การตรวจเชื้อเลปโตสไปร่าด้วยวิธีทางซีรั่มวิทยาและอณูชีวโมเลกุล
จากสัตว์เลี้ยงในจังหวัดน่าน ประเทศไทย

นายอลงกรณ์ ขุริลัง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาพยาธิชีววิทยาทางสัตวแพทย์ ภาควิชาพยาธิวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

LEPTOSPIRA SPP. DETECTION BY SEROLOGICAL AND MOLECULAR ASSAYS IN DOMESTIC ANIMALS IN NAN PROVINCE, THAILAND

Mr. Alongkorn Kurilung

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Pathobiology
Department of Veterinary Pathology
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2015

Thesis Title	*LEPTOSPIRA* SPP. DETECTION BY SEROLOGICAL AND MOLECULAR ASSAYS IN DOMESTIC ANIMALS IN NAN PROVINCE, THAILAND
By	Mr. Alongkorn Kurilung
Field of Study	Veterinary Pathobiology
Thesis Advisor	Associate Professor Nuvee Prapasarakul, Ph.D.
Thesis Co-Advisor	Instructor Pattrarat Chanchaithong, Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Veterinary Science
(Professor Roongroje Thanawongnuwech, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Wijit Banlunara, Ph.D.)

.....Thesis Advisor
(Associate Professor Nuvee Prapasarakul, Ph.D.)

.....Thesis Co-Advisor
(Instructor Pattrarat Chanchaithong, Ph.D.)

.....Examiner
(Janjira Thaipadungpanit, Ph.D.)

.....External Examiner
(Assistant Professor Piengchan Sonthavanon, Ph.D.)

อลงกรณ์ ขุริลัง : การตรวจเชื้อเลปโตสไปร่าด้วยวิธีทางซีรั่มวิทยาและอณูชีวโมเลกุลจากสัตว์เลี้ยงในจังหวัดน่าน ประเทศไทย (*LEPTOSPIRA* SPP. DETECTION BY SEROLOGICAL AND MOLECULAR ASSAYS IN DOMESTIC ANIMALS IN NAN PROVINCE, THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร.ณุวีร์ ประภัสระกูล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. น.สพ. ดร.ภัทรรัฐ จันทร์ฉายทอง{, หน้า.

จังหวัดน่าน เป็นพื้นที่หนึ่งที่มีการศึกษาโรคเลปโตสไปโรซิสในประเทศไทยและพบความสัมพันธ์ของเชื้อเลปโตสไปร่าที่ก่อโรคระหว่างคนและหนู แต่อย่างไรก็ตามยังขาดข้อมูลความชุกของโรคเลปโตสไปโรซิสและการเป็นพาหะของโรคในสัตว์เลี้ยง เช่น โค สุกร และ สุนัข การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาเชื้อเลปโตสไปร่า ด้วยวิธีทางซีรั่มวิทยา Microscopic Agglutination Test (MAT) อณูชีววิทยา *rrs* nested polymerase chain reaction (PCR) และการเพาะแยกเชื้อเลปโตสไปร่าที่ก่อโรคในสัตว์เลี้ยงจังหวัดน่าน ระหว่างปี 2556 ถึง 2558 จากตัวอย่างเลือดโค (n=160) และ สุนัข (n=50) จำนวน 210 ตัวอย่าง และ ตัวอย่างปัสสาวะโค (n=131) สุกร (n=152) และ สุนัข (n=58) จำนวน 341 ตัวอย่าง จากจำนวน 20 หมู่บ้านของ 3 อำเภอ ได้แก่ อำเภอเมืองน่าน อำเภอเชียงกลาง และอำเภอท่าวังผา

ด้วยวิธีทางซีรั่มวิทยา (titer > 1:80) พบความชุกของโรคเลปโตสไปโรซิส 8.09% (17/210) โดยพบความชุกในโค 10.62% (17/160) และไม่พบความชุกในสุนัข ซีโรกรุ๊ปที่พบมากในพื้นที่คือ Shermani, Sejroe และTarassovi ตามลำดับ

ด้วยวิธีทางอณูชีววิทยา *rrs* nested PCR พบความชุกของโรค 9.97% (34/341) พบเชื้อเลปโตสไปร่าที่ก่อโรคในโค 12.21% (16/131), สุกร 7.89% (12/152) และ สุนัข 10.34% (6/58) จากการวิเคราะห์แผนภูมิต้นไม้ไฟโลเจเนติกระดับโมเลกุลของตัวอย่างที่ให้ผลบวกต่อ *rrs* nested PCR จำนวน 34 ตัวอย่าง พบว่าเชื้อเลปโตสไปร่าที่ตรวจพบอยู่ในกลุ่ม *L. interrogans* (n=9), *L. weilii* (n=22) และ unidentified *Leptospira* spp. (n=3) พบเชื้อ *L. interrogans* ในโค (n=2), สุกร (n=3) และสุนัข (n=4) พบเชื้อ *L. weilii* ในโค (n=11), สุกร (n=9) และ สุนัข (n=2) และพบ unidentified *Leptospira* spp. ในโค (n=3). เชื้อ *L. weilii* เป็นเชื้อเลปโตสไปร่าก่อโรคที่พบมากในการศึกษาครั้งนี้ และ เป็นเชื้อที่มีการรายงานครั้งแรกในสัตว์เลี้ยง ประเทศไทย นอกจากนั้นสามารถแยกเชื้อเลปโตสไปร่าบริสุทธิ์ได้จากสุนัข (n=4) เป็นเชื้อ *L. interrogans* (n=2) และ *L. weilii* (n=2) โดยพื้นที่ที่พบความชุกของโรคเลปโตสไปโรซิสในสัตว์เลี้ยงมากที่สุด คือ อำเภอท่าวังผา

การศึกษาครั้งนี้ให้ข้อมูลที่สำคัญเชิงระบาดวิทยาของโรคเลปโตสไปโรซิสในสัตว์ในพื้นที่ที่มีการระบาด ซึ่งนำไปสู่กระบวนการควบคุมและป้องกันโรคในพื้นที่ต่อไป

ภาควิชา	พยาธิวิทยา	ลายมือชื่อนิสิต .....
สาขาวิชา	พยาธิชีววิทยาทางสัตวแพทย์	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
ปีการศึกษา	2558	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5775314531 : MAJOR VETERINARY PATHOBIOLOGY
KEYWORDS: LEPTOSPIRA SPP., SEROLOGICAL ASSAY, MOLECULAR ASSAY, DOMESTIC ANIMALS, NAN PROVINCE

ALONGKORN KURILUNG: *LEPTOSPIRA* SPP. DETECTION BY SEROLOGICAL AND MOLECULAR ASSAYS IN DOMESTIC ANIMALS IN NAN PROVINCE, THAILAND. ADVISOR: ASSOC. PROF. NUVEE PRAPASARAKUL, Ph.D., CO-ADVISOR: INSTRUCTOR DR.PATTRARAT CHANCHAITHONG, Ph.D.{, pp.

Nan province is an index area of leptospirosis study model in Thailand where provided the evidence linkage of infecting pathogenic *Leptospira* spp. between human and rodents but lack of prevalence and animal carriage data of leptospirosis in domestic animals such as cattle, pigs and dogs. This study aimed to identify the pathogenic *Leptospira* spp. by serological assay, Microscopic Agglutination Test (MAT), molecular assay, *rrs* nested polymerase chain reaction (PCR), and culture from domestic animals in Nan province during 2013 to 2015. A total of 210 blood samples were collected from cattle (n=160) and dogs (n=50) and 341 urine samples were collected from cattle (n=131), pigs (n=152) and dogs (n=58) in 20 villages from three districts; Muang Nan, Chiang Klang and Tha Wang Pha.

Overall, the seroprevalence (titer > 1:80) to leptospiral detection by MAT was 8.09% (17/210). The seropositive MAT was found in cattle with a prevalence 10.62% (17/160) but all were negative in dogs. The major leptospiral serogroup was Shermani and the minor serogroup were Sejroe and Tarassovi, respectively.

By *rrs* nested PCR 9.97% (34/341) of urine samples were positive to pathogenic *Leptospira* spp. from cattle 12.21% (16/131), pigs 7.89% (12/152) and dogs 10.34%(6/58). Phylogenetic analysis that confirmed the 34 positive samples were clustered in a branch of *L. interrogans* (n=9), *L. weilii* (n=22) and unidentified *Leptospira* spp. (n=3). *L. interrogans* were detected in cattle (n=2), pigs (n=3) and dogs (n=4), *L. weilii* were found in cattle (n=11), pigs (n=9) and dogs (n=2) and unidentified *Leptospira* spp. were found in cattle (n=3). *L. weilii* were the most common pathogenic leptospiral species in this study and being the first report of *L. weilii* in animal in Thailand. Moreover, we could successfully isolate four leptospires from dogs; *L. interrogans* (n=2) and *L. weilii* (n=2). Areas with the most prevalence of animal leptospirosis were Tha Wang Pha district.

In conclusions, our study provides an important epidemiological information of animal leptospirosis in an endemic area that may be continually useful for disease control and prevention strategies.

Department:	Veterinary Pathology	Student's Signature	.....
Field of Study:	Veterinary Pathobiology	Advisor's Signature	.....
Academic Year:	2015	Co-Advisor's Signature	.....

ACKNOWLEDGEMENTS

First of all, I would like to thank Chulalongkorn University Graduate Scholarship to commemorate the 72th Anniversary of His Majesty King Bhumibol Adulyadej and National Science and Technology Development Agency (NSTDA) to support this work.

This thesis was successful by the guidance and comment by my advisor Associate Professor Dr. Nuvee Prapasarakul. I would like to express my gratitude for his kindness and opportunity for study. I would like to thank Instructor Dr. Pattrarat Chanchaithong my thesis co-advisor for giving me a great consultation. I would like to acknowledge all staffs and graduate students at Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University who support me in every occasions.

I am grateful to thank Instructor Vanaporn Wuthiekanun and all staffs at Mahidol Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University for a valuable knowledge of leptospiral isolation. I am also thank Dr. Patharapol Piamsomboon, Faculty of Veterinary Science, Prince of Songkla University for help me in map designation and Dr. Tippawan Deeinta, Center Learning Network for the Region, Nan province for conveniently support during sample collections in Nan province.

This initiates success is dedicated to my parent and my friends who always give encouragement and great attitude to me throughout my study.

CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

bp	=	Base pair
°C	=	Degree Celsius
EMJH media	=	Elinghauson McCullough Johnson and Harris media
g	=	Gram
LVW agar	=	Leptospira Vanaporn Wuthiekanun agar
LPS	=	Lipopolysaccharide
µm	=	Micrometer
ml	=	Milliliter
MAT	=	Microscopic agglutination test
n	=	Number
PCR	=	Polymerase chain reaction

CHAPTER I

INTRODUCTION

1. Importance and Rationale

Leptospirosis is a worldwide important zoonotic disease (Adler and de la Pena Moctezuma, 2010; Evangelista and Coburn, 2010b; Boonsilp et al., 2011), including Thailand, caused by spirochete bacteria in genus *Leptospira* that divides into more than 250 serovars (Levett, 2001; Adler and de la Pena Moctezuma, 2010). Naturally, *Leptospira* spp. colonizes and persists in proximal tubule of the mammalian's kidney and the important reservoir host is rodent, but other animals such as cattle, buffalo, pig and dog can also maintain *Leptospira* spp. and potentially being a source of human leptospirosis (Palaniappan et al., 2007; Evangelista and Coburn, 2010b; Sykes et al., 2011). Human and animals can be infected by direct contact via urine of reservoir hosts and by indirect expose via water and soil in the endemic area (Levett, 2001; Palaniappan et al., 2007).

