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Malaria Pre-elimination Assessment in Eastern Indonesia

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Abstract

About 4.8 million Indonesians were infected with malaria in 2013 while the infections were mostly in Eastern Indonesia. Indonesia Ministry of Health announced that malaria pre-elimination stage should be reached by 2015-2020 and free of malaria transmission by 2030 in Eastern Indonesia. Although detailed maps of epidemiological distribution, prevalence and risk factors for malaria were needed, there was limited access to routine surveillance data such as annual parasitic incidence and surveillance report. Hence, this study aimed to assess the epidemiological situation of this area. Total 555 people who aged 14 years and above, and lived in one of five districts in Eastern Indonesia were enrolled in this study. About 32.6% (181/555) were tested positive for malaria by nested polymerase chain reaction, with *Plasmodium vivax* as the dominant species. Not using bed nets was the most significant risk factors. Spatial analysis indicated three distinguished clusters (180, 229 and 88 cases). Hence, malaria pre-elimination program in Eastern Indonesia should be postponed as the situation did not meet the criteria yet. Routine treatment to stop silent transmission, scaling up control measures and improving laboratory surveillance were needed before pre-elimination.

Key words: epidemiology, malaria pre-elimination program, Eastern Indonesia

Introduction

In 2013, there were about 4.8 million of malaria cases reported in Eastern Indonesia, with *Plasmodium falciparum* (62%) and *P. vivax* (33%) as being the most common species.[1,2] In this region, malaria was the leading cause of mortality with 5.3% deaths (23,483/441,155). South Central Timor (SCT) District had the highest malaria prevalence with annual parasitic incidence (API) 15.6 per 1,000 population for more than 10 years, and maternal mortality rate and infant mortality rate were two times higher than that of the average national rates.[3]

Currently, Indonesia Ministry of Health announced that malaria pre-elimination stage should be reached by 2020 and free of malaria transmission by 2030 to achieve the goal of "an Asia-Pacific free of malaria by 2030".[4] To reach malaria pre-elimination stage, malaria incidence must be zero with API less than one per 1,000 population and there must be no local transmission or relapsing cases for three consecutive years.[5,6]

About 75.4% (49/61) of countries had failed to achieve malaria elimination in 2010, including Angola, Botswana and Swaziland[7,8]. Philippines and Thailand have successfully pre-eliminated malaria, and stated that control activities such as active and passive case finding, vector control, drugs, surveillance systems and outbreak control must be refined and continued strictly in order to achieve the pre-elimination state.[9,10]

There was limited access to routine surveillance information such as API, surveillance report and mapping of malaria cases in Eastern Indonesia although detailed maps of epidemiological distribution, prevalence and risk factors for malaria were still in need. This study aimed to assess the epidemiological situation in this area as the routine surveillance data were too limited to assess whether Eastern Indonesia should proceed with the activities for malaria pre-elimination.

Methods

A survey was conducted from August 2013 to September 2014 to identify malaria infected cases. Sample size was calculated using a malaria prevalence (15%) in Eastern Indonesia during 2013 [11,12]. Malaria case definition in this study was an individual who had positive *Plasmodium* from nested polymerase chain reaction (PCR) and/or microscopic examination. Criteria for inclusion were people who were 14 years and older, tested negative by a tuberculosis rapid test[13] and a typhoid rapid test[14], and with hemoglobin level more than 10 g/dL using HemoCue[15].

All survey participants were selected by systematic random sampling and a written consent form was signed. The ethical committee from Faculty of Medicine, University of Gadjah Mada, Indonesia approved the survey (reference No: KE/FK/85/EC). Participants were interviewed face-to-face with a standard questionnaire. Household locations were obtained by global position system[16].

Physical examination was performed to all participants by local health practitioners. During the interview, thick and thin blood smears (Giemsa 5%) were conducted for identification of malaria parasites according to the protocol from Eijkman Institute,

Table 1. Distribution of malaria cases by results of nested polymerase chain reaction in Eastern Indonesia, 2013-2014

Variable	Nested polymerase chain reaction result				Total (%) (n=555)
	P. falciparum (%) (n=57)	*P. vivax* (%) (n=94)	*P. falciparum* & *P. vivax* (%) (n=30)	Total infected (%) (n=181)	
Sex					
Male	23 (29.5)	43 (55.1)	12 (15.4)	78 (34.1)	229 (41.3)
Female	34 (33.0)	51 (49.5)	18 (17.5)	103 (31.6)	326 (58.7)
Age group (year)					
≤ 15	1 (33.3)	2 (66.7)	0 (0)	3 (27.3)	11 (2.0)
16-20	3 (75.0)	0 (0)	1 (25.0)	4 (28.6)	14 (2.5)
21-30	6 (33.3)	7 (38.9)	5 (27.8)	18 (22.2)	81 (14.6)
31-40	15 (31.9)	25 (53.2)	7 (14.9)	47 (30.7)	153 (27.6)
41-50	8 (18.2)	30 (68.2)	6 (13.6)	44 (33.1)	133 (24.0)
≥ 51	24 (36.9)	30 (46.2)	11 (16.9)	65 (39.9)	163 (29.4)
District					
Oinlasi	2 (8.0)	20 (80.0)	3 (12.0)	25 (25.0)	100 (18.0)
Oe Ekam	17 (37.8)	18 (40.0)	10 (22.2)	45 (45.0)	100 (18.0)
Panite	11 (44.0)	10 (40.0)	4 (16.0)	25 (25.0)	100 (18.0)
Batu Putih	7 (16.3)	29 (67.4)	7 (16.3)	43(35.8)	120 (21.6)
Oenino	20 (46.5)	17 (39.5)	6 (14.0)	43 (31.9)	135 (24.3)
Ethnic					
Timorese	54 (32.5)	84 (50.6)	28 (16.9)	166 (32.4)	512 (92.3)
Sabunese	1 (33.3)	2 (66.7)	0 (0)	3 (42.9)	7 (1.3)
Rote	2 (18.2)	8 (72.7)	1 (9.1)	11 (42.3)	26 (4.7)
Bugis	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)
Sumbanese	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.5)
Others (Sundanese, Sundanese, Batak, Chinese)	0 (0)	0 (0)	1 (100)	1 (16.7)	6 (1.1)
Length of stay (year)					
≤ 5	50 (31.4)	82 (51.6)	27 (17.0)	159 (32.9)	483 (87.0)
≥ 6	7 (31.8)	12 (54.5)	3 (13.6)	22 (30.6)	72 (13.0)
Haemoglobin (Hb)					
Anemia (≤10 g/dL)	34 (30.4)	58 (51.8)	20 (17.9)	112 (32.0)	350 (63.1)
No anemia (>10 g/dL)	23 (33.3)	36 (52.2)	10 (14.5)	69 (33.7)	205 (36.9)
Anti-malarial drug history					
Yes	5 (38.5)	6 (46.2)	2 (15.4)	13 (33.3)	39 (7.0)
No	52 (31.0)	88 (52.4)	28 (16.7)	168 (32.6)	516 (93.0)

Remark: all surveyed participants were tested negative for P. malariae and P. ovale.

Ministry of Health, Indonesia. Microscopic reading was done at least 100 oil fields by 100 objective lens[17].

Whole blood sample was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant tube (BD vacutainer 5 mL)[18] and stored at -20°C for molecular assessment. DNA was extracted from 200 µL of whole blood by nucleon genomic DNA extraction kit[19]. All five *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*) were tested with nested PCR using protocols from Promega, Madison, USA[20] with PCR conditions followed from Snounou et al[21]. Electrophoresis was done with 1% Agarose gel[22] containing 5 µl/mL ethidium bromide[23]. Bands visualized by ultra violet illumination with DNA ladder[24].

Nested PCR confirmed cases were mapped using Arc GIS version 9.1. Spatial analysis was conducted by spatial, temporal and space time cluster analysis (SatScan)[25,26]. Univariate and bivariate statistical analyses, including prevalence ratio (PR) and 95% confidence interval (CI), were performed with statistical software.

