Detection of *Leptospira* in Rats Trapped from Households in Phraroj Village, Muang Sam Sip District, Ubon Ratchathani Province Using Polymerase Chain Reaction Technique

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Background: *Leptospirosis, a zoonotic disease caused by Leptospira, has been a health problem in Thailand for several years. Rats are a major reservoir host for Leptospira, and the people who are usually in contact with environments contaminated with rats' urine are at risk of infection. The prevalence rate of Leptospira infection in rats may result in the spread of leptospirosis in humans.*

Objective: *This study aimed to determine the prevalence rate of Leptospira infection in a total of 28 rats and develop a spatial database for leptospirosis surveillance in Phraroj village in Muang Sam Sip District, Ubon Ratchathani Province.*

Material and Method: *The positions of the households and the rat-trapping area were tagged by using of a Global Positioning System (GPS). DNA samples were isolated from rats' kidneys. The polymerase chain reaction (PCR) technique was used for the detection of 16s rRNA and LipL32 genes specific to genus and pathogenic Leptospira respectively. All of the data were used to develop a geo-data base by the connection of spatial data and attributed data to be used for query and retrieval.*

Results: *A map of the positions of the households and the rat-trapping area in Phraroj village was created. No rats were found to be infected in the Leptospira survey.*

Conclusion: *There was no trapped rat infected with Leptospira in Phraroj village. This result may involve unreported leptospirosis in patients in this village. The Leptospira survey in rats and the geo-database will be used as a primary resource to support and make decisions about surveillance, prevention, and control of leptospirosis.*

Keywords: *Leptospirosis, Leptospira, 16s rRNA, LipL32, Polymerase chain reaction, Geo-database, GPS*

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Leptospirosis is an emerging disease in Thailand; the highest incidence rate from 2006 to 2010 was found in North-East Thailand[1,2]. The finding was also reported in the same area in 2011 and 2013[3,4]. In Ubon Ratchathani Province, lepspirosis is a crucial problem, with 84, 44, and 105 patients with leptospirosis reported from 2011 to 2013[3-5]. During 2009-2011, Nong Sang village in Muang Sam Sip district in Ubon Ratchathani Province was high incidence of leptospirosis, while, Phraroj village which locate in the same district was no reports of leptospirosis[6]. In 2013, Lao Seua Gohk, Sirindhorn, Muang Sam Sip, and Buntrarik districts were reported as having high incidence rates of leptospirosis[7].

Leptospirosis caused by *Leptospira* infection affects humans and several animal species[8,9]. Rodents and domestic animals such as cattle, dogs, and pigs are the common reservoir hosts[10]. The carrier animals retain *Leptospira* in their kidneys and excrete the bacteria into the environment via urine. Direct contact with contaminated areas increases the risk of *Leptospira* infection. The prevalence rates of *Leptospira* infection in rodents from1998 to 2000 were 7.1%, 4.9%, 4.3%, and 3.0% in the North-East, Northern, Central, and Southern regions respectively[11]. *Leptospira* infected rats were not found in any villages that had no reports of patients with leptospirosis, while 8.7% of infected rats presented in endemic villages[12]. The prevalence rate in rats may be involved in the spread of leptospirosis in humans. Therefore, the objective of this study was to determine the prevalence rate of *Leptospira* infection in rats in

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Phraroj village, which had had no reports of patients with leptospirosis. This village is located in Muang Sam Sip district, which is a leptospirosis-risk area in Ubon Ratchathani Province. In addition, a model of the spatial distribution of households and trapped rats was developed. These data can be used for decision-making, surveillance, and the epidemiological control of leptospirosis. Phraroj village was selected to support the previous data from Si Saket Province, which has no *Leptospira*-infected rats in villages with no leptospirosis cases[12]. This preliminary result will be used for further study.

Material and Method

Study areas

Phraroj village located in Muang Sam Sip District, Ubon Ratchathani was selected. The study site contained 139 households. The Huai Phraroj irrigation canal runs through the area all year (Fig. 1).

Data collection

One hundred thirty-nine household positions were tagged by the use of a global positioning system (Garmin GPSMP 60CSx) tool. Geometric correction was manipulated based on a topographical map scale of 1:50,000 of the Royal Thai Survey Department (RTSD), series L7018, with grid coordinate references of UTM zone 48 and spheroid datum of WGS-1984.