The leptospirosis distribution is globally found, but geographical difference may be influential beneath a variety of serovars that becomes a diagnostic marker related to post-infection immunity response (Levett, 2001; Adler and de la Pena Moctezuma, 2010; Evangelista and Coburn, 2010b).

In Thailand, there are many reports of leptospirosis in human and animals including cattle, pig, dog and rodent (Heisey et al., 1988; Kositanont et al., 2003;

Doungchawee et al., 2005; Meeyam et al., 2006; Silva et al., 2009; Suepaul et al., 2011; Suwancharoen et al., 2013). However, the situation of animal leptospirosis derived from the microscopic agglutination test (MAT) results is limited to represent an individual infection and cannot identify a presence of animal reservoirs (Sykes et al., 2011). Thus, screening by serological test (MAT) and further urine analysis by polymerase chain reaction (PCR) is suitable for detection of renal carriers of leptospires in animals (Otaka et al., 2012; Director et al., 2014).

The Bureau of Epidemiology reported the most case of human leptospirosis in 2014 in the Northeastern (5.15 cases/100,000 inhabitants), Southern (4.93 cases/100,000 inhabitants) and Northern parts of Thailand (1.96 cases/100,000 inhabitants), respectively , including Nan province. Nan province locate as a separated area surrounding with high mountains where the north boundary connected to Laos and processing the source of river from China and Laos origin downstream to the capital city, Bangkok. Leptospirosis is an endemic disease in Nan province documented in annual reports by the Bureau of Epidemiology in 2014 at average 7.07 cases/100,000 inhabitants, and the most cases occur during rainy season (June to October). According to the human leptospirosis in Nan province, this leads us to the conception that animals including livestock and companion may associate with the persistence of *Leptospira* spp. in this area. Even though, animals maintain and transfer pathogenic *Leptospira* spp. to human, but the situation of animal

reservoir has not much been investigated once leptospirosis patient (index case) is often report.

The aim of this study is to investigate prevalence of leptospirosis in domestic animals from Nan province, Thailand during 2013 to 2015.

2 Objectives

- To survey seroprevalence of leptospirosis in domestic animals by microscopic agglutination test (MAT) in Nan province, Thailand
- To detect and identify pathogenic *Leptospira* spp. from animal urine by nested PCR and/or culture.

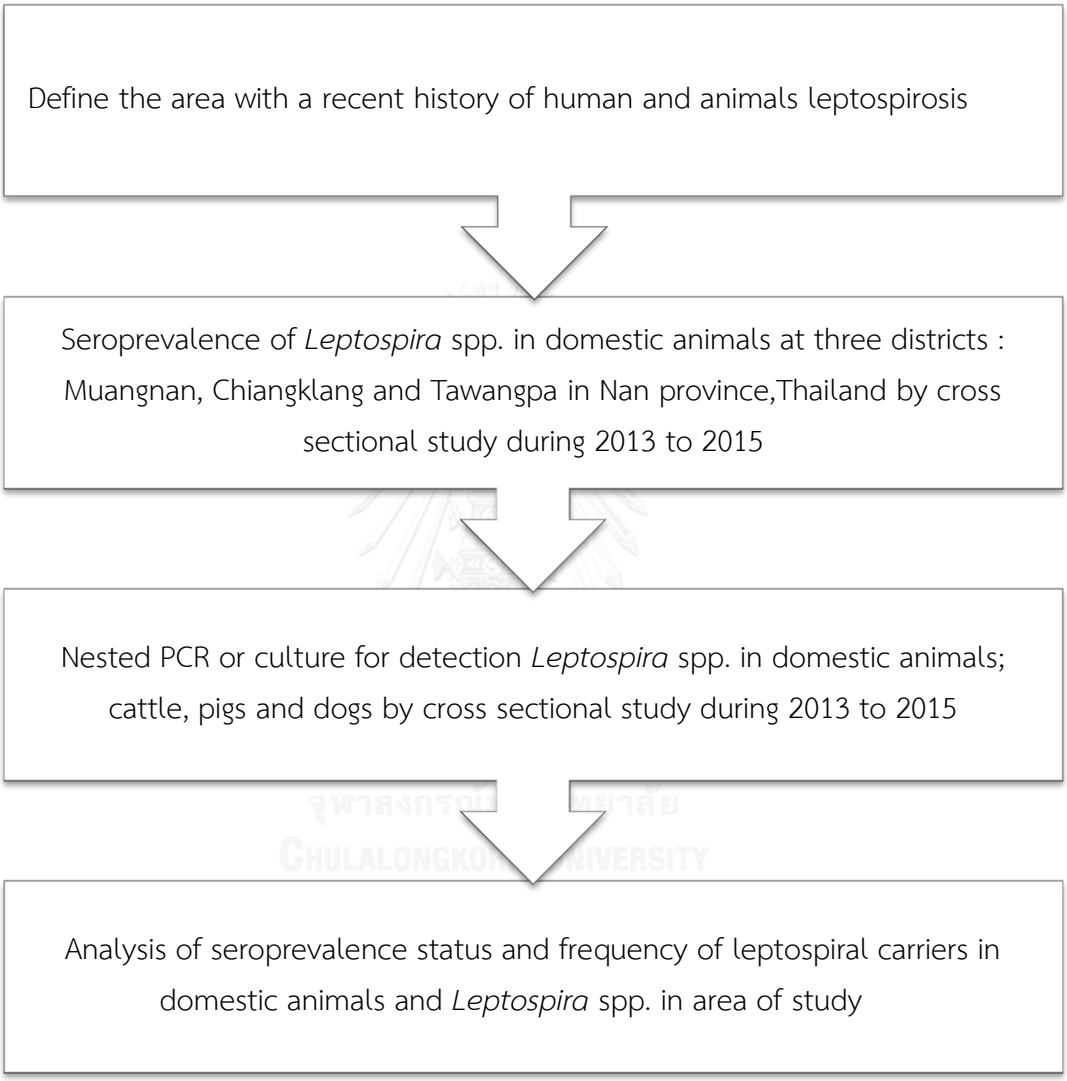
3 Hypothesis

- In Nan province, domestic animals present a high seropositive number and titer to *Leptospira* spp. by microscopic agglutination test (MAT)
- Domestic animals in Nan province can be a carrier of pathogenic *Leptospira* spp. That can be detected by a nested PCR and/or culture

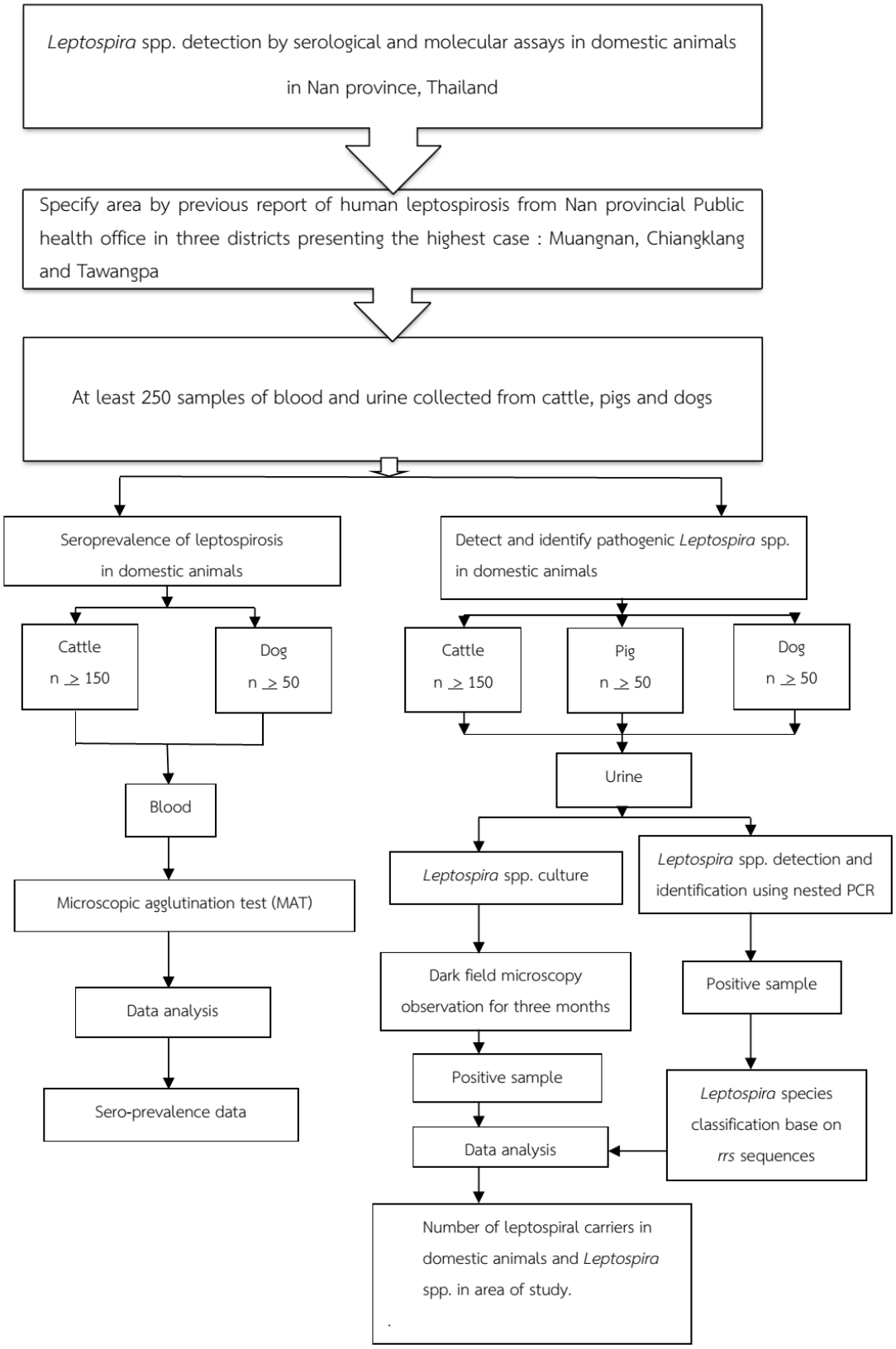
4. Conceptual framework

Leptospira spp. detection by serological and molecular assays

in domestic animals in Nan province, Thailand



5 Research plan



CHAPTER II

LITERATUER REVIEW

1. Taxonomy and classification

Leptospira is spirochete bacteria belonging to genus *Leptospira*, order *Spirochaetales* (Faine S, 1999) comprising both of saprophytic and pathogenic species (Adler and de la Pena Moctezuma, 2010). Currently, pathogenic *Leptospira* consists of 13 species: *L. alexanderi, L. alstonii , L.borgpetersenii, L. inadai, L. interrogans, L. fainei, L. kirschneri, L. licerasiae, L. noguchi, L. santarosai, L. terpstrae, L. weilii* and *L. wolffii*. On the other hand, the saprophytic *Leptospira* contains *L. biflexa, L. meyeri, L .yanagawae, L. kmetyi, L. vanthielii* and *L. wolbachii* (Levett, 2015). Regarding to genetic relation, phylogenetic tree analysis of *16S rRNA* gene is categorized among *Leptospira* spp. into 3 groups, pathogenic, intermediate pathogenic and saprophytic *Leptospira* (Yersin et al., 1998; Levett, 2001; Levett, 2015)(Figure 1). Moreover, *Leptospira* spp. can be divided into 24 serogroups and more than 250 serovars (Palaniappan et al., 2007) by the difference of carbohydrate structure of lipopolysaccharide (LPS) in outer membrane (Levett, 2001; Silva et al., 2009; Adler and de la Pena Moctezuma, 2010).