Results

Total 555 (99.5%) out of 558 individuals completed the questionnaire and provided blood samples. Of 181 (32.6%) cases tested positive for malaria by nested PCR, *P. vivax* (51.9%, 94/181) was the most common species. Microscopic results revealed only 1.6% (9/555) positive for malaria, including three for *P. falciparum* and six for *P. vivax*.

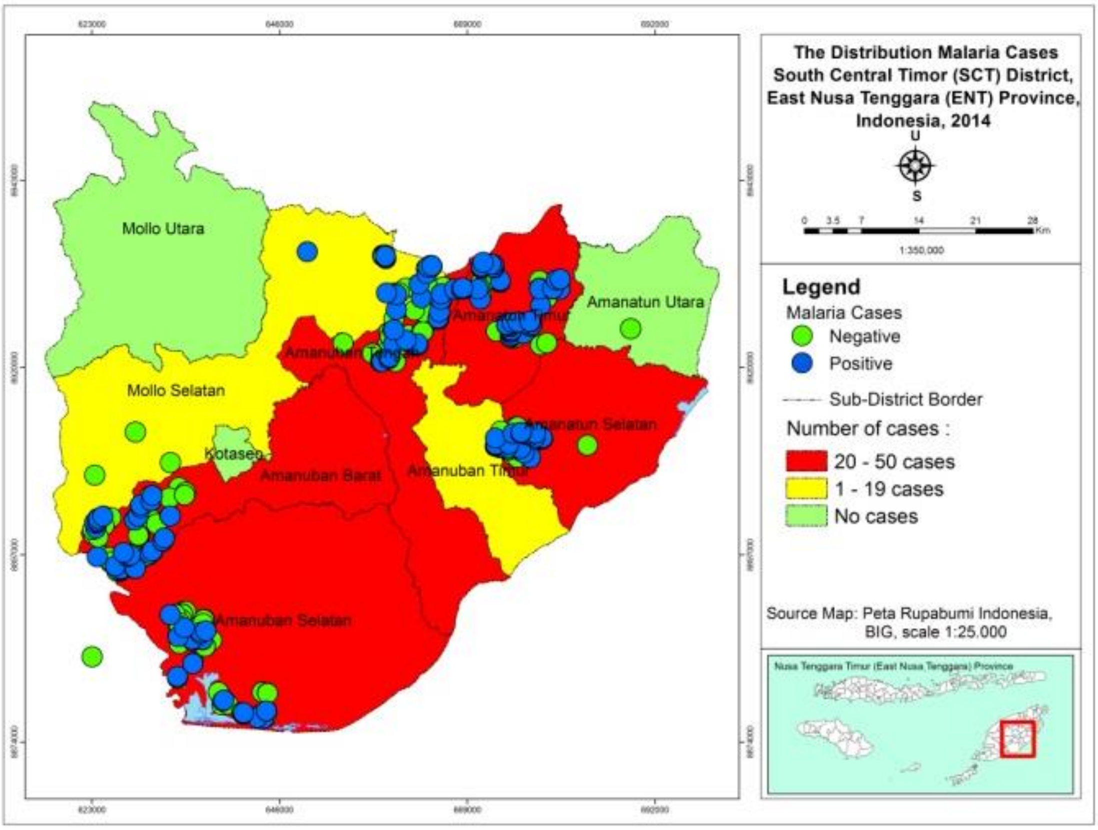
Malaria prevalence was similar for females (34.1%) and males (31.6%). Malaria prevalence increased for older age groups, with the highest in 51 years old and above (Mantel-Haenszel chi-square for linear trend = 6.67, p-value = <0.01). About 32.6% (181/555) had clinical manifestations such as fever, shiver, headache, extreme pain and nausea while 67.4% were asymptomatic.

Malaria cases were found in all districts, including districts with low case incidence. About 61.9% of people with malaria had anemia (Table 1). Statistical analysis for risk factors identification showed only one low significant variable which was not using bed nets (PR = 1.34, 95% CI = 0.98-1.84) (Table 2).

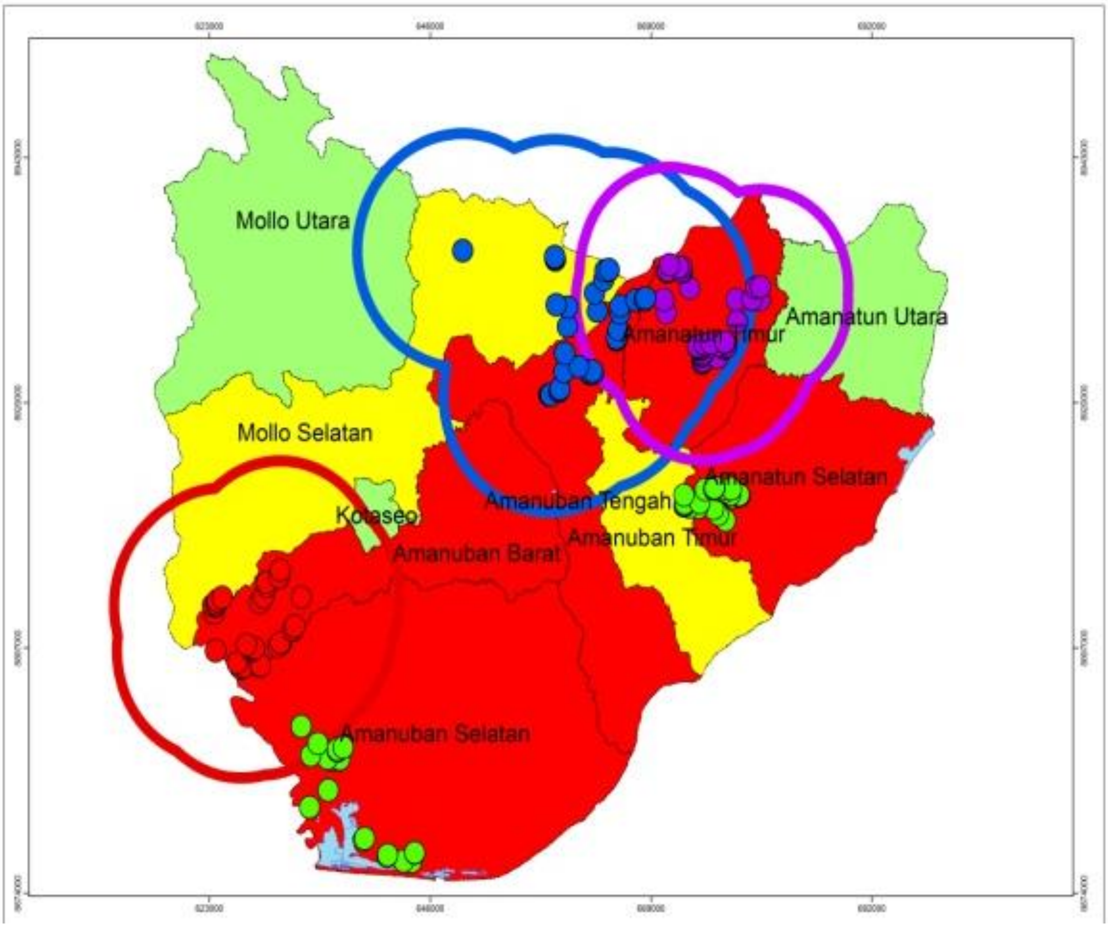
Retrospective space time series analysis identified three clusters in 183 villages with at least one malaria case. Primary cluster had radius of 10.00 km with 180 cases, followed by secondary cluster with 10.98 km (229 cases) and third cluster with 9.15 km (88 cases) (p-value ≤0.05). This result showed that malaria transmission was still occurring in high level (Figure 1).

