



Outbreak, Surveillance and Investigation Reports

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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.

Keywords: 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 Africa². 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 Asia⁴ while rabies viruses found in Thailand, Vietnam, Cambodia, Lao PDR, Myanmar,

Philippines, Indonesia and Malaysia belong to Asian group⁵⁻⁷.

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, 2013⁸. 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 QGIS¹⁰ 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 animals¹¹. Moreover, another study conducted in Thailand

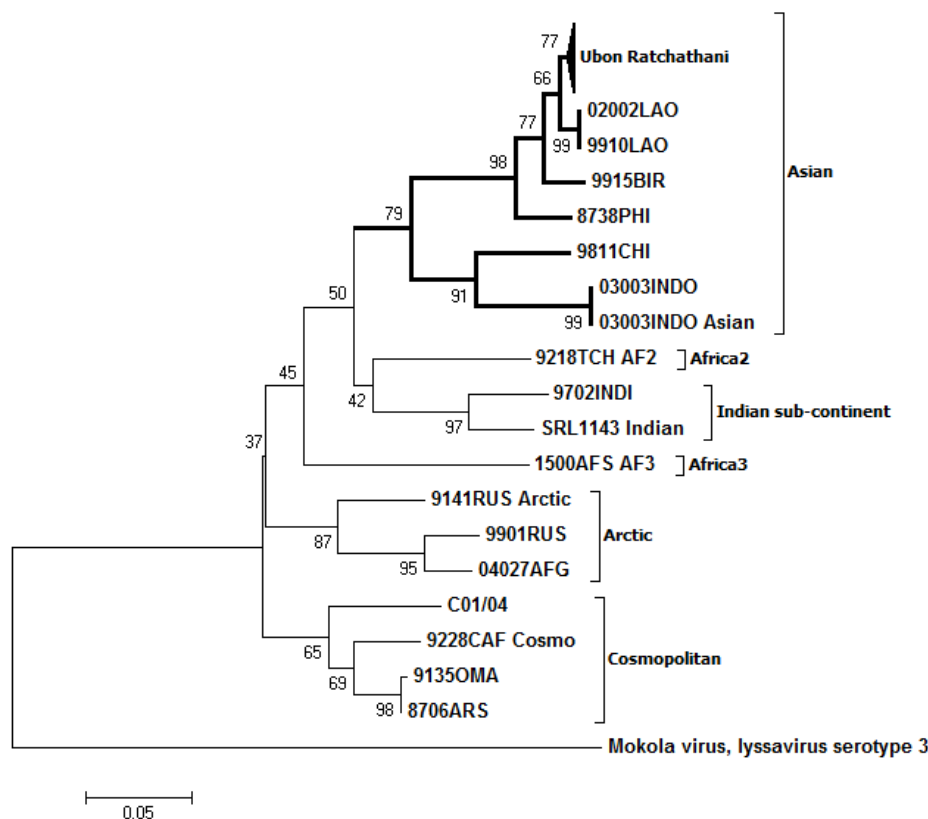


Figure 1. Classification of rabies virus in phylogenetic tree of nucleoprotein gene (DNA sequence 1013-1536) from 22 dog brain samples in Ubon Ratchathani Province, Thailand compared with gene sequences from various countries