Trapping of rats and positioning of trapped area

The trapping of rats was completed from April 2013 to July 2013 in household areas by the use of rat traps left out overnight. The attribute data created a primary data field address and geo-referenced by GPS in each trapped rat in a household. The database of trapping of rats created a buffer zone of 50 meters for the distribution of *Leptospira* infection. The buffer zone distance at 50 meters was considered depending on of living area of rat which was range 100-150 feet[13]. Live rats were anesthetized by the use of diethyl ether. Animal protocols were approved by Ubon Ratchathani University Animal Care and Use Committee (ID#2/2556/ Research).

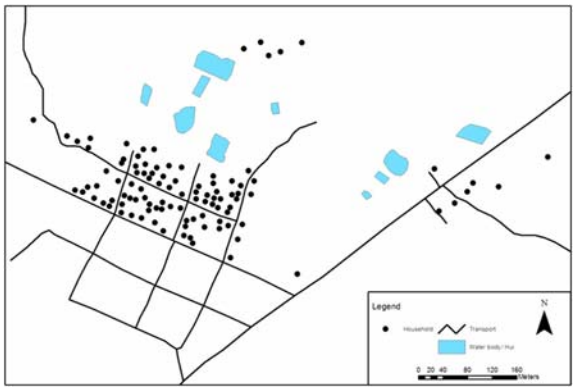


Fig. 1 Location of households in Phraroj village, Muang Sam Sip District, Ubon Ratchathani Province.

DNA extraction from rats' kidneys

Rats' kidneys were collected for DNA extraction using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. Extracted DNA was stored at -20°C following previous protocol[12].

Detection of Leptospira DNA by polymerase chain reaction

Leptospira infection in humans and animals was diagnosed by the use of *16srRNA* and *LipL32* genes[14-18]. *LipL32* gene was used for pathogenic *Leptospira* detection[19,20]. All samples were detected for the *16srRNA* gene specific for genus *Leptospira* using primers reported by Merien et al[14] (*16srRNA*-F: 5'-GGCGGCGCGTCTTAAACATG-3' and *16srRNA*-R: 5'-TTCCCCCCATTGAGCAAGATT-3'). *LipL32* primers specific for pathogenic *Leptospira* (*LipL32*-F: 5'-GGACGGTTTAGTCGATGGAA-3' and *LipL32*-R: 5'-GCATAATCGCCGACATTCTT-3') were designed by Jittimanee and Wongbutdee[12].

The PCR mixing was prepared following a protocol by Jittimanee and Wongbutdee[12]. DNA amplification was performed under conditions as follows: pre-amplification for one cycle at 95°C for 2 min, and PCR amplification for 35 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec. *Leptospirainter rogans* serovar Pomona DNA was used as a positive control. Amplification products were analyzed by 2% agarose gel electrophoresis.

Results

Positions of rats trapped in rat-trapping area

The spatial data showed the positions and buffer zone (50 meters) of trapped rats in households in Phrajoj village (Fig. 2).

Detection of Leptospira in rats' kidneys by polymerase chain reaction

All DNA samples isolated from the 28 rats' kidneys were subjected to PCR to amplify *16s rRNA* (331 bp) and *LipL32* (227 bp). There was no positive

band of the two specific genes from any of the 28 samples; only positive control (DNA from *L. interrogans* serovar Pomona) was found (Fig. 3).

Discussion

Leptospirosis is a health problem in several parts of Thailand. In the Northeast, this disease presented the nation's highest incidence rate from 2006 until 2013[1-5]. In Ubon Ratchathani Province, lepspirosis is a serious health problem and cases increased to 105 in 2013[4], especially in Lao Seua Gohk, Sirindhorn, Muang Sam Sip, and Buntrarik districts[7].