Table 2. Bivariate analysis on risk factors for malaria in Eastern Indonesia, 2013-2014 (n=555)

Variable	Nested polymerase chain reaction (*Plasmodium* identification)		Prevalence ratio	95% CI
	Positive (%)	Negative (%)		
Sex				
Male	78 (14.1)	151 (27.2)	1.08	0.85-1.37
Female	103 (18.6)	223 (40.2)		
Visited other district				
Yes	76 (13.7)	154 (27.7)	1.02	0.80-1.30
No	105 (18.9)	220 (39.6)		
Not used bed nets				
Yes	146 (26.3)	274 (49.4)	1.34	0.98-1.84
No	35 (6.3)	100 (18.0)		
Slept outside the house at night (field) (n=554)				
Yes	46 (8.3)	94 (17.0)	1.02	0.77-1.34
No	134 (24.2)	280 (50.5)		
Used ventilation screen (n=553)				
Yes	3 (0.5)	2 (0.4)	1.86	0. 90-3.84
No	177 (32.0)	371 (67.1)		
Lived near cattle shed				
Yes	124 (22.3)	251 (45.2)	1.04	0.81-1.35
No	57(10.3)	123 (22.2)		
Had mosquitoes breeding places near home				
Yes	144 (25.9)	306 (55.1)	0.91	0.68-1.22
No	37 (6.7)	68 (12.3)		



(a)



(b)

Figure 1. (a) Distribution of malaria cases based on annual parasites incidence level, (b) Three clusters of malaria cases identified in Eastern Indonesia, 2013-2014

Discussion

Prevalence of malaria based on the nested PCR results (32.6%) was still high compared to that of the microscopic results (1.6%). *P. vivax* was dominant in Eastern Indonesia although *P. falciparum* was the dominant malaria infection in the whole country. Although WHO indicator stated that API should be less than one case per 1,000 population, the findings from our study were unfortunately not consistent with the national plan for pre-elimination by 2015 to 2020. Thus, the malaria pre-elimination activities in Eastern Indonesia should be postponed[2,27].

High asymptomatic infection (67.4%) revealed in this study implied with the fact that asymptomatic malaria is common in high malarious areas and highlighted that low parasitemia or asymptomatic cases should be identified during implementation of the malaria elimination program as well.

Malaria had been a major public health burden in Eastern Indonesia, affecting vast regions based on reports from local health offices in 2005 to 2010. There were outbreaks in 19 provinces, covering 65 districts with 58,152 reported cases and 536 deaths (case fatality rate 0.9%)[28,29]. To address malaria pre-elimination, many interventions were taken place, including residual spraying with insecticides (initial DDT), rapid diagnostic testing, artemisinin-based combination therapy (ACT) and surveillance introduced since 2005[30-32].

Lesson learned from the successful malaria elimination in Central Java, Jembrana District in Bali and Sabang District in Aceh was that they focused largely on seven steps recommended for control in malaria endemic districts. The steps recommended were producing a map of endemicity, identifying foci of malaria, finding feasibility for collaboration between communities and government, developing strategic plan for malaria control, obtaining support from district health office and legislative council, developing and implementing an integrated working plan for malaria, and monitoring and evaluating the strategy and progresses made[33].

Based on our study results, as the situation was entirely different to other provinces in Indonesia, we suggested four priorities: active and passive case finding coupled with periodic mass blood surveys, effective drugs management, vector control, and good surveillance. Additional work plan would be need to reduce the risk in remote areas where health care facilities were less developed. Such work plan should include prompt diagnosis and effective treatment, including accessibility to services[34].

As a vector control intervention, long-lasting insecticide-treated nets (LLINs) should be distributed to people at risk of malaria such as pregnant women and children living in malaria endemic areas[35]. Cost estimates of scaling up LLIN coverage could also be conducted by considering on distribution of *Anopheles* vectors and their bionomics, which should be evaluated before scaling up any intervention[36].

The spatial analysis showed that transmission was not homogeneous as risk of malaria was spreading throughout the whole study area (9-10 km). These maps could provide basic information in designing the surveillance strategy and identifying hotspots to target control activities. The endemic maps of *P. falciparum* and *P. vivax* could be used to estimate incidence in the areas where activities for pre-elimination were underway.

Public Health Actions and Recommendations

This study suggested that Eastern Indonesia was not yet eligible for elimination and the country has to scale-up the control measures (intensified malaria control) in order to reach the pre-elimination and elimination phases as soon as possible. Routine treatment to stop silent transmission, scaling up control measures, further researches and more sensitive laboratory support were needed before conducting the pre-elimination program. Many factors such as ongoing baseline transmission of malaria, lack of communication between health systems and lack of effective vector control measures prevented Eastern Indonesia from meeting the WHO requirements.

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Epidemiological and Genetic Characteristics of Rabies Virus in Ubon Ratchathani Province, Thailand, 2011-2014

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Abstract

Total 105 human rabies cases were reported in Thailand during 2006 to 2012. The objectives of this study were to describe epidemiology of animal rabies and genetic characteristics of the viruses found in Ubon Ratchathani Province where the highest number of animal rabies reported in 2011-2015. All 120 animal brain samples submitted to Veterinary Research and Development Center during 2011 to 2014 were included in this study. Epidemiological information was obtained from the sample submitter interview. The virus was identified with direct fluorescent antibody assay and characterized by neighbor-joining analysis. Muang (30.7%), Khemarat (23.1%) and Trakarn Puetpon (10.3%) districts had the highest incidence. The majority of the positive samples were from dogs (92.3%) and samples from dogs were 3.7 times more likely to be found positive, compared to those of other animals. The genetic analysis revealed close relationship between isolates from this study and other rabies viruses isolated in Asia. Rabies viruses in the province appeared to be localized and there was no evidence of transmission from China or Philippines. Determination of epidemiology and genetic characterization of rabies virus in specific areas or nationwide should be performed continuously to monitor the disease trend.

Key words: molecular epidemiology, rabies virus, Ubon Ratchathani, Thailand

Introduction

Rabies is an important fatal zoonotic disease that caused more than 55,000 human deaths around the world every year,[1] with more than 99% of all human deaths from rabies occurring in Asia and Africa[2]. Dogs were reported as the main carrier for human rabies in Asia, including Thailand.[2] There were 105 human rabies cases reported in Thailand during 2006 to 2012.[3] Although no human cases were reported in Ubon Ratchathani Province during 2011 to 2015, the highest number of rabies positive animals were identified in the province when compared to other lower northeastern provinces in Thailand. Approximately 10 animal samples were tested positive for rabies virus annually and mostly found near the border areas with the neighboring countries, Cambodia and Lao PDR.

Classical rabies viruses can be classified into six groups, namely Africa 2, Africa 3, Arctic-related, Asian, Cosmopolitan and Indian subcontinent. Arctic-related, Asian, Cosmopolitan and Indian subcontinent groups are found in Asia[4] while rabies viruses found in Thailand, Vietnam, Cambodia, Lao PDR, Myanmar, Philippines, Indonesia and Malaysia belong to Asian group[5-7].

We explored the epidemiological and genetic characteristics of rabies viruses in Ubon Ratchathani Province. The results from this study such as in-depth information of the virus ribonucleic acid (RNA) and spreading pattern could be used in creating guidelines for rabies control and monitoring.

Methods

Study Samples

Animal brain samples submitted by provincial veterinary officers in Ubon Ratchathani Province to the Veterinary Research and Development Center (VRDC) for lower northeastern region in Surin Province during 2011 to 2014 were recruited into this study. These samples included suspected animals died with clinical signs of nervous system and/or human exposure (passive surveillance) and samples obtained from an animal rabies project (active surveillance).

Data Analysis

Case definition was classified according to the outcome from direct fluorescent antibody (DFA) test, following the guidelines set in the manual of diagnostic tests and vaccines for terrestrial animals, 2013[8]. Relevant data, including vaccination history, stray or owned, sex, approximate age and district location of the submitted samples, were extracted from the sample submission forms. Descriptive statistics was employed to describe the rabies situation. Odds ratio (OR) was calculated using logistic regression method, and association between rabies and independent variables were determined as well.