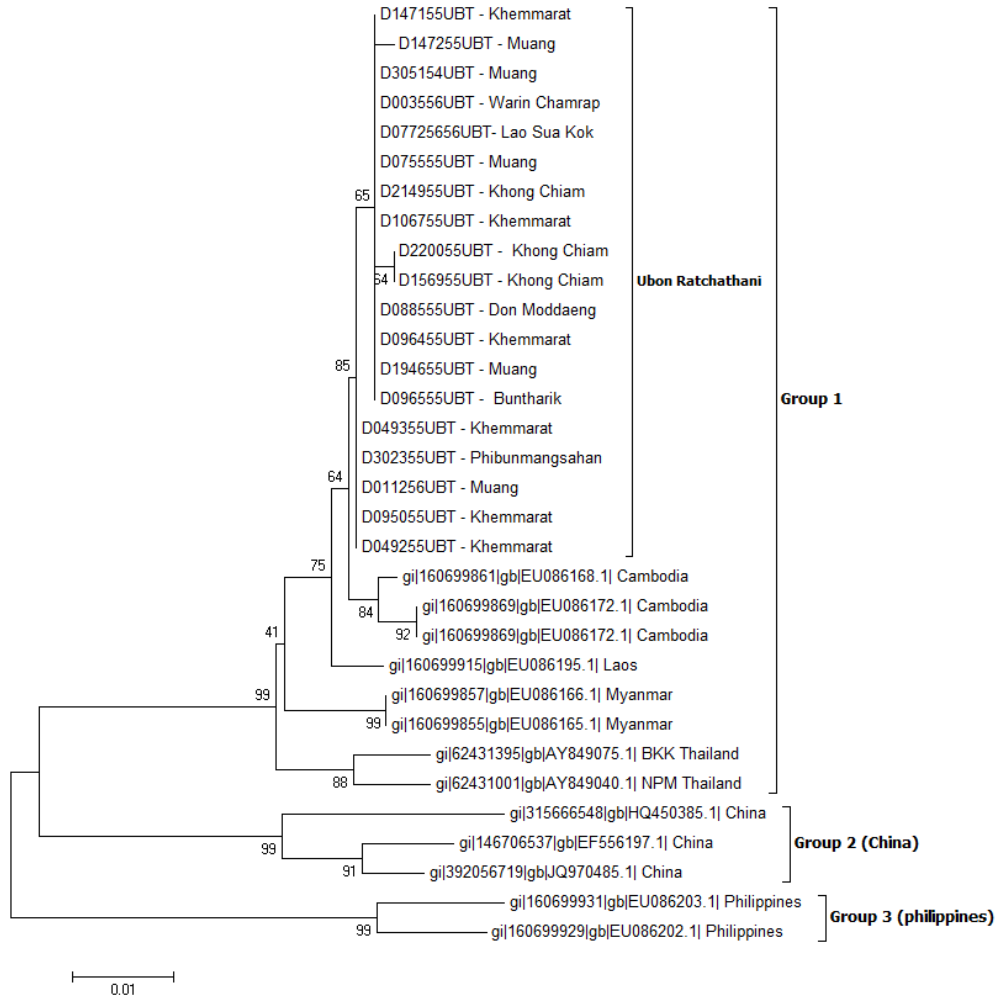


Figure 2. Classification of rabies virus in phylogenetic tree of nucleoprotein gene (DNA sequence 1013-1536) from 22 dog brain samples in Ubun Ratchathani Province, Thailand compared among groups of Asian gene sequences

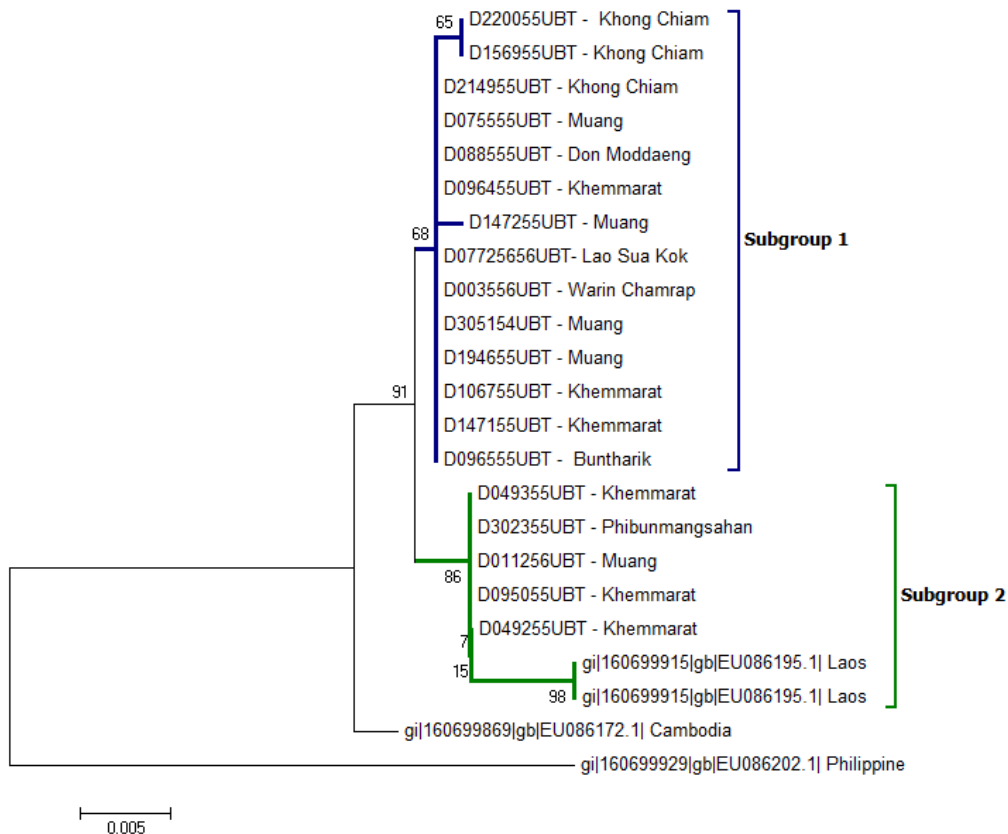


Figure 3. Classification of rabies virus in phylogenetic tree of nucleoprotein gene (DNA sequence 1013-1536) from 22 dog brain samples in Ubun Ratchathani Province, Thailand compared among subgroups 1 and 2