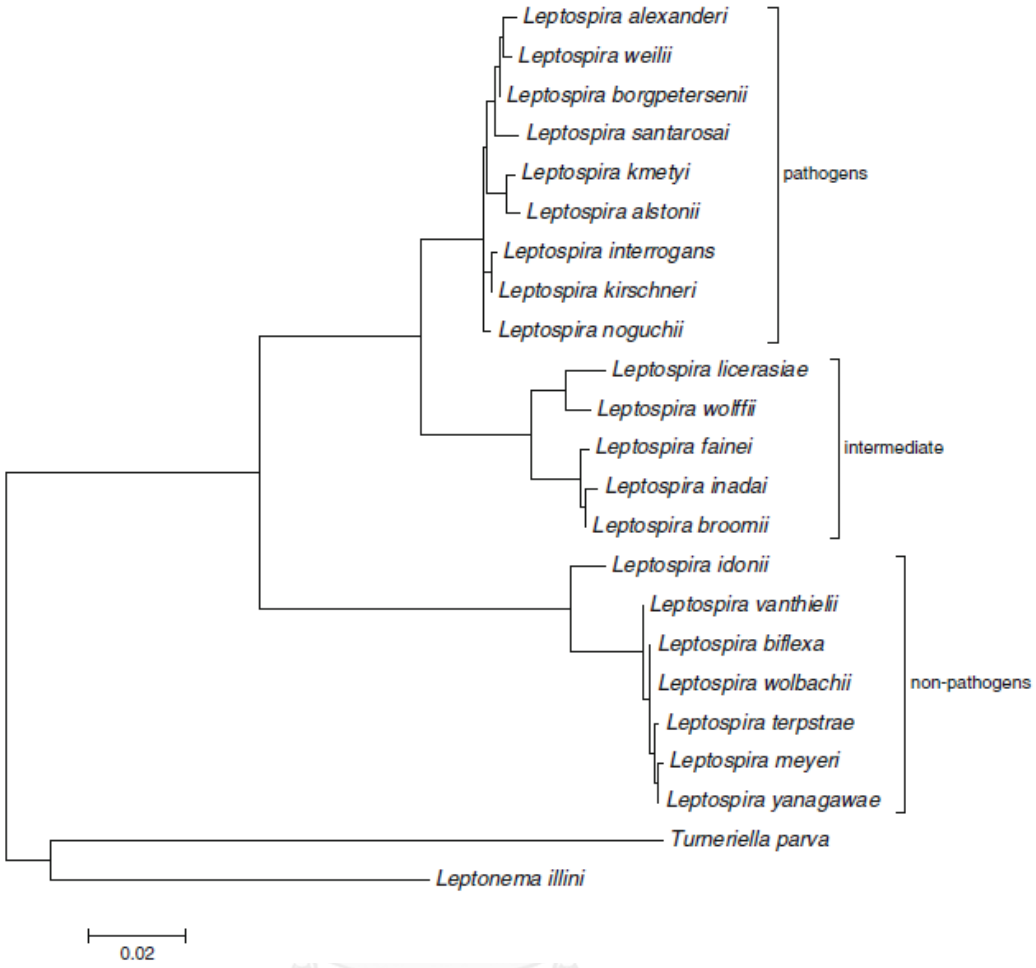


Figure 1. Molecular phylogenetic of *Leptospiraceae 16S rRNA* gene sequence by maximum likelihood method (Levett, 2015).

Table 1. Serogroups and serovars of *L. interrogans* (Levett, 2001)

L. interrogans	
Serogroup	Serovar(s)
Icterohaemorrhagiae	Icterohaemorrhagiae, Copenhageni, Lai
Hebdomadis	Hebdomadis, Jules
Authumnalis	Authumnalis, Bim
Pyrogenes	Pyrogenes
Bataviae	Bataviae
Grippotyphosa	Grippotyphosa, Canalzonae
Canicola	Canicola
Australis	Australis, Bratislava
Pomona	Pomona
Javanica	Javanica
Sejroe	Sejroe, Hardjo
Panama	Panama, Mangus
Cynopteri	Cynopteri
Djasiman	Djasiman
Sarmin	Sarmin
Mini	Mini
Tarassovi	Tarassovi
Ballum	Ballum
Celledoni	Celledoni
Louisiana	Louisiana
Ranarum	Ranarum
Manhao	Manhao
Shermani	Shermani
Hurstbridge	Hurstbridge

2. Biology of leptospires

Leptospira spp. is a gram-negative obligate aerobic bacteria, spiral shape with hooked end, 6-20 μm in length and 0.1 μm in diameter. Periplasmic flagella tapered among protoplasmic cylinder and covering with sheath of outer membrane make a corkscrew-liked motility of *Leptospira* spp. (Adler and de la Pena Moctezuma, 2010). Especially, outer membrane mainly contains lipopolysacharide (LPS), which is the important antigen of *Leptospira* spp. and direct response to the specific humoral immunity (Faine S, 1999).

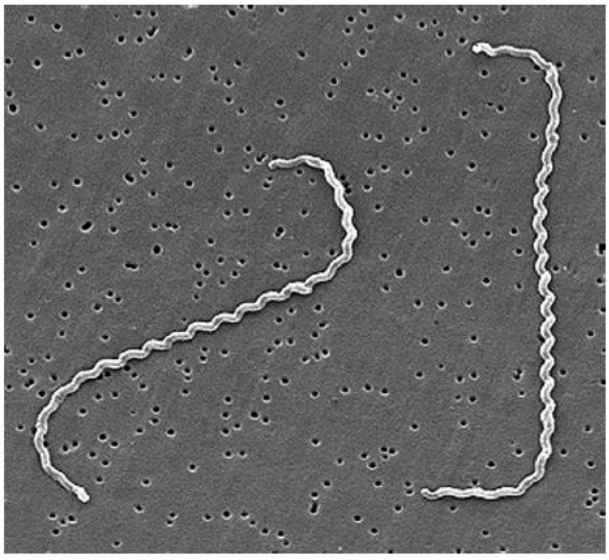


Figure 2. Electron microscope of leptospires revealed a spiral shape with hooked end microorganism (Levett, 2001)

The growing rate of *Leptospira* spp. is quite slower than other bacteria such as *Brachyspira*, the intestinal spirochete or *Enterobacteraceae* family. *Leptospira* spp. grow in optimal temperature ranging from 28-30 ℃., and also need the particular long chain fatty acid, vitamins B1, B12, ammonium salt and pH 6.8-7.6 for growing in their specific media. In primary isolation, the growth may prolong to 13 weeks (Levett, 2001). Currently, Ellinghauson McCullough Johnson and Harris (EMJH) media is the most widely used for culture *Leptospira* spp. and was modified into many formula by adding antimicrobials; 5-fluorouracil, nalidixic acid or rifampicin (Faine S, 1999) for inhibition of contaminated bacteria in clinical samples. In 2012, Wuthiekanun et al. developed solid media called Leptospira Vanaporn Wuthiekanun (LVW) Agar for culture and isolation of leptospires. LVW agar can promote the growth of single colony of leptospires within 7 days under initial incubation in 30 ℃ with 5% CO_2 for 2 days following 30 ℃ in air incubation (Wuthiekanun et al., 2013). Moreover, LVW agar can maintain leptospires for 12 months without subculture (Wuthiekanun et al., 2014).

3. Clinical manifestations

Leptospirosis is a global zoonotic disease, including Thailand. *Leptospira* spp. infects human and animals by direct contact via carrier's urine or indirect expose water or soil contamination (Silva et al., 2009). *Leptospira* spp. colonizes and persists in proximal tubules of mammalian's kidney and then repelled simultaneously from animal urine. The reservoirs including cow, buffalo, goat, pig, dog and rat (Doungchawee et al., 2005; Niwetpathomwat et al., 2005; Evangelista and Coburn, 2010), can possess *Leptospira* spp. without clinical sign and intermittently shed leptospires for several months to years or lifelong in leptospiral carriers. While the human is an incidental host that can be infected and developed jaundice, kidney failure, severe hemorrhagic pneumonia and fatalty (Silva et al., 2009; Adler and de la Pena Moctezuma, 2010).

For animal leptospirosis, presence of clinical signs depend on stage of infection, dose of infection and host tolerance (Faine S, 1999). In general, clinical sign in acute infection usually notices from high fever, septicemia, abortion, pneumonia, anemia, jaundice and renal failure (Levett, 2001). However, the clinical sign in chronic state cannot specifically observed (Lilenbaum and Martins, 2014). Leptospiral infection and transmission is shown in Figure 3.

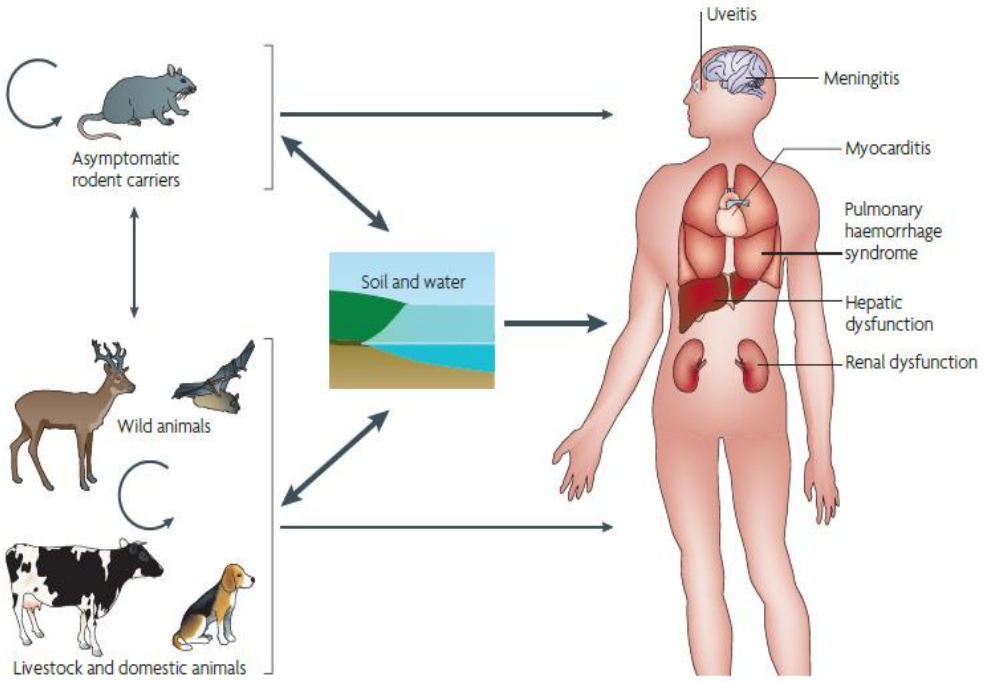


Figure 3. Schematic depiction of the cycle of leptospiral infection (Ko et al., 2009).