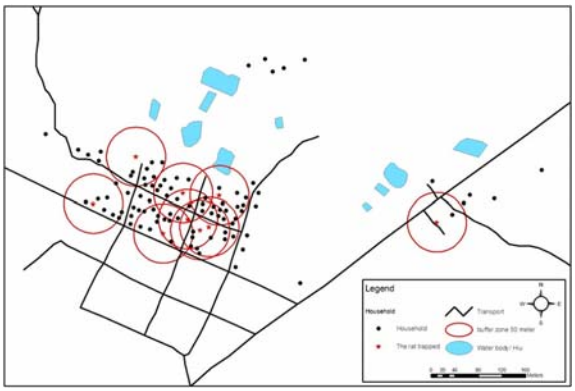
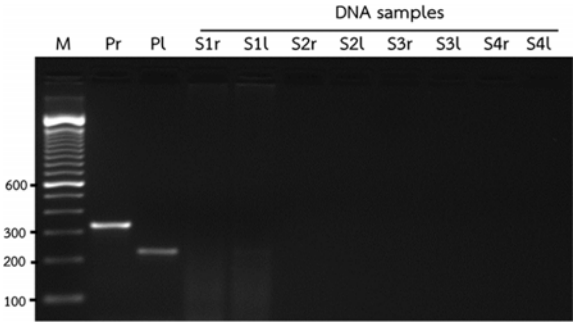


Fig. 2 Positions of buffer zone and trapped rats in Phraroj village, Muang Sam Sip District, Ubon Ratchathani Province.



M = 100 bp DNA ladder; Pr = positive control of *16s rRNA*; Pl = positive control of *LipL32*; S = DNA samples extracted from rats' kidneys

Fig. 3 Agarose gel electrophoresis analysis of *16s rRNA* (331 bp) and *LipL32* (227 bp) genes specific to *Leptospira* in DNA samples isolated from rats' kidneys. There was no band in any of the 28 DNA samples extracted from rats' kidneys. Positive bands of both genes appeared in the positive control (DNA sample from *Leptospira interrogans* serovar Pomona).

Rats are an important source of leptospirosis transmission to humans[10,21]. In this study, no *Leptospira* infected rats were found in Phraroj village. This result supports previous reports in Si Saket Province from June 2011 to January 2012 that found no infected rats in villages, which had no incidence of leptospirosis in human, whereas 8.7% of *Leptospira*-infected rats were found in villages with high incidence rates of patients with leptospirosis[12]. In 2011 and 2012, the incident rates of patients with leptospirosis in Si Ssaket were 29.4 and 24.53 per 100,000 of the population, respectively[3,5]. High incidence rates of leptospirosis cases were also found in Buriram and Nakhon Ratchasima (over 50 per 100,000 of the population) from 1999 to 2001[22]. The prevalence rates of *Leptospira*-infected rats in Buriram, Udonthani and Nakhon Ratchasima Provinces from October 1998 to April 2000 were 12.5%, 6.7%, and 3.9%, respectively[11].

The development of a 50 meter buffer zone shows a clear pattern of spatial distribution, which can be used as a model in leptospirosis prevention. If the *Leptospira* is detected in rats, the buffer zone model will be beneficial as it gives information of the distribution and positions of carrier rats, surrounding households in the buffer zone, the distances between households, and the surface water sources that may be necessary for the survival of rats.

In conclusion, there was no trapped rat infected with *Leptospira* in Phraroj village. This result may involve in leptospirosis in patient, which was not reported in this village, although, Phraroj village is geography similar to Nong Sang village, which is a leptospirosis, risk area. However, the small number of rat may limit our results; the further study with high population will be done. The *Leptospira* survey and the geo-database will be used as a primary resource to support and make decisions about surveillance, prevention, and control of leptospirosis.

What is already known on this topic?

Leptospirosis caused by *Leptospira* infection affects humans and several animal species[8,9]. Rodents and domestic animals such as cattle, dogs, and pigs are the common reservoir hosts of this *Leptospira*[10]. In Thailand, the prevalence rates of *Leptospira* infection in rodents from1998 to 2000 were 12.5%, 6.7%, and 3.9% in Buriram, Udonthani, and Nakhon Ratchasima Provinces, respectively[11]. Recently the report in Si Saket Province, 8.7% of *Leptospira* infected rats was found only in villages that had report of leptospirosis

patients, but not in villages that had noreport of leptospirosis patients[12].

What this study adds?