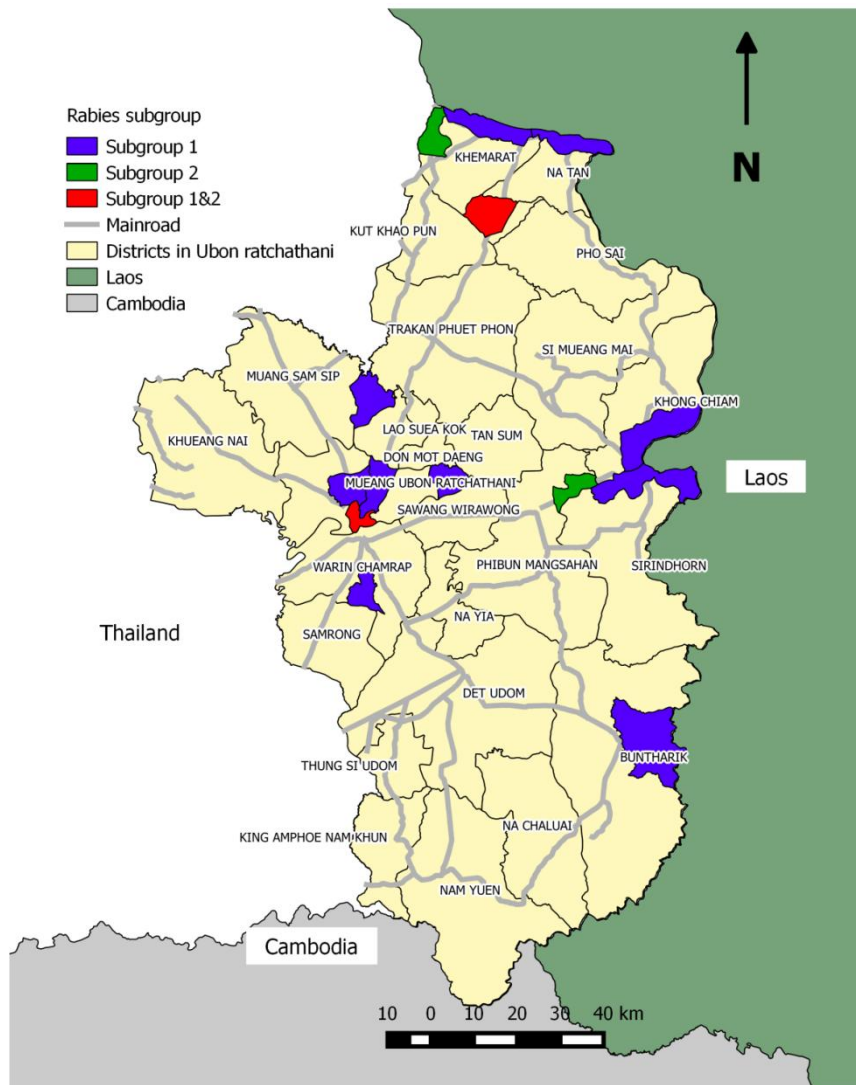


Figure 4. Geographical distributions of nucleoprotein gene subgroups 1 and 2 from 22 dog brain samples in districts of Ubol Ratchathani Province, Thailand

during 2014 found statistically significant association between animal rabies and two risk factors that were connecting borders and presence of main road through sub-districts¹². That study included 379 samples from nine provinces in the lower northeastern Thailand during 2011-2013, with Ubol 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 transportation¹³. 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 Vietnam⁵⁻⁷. 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 well⁴. 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 al⁷ and Nguyen et al¹⁵. 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 areas¹⁶. 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

The study was part of the “Sub-project Field Veterinarian and Epidemiologist Development” program organized by Bureau of Disease Control and Veterinary Services, Department of Livestock Development, Thailand. We would like to thank Dr. Supaporn Wacharapluesadee and staff from Molecular Biology Laboratory for Neurological Disease, King Chulalongkorn Memorial Hospital, Faculty of Medicine and Chulalongkorn University for providing a set of specific primers for rabies detection.

Suggested Citation

Puyati B, Senayai S, Chanachai K, Panichabhongse P. Epidemiological and genetic characteristics of rabies virus in Ubon Ratchathani Province, Thailand, 2011-2014. OSIR. 2016 Mar;9(1):8-14. <<http://www.osirjournal.net/issue.php?id=94>>.

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