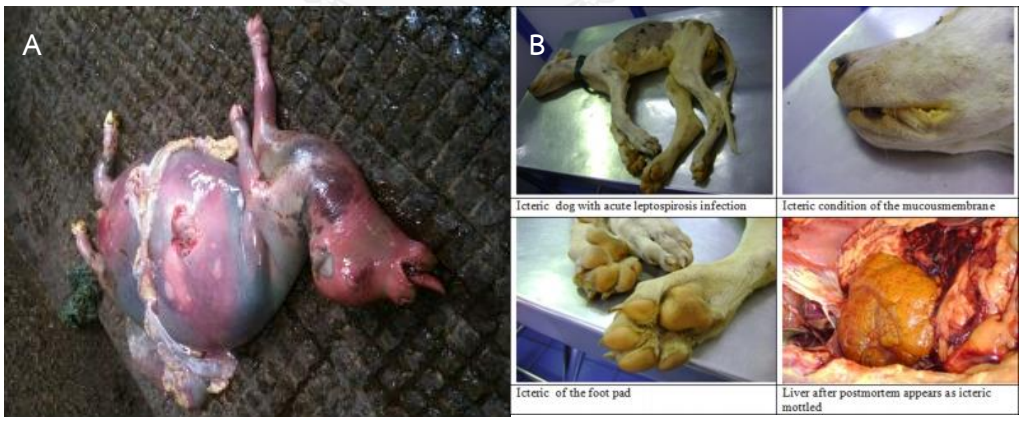


Figure 4. Clinical sign of acute animal leptospirosis between livestock and dogs are different. Livestock become reproductive failure and abortion (A). Dog manifest jaundice, hepatic and renal failure (B) (http://www.farmacy.co.uk/mobile/products/78-bovilis-ibr-marker-live-50-dose-with-applicators; Khan et al., 2009)

4. Epidemiology in Thailand

In previous reports, the incidence of leptospirosis was commonly found in tropical regions such as South East Asia, Pacific Island, Indian subcontinent and Latin America. The factors related to leptospiral incidence include climate, flooding, natural disaster and poor hygienic management especially rodent control program (Pappas et al., 2003). In geographical study of human leptospirosis in Thailand, Northeastern part were the most incidence of leptospirosis and relevant with report of human leptospirosis in 2014 by The Bureau of Epidemiology that found 5.15, 4.93 and 1.96 cases of human leptospirosis per 100,000 inhabitants in the Northeastern, Southern and Northern parts of Thailand, respectively.

The distribution of *Leptospira* spp. based on seroprevalence and PCR detection are varied in each area of investigations. To determine the valid number of leptospirosis, serological and molecular diagnosis should be simultaneously compared and recommended to each suitable indication of detection; herd health status or individual identification (Kositanont et al., 2003; Victoriano et al., 2009; Evangelista and Coburn, 2010a; Suepaul et al., 2010; Sykes et al., 2011).

To survey animal leptospirosis in Thailand, there have been previous reports by serological detection from livestock (Heisey et al., 1988; Suwancharoen et al., 2013), rat (Kositanont et al., 2003; Doungchawee et al., 2005) and dog (Meeyam et al., 2006). In 2001, National Institute of Animal Health, Thailand, reported that

seroprevalence of *Leptospira* spp. in buffalo, cow, goat/sheep and pig were 30.5%, 9.9%, 12.6% and 10.8%, respectively (Suwancharoen et al., 2013). The common leptospiral serovars in livestock in Thailand were Ranarum, Sejroe and Bratislava (Suwancharoen et al., 2013).

5. Diagnostic tests

Serological diagnosis

Microscopic agglutination test (MAT) is a reference method to diagnose leptospirosis by observed agglutination reactivity of live leptospires with patient's antibodies under dark filed microscope and end point titer of this test is 50% agglutination in the highest serum dilution (Levett, 2003). MAT requires live leptospires that play as a representative in which serogroup and, in epidemiological study, recruitment of local strain in panel must be considered. However, the panel of serovar antigens using in MAT cannot predict the actual serovar identification in individual animals since there is cross-reactivity among *Leptospira* spp. serogroup/serovar during the first week of infection. Thus, paired serum titer was confirmed the infection by increase a four-fold titers within two weeks interval (Faine S, 1999; Levett, 2001; Levett, 2003). Although MAT is complexity to test, complicate to interpret the results, MAT is still an approved serological diagnosis standard detection of OIE to determine a probable number of leptospirosis in herd scale (Lilenbaum and Martins, 2014; OIE, 2014). Thus, MAT can be used for screening of

leptospirosis, but it could not routinely identify serogroups/serovars typing because of their cross-reaction in LPS **(**Adler and de la Pena Moctezuma, 2010; Lilenbaum and Martins, 2014). Because of complication in MAT, the alternative immunological assays can be used to detect antibodies, especially IgM during acute phase. IgM detection has more sensitive than MAT and has been commercially developed as a quantitative method by Enzyme-linked immunosorbent assays (ELISAs). ELISAs have several platforms such as plate ELISAs, dipstick ELISAs and dot-ELISAs. ELISAs can detect anti-leptospiral antidodies by using leptospiral antigen. Antigenic selection for ELISAs depend on the purpose of study. For instance, outer membrane protein (OMPs) can be applied because OMPs can specifically react with all pathogenic *Leptospira* spp.. While in epidemiological study in veterinary field, lipopolysaccharide (LPS) is suitable for detection because they provide a specific serogroup/serovar information (OIE, 2014). Other antibodies detection assays such as indirect hemagglutination assay (IHA), latex agglutination, lateral flow assay can be the optional detections (Adler and de la Pena Moctezuma, 2010).

Culture and isolation of leptospires

Culture and isolation of leptospires is definitive or gold standard for diagnose of leptospirosis (Wuthiekanun et al., 2007). This method is such time consuming and strictly require fresh specimen. Blood culture can be successful during the first week of illness. Blood sample is dropped into culture media for human sample but not

animals that usually have asymptomatic infection acted as a maintenance hosts or reservoir during healthy stage and develop leptospirosis during immunocompromised stage (Wuthiekanun et al., 2007; Lilenbaum and Martins, 2014) thereby urine sample is suitable for culture and isolation of leptospires. However, intermittent shedding of leptospires and also low number of leptospires in animal urine with high contamination with other bacteria becomes the great limitation for detection.

Molecular diagnosis

Currently, molecular assays are increasingly applied to detect nucleic acid of leptopires especially polymerase chain reaction (PCR).

PCR is a routine diagnostic technique specific for conserved gene regions, which is faster than serology and cultivation (Wangroongsarb P, 2014). Not only rapid, the advantage of PCR is also concise to diagnose from patient sera during leptospiremia at acute phase when serological result are negative making a treatment plan with antimicrobial is effectiveness (Levett, 2001).

PCR technique has been satisfied on susceptibility and specificity and can be used for nucleic screening in clinical samples including blood samples either during the onset of disease or after antimicrobial administration (Boonsilp et al., 2011). Moreover, PCR can be used to detect carrier stage of animal in urine samples (Merien et al., 1992).

To date, the particular genes used for detection of *Leptospira* spp. are divided in to two categories i.) common genes presenting in all *Leptospira* spp. such as *gyrB*, *rrs* and *secY* (Adler and de la Pena Moctezuma, 2010). ii.) genes that restrict to pathogenic *Leptospira* spp. such as *lipL21*, *lipL32*, *lipL41*, *ligA* and *ligB* (Thaipadungpanit et al., 2011).

Alternative detection methods are real-time quantitative polymerase chain reaction (qPCR), immunofluorescence, antigen ELISA immunoprecipitation, Matrix-assisted laser desorption/ionization-Time of flight (MALDI-TOF) and immuno-labelling by gold nano-particle (Kositanont et al., 2003; Adler and de la Pena Moctezuma, 2010) has been developed to detect leptospires.

6. Phylogeny

Phylogenetic analysis by *rrs* gene sequencing can be clustered the three major groups of *Leptospira* spp. including non-pathogenic, intermediate and pathogenic *Leptospira* spp. (Boonsilp et al., 2011; Levett, 2015). The other genes such as *rpoB* (Balamurugan et al., 2013), *gyrB* (Slack et al., 2006) and *ligB* (Cerqueira et al., 2009) can also be used for species differentiation and generated results similar to that obtained by *rrs* sequences in term of comparing the strain into three group (Balamurugan et al., 2013). On the other hand, DNA fingerprint analysis such as restriction endonuclease analysis (REA), ribotyping, randomly amplified polymorphic

DNA (RAPD) and pulsed-field gel eletrophoresis (PFGE) (Sehgal et al., 2003) are also approved for genetic characterization of *Leptospira* spp.

CHAPTER III

MATERIALS AND METHODS

1. Study sites and sample collections

Community hospital informed the Nan provincial Public health office that human leptospirosis cases occurred in Nan province. Inclusion criteria for selected study area based on i.) Area of human leptospirosis screening by routine diagnostic test. ii.) Area with history of number of human leptospirosis in Nan province by the Bureau of Epidemiology in 2014. iii.) Area with agriculture and animal husbandry were employed by human. However, area with unable for access sample collections were excluded from this study. Three different districts; Muang Nan, Chiang Klang and Tha Wang Pha were chosen in this study. Map of Nan province with study area is shown in Figure 5. Standardized questionnaire had been used to obtain the sociodemography of the animals including cattle, pigs and dogs around the leptospirosis human (index human).

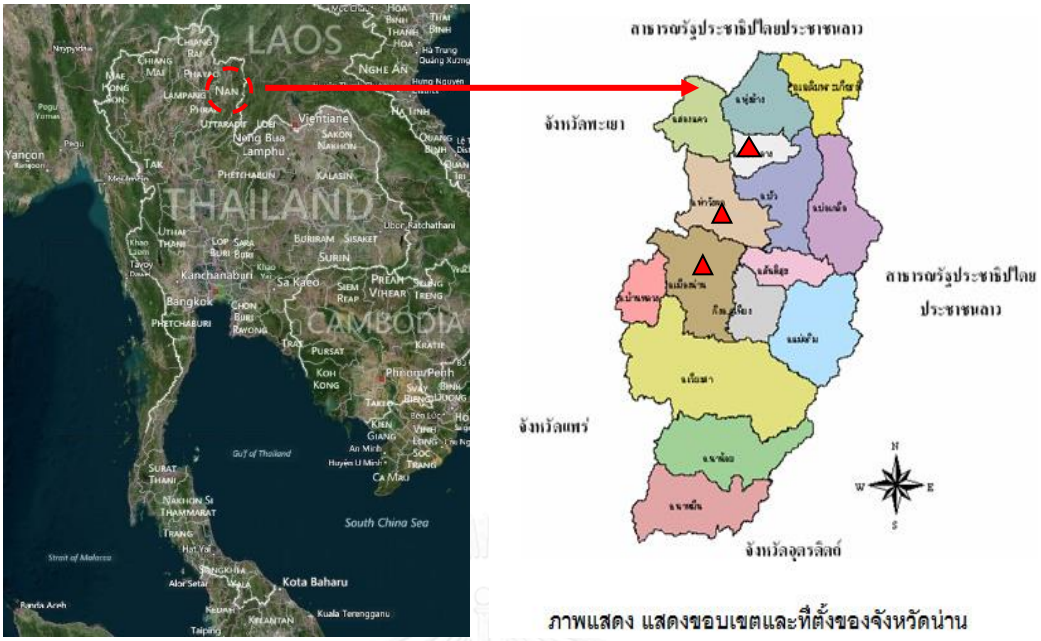


Figure 5. Map of Thailand and Nan province. Red triangles are study area. Adapted from Epi info 7.0 and www.brrd.in.th

Urine and/or blood samples were collected from cattle (n≥150), pigs (n≥50) and dogs (n≥50) whose without clinical sign and/or history of leptospirosis such as jaundice and reproductive failure; abortion. This sample size was calculated by Epi info 7.0 with the prevalence 1.5% (Suwancharoen et al., 2013). Number of animals and samples collection is shown in Table 2.

Animal sampling protocol was approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC) protocol No. 1531076. Urine samples were collected at least 15 ml in cattle and pigs by voiding and in dogs by catheterization. Blood sample were collected at least 3 ml in cattle at Jugular vein

and in dogs at Cephalic vein. All samples were kept at 4 ℃ before further processes in laboratory within 3 hours.

Table 2. Number of animals and samples in this study

Animal	Number	Sample	
		Blood	Urine
Cattle	291	160	131
Pigs	152	NC	152
Dogs	108	50	58
Total	551	210	341

NC: Not collection

2. Microscopic agglutination test (MAT)

Blood samples were centrifuged at 20,000 x g for 5 minutes and the serum was collected for MAT. MAT was processed at the standard laboratory accreditation for *Leptospira* spp. sero-detection, National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand. A total of 23 serogroups live reference of *Leptospira* comprising of Bratislava, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Pomona, Pyrogenes, Ranarum, Sarmin, Sejroe, Shermani and Tarassovi, and 1 serogroup of *L. biflexa* (Patoc I) were used in the assay. The positive titer presenting at least 50% of agglutination under dark-filed microscopy was defined. The normal saline was used as negative control confirming no auto-agglutination. Sera were judged to be positive if the titer was reached $\geq$ 1:80.

