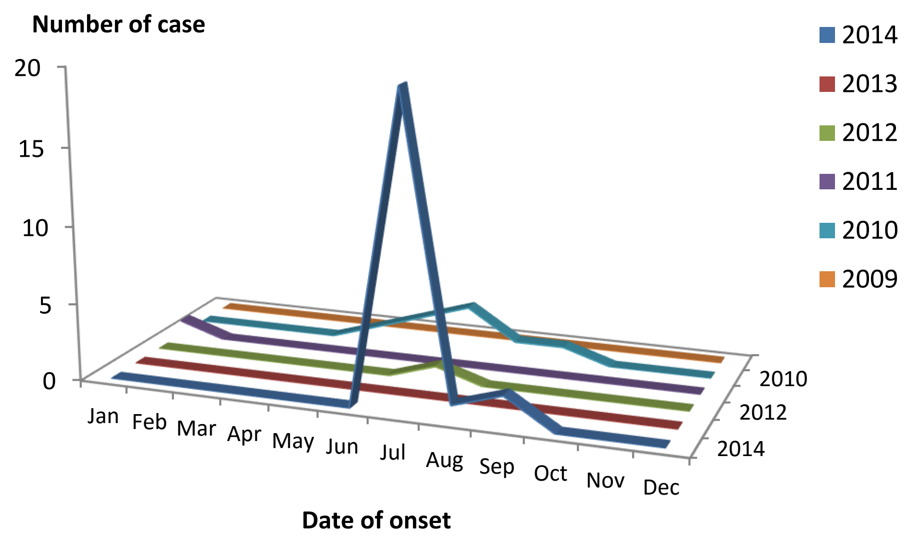


Figure 4. Number of Japanese encephalitis cases in Rakhine State reported to Vector Borne Disease Control Unit, Myanmar, 2009-2014

Potential Risk Factor, Reservoir and Vectors

According to the information from the local authority, basic health staff and families of JE patients, two months prior to this outbreak, there were mass causalities of pigs (more than 20-1000 pigs died), and still-born or mummified fetuses, abortion and infertility of pigs in some villages of Kyauktaw and Minbya Townships. Later, JE cases occurred sporadically in Pauktaw, Ponnagyun, Sittwe, Rathedaung, Mrauk-U, Munaung and Toungup Townships in Rakhine State. Although most villagers suspected that the cause of death in many pigs was due to swine fever, there was no definite evidence or laboratory confirmation.

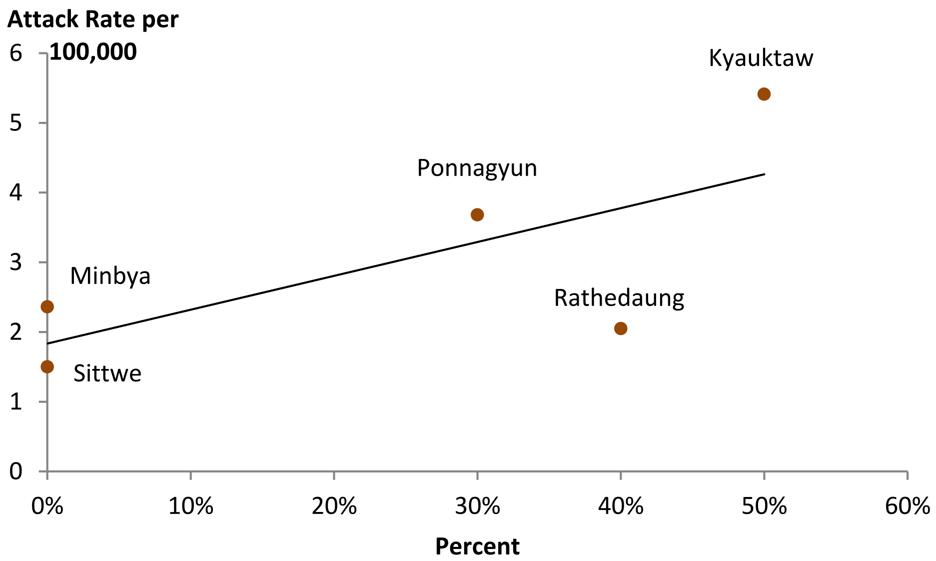
From the observation, there were also domestic or commercial poultry raised in the villages. The domestic pigs and cattle were kept under or in front of most of the houses in JE affected villages. Rice paddies were situated in most of the affected villages.