Genetic Characteristic Analyses

Out of the original 39 DFA positive brain samples kept at -20°C, 17 samples were decomposed and only 22 samples were included in the nucleoprotein gene sequencing analysis. The amplified products of 523 bp (nt 1013-1536) of complementary DNA (cDNA) were obtained by direct one-step reverse transcription polymerase chain reaction (RT-PCR) amplification of the nucleoprotein (N) gene fragment and characterized by sequencing. RT-PCR was conducted at the laboratory in VRDC (lower northeastern region) and sequencing procedures were conducted using Sanger sequencing at Solgent Laboratory in Korea. One set of primers was used for RT-PCR sequencing reaction.[9]

Total 18 worldwide N sequences, including Asian, Africa 2, Africa 3, Indian subcontinent, Arctic and Cosmopolitan groups, were retrieved from GenBank database. These gene sequences were compared with the samples recruited in this study, and genes from the same region and with similar length of DNA data were selected. All sequences were aligned together using program Clustal X. Genetic relationship between these N gene sequences was examined and a tree diagram was drawn using neighbor-joining method. The phylogenetic analyses were performed with program Mega version 6. Robustness of the tree was assessed with branch supporting values from bootstrap statistical analyses (1,000 replicates).

Total 12 Asian N sequences, including Cambodia, Lao PDR, Myanmar, China and Philippines viruses, were retrieved from GenBank database for Asian phylogenetic analysis as well.

Four N sequences from Cambodia, Lao PDR and Philippines retrieved from GenBank were used for within-province phylogenetic analysis in order to clearly categorize the viruses from the members of Asian group. Geographical locations of the samples were mapped with QGIS[10] version 2.2.0 and compared among the subgroups.

Results

Total 120 brain samples (98 dogs, 11 cats, five cows, five mice and one rabbit) submitted to the VRDC during 2011 to 2014 were included in this study, including 108 samples (92 dogs, 11 cats and five cows) from passive surveillance and 12 samples (six dogs, five mice and one rabbit) from active surveillance.

The districts that submitted the highest number of samples were Muang (27.5%), followed by Khemarat (15.0%), Trakarn Puetpon (8.3%) and Warin Chamrap (8.3%) (Table 1). Among all rabies positive samples, 30.7% were from Muang, 23.1% from Khemarat, 10.3% from Trakarn Puetpon and 2.6% from Warin Chamrap. About 11% (13/120) of samples were sent from the sub-districts without main road passing through.

Table 1. Diagnostic results of dog brain samples from Ubon Ratchathani Province, Thailand, 2011-2014

District	Total	Positive	Percent
Muang	33	12	36.4
Khemarat	18	9	50.0
Trakarn Puetpon	10	4	40.0
Warin Chamrap	10	1	10.0
Khueng Nai	7	1	14.3
Boontarik	7	2	29.6
Nam Khun	5	0	0
Lao Sue Kok	4	1	25.0
Khong Chiam	4	3	75.0
Don Moddaeng	4	2	50.0
Nam Yuen	4	0	0
Pibun Mangsahan	4	2	50.0
Tansoom	2	0	0
Sri Muengmai	2	1	50.0
Sirindhorn	2	1	50.0
Det-udom	1	0	0
Najaluay	1	0	0
Na Tan	1	0	0
Muang Samsib	1	0	0
Total	120	39	32.5

Total 36 (92.3%) samples from dogs and three (7.7%) from cows were tested to have rabies virus. Among these positive samples, three were from dogs aged 3-5 years that were vaccinated 11 days, 10 months and 11 months ago. The analysis showed that the virus could be found in dog samples 3.7 times more than other animal samples (OR = 3.7, 95% CI = 1.02-13.29) and samples from animals older than two years were more likely to be positive than the younger animals (Table 2).

Table 2. Association between independent variables and rabies virus exposure in Ubon Ratchathani Province, Thailand, 2011-2014

Factor	Case		Non-case		Odds ratio (95% CI)
	Exposed	Unexposed	Exposed	Unexposed	
Sub-district not connected to the main road	5	32	6	74	1.9 (0.54-6.77)
Stray animal	10	28	16	58	1.3 (0.52-3.22)
Having been vaccinated	32	3	55	17	3.3 (0.89-12.13)
Samples from dog	36	3	62	19	3.7 (1.02-13.29)
Male	25	9	40	23	1.6 (0.64-4.00)
Age of animal (year)					
< 1	22	12	47	17	0.7 (0.28-1.64)
1-2	2	32	9	55	0.4 (0.07-1.88)
> 2	10	24	8	56	2.9 (1.02-8.30)

The phylogenetic analysis was conducted based on nucleoprotein gene (523 bp) of rabies viruses in the province by comparing with worldwide rabies viruses and was classified into the Asian group (Figure 1). Rabies viruses in Southeast Asia and China were then classified into three groups. Group 1 consisted of Thailand, Cambodia, Lao PDR and Myanmar while group 2 was China and group 3 was Philippines (Figure 2). Rabies virus in the province was recruited into group 1. The virus in the province was then classified into subgroups 1 and 2 by categorizing number of roots in phylogenetic tree and the roots with bootstrap greater than 50 was determined as a subgroup (Figure 3). Both subgroups were found in Muang and Khemarat Districts while other districts found only one (Figure 4).

Discussion

After exploring the epidemiological and genetic characteristics of rabies virus in Ubon Ratchathani Province during 2011-2014, the analytical results identified that the virus could be found more commonly in dog samples, which was consistent with a study in 2012 which revealed that dog had higher chance to be infected by rabies than other animals[11]. Moreover, another study conducted in Thailand

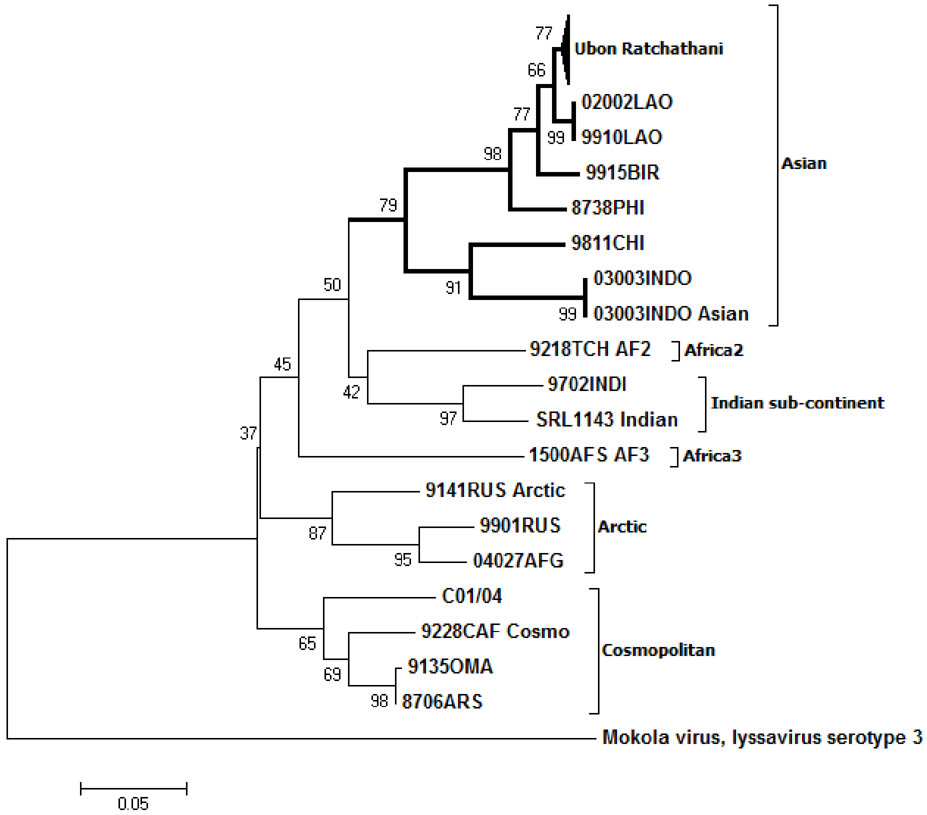

Figure 1. Classification of rabies virus in phylogenetic tree of nucleoprotein gene (DNA sequence 1013-1536) from 22 dog brain samples in Ubon Ratchathani Province, Thailand compared with gene sequences from various countries