3. *Leptospira* spp. culture

After placing urine for 30 minutes, 0.5 ml of subsurface urine sample were inoculated in the modified Ellinghauson McCullough Johnson and Harris (EMJH) semisolid, and the remained urine (14.5 ml) were centrifuged at 3,500 x g for 15 minutes. At least 1 ml of solid pellet was used a 10-fold serially diluted technique in EMJH broth (WHO, 2003) that containing 5-fluorouracil, rifampicin and neomycin (Adler et al., 1986). The inoculated media was incubated at 28-30 ℃, and the presence of *Leptospira* spp. was observed under dark field microscope once a week for three months. Positive sample was filtrated in 0.2 micrometers filter and 100 µl of filtrate were spread on Leptospira Vanaporn Wuthiekanun (LVW) agar (Wuthiekanun et al., 2013) for leptospiral isolation and further detection by nested polymerase chain reaction (nested PCR) technique.

4. *Leptospira* spp. detection

By molecular detection, at least 2 ml of urine solid pellet after centrifugation were used for DNA isolation by Nucleospin® extraction kit (Macherey-Nagel, Germany) following manufacturer's instruction.

To detect pathogenic and intermediate pathogenic *Leptospira* spp., nested PCR was performed to amplify 547 bp of *rrs* (Boonsilp et al., 2011). Primer list for *Leptospira* spp. detection is shown in Table 3 and *rrs* nested PCR conditions are show in Table 4. PCR product were detected by 1.5% agarose gel electrophoresis

strained with Redsafe™ (iNtRON Biotechnology, USA) and visualized by UV transilluminator.

Table 3. Primer for *Leptospira* spp. detection by nested PCR.

Target genes	Primer name	Oligonucleotide primer (5'→3')	Product sizes (bp)
rrs	rrs-outer-F	5'-CTCAGAACTAACGCTGGCGGCGCG-3'	547
	rrs-outer-R	5'-GGTTCGTTACTGAGGGTTAAAACCCCC-3'	
	rrs-inner-F	5'-CTGGCGGCGCGTCTTA-3'	
	rrs-inner-R	5'-GTTTTCACACCTGACTTACA-3'	

Table 4. Condition for *rrs* nested PCR

Step	Temperature	Time
Initial denaturation	98 °C	2 minutes
40 cycles of outer primer		
Denaturation	95 °C	10 seconds
Annealing	70 °C	15 seconds
Extension	72 °C	30 seconds
40 cycles of inner primer		
Denaturation	95 °C	10 seconds
Annealing	58 °C	15 seconds
Extension	72 °C	30 seconds
Final extension	72 °C	7 minutes

5. DNA sequencing

rrs amplicons resulted from nested PCR were purified using Nucleospin® Gel and PCR Clean up (Macherey-Nagel, Germany) according to manufacturer's

procedure. All DNA products were submitted for DNA sequencing via a commercial available service (1st BASE Pte Ltd, Singapore).

6. Molecular analysis

For phylogenetic analysis, nucleotide sequences of *rrs* amplicons 547 bp were trimmed to 443 bp. The 443 bp region was aligned and defined species with *rrs* gene sequences of *Leptospira* spp. in GenBank database (Boonsilp et al., 2011) and constructed a neighbor-joining tree by MEGA version 6 (Tamura et al., 2007). Neighbor-joining tree was performed using Kimura's two-parameter model with 1,000 bootstrap replications. The tree was reconstructed by Figtree program (http://tree.bio.ed.ac.uk/sofeware/figtree/)

7. Data analysis

Seroprevalence and prevalence of leptospiral carriers in domestic animals detected by nested PCR and/or culture obtained from cross sectional study during 2013 to 2015 were analyzed by descriptive analysis. The agreement of leptospiral detection by culture and nested PCR were compared and analyzed by Cohen's Kappa analysis which value range 0 indicated no agreement, 0.1-0.2 indicated low agreement, 0.21-0.4 indicated fair agreement, 0.41-0.6 indicated moderate agreement, 0.61-0.8 indicated substantial agreement and 0.81-0.99 indicated perfect agreement among two tests (Viera and Garrett, 2005). Chi-square was analyzed relationship between culture and nested PCR results that the value of $P<0.05$ was defined as statistical significance. Distribution of leptospirosis in domestic animals was

mapped in area of study. Map was generated by ArcGIS 10.2 software (ESRI, Redland, CA)

CHAPTER IV

RESULTS

1. Sample collections

During 2013 to 2015, a total of 551 samples were collected from domestic animals in Nan province including 210 blood samples and 341 urine samples (Table 5). All samples were recruited from 20 villages in Muang Nan, Tha Wang Pha and Chiang Klang districts. Locality of sample collections are shown in figure 6 and geographical co-ordinates are shown in Table 6.

Table 5. Number and locality of sample collections divided by host and specimen type.

Districts	Animals						Total
	Cattle		Pigs		Dogs		
	Blood	Urine	Blood	Urine	Blood	Urine	
Chiang Klang	0	0	NC	20	0	5	25
Muang Nan	160	30	NC	4	36	14	228
Tha Wang Pha	0	101	NC	128	14	39	298
Total	160	131	NC	152	50	58	551

NC: Not collection

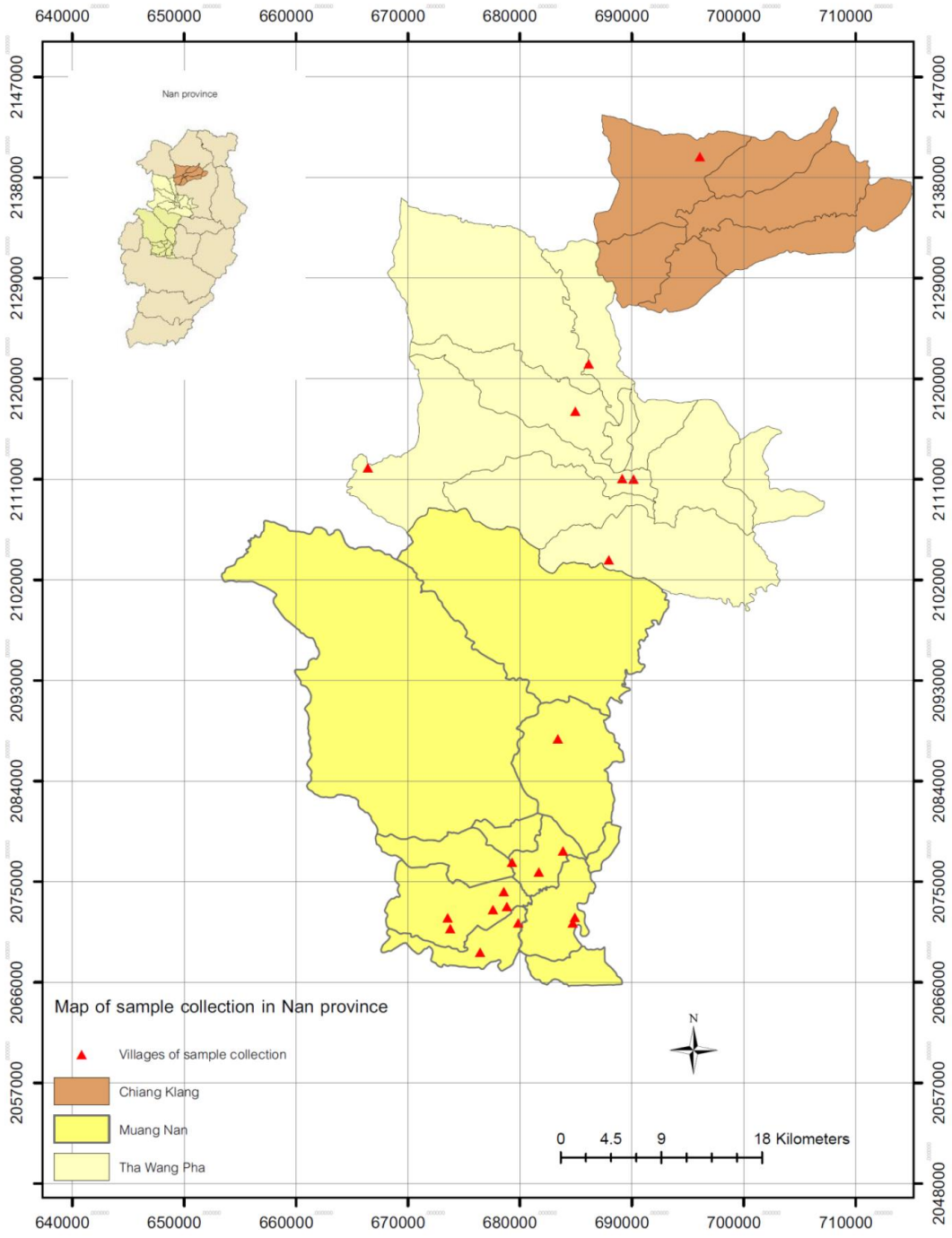


Figure 6. Map of study area and sample collection in Nan province. Map were designed by ArcGIS 10.2 software (ESRI, Redland, CA) with coordinate system WGS 1984 UTM zone 47N. Red triangle represents coordinating of 20 villages in three districts; Chiang Klang (Brown color), Muang Nan (Yellow color) and Tha Wang Pha (Light Tan color)

Table 6. Geographical co-ordinates of 20 villages in Nan province modified location from GoogleMap to coordinate system WGS 1984 UTM zone 47N by ArcGIS

Number	X	Y	Villages
1	678581.283592239	2074183.788449700	Ban Suak
2	677605.862259862	2072565.729963210	Muang Charoen Rad
3	673542.628308011	2071835.633343960	Na Pong Pathana
4	678845.063696664	2072849.452141540	Nong Tonm
5	679866.245847805	2071381.099961470	Na Sua
6	676477.692369381	2068749.294641800	Sa Mai
7	684915.112023428	2071891.217054220	Doo Tai
8	684744.447600459	2071366.830353730	Doo Ton Hang
9	681719.245356698	2075925.344695690	Sri Kerd
10	683858.601465537	2077840.092159880	Ban God
11	679286.946280276	2076813.294745380	Ban Fang
12	683413.191715929	2087840.202947670	Pha Singh
13	687961.836953051	2103853.466742250	Don Keng
14	686147.742868257	2121393.795198710	Wang Thong
15	684962.578565800	2117146.116051630	Huak
16	673781.895110218	2070845.280305400	Chiang Yean
17	690152.521031520	2111079.237491970	Fai Moon
18	666450.694012050	2112115.377454050	Sop Khun
19	689160.529189482	2111142.315745820	Ton Hang
20	696119.471488945	2139923.538475680	Num Aor

2. Microscopic agglutination test (MAT)

A total of 210 serum samples from cattle and dogs were collected in Muang Nan and Tha Wang Pha districts. At the titer ≥ 1:80 by MAT, a total of 17 of 210 sera were positive for *Leptospira* spp. at the prevalence of 8.09%. The seroprevalence detection in cattle was 10.62% (17/210) but it was negative in dogs as shown in Table 7. Among 13 serum samples, MAT was positive with single *Leptospira* serogroup comprising of Shermani (n=10), Sejroe (n=2) and Tarasovi (n=1). Four serum samples were positive multiple serogroups; Shermani and Tarassovi or Shermani and Icterohaemorrhagiae. A range of titer from 80 to 320 of single and multiple positive samples are described in Table 8. In this study, Shermani was the most common serogroup followed by Sejroe and Tarasovi, respectively. Distribution of *Leptospira* serogroup is demonstrated by mapping in Nan area as shown in Figure 7.