About 90% of people used ordinary bed net while only 10% of them applied the long-lasting insecticide-treated net. All people and pigs had no prior vaccination for JE. All the villagers did not have awareness about JE or knowledge related to transmission and prevention of the disease.

From the entomological survey, two major species of mosquitoes, *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*, were identified. The highest proportion of *Cx. tritaeniorhynchus*, the main vector of JE, was found to be 80% in Nagoomay Village, Kyauktaw Township (Table 3). A scatter plot between JE attack rate and proportion of *Cx. tritaeniorhynchus* mosquitoes surveyed from five townships suggested positive association. Despite

Table 3. Results of entomological survey among adult mosquitoes in Rakhine State, Myanmar, 3-18 Aug 2014

Name of village (Township)	Collection method	Number of mosquito	Type of adult *Culex* found	Percent of adult *Cx. tritaeniorhynchus*
Northern Sanpya (Sittwe)	- Indoor in the morning - Light trap - Outdoor human landing catch	48	*Cx. quinquefasciatus*	0
Balepound (Minbya)	Spray sheet	33	*Cx. quinquefasciatus, Cx. tritaeniorhynchus*	40
Athetmyintlet (Ponnagyun)	Spray sheet	28	*Cx. quinquefasciatus, Cx. tritaeniorhynchus*	30
Laydaungkan (Rathedaung)	Spray sheet	42	*Cx. quinquefasciatus*	0
Kathettaw (Kyauktaw)	Spray sheet	8	*Cx. quinquefasciatus, Cx. tritaeniorhynchus*	50
Nagoomay (Kyauktaw)	Light trap	4	*Cx. quinquefasciatus, Cx. tritaeniorhynchus*	80



Remark: Linear regression coefficient = 4.9, p-value = 0.18

Figure 5. Comparison between attack rate of Japanese encephalitis and proportion of adult *Cx. tritaeniorhynchus* in Rakhine State, Myanmar, 3-18 Aug 2014

that, the association was not statistically significant by linear regression (Figure 5).

Discussion

The investigation highlighed that an outbreak of JE occurred in Rakhine State during 2014 as 21 JE confirmed cases, which increased highly compared to those of the previous five years. JE outbreak was observed with case fatality rate 20.4% (10/49) as the JE patients died within a short period after appearance of the symptoms. Almost all JE cases were under 15 years old (90%).

Cx. tritaeniorhynchus which can spread JE was collected from the entomological study in three JE affected townships. Animal husbandry and paddy fields were found to be closely located near the residence of the cases. JE outbreak was most likely caused by mosquito bite, starting around early June 2014. According to interviews, many pigs died before JE outbreak in humans. JE virus can maintain in an enzootic cycle between mosquitoes and amplifying vertebrate hosts, primarily pigs.[4] The domesticated pigs under the patients' houses were most likely to be reservoirs of JE infection. Veterinarians could not find the cause of death of many pigs. Moreover, there was no established information sharing system for zoonotic diseases between veterinarians and public health officers in Rakhine State.

Many villagers in the study areas had no awareness about JE transmission and prevention. If early notification of JE cases could be carried out by the villagers, it would have been helpful for timely control of the outbreak and saved the lives. Even though JE vaccination for pig is available, it is not the best solution since birds or other animals could also act as JE amplifying hosts. In endemic area, human vaccination could be more effective long-term control measure. On the other hand, this outbreak was difficult to control as most of the villagers could not afford for JE vaccine.

Limitations

Environmental and entomological studies were not be able to conduct in all JE affected villages because of unfavorable weather condition, poor transportation, insecurity and budget constraints. Despite outdoor human landing collection, using animal bait is the most suitable method for catching *Culex* mosquitoes[6]. However, this method could not be applied in all JE affected townships due to heavy rain. In addition, specimens from 14 suspected cases could not send for laboratory confirmation because of transportation barriers, inability to do blood test in the villages and higher expenses. Some patients had died before collection of blood samples.

Public Health Action and Recommendations

Despite the fact that JE vaccination have 90-97% efficacy,[9] it was costly and did not include in the expanded programme on immunization (EPI) in Myanmar. Hence, many people could not be vaccinated.

Advocacy meetings were conducted at the central level as well as in Rakhine State. Utilization of insecticide-impregnated bed nets, and long-sleeved shirts and pants were promoted to avoid mosquito bites[10]. Health education on disease transmission and prevention was provided to all affected villages as well. In addition, searching for more patients, opening of mobile clinic and vector control such as fogging were performed in the affected areas, in collaboration with the central VBDC team, VBDC team in Rakhine State and the local authorities.