In our study, there was no *Leptospira* infected rats found in Phraroj village in Ubon Ratchathani Province, this result support previous study in Sisaket Province that was no *Leptospira* infected rats found in villages, which were no leptospirosis patients[12]. The prevalence rate of *Leptospira* infected rats was assumed to be involved in the incidence rate of leptospirosis patients. The prevalence rate of *Leptospira* infected rats may be benefit for making decision to surveillance and the epidemiological control of leptospirosis in study area.

Acknowledgement

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Potential conflicts of interest

None.

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การตรวจหาเชื้อเลปโตสไปร่าในหนูที่ดักจับจากบ้านเรือนในหมู่บ้านพระโรจน์ อำเภอม่วงสามสิบ จังหวัดอุบลราชธานี โดยอาศัยเทคนิคโพลิเมอเรสเชนรีเอกชัน

จารุวรรณ์ วงบุตดี, จุฑารัตน์ จิตติมณี

ภูมิหลัง: *โรคเลปโตสไปโรสิส (leptospirosis) จัดเป็นโรคติดต่อจากสัตว์สู่มนุษย์ซึ่งมีสาเหตุมาจากการติดเชื้อเลปโตสไปร่า (Leptospira) โรคนี้เป็นปัญหาสุขภาพของประเทศไทยมายาวนานโดยมีหนูเป็นแหล่งรังโรคหลักของเชื้อเลปโตสไปร่า ซึ่งผู้ที่สัมผัสกับสิ่งแวดล้อมที่มีการปนเปื้อนจากปัสสาวะของหนูอยู่เป็นประจำจะมีความเสี่ยงต่อการติดเชื้อ อัตราความชุกของการติดเชื้อเลปโตสไปร่าในหนูอาจจะส่งผลต่อการแพร่กระจายของโรคเลปโตสไปโรสิสในมนุษย์ได้*

วัตถุประสงค์: *สำรวจหาอัตราความชุกของเชื้อเลปโตสไปร่าในหนูทั้งหมดจำนวน 28 ตัว และทำการพัฒนาข้อมูลเชิงพื้นที่เพื่อเฝ้าระวังโรคเลปโตสไปโรสิสในหมู่บ้านพระโรจน์ อำเภอม่วงสามสิบ จังหวัดอุบลราชธานี*

วัสดุและวิธีการ: *ทำการกำหนดตำแหน่งของบ้านเรือนและบริเวณพื้นที่ดักจับหนูโดยใช้ระบบกำหนดตำแหน่งบนโลก (Global Positioning System; GPS) สกัดแยก DNA ตัวอย่างออกมาจากไตของหนู จากนั้นใช้เทคนิคโพลิเมอเรสเชนรี เอกชัน (PCR) สำหรับตรวจหาจีน 16srRNA และ LipL32 ซึ่งเป็นจีนที่จำเพาะสำหรับเชื้อเลปโตสไปร่า จากนั้นนำข้อมูลทั้งหมดมาพัฒนาเป็นฐานข้อมูลเชิงพื้นที่ (geo-database) โดยเชื่อมโยงข้อมูลเชิงพื้นที่กับข้อมูลอรรถาธิบายเพื่อใช้ต่อไปในอนาคต*

ผลการศึกษา: *มีการสร้างแผนที่กำหนดตำแหน่งของบ้านเรือนและบริเวณที่ดักจับหนูในหมู่บ้านพระโรจน์ และตรวจไม่พบหนูติดเชื้อในการสำรวจเชื้อเลปโตสไปร่า*

สรุป: *ไม่พบหนูที่ดักจับได้ในหมู่บ้านพระโรจน์มีการติดเชื้อเลปโตสไปร่า ผลการทดลองนี้อาจมีความเกี่ยวข้องกับการไม่พบผู้ป่วยโรคเลปโตสไปโรสิสในหมู่บ้านพระโรจน์ ผลการสำรวจเชื้อเลปโตสไปร่าในหนูและฐานข้อมูลเชิงพื้นที่จะถูกใช้เป็นข้อมูลเบื้องต้นที่ช่วยสนันสนุน และตัดสินใจในการเฝ้าระวังป้องกัน และควบคุม โรคเลปโตสไปโรสิส*