Table 7. Prevalence and distribution of positive animals to *Leptospira* by MAT (titer ≥ 1:80)

District	Prevalence of *Leptospira* positive animals.		Total (n=210)
	Cattle (n=160)	Dogs (n=50)	
Muang Nan	10.62% (17/160)	0	8.09% (17/210)
Tha Wang Pha	0	0	0
Total	10.62% (17/160)	0	8.09% (17/210)

Table 8. Number of seropositive animals to *Leptospira* at the titers ranged from 1:80 to 1:320.

Serogroup	No. of animals seropositive titers (n=17)			Frequency
	1:80	1:160	1:320	
Single				
Sejroe	2			0.94% (2/210)
Shermani	6	3	1	4.76% (10/210)
Tarasovi	1			0.47% (1/210)
Multiple				
Djasiman/Grippotyphosa†	1	1		0.47% (1/210)
Icterohaemorrhagiae/ Sarmin/Sejroe	1			0.47% (1/210)
Shermani/Tarasovi	1			0.47% (1/210)
Shermani/Icterohaemorrhagiae	1			0.47% (1/210)
Total				8.09% (17/210)

† Positive with titer with 1:80 and 1:160

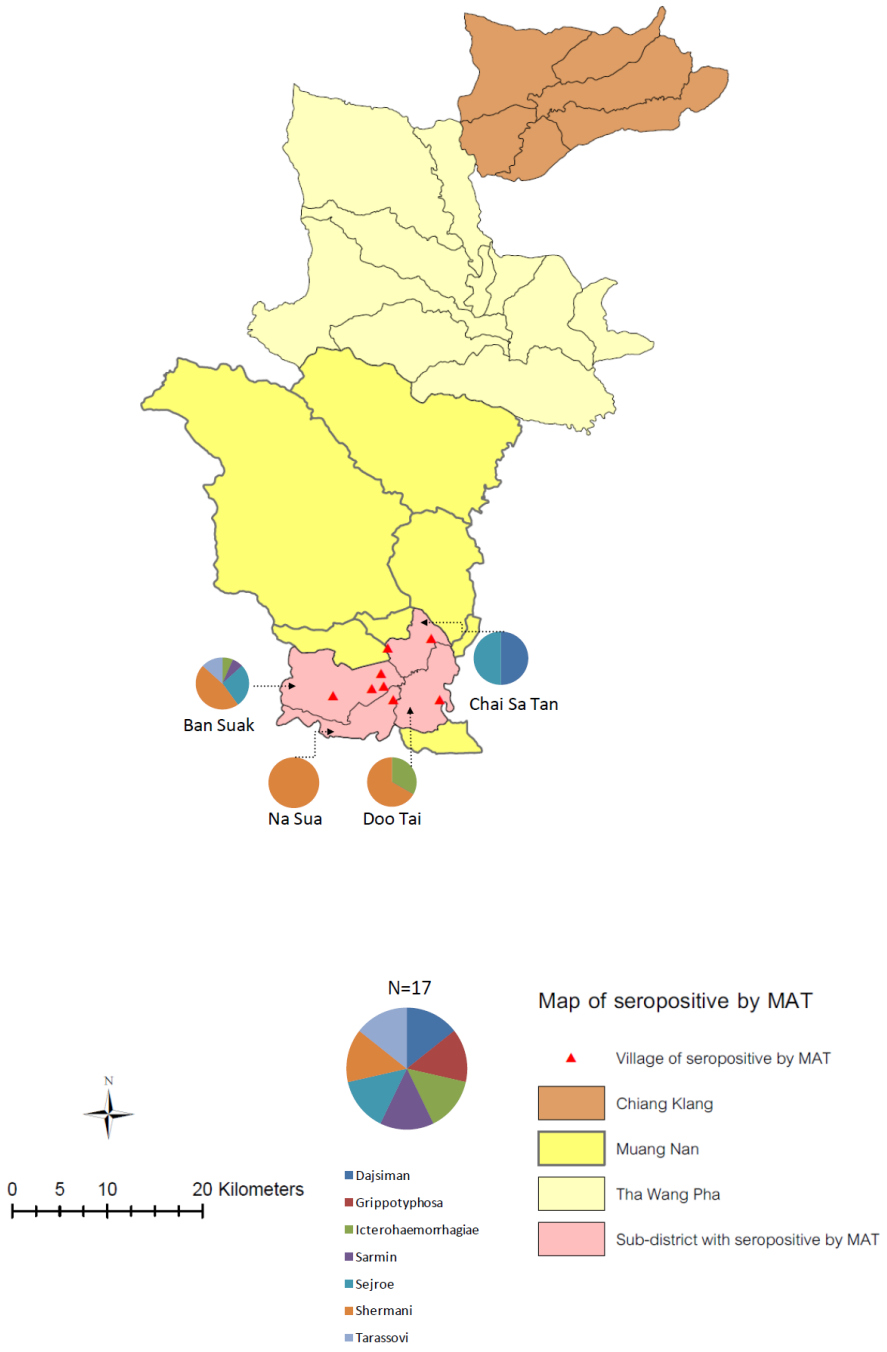


Figure 7. Geographical illustration represents the area containing animals with seropositive to *Leptospira* by Microscopic Agglutination Test (MAT) in four sub-districts; Ban Suak, Na Sua, Doo Tai and Chai Sa Tan, Nan province.

3. *Leptospira* detection by culture and molecular assays.

Of 341 urine samples, *Leptospira*-like microorganism were observed in 11 samples comprising of from cattle (n=1), pigs (n=6) and dogs (n=4). All positive samples were confirmed by nested PCR with a specific product. Four pure leptospiral isolates (1.17%) were obtained from dog urines by using LVW agar (Figure 8) whereas the other urine samples from cattle and pigs were contaminated and eventually could not be isolated.

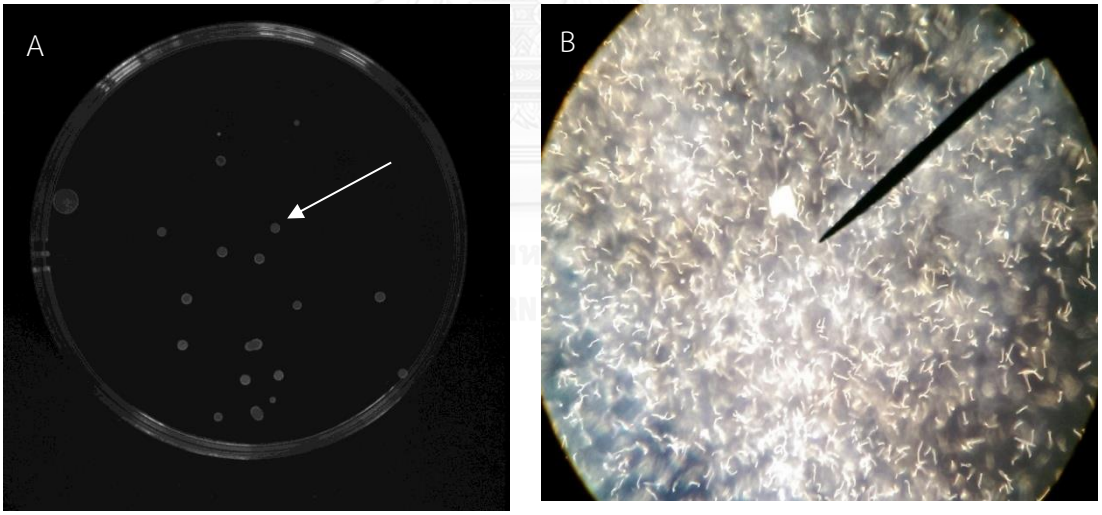


Figure 8. Presence of leptospiral colony on LVW agar and leptospiral-like microorganism under 40X dark field microscope. Arrow indicates leptospiral colony (A) and leptospires in semi-solid EMJH under (B).

By *rrs* nested PCR directed detection from urine samples, thirty-four samples were positive to pathogenic or intermediate *Leptospira* spp. (Figure 9) by possessing the 547 bp DNA product. The prevalence of *Leptospira* spp. in urine samples was 9.97% (34/341) that derived from cattle (n=16), pigs (n=12) and dogs (n=6). Prevalence of *Leptospira* spp. positive animals by *rrs* nested PCR are shown in Table 9.

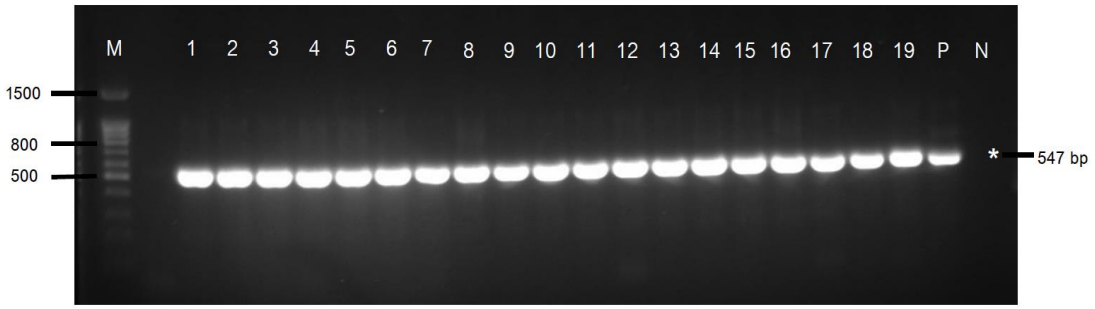


Figure 9. Representative nested PCR products of *rrs* gene separated in 1.5% agarose gel electrophoresis. Lane M represented 1500-bp molecular weight maker. Lane 1 to 19 represented amplified nested PCR products from urine of domestic animals in Nan province, Thailand. Lane P represented *L. interrogans*. Lane N represented Negative control. Asterisk represented *rrs* nested PCR product size.

Table 9. Comparison of *Leptospira* prevalence in urine animals by *rrs* nested PCR detection.

Animal	n	Total *rrs* nested PCR positive (n=34)			
		L. interrogans	*L. weilii*	Unidentified *Leptospira* spp.	Total
Cattle	131	1.52% (2/131)	8.36% (11/131)	2.29% (3/131)	12.21% (16/131)
Pigs	152	1.97% (3/152)	5.92% (9/152)	0	7.89% (12/152)
Dogs	58	6.89% (4/58)	3.44% (2/58)	0	10.34% (6/58)
Total	341	2.63% (9/341)	6.45% (22/341)	0.87% (3/341)	9.97% (34/341)

4. DNA sequencing and phylogenetic analysis

The 443-bp of nested PCR product was submitted for DNA sequencing and analyzed the species identification and relation by Neighbor-joining phylogenetic analysis (Figure 10). All sequences were submitted in GenBank Database (Accession number KU854349-KU854387). Nine samples from 2 cattle, 3 pigs and 4 dogs were clustered in the branch of *L. interrogans*. On the other hand, 22 samples from 11 cattle, 9 pigs, 2 dogs clustered in a branch of *L. weilii* and three samples from cattle were the unidentified *Leptospira* species (CUB9, CUB11 and CUB15)