We recommended that awareness about JE disease and personal protection should be raised in the communities before JE transmission season commences in the endemic areas. Local health authority should develop an early warning system of JE outbreak in case of abortion and fatalities in pigs through network among villagers, veterinarians and local health authorities.

The VBDC in Rakhine State should provide timely and immediate response such as health education on awareness and JE prevention and control. They should also encourage collaboration between physicians and veterinarians for information sharing as early as possible. Information obtained from this study was shared with the responsible persons and discussed for improvement of strategies and better support.

At the central level, introduction of JE vaccination to high risk group in collaboration with EPI program should be considered as an epidemiological study or a cost-benefit study. Thus, political commitment, financial resources and inter-sectoral collaboration between Ministry of Health and other stakeholders to set up JE vaccination programs for young children as well as rigorous monitoring and surveillance should be considered in prevention and control of JE.

Conclusion

An outbreak of JE was identified in Rakhine State during 2014. Total 21 confirmed cases out of 49 JE suspected cases were reported from 46 villages of nine townships, including 10 fatal cases. *Cx. tritaeniorhynchus* were found in most of the JE affected townships. Collaboration among physicians, veterinarians, local health providers, local health authority and community should be strengthened for better control measure in the future. JE vaccination should be considered for high risk population. Awareness raising about JE disease such as JE transmission, prevention and control should be started before the rainy season in Rakhine State to enhance knowledge and practice among the villagers.

After the study period which ended on 24 Sep 2014, two more JE cases were confirmed by IgM ELISA, with one each from Pauktaw Township and Myebon Township in Rakhine State. No more cases were reported since October 2014.

Acknowledgement

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Suggested Citation

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Seroprevalence of Q Fever among Dairy Cattle in Nongpho Sub-district, Ratchaburi Province, Thailand, 2015

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Abstract

Q fever, a zoonosis caused by *Coxiella burnetii*, is an important occupational health risk for livestock farmers, veterinarians and public health officers. However, information about Q fever was limited in Nongpho Sub-district which had the highest dairy cattle density in Ratchaburi Province. This cross-sectional study aimed to assess the seroprevalence of antibody against *C. burnetii* among dairy cattle in Nongpho Sub-district. Serum samples were collected from all 10 villages of the sub-district during 21 April to 26 May 2015, including a total of conveniently selected 135 cattle in 27 dairy farms. The sera were tested for antibody against *C. burnetii* using enzyme-linked immunosorbent assay. An investigation team administered a structured questionnaire to collect information of the seropositive herds. The seroprevalence of individual cattle was 5.2% while seroprevalence at the herd level was 25.9%. Among the seropositive farms, farmers had limited knowledge and practices on biosecurity and sanitation. Animal health authority should develop an integrated strategy to improve biosecurity and sanitation practice in dairy cattle farms.

Keywords: Q fever, dairy cattle, seroprevalence, Nongpho Sub-district, Thailand

Introduction

Q fever is a zoonotic disease caused by *Coxiella burnetii* which is a Gram-negative, obligate intracellular bacterium. *C. burnetii* infections have been reported in humans, farm animals, pets, wild animals, birds and arthropods worldwide. Q fever is transmitted by aerosol, direct contact or ingestion.[1] Shedding of bacteria occurs through secretion, excretion, vaginal discharge, milk, feces and urine of infected animals. During birthing and abortion, the bacteria are excreted massively with genital secretion, placenta and fetal fluid.[2] Arthropods, mainly ticks, are reservoirs for Q fever transmission while the risk of transmission is associated with wildlife.[3]

In humans, the infection can manifest as either in acute or chronic form. The acute form commonly manifests as flu-like syndrome, pneumonia, or granulomatous hepatitis, and can resolve quickly after antibiotic therapy. Chronic Q fever occurs almost exclusively in patients with predisposing conditions, including those with heart valve lesions, vascular abnormalities and immunosuppression. Clinical signs of the chronic form are vascular infections, hepatitis and chronic fatigue syndrome. This form requires prolonged antibiotic therapy for 18 months or more. Complications of the chronic form might be severe to fatal if the patient does not receive the appropriate antibiotic treatment.[4] Patients with asymptomatic infection carry a risk for progression to chronic form of the disease. After either symptomatic or asymptomatic infection, antibodies may remain detectable for months, years or lifelong.[5] Moreover, Q fever infection in pregnant women can provoke placentitis, and lead to premature birth, restricted growth, spontaneous abortion or fetal death.[4] Q fever infection in cattle usually remains asymptomatic. However, it may present with reproductive disorders such as abortion, metritis and infertility.[6]