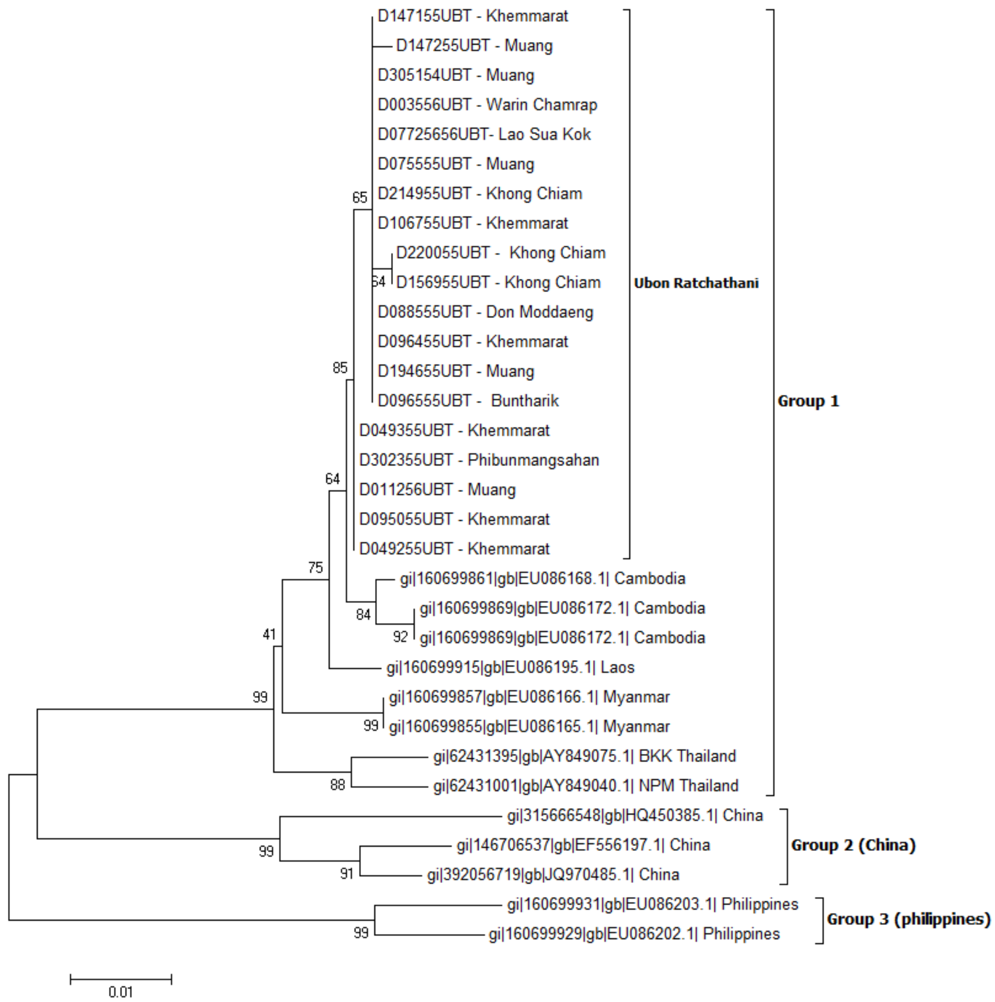

Figure 2. Classification of rabies virus in phylogenetic tree of nucleoprotein gene (DNA sequence 1013-1536) from 22 dog brain samples in Ubon Ratchathani Province, Thailand compared among groups of Asian gene sequences

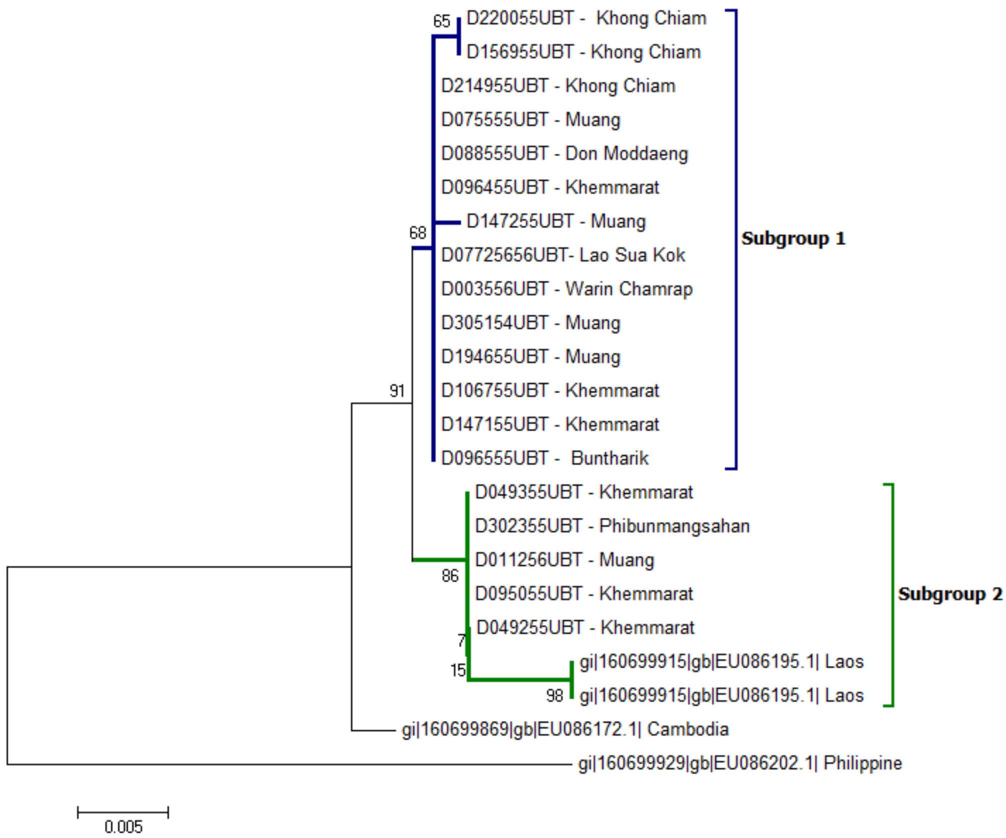

Figure 3. Classification of rabies virus in phylogenetic tree of nucleoprotein gene (DNA sequence 1013-1536) from 22 dog brain samples in Ubon Ratchathani Province, Thailand compared among subgroups 1 and 2