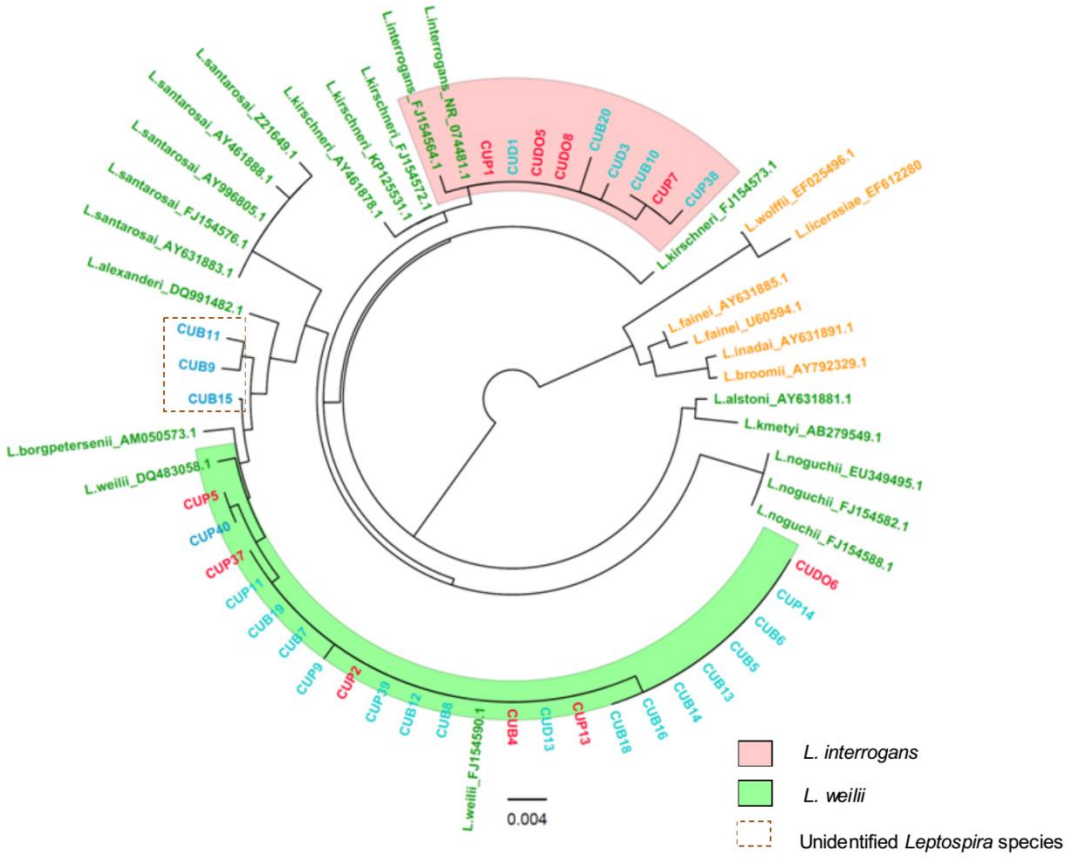


Figure 10. Phylogenetic analysis by neighbor-joining method of 443 bp partial *rrs* of 34 isolates with 14 *Leptospira* spp. Blue letter indicated direct *rrs* nested PCR positive samples, Pink letter indicate direct *rrs* nested PCR positive sample with culture positive, Green letter indicated pathogenic *Leptospira* spp. and Orange letter indicated intermediate *Leptospira* spp. from GenBank database.

Because of the CUB9, CUB11 and CUB15 were pended among the branch of *L. weilii*, *L. borgpetersenii*, *L. santorosai* and *L. alexanderi*, the polymorphic position within 443 bp were further analyzed and compared with the nine *rrs* alleles of four *Leptospira* species (Figure 11) (Boonsilp et al., 2011).CUB9 revealed four polymorphic bases at positions 122 (T), 164 (G), 175 (A) and 213 (A), especially bases at position 122 (T) were distinguished from the nine leptospiral references. CUB11 showed five polymorphic bases at position 149 (A), 164 (G), 175 (A), 213 (A) and 224 (T). Base A at position 149 from CUB11 differed from the others. CUB15 also showed two polymorphic base at position 164 (G) and 213 (A). CUB9 and CUB11 were closely related to *L. alexanderi* whereas CUB11 were closely related to *L. borgpetersenii*.

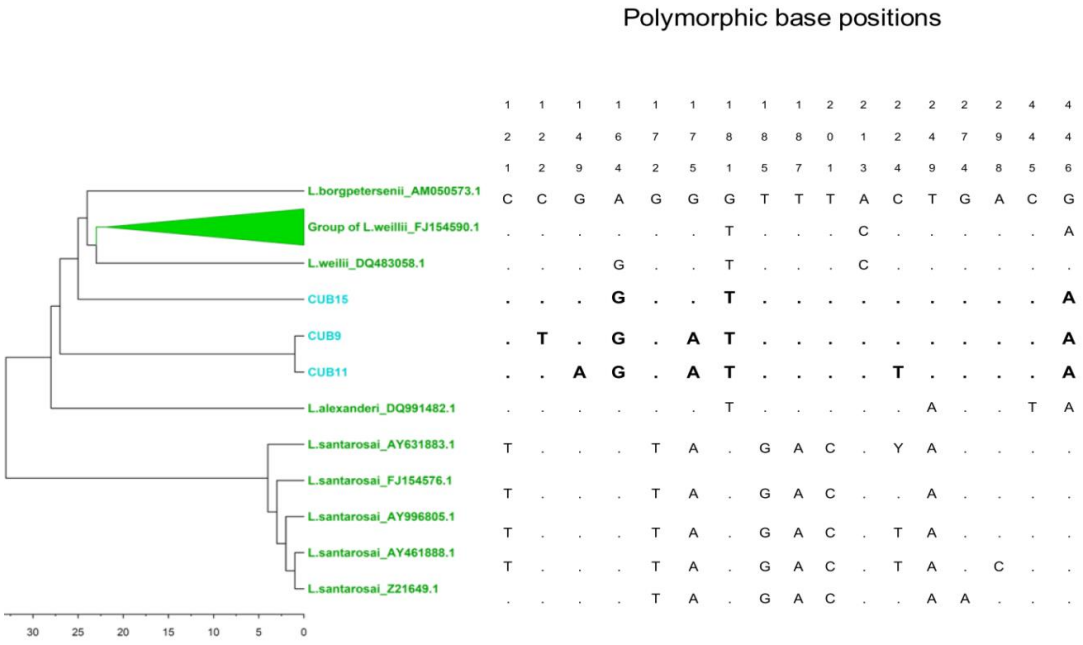


Figure 11. Polymorphic base positions of unidentified *Leptospira* spp. from three cattle urine samples. Green letter indicates pathogenic *Leptospira* spp. in GenBank database. Blue letter indicates unidentified *Leptospira* species. (CUB9, CUB11 and CUB15).

5. Statistical analysis and *Leptospira* spp. distribution

By Cohen's Kappa analysis, detection of *Leptospira* spp. showed a moderated agreement between culture and *rrs* nested PCR at Kappa value = 0.463 and significant relationship between culture and nested PCR ($P<0.05$). Positive rates of leptospiral detection by culture and *rrs* nested PCR are shown in Table 10.

Table 10. Comparison of leptospiral positive rates detected by culture and nested PCR in urine sample

Animal	n	*Leptospira* like-microorganism culture positive (n=11)	*rrs* nested PCR positive (n=34)
Cattle	131	0.76% (1/131)	12.21% (16/131)
Pigs	152	3.94% (6/152)	7.89% (12/152)
Dogs	58	6.89% (4/58)	10.34% (6/58)
Total	341	3.22% (11/341)*	9.97% (34/341)*

*Cohen's Kappa analysis (95%CI), Chi-square significant *P* value at $P<0.05$

Kappa value = 0.463 (moderated agreement), $P<0.05$

Geographical distribution of *Leptospira* spp. in the study area is shown Figure 12. The highest positive of *Leptospira* spp. infection in domestic animals were found in three villages including Sop Khun, Fai Moon and Ton Hang villages at Tha Wang Pha district. While, Pha Singh villages from Muang Nan district and Nom Aor village from Chiang Klang district were found three and one animal infection in each area, respectively (Figure 12).

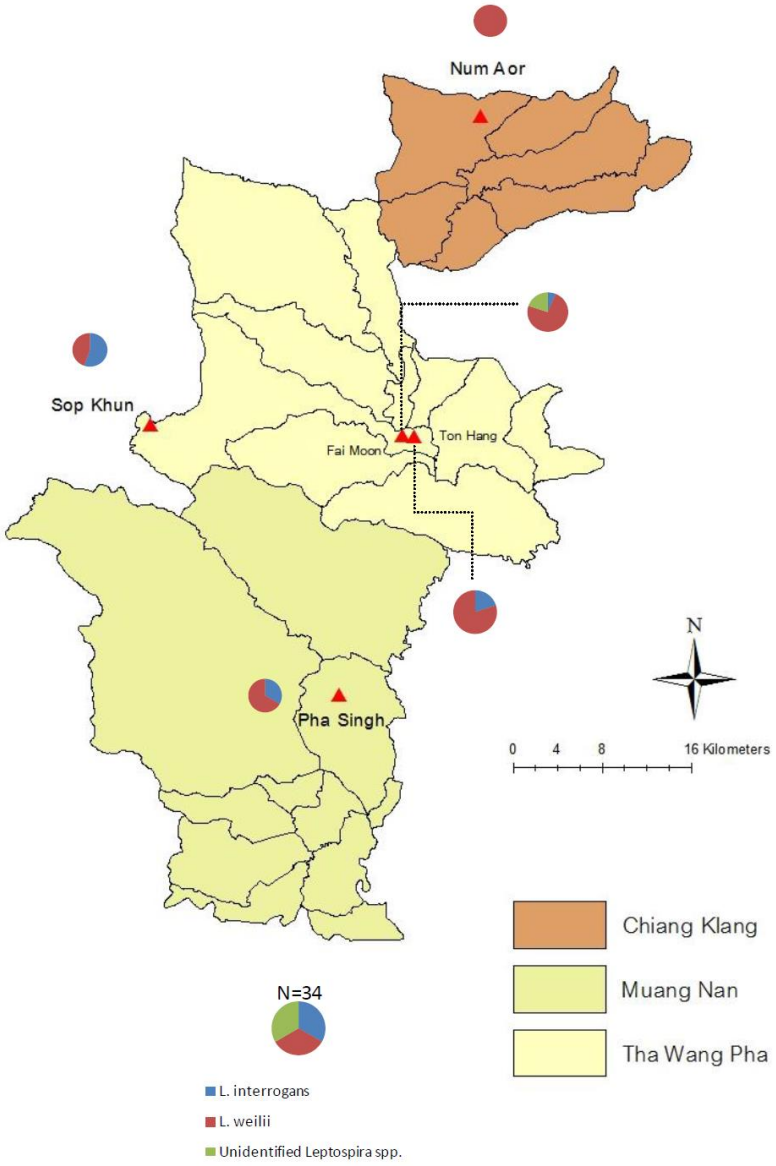


Figure 12. Geographical distribution of *Leptospira* spp. in domestic animals in Nan province, Thailand.

CHAPTER V

DISCUSSIONS

Nan province is one of endemic area of human leptospirosis (Cosson et al., 2014; Della Rossa et al., 2015). Domestic animals presented a high seropositive number in the moderated titer to *Leptospira* spp. by microscopic agglutination test (MAT). In term of disease distribution, the results obtained between serological detection and molecular detection was incongruent within area of study. This study could strongly confirm that domestic animals in Nan province could be a carrier of pathogenic *Leptospira* spp. resulting from the nested PCR and culture. It directly provides the knowledge perceptions in public.

Microscopic agglutination test (MAT) is a reference tool that commonly uses to detect reaction between unknown serum antibodies and live standard leptospires (OIE, 2014). MAT reflects the situation of clinical leptospirosis especially by using pair serum that can differentiate of current or past of infection (Villanueva et al., 2010). In this study, only single blood collection from animals with normal appearance was performed by using the available service for MAT. The seroprevalence did not reflect clinical stage in individual animals but determine a probable number of leptospirosis in herd level (Levett, 2001; Lilenbaum and Martins, 2014; OIE, 2014).To date, there has not been a report of consensual identification by cut-off titer in animal

leptospirosis. In Thailand, there were various proposed cut-off MAT titers for diagnosis of animal leptospirosis and disease surveillance. The previous reports in livestock animals used titer ≥ 1:50 as a positive for leptospirosis in livestock (Suwancharoen et al., 2013), whereas another used titer ≥ 1:100 for leptospirosis in pigs (Niwetpathomwat et al., 2006). Our study did consider at the titer ≥ 1:80 since this was in the set of serial dilution of laboratory provider (1:20 to 1:640). By using the lower cut-off titer (1:50 or 1:100), this MAT cut-off titers was assumed for the history of disease exposure (da Silva Pinto et al., 2016) and this has been recommended by the World Organization of Animal Health (OIE, 2014).