In Thailand, reports of Q fever revealed widespread infection since 1966. The prevalence of Q Fever among cattle in Thailand was reported to be 2-7%.[7,8] According to a previous study, prevalence of Q fever among people working in slaughterhouses was estimated to be 1%.[7] Recently, Q fever infection was reported among people living in the northeastern part of Thailand[9] where endocarditis human cases caused by Q fever were identified during 2012[10]. In Chiang Mai, the northern province of Thailand, proportions of seropositive dairy cattle at herd and individual levels were 62% and 5% in 2012.[11]

This study was conducted to determine the seroprevalence of Q fever in a western province of Thailand and investigate the potential factors for disease transmission among dairy cattle herds based on farmers practices. Q fever seroprevalence in dairy cattle and the potential reservoir for human infection could provide useful information to assess the risk of Q fever among both farmers and animals in Ratchaburi.

Methods

We conducted a descriptive cross-sectional study in Nongpho Sub-district, Ratchaburi Province, the western province of Thailand. Nongpho Sub-district had the highest population density of dairy cattle in the province. Totally 6,447 dairy cattle were raised in 290 farms in the whole area of all 10 villages of Nongpho sub-district. A total of 135 cattle from 27 herds were selected using convenience sampling.

Blood samples were collected from the median caudal vein of individual cattle from 21 Apr to 26 May 2015. The blood samples were centrifuged to extract 3 cc of serum which was then tested for immunoglobulin G (IgG) phase one and phase two specific antibodies of *C. burnetii* using a commercial indirect enzyme-linked immunosorbent assay (ELISA) kit, LSIVET ruminant milk/serum Q fever ®. Sensitivity of the ELISA kit was 87% and specificity was 100% according to the manufacturer's data.[12] The laboratory testing was conducted at the Veterinary Research and Development Center for Western Region in Ratchaburi Province.

A herd was considered to be a seropositive herd for Q fever if at least one animal in the herd yielded positive result for Q fever. A questionnaire was administered to owners of the seropositive herds to collect information on cattle such as age, gender, history of abortion and farm management in July 2015. The data obtained were analyzed using statistical software. Prevalence of seropositivity at cattle and herd levels were calculated. In the seropositive farms, characteristics of the seropositive cattle were compared with the seronegative cattle.

Results

Nongpho Sub-district is located at the eastern part of Photharam District in Ratchaburi Province. Geographic distribution of farms with positive results for *C. burnetii* was illustrated in figure 1.

Total 27 farms and 135 dairy cattle in all 10 villages of Nongpho Sub-district were included in this study. All animals in this study were tested negative for brucellosis. Size of the farm area in average was 565.4 m^2.

There were seven dairy cattle from seven different farms in five villages that were positive against *C. burnetii*. The prevalence of *C. burnetii* in dairy cattle at the individual animal level was 5.2% (7/135) and at the farm level was 25.9% (7/27). Villages 3 and 9 had the highest proportion of seropositive individual cows (25.0%) (Table 1).