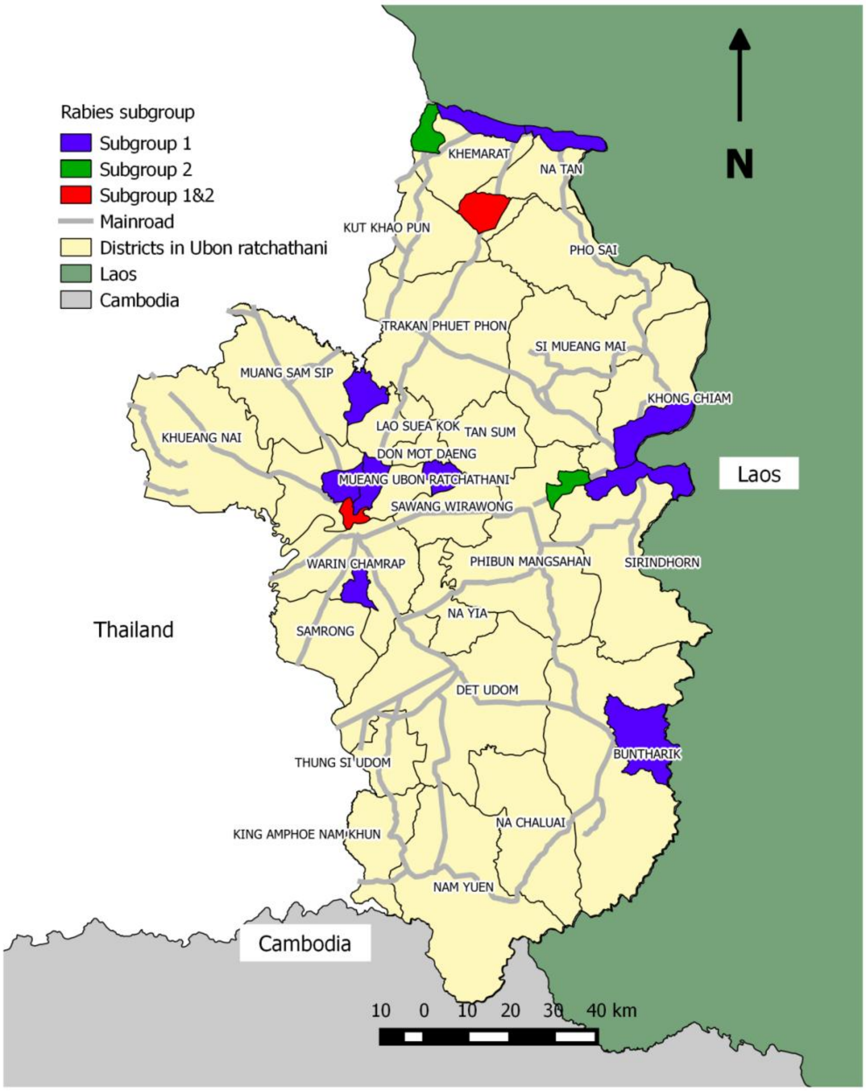

Figure 4. Geographical distributions of nucleoprotein gene subgroups 1 and 2 from 22 dog brain samples in districts of Ubon Ratchathani Province, Thailand

during 2014 found statistically significant association between animal rabies and two risk factors that were connecting borders and presence of main road through sub-districts[12]. That study included 379 samples from nine provinces in the lower northeastern Thailand during 2011-2013, with Ubon Ratchathani as one of nine provinces. In 2012, Theerapong's study stated that unvaccinated animals had higher chance to infect rabies than that of vaccinated animals.[11] Inconsistent findings with the previous studies might be caused by small sample size in our study. We also observed that only 11% of samples were sent from sub-districts without main road passing through, which was possibly resulted from inconvenient transportation and might cause under-reporting of animal rabies cases. Hence, awareness to send animal samples for testing whenever people find clinically suspected cases should be raised in the communities.

In addition, three samples from dogs aged 3-5 years were found to have history of vaccination within one year. Several factors might influence vaccine effectiveness such as health condition of animals at the time of vaccination, and cold chain system during storage and transportation[13]. Hence, history taking and health examination should be conducted carefully before vaccination. As for the rabid dog that died 11 days after being vaccinated, the immunity from vaccination might not reach the protective level yet.

Rabies virus found in this region was classified into Asian group, which was consistent with finding from the previous studies in Philippines, Indonesia and Vietnam[5-7]. In contrast, a study in Nepal stated that the Indian sub-continent group was commonly found in southern India and Sri Lanka.[14] Arctic group, which is generally found in Afghanistan, Russia, Greenland and United States, could be found in Nepal as well[4]. These findings showed that rabies virus could spread from one region to another and highlighted the importance of genetic analysis. Despite that being said, rabies from other regions has not been reported in Thailand.

Results of phylogenetic analysis on rabies virus in the province were consistent with other studies in Southeast Asia by Yamagata et al[7] and Nguyen et al[15]. Our study also revealed that rabies virus in the province could be classified into the same group as Cambodia, Lao PDR and Myanmar.

Concerning the variety of virus, Muang and Khemarat Districts found both subgroups 1 and 2 while other districts found only one of them. The highest number of samples was submitted from Muang District, followed by Khemarat District. Large amount of samples submitted might lead to higher chance of finding high variation of the virus. Muang District is the economic center of the province with convenient in transportation, and more human and animal movement. These might facilitate transmitting a variety of rabies virus in the area. The result was consistent with a study by Denduangboripant et al which demonstrated that rabies virus subgroups TH1 and TH2 were found in Bangkok and central provinces while only TH2 was found in other areas[16]. In addition, another study conducted in Bangkok and its surrounding provinces found that Bangkok Metropolitan had larger variety of rabies virus than other rural provinces such as Chaiyaphum and Kanchanaburi.[17]

The rabies virus in the province was still the same as the virus circulating in the country, with no evidence of transmission from China or Philippines. Nevertheless, determination of epidemiology and genetic characterization of rabies virus in specific areas or nationwide should be performed continuously to monitor the disease trend.

Acknowledgements

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Census versus Capture-recapture Method to Estimate Dog Population in Lumlukka District, Pathum Thani Province, Thailand, 2010

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Abstract

Although reliable data for dog population is essential for designing an effective strategy for rabies vaccination, it is difficult to precisely estimate the dog population, especially the stray dogs. This study estimated the dog population by census and capture-recapture method (CR), characterized dog population, described practicality and feasibility, and estimated the rabies vaccination coverage. Ten urban and rural areas in Lumlukka District, Pathum Thani Province were randomly selected. Results showed that stray dog population from census was lower than CR estimates in both urban and rural areas. The census showed that the majority of dogs were confined owned dogs in the urban area (70%) and unconfined owned dogs in the rural area (96%). The stray dog population from census was 8.0% in the urban and 4.4% in the rural areas. Rabies vaccination coverage among dogs in the urban was 84% and in the rural was 65%. Although CR method used less time and people than census, it was more complicated. The census method might underestimate the number of stray dogs while the CR failed to include the confined owned dogs. Therefore, the census method could be a preferable method to collect data of owned dogs and CR could provide a better estimate of stray dog population. Both methods could be used to monitor the rabies control program and plan for effective strategy to eradicate rabies in Thailand.

Key words: Capture-recapture, census, dog population, rabies, vaccination coverage, Thailand

Introduction

Rabies is a zoonosis with almost 100% fatality in both humans and animals. Human deaths due to rabid dog bite occurred mostly in developing countries.[1] Over 96% of the reported rabies cases in animals were dogs while about 95% of human rabies deaths were from the bite of rabid dogs.[2] World Health Organization (WHO) recommends immunization of at least 70% of dog population in each area in order to reach a herd immunity level to prevent rabies outbreaks, along with the integrated management approach such as dog population control and public education.[3]

In Thailand, the first human rabies was reported during 1929. Over the past three decades, the number of human and dog rabies cases have dramatically decreased as a result of the national rabies control program.[4] However, there has been continuous occurrence of rabies infection among dogs and humans.[5] Between January 2008 to 2010, a total of 47 confirmed human rabies cases were reported[6] while there were 489 animal rabies cases from January 2009 to June 2010, with 90% of them were dogs[7]. Major constraints to rabies control in Thailand included failure to reach the national goal of 80% rabies vaccination coverage in dogs, lack of effective dog population management and control, and limited participation of certain local administrations. Furthermore, the current vaccination campaign in dogs was restricted by lack of accurate information on targeted dog population and social-economic factors which might in turn influence owners' decision to vaccinate their dogs.[8]

It is essential to improve population management and rabies vaccination campaigns in dogs to achieve the ultimate goal of rabies free area. Reliable estimates of size and composition of the entire dog population are necessary to plan for effective control activities. Conducting a census is the standard method to estimate dog population size and composition while other methods are registration and identification. Nevertheless, these methods tend to omit estimates of stray dog population. Stray dogs are common and constitute a significant proportion of dog population in many developing countries, including Thailand which accounted for 70-80% of dogs that bite humans. Studies in Philippines[9], Japan[10] and Sri Lanka[11] applied the capture-recapture method (CR), a method developed to estimate wild animal population. CR is now used to estimate dog population in settings with large number of stray dogs. This approach was used to estimate dog population in Songkhla Province, Thailand as well[12]. The principal aims of this study were to characterize and estimate the dog population by census and CR, determine rabies vaccination coverage in Lumlukka District, Pathum Thani Province, and describe practicality and feasibility of the CR method.