In this study, the seroprevalence of animal leptospirosis in Nan province (8.09%) was higher than the previous country surveillance belonging to Department of Livestock Development (DLD) data in Thailand (Suwancharoen et al., 2013). As Shermani, Sejroe and Tarassovi were the major serovars which uncorrelated with the previous report that found Ranarum and Batislava (Suwancharoen et al., 2013). Serovar Sejroe was found from cattle in Nan as well as other parts in Thailand (Suwancharoen et al., 2013). Four sera showed multiple serogroups at titer ≥ 1:80. The explanation of these phenomena are i.) cross-reaction of various serogroups ii.) animals may infect with one serogroup in the past and other serogroups in present (Chirathaworn et al., 2014). The factors that effected to serovar and seroprevalence of leptospirosis was the different of geographical location and duration of study.

There was no seropositive found in dog sera in this study might reflect an immune evasion during chronic leptospiral infection in dog carrier that presenting very low antibody in blood stream (Faine, 1999) or caused by difference between the infected serovar and the serovars for MAT panel (Villanueva et al., 2010). However, the reason of area where are very far from province center and underdeveloped, it was such hard to follow up the sample collection and some was lost during transportation.

This study successfully isolated leptospiral microorganism from four dog urine samples, these living isolates on LVW agar were the first animal isolates in Nan province, Thailand. Unfortunately, isolation of leptospires from urine samples had been the major problem in world-wide laboratories. The most of positive cultures were lost during subculture due to overgrowth of saprophytic bacteria and yeast in urine (Esfandiari et al., 2015), even thought, the antimicrobial agents and paper filter were added into culture procedure. This is the great obstacle of leptospirosis study in animals. To invent a proper selective media used for initial culture and isolation from animal specimen is hope to expand the knowledge of animal leptospirosis and one health approach. Despite of isolation is a definitive method to diagnose leptospirosis, but Polymerase chain reaction (PCR) was an alternative tool with high sensitivity and specificity for epidemiological study (Boonsilp et al., 2011). Use of nested PCR is very useful in case of leptospiremia detected from only one milliliter

of human blood (Boonsilp et al., 2011). The nested PCR could be used for detection of pathogenic *Leptospira* in urine of animal reservoirs in this study.

Nan province was chosen due to its annually high incidence of human leptospirosis (Della Rossa et al., 2015), where farmers are thought to be exposed to leptospires via standing water in rice fields and rivers. We restricted the screening of leptospires to domestic animals that directly involved as carriers of leptospirosis, whereas the reservoir rodents have already been confirmed in the area (Cosson et al., 2014; Della Rossa et al., 2015). Beside rodents, our study revealed that asymptomatic domestic animals including cattle, pigs and dogs rearing in the area possessed pathogenic leptospires in their urine that could be the significant source of leptospirosis to human patients.

Among 34 positive PCR samples were composed of two major pathogenic *Leptospira* spp., *L. weilii* and *L. interragans* and unidentified *Leptospira* species. .*L. interragans* was detected from all collected animal hosts that could be maintained and become the source of contamination to environment and infection to human. *L. weilii* was the most common pathogenic *Leptospira* spp. in cattle urine. Our finding confirmed the large ruminants are the major reservoir for *L. weilii* and accorded with previous report (Corney et al., 2008). Interestingly, detection of *L. weilii* in pigs and dogs in this study is uncommon and could provide as update information. By 443 bp of *rrs* product, three unidentified samples from cattle urine showed the distinct of polymorphic base position compared to *rrs* gene in the data base, these also could

not be grouped in any species within pathogenic leptospiral member. It is speculated that there was the new variant of animal *Leptospira* spp. in Nan province. However, a little difference of allele might be inadequate to speciate *Leptospira* species (Boonsilp et al., 2011).

The prevalence of *Leptospira* spp. in domestic animals were major detected in three villages at Tha Wang Pha district. Our data strongly related with the history of human leptospirosis in this area during 2013 to 2016 by Nan Provincial Public Health Office. At Sop Khun village, all pure leptospires were retrieved from four asymptomatic dogs that dwell at the household of folk with history of leptospirosis. This may be an important connection between human infection and animal carriage especially from asymptomatic subjects.

In conclusion, our data revealed that the prevalence in asymptomatic animals in Nan province was 8.06% and 9.97% by MAT and nested PCR, respectively. Existence of *L. interragans* in urine source was confirmed in dog, pig and cattle. *L. weilii* was firstly reported in animals in Thailand and became the most prevalence in domestic animals at the study area. Our study provides a great awareness of asymptomatic animal reservoirs in the endemic area that concretely links to the folk with a history of leptospirosis

REFERENCES

Adler B and de la Pena Moctezuma A. 2010. *Leptospira* and leptospirosis. Vet Microbiol. 140(3-4): 287-296.

Adler B, Faine S, Christopher WL and Chappel RJ. 1986. Development of an improved selective medium for isolation of leptospires from clinical material. Vet Microbiol. 12(4): 377-381.

Balamurugan V, Gangadhar NL, Mohandoss N, Thirumalesh SR, Dhar M, Shome R, Krishnamoorthy P, Prabhudas K and Rahman H. 2013. Characterization of leptospira isolates from animals and humans: phylogenetic analysis identifies the prevalence of intermediate species in India. Springerplus. 2: 362.

Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Chierakul W, Limmathurotsakul D, Day NP and Peacock SJ. 2011. Molecular detection and speciation of pathogenic *Leptospira* spp. in blood from patients with culture-negative leptospirosis. BMC Infect Dis. 11: 338.

Cerqueira GM, McBride AJ, Picardeau M, Ribeiro SG, Moreira AN, Morel V, Reis MG, Ko AI and Dellagostin OA. 2009. Distribution of the leptospiral immunoglobulin-like (*lig*) genes in pathogenic Leptospira species and application of *ligB* to typing leptospiral isolates. J Med Microbiol. 58(Pt 9): 1173-1181.

Chirathaworn C, Inwattana R, Poovorawan Y and Suwancharoen D. 2014. Interpretation of microscopic agglutination test for leptospirosis diagnosis and seroprevalence. Asian Pac J Trop Biomed. 4(Suppl 1): S162-164.

Corney BG, Slack AT, Symonds ML, Dohnt MF, McClintock CS, McGowan MR and Smythe LD. 2008. *Leptospira weilii* serovar Topaz, a new member of the Tarassovi serogroup isolated from a bovine source in Queensland, Australia. Int J Syst Evol Microbiol. 58(Pt 10): 2249-2252.

Cosson JF, Picardeau M, Mielcarek M, Tatard C, Chaval Y, Suputtamongkol Y, Buchy P, Jittapalapong S, Herbreteau V and Morand S. 2014. Epidemiology of

leptospira transmitted by rodents in southeast Asia. PLoS Negl Trop Dis. 8(6): e2902.

da Silva Pinto P, Libonati H, Penna B and Lilenbaum W. 2016. A systematic review on the microscopic agglutination test seroepidemiology of bovine leptospirosis in Latin America. Trop Anim Health Prod. 48(2): 239-248.

Della Rossa P, Tantrakarnapa K, Sutdan D, Kasetsinsombat K, Cosson JF, Supputamongkol Y, Chaisiri K, Tran A, Supputamongkol S, Binot A, Lajaunie C and Morand S. 2015. Environmental factors and public health policy associated with human and rodent infection by leptospirosis: a land cover-based study in Nan province, Thailand. Epidemiol Infect. 1-13.

Director A, Penna B, Hamond C, Loureiro AP, Martins G, Medeiros MA and Lilenbaum W. 2014. Isolation of *Leptospira interrogans* Hardjoprajitno from vaginal fluid of a clinically healthy ewe suggests potential for venereal transmission. J Med Microbiol. 63(Pt 9): 1234-1236.

Doungchawee G, Phulsuksombat D, Naigowit P, Khoaprasert Y, Sangjun N, Kongtim S and Smythe L. 2005. Survey of leptospirosis of small mammals in Thailand. Southeast Asian J Trop Med Public Health. 36(6): 1516-1522.

Esfandiari B, Pourshafie MR, Gouya MM, Khaki P, Mostafavi E, Darvish J, Bidhendi SM, Hanifi H and Nahrevanian H. 2015. An epidemiological comparative study on diagnosis of rodent leptospirosis in Mazandaran Province, northern Iran. Epidemiol Health. 37: e2015012.

Evangelista K and Coburn J. 2010a. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. Future Microbiol. 5(9): 1413-1425.

Faine S AB, Bolin C and Perolat P 1999. *Leptospira* and leptospirosis. Medisci, Melbourne. 2: 272.

Heisey GB, Nimmanitya S, Karnchanachetanee C, Tingpalapong M, Samransamruajkit S, Hansukjariya P, Elwell MR and Ward GS. 1988. Epidemiology and characterization of leptospirosis at an urban and provincial site in Thailand. Southeast Asian J Trop Med Public Health. 19(2): 317-322.

http://www.farmacy.co.uk/mobile/products/78-bovilis-ibr-marker-live-50-dose-with-applicators.

Khan S, Hassan M and Yasin G. 2009. Acute Leptospirosis in Dog- A case report. The Internet Journal of Veterinary Medicine. 7(2): 1-4.

Ko AI, Goarant C and Picardeau M. 2009. *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol. 7(10): 736-747.

Kositanont U, Naigowit P, Imvithaya A, Singchai C and Puthavathana P. 2003. Prevalence of antibodies to Leptospira serovars in rodents and shrews trapped in low and high endemic areas in Thailand. J Med Assoc Thai. 86(2): 136-142.

Levett PN. 2001. Leptospirosis. Clin Microbiol Rev. 14(2): 296-326.

Levett PN. 2003. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. Clin Infect Dis. 36(4): 447-452.

Levett PN. 2015. Systematics of *Leptospiraceae*. Curr Top Microbiol Immunol. 387: 11-20.

Lilenbaum W and Martins G. 2014. Leptospirosis in cattle: a challenging scenario for the understanding of the epidemiology. Transbound Emerg Dis. 61 Suppl 1: 63-68.

Meeyam T, Tablerk P, Petchanok B, Pichpol D and Padungtod P. 2006. Seroprevalence and risk factors associated with leptospirosis in dogs. Southeast Asian J Trop Med Public Health. 37(1): 148-153.

Merien F, Amouriaux P, Perolat P, Baranton G and Saint Girons I. 1992. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. J Clin Microbiol. 30(9): 2219-2224.

Niwetpathomwat A, Luengyosluechakul S and Geawduanglek S. 2006. A serological investigation of leptospirosis in sows from central Thailand. Southeast Asian J Trop Med Public Health. 37(4): 716-719.

OIE. 2014. LEPTOSPIROSIS. OIE Terrestrial Manual 2014

Otaka DY, Martins G, Hamond C, Penna B, Medeiros MA and Lilenbaum W. 2012. Serology and PCR for bovine leptospirosis: herd and individual approaches. Vet Rec. 170(13): 338.

Palaniappan RU, Ramanujam S and Chang YF. 2007. Leptospirosis: pathogenesis, immunity, and diagnosis. Curr Opin Infect Dis. 20(3): 284-292.

Pappas G, Akritidis N, Christou L, Mastora M and