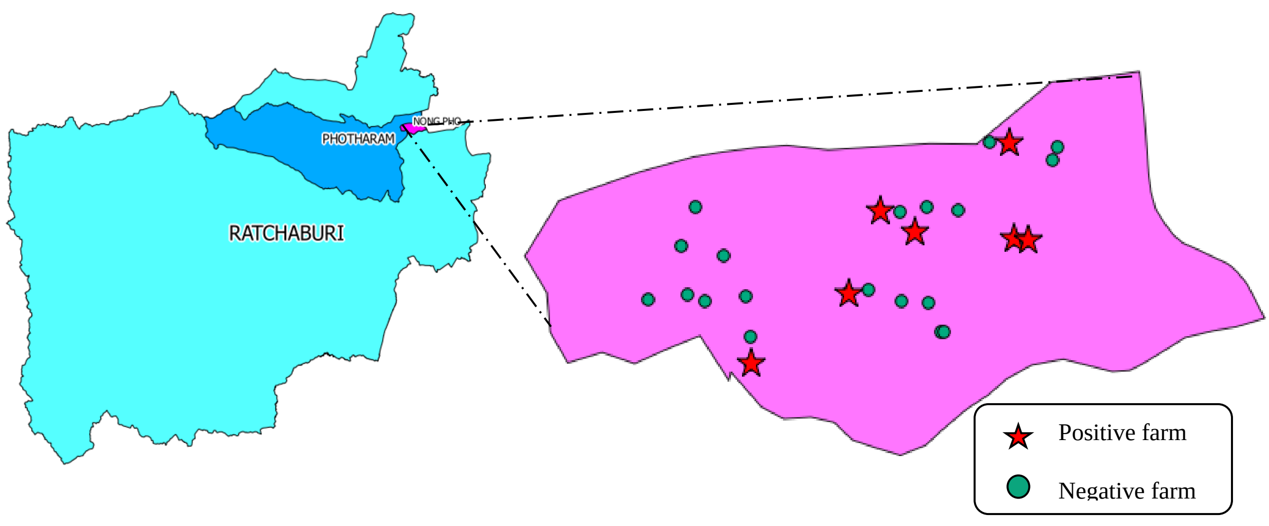

Figure 1. Geographic distribution of cattle farms where serum samples tested against *Coxiella burnetii* in Nongpho Sub-district, Photharam District, Ratchaburi Province, Thailand, 2015

Table 1. Proportion of seropositive results against *Coxiella burnetii* at individual and herd levels in Nongpho Sub-district, Photharam District, Ratchaburi Province, Thailand, 2015

Village	Number tested		Number positive		Percent of proportion positive	
	Animal	Herd	Animal	Herd	Animal	Herd
1	7	4	0	0	0	0
2	3	1	0	0	0	0
3	8	4	2	2	25.0	50.0
4	30	4	1	1	3.3	25.0
5	17	3	0	0	0	0
6	15	2	1	1	6.7	50
7	10	2	0	0	0	0
8	27	4	2	2	7.4	50.0
9	4	1	1	1	25.0	100
10	14	2	0	0	0	0
Total	135	27	7	7	5.2	25.9

Only one out of seven seropositive farms had a resting stall for new cattle (14.3%). In three farms (42.9%), a surrounding fence existed and disinfecting equipment before or after using with cattle was observed. Although 42.9% of seropositive farms had one separate stall for sick or calving cows, the normal and sick stalls were in the same area. About 57.1% of seropositive farms applied the external parasite prevention program two times per year whereas other farms conducted it annually. All seropositive farms raised other kinds of pet on the farm areas (Table 2).

Among the seropositive herds, most farmers (71.4%) cleaned the stall only two times per day. All of them used chlorine to disinfect the stall floor. Some of the seropositive farms (28.6%) sold the aborted fetus and all of them sold the placenta waste. Majority of the farmers (85.7%) kept the dry manure less than five meters from the farm area and all of them sold the dry manure. Most of the farmers did not use gloves while removing aborted fetus or placenta waste (71.4%), placenta (85.7%) or assisting with birth (100%) (Table 2).

All farmers in the seropositive herd neglected to disinfect their vehicles, visitor's footwear and equipment before entering into the farm area. In addition, water treatment was not observed in the farms. None of the farmers or their family members had heart disease, pregnancy or abortion before. All the farmers used to drink unpasteurized milk.

Table 2. Characteristics of farm management in *Coxiella burnetii* seropositive farms, Nongpho Sub-district, Photharam District, Ratchaburi Province, Thailand, 2015 (n=7)

Variable	Number	Percent
Days open > 150 days (n=6)	2	33.3
Prevention from outside		
Having stall for new cattle	1	14.3
Having surrounding fence	3	42.9
Drinking and using underground water in farm	6	85.7
Animal management		
Always disinfecting before or after using equipment with another dairy cattle	3	42.9
Having stall for sick dairy cattle	3	42.9
Having stall for calving cattle	3	42.9
Culling cattle	4	57.1
Conducting program for prevention external parasite for 2 times/year	4	57.1
Having at least one other pet on farm area	7	100.0
Disposal management		
Cleaning stall 2 times per day	5	71.4
Selling abortion waste	2	28.6
Selling placenta waste	7	100.0
Distance between farm and place to keep dry manure (< 5 meter)	6	85.7
Selling dry manure	7	100.0
Sanitation practice		
Not using glove for removal of abortion or placental waste	5	71.4
Not using glove for removal placenta	6	85.7
Not using glove for birth assistance	7	100.0

Physical examination of cows in the seropositive farms did not find any ticks. Survey of the farms with seropositive dairy cattle found that the median body condition score (BCS) was three in seropositive cattle and 3.5 in seronegative cattle. The average milk production was 8.3 kg/cow/day in seropositive cattle and 10.0 kg/cow/day for the seronegative cattle. Milking machines were used in all farms to produce milk twice per day.