Methods

The study area was Lumlukka District in Pathum Thani Province which ranked the second highest number of animal rabies cases in 2009, with 30% of cases in Lumlukka District.[7] This district covers 297.7 km^2, and comprises of 49 communities in the urban municipal area (0.2 km^2) and 103 villages in the rural area (2.6 km^2). A total of 10 areas were randomly selected, including five communities in the urban area and five villages in the rural area.

All domestic dogs in Lumlukka District were included in this study. Dogs were classified into two main groups: the owned dog and the stray dog. An owned dog was defined as a dog that belonged to and was regularly fed by a household. It was then subdivided further into either a confined owned dog which was totally confined or let roam free for less than two hours a day, or an unconfined owned dog which was allowed to roam free for two hours or more per day. A stray dog referred to a dog without any defined owner.

Census Survey

Data of households and a map of selected communities and villages were obtained from Lumlukka Administration Office. Dog population census was performed by interviewing the dog owners at home or a public site in the village. Data on number of dogs in household, gender, breed, type, confinement, and history of rabies vaccination and surgical sterilization were collected. In addition, a rabies vaccination campaign was set up and dogs were provided with rabies vaccination at a public site in the village. The census was conducted in two days, including one working day and a day in weekend as an effort to boost cooperation from dog owners in the communities and villages. Stray dogs were identified by observing and interviewing people in the communities or villages.

The dogs included in the census survey were photographed, and collars with different colors were put on them to differentiate between owned and stray dogs on the same day (Figure 1). A photograph was taken to confirm the identity of each dog in case dogs

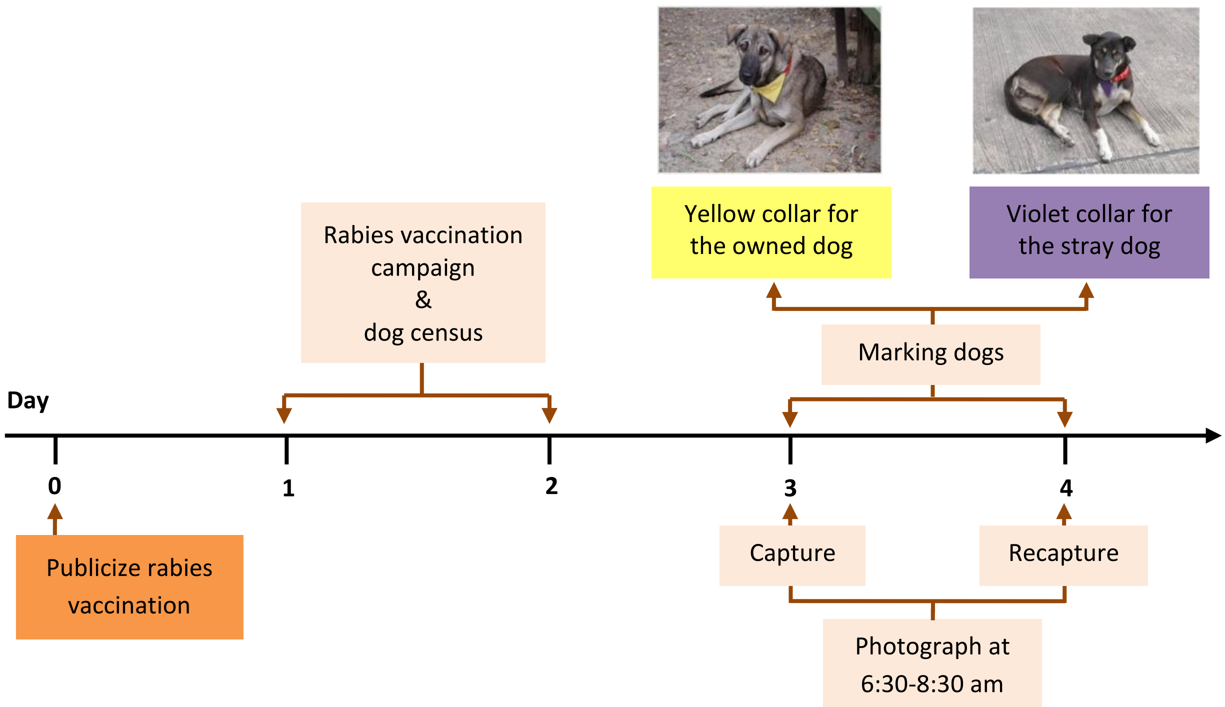

Figure 1. Diagram illustrating steps and workflow of dog population survey by census and capture-recapture method in Lumlukka District, Pathum Thani Province, Thailand, 2010

would lose the collars or when there was an aggressive dog that no one could put a collar on.

Capture-recapture (CR) Method

After the census survey, the CR was conducted for two consecutive days. Capture (by photograph) took place on the first day at 6:30-8:30 am when most stray dogs were present in the public areas[10]. The researcher photographed any visible dogs while walking through the communities or villages. Locations and number of dog were also recorded. Similar activities were repeated at the same period of time on the second day for recapture. Dogs were then identified by characteristics, including type of fur, body, tail and color seen in the photographs as well as color of the collar. However, confined owned dogs were not included in the CR since the method only allows counting the dogs that can be captured outside the houses while the researcher was walking across the community.

The number of dogs derived between capture and recapture were differentiated, counted and recorded. Information on time spent and the number of staff assisting in each survey method was also recorded.

Data Analysis

Information obtained was characterized and vaccination coverage was estimated. The time spent and man-power used for both survey methods per study site were described by person-hours (the number of staff multiplied by working hours for each person). An estimated dog population by CR ($\hat{N}$) was calculated with 95% CI using the Chapman estimator from the survey toolbox[12].

$$\hat{N} = \frac{(n+1)(M+1)}{(m+1)} - 1$$

where
M = Total number of dog found on the 1st day (Capture)
n = Total number of dogs found on the 2nd day (Recapture)
m = Number of dogs found on both days

The percent difference (% Difference) between estimates from census and CR data was calculated by the below formula.[13]

$$\% \text{ Difference} = \frac{N_C - N_{CR}}{N_C} \times 100$$

where
N_C = Census population
N_{CR} = Estimated population from CR

Results

Characteristics of Dog Population and Rabies Vaccination Coverage

The census estimated a total of 1,680 dogs in 10 study sites, including 820 dogs in the urban area and 860 dogs in the rural area. In the urban area, majority of the owned dogs were confined (69.6%) which was in contrast with the rural area where all of them were reported as unconfined (95.6%). The stray dogs found in the urban and rural areas were 8.0% and 4.4% respectively. Medium-sized dogs (approximate 11-25 kg) were most common in both areas. Surgically sterilized dogs were accounted for 22.2% in the urban and 5.2% in the rural areas (Table 1). The age-sex population pyramid showed that most dogs aged between one day to two years, followed by 2-4 years (Figure 2).

Table 1. Characteristics of dogs in Lumlukka District, Pathum Thani Province, Thailand, 2010 (n=1,680)

Characteristic	Urban		Rural	
	Number	Percent	Number	Percent
Gender (n=1,599)				
Male	422	53.6	430	53.0
Female	366	46.4	381	47.0
Type of dog				
Confined owned	571	69.6	0	0
Unconfined owned	183	22.3	822	95.6
Stray	66	8.0	38	4.4
Breed (n=1,667)				
Large	31	3.8	9	1.0
Medium	584	72.4	784	91.2
Small	192	23.8	67	7.8
Received rabies vaccination in the previous year				
Yes	621	75.7	505	58.7
No	115	14.0	271	31.5
Do not know	84	10.2	84	9.8
Surgically sterilized				
Yes	182	22.2	45	5.2
No	542	66.1	755	87.8
Do not know	96	11.7	60	7.0

Rabies vaccination coverage in the previous year was 84.4% in the urban and 65.1% in the rural areas. The vaccine coverages in the urban and rural areas were similarly low for unconfined owned dogs (66-67%) and stray dogs (45-48%). However, the owned dogs had vaccination coverage higher than the stray dogs in both urban (OR = 8.39, 95% CI = 4.67-15.07) and rural (OR = 2.07, 95% CI = 0.93-4.60) areas,

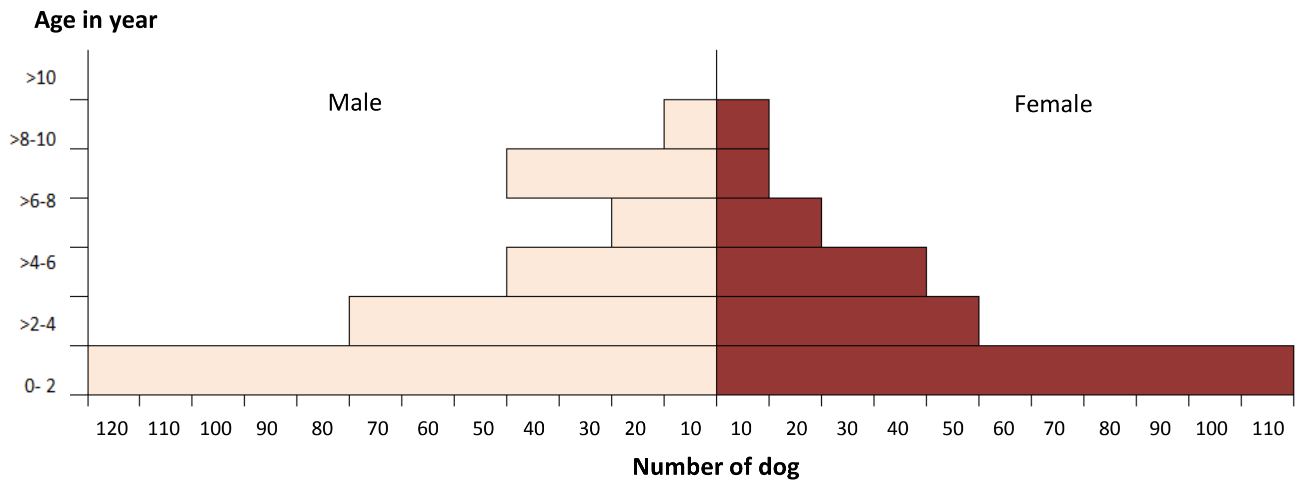

Figure 2. Age-sex pyramid of dog population from census method in Lumlukka District, Pathum Thani Province, Thailand, 2010

especially among the confined owned dogs (Table 2). In the urban area, ratio of dog per household was 1:1.2 (820:973) and ratio of dog per human population was1:3.9 (742:2,916). In the rural area, the ratio of dog per household was 1:0.9 (860:781) and the ratio of dog per human population was 1:3.0 (860:2,583).

Dog Population Estimation by Census and CR

Total 571 confined owned dogs were identified by the census survey in the urban area. Unconfined owned-dog population estimated by CR (288) was higher than that of the census (183) in the urban area and was inversed in the rural area. The owned dogs estimated by CR was 57.4% higher in the urban and 8.5% lower in the rural area compared with the census population. The estimated stray dog population by CR was higher than that of from census in both areas, with 18.2% higher in the urban and 76.3% higher in the rural area. Total population estimated by CR was 47.0% more than that of the census in the urban area. The results estimated by CR and census were very similar while the estimate by CR was 4.8% lower than that of the census in the rural area (Table 3).

Practicality and Feasibility of Census versus CR

Time spent on performing census and CR per study site was 12 hours (6 hours per day) and four hours (2 hours per day) respectively. Person-time consumed per study site was 96 person-hours for census and 18 person-hours for CR. Census was performed during working hours while CR was done in the early morning (6:30-8:30 am). Personnel carrying out the census were veterinarians and assistants (livestock volunteers, municipality officers and community health volunteers). As for CR, the team members of the survey were researchers and community health volunteers.

Some constraints arose from performing CR were inability to put collar on aggressive dogs, missing collars, difficulty to see the collars due to long dog fur, and unable to take clear photographs for the running dogs. We were not able to mark 11.7% (196/1,680) of dogs by a collar or photographed on the day of census.

Table 2. Rabies vaccination coverage among different types of dog in the urban and rural areas from census method, Lumlukka District, Pathum Thani Province, Thailand, 2010

Area	Total	History of vaccination			Vaccine coverage among dogs with known vaccination history
		Yes	No	Do not know	
Urban					
Confined	571	488	31	52	94.0
Unconfined	183	109	55	19	66.5
Stray	66	24	29	13	45.3
Total	*820*	*621*	*115*	*84*	*84.4*
Rural					
Unconfined	822	493	258	71	65.7
Stray	38	12	13	13	48.0
Total	*860*	*505*	*271*	*84*	*65.1*

Table 3. Dog population estimated by census and capture-recapture method in Lumlukka District, Pathum Thani Province, Thailand, 2010

Type of dog	Population from census	Capture	Recapture		Estimated population from CR	95% CI	% Difference
			Total	Marked			
Urban Area							
Unconfined owned	183	178	199	123	288	270.42-305.01	-57.4
Stray	66	52	39	26	78	65.91-89.13	-18.2
Total	*249*	*230*	*238*	*149*	*366*	*345.85-388.27*	*-47.0*
Rural Area							
Unconfined owned	822	562	573	428	752	742.48-762.10	8.5
Stray	38	45	54	36	67	61.88-72.88	-76.3
Total	*860*	*607*	*627*	*464*	*819*	*816.89-823.57*	*4.8*

Discussion

Stray dog population identified from the census survey in both urban and rural areas were lower than the estimates from CR, which supported the results from the previous studies that estimated dog population by census survey[11]. However, CR cannot be used to count the confined owned dogs. Although the method cannot distinguish types of dog without having sign or mark, this study conferred the particular limitation by putting different colors of collar for owned and stray dogs, and performing CR survey soon after. However, reporting bias might still exist.

The unconfined owned dog population estimated by CR was higher than that of census in the urban and lower in the rural area, which might be due to the fact that some confined owned dogs released for daily excretion in the urban area might include in the estimated population. On the other hand, in the rural area, all owned dogs were defined as unconfined and this might fail to cover all of them. Thus, in the future CR surveys, confined and unconfined owned dogs should be marked differently, and the definitions for confined and unconfined owned dogs should be reviewed to improve the survey method.

Dog population census during the time of rabies vaccination campaign had gained some advantages. It reduced time of house-to-house visit to collect data, promoted people cooperation, and obtained general information (demographic, management practices and history of vaccination) and data to evaluate the vaccination coverage. Data from census showed that there were more male dogs than females. Although it was consistent with another study[14], it might show a bias of population in male dogs. The population composition consisted of dogs under two years old as the majority, implying the increasing trend of dog population and the need to control by the appropriate method. In circumstance when there is no reliable data available, dog population was estimated in relation to human population. The dog population per human population in this study was closed to the estimated ratio reported from the studies in Philippines[9] and Japan[10].

Data obtained in the census survey also showed the distinguished characteristics of dog population, and the management between the urban and rural areas. When compared with the rural area, the urban had higher proportion of small breed dogs, confined owned dogs, dogs received the rabies vaccination in the previous year and surgically sterilized dogs. Furthermore, dog proportion that received the rabies vaccination was higher among the confined owned dogs than that of the unconfined owned dogs. A practice of dog confinement appeared to correlate with receiving the rabies vaccination and being surgically sterilized. In fact, those unconfined owned dogs are at higher risk of infecting rabies by exposing to stray dogs which pose a major source of rabies transmission[15].

The rabies vaccination coverages in dogs from this study were 84.4% in the urban and 65.1% in the rural area. The vaccination coverages in the rural was still lower than the national goal of 80% and have not met the WHO recommendation yet[3]. Low vaccination coverage could be a potential factor for high occurrence of rabies in the area. Besides, this estimated vaccination coverage might be over-estimated if most of stray dogs were unvaccinated and some owners made the false report.

Conclusion

Application of census and CR to estimate dog population required some additional technical adjustment and prior knowledge on the characteristics of dog population and the environment. No single methods could truly estimate the dog population in the setting similar to the study area in

Thailand. However, these two methods could complement each other. The findings from this study provided important information to monitor rabies control program and plan for effective strategy for rabies control and eradication in Thailand.

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