The average age was five and 4.8 years in seropositive and seronegative cattle respectively. Reproductive disorder problems were regularly found in this sub-district. About 28.6% of the cows had history of abortion when compared to the seronegative ones (5.9%). However, there was no confirmation on the cause of abortion. Other characteristics of the cows such as infertility were not different between seropositive and seronegative cattle (Table 3).

Table 3. Comparison of characteristics among individual cattle in *Coxiella burnetii* seropositive farms, Nongpho Sub-district, Ratchaburi Province, Thailand, 2015

Variable	Positive (n=7)		Negative (n=34)	
	Number	Percent	Number	Percent
Age > 5 years	3	42.9	11	32.4
Abortion history	2	28.6	2	5.9
Pregnancy	1 (n=6)	16.7	13 (n=32)	40.6
Infertility	2	28.6	8 (n=32)	25.0

Discussion and Conclusion

The antibody against *C. burnetii* was described for the first time in dairy cattle from Nongpho Sub-district. Seven cows from seven seropositive farms were found to have *C. burnetii*. Although low seroprevalence (5.2%) in dairy cattle was demonstrated, there was evidence of *C. burnetii* circulating in these five villages, implying that abortion and sickness in both cows and farmers should be monitored regularly. Most of the seropositive farms had no resting stall for replacement cattle or surrounding fence partly due to limited budget for the construction and partly because they thought that it was not important.

This survey found that the farmers did not clearly understand about the biosecurity system. Although there was no evidence found in this study that using the same equipment could transfer *C. burnetii* infection from infected cattle to uninfected ones, lack of precaution for disinfecting vehicles, visitors' footwear and equipment before entering into the farm area might increase the risk of *C. burnetii* infection in dairy cattle as fomites can transfer the pathogen[13,14].

Tick infestation can be one of the biological transmitters.[2] The external parasite control program yielded good results in this sub-district as no tick was found in all farms. All of the farms had the external parasite control program at least once a year and this should be maintained regularly to prevent the infection.

All seropositive farms allowed dogs to come into contact with cattle on the farms. The previous study found that domestic animals had the highest prevalence of Q fever infection, especially in dogs[7]. Hence, farmers in dairy cattle farms should be informed not to allow dogs to contact with cattle.

In addition, dry manure beside the farm area could increase the risk of Q fever infection in seropositive farms, and selling the infected aborted or placenta waste could spread the infection to the carcass shop as well[15].

Sanitation practice of the farmers might not be good enough to prevent Q fever infection. In case of performing the high risk activities such as birth assistance, placenta removal, handling of carcass and cleaning birth fluid, gloves, mask and goggle should be used.

Abortion history and infertility were not different between seropositive and normal cattle. The normal cattle might have other underlying problems for infertility that might never been diagnosed and treated in appropriate ways as most of the farmers had less concern on long drying period, infertility and abortion occurred in their cattle, and rarely utilized the veterinary services. Therefore, there is remained possibility that the other 34 cattle that lived in the same seropositive farms will get Q fever and undetected.

The survey was not carried out in the seronegative farms due to time limitation. Moreover, we did not collect samples from dogs in the positive farms. In this cross-sectional study, we were not certain of the temporal relationship between risk factors and Q fever infection. Another study showed that poor sanitation and inappropriate sanitation practice in the farms posed a risk of transmitting Q fever from the dairy cattle to human[16]. The nature of convenience sampling prevented us from validating the Q fever situation of all cattle in Ratchaburi Province. Despite that, as the data on Q fever in Thailand was limited, this study was one of the few studies that provided baseline information for future studies to identify the causal factors.

Although no acute human case was reported during the study period, the local people might have the chronic disease as *C. burnetii* had been circulating in the villages. Thus, people who worked with dairy cattle such as farmers, veterinarians and livestock officers should use personal protective equipment to reduce the risk of Q fever infection from dairy cattle. Biosecurity in farms should also be improved through communication and public education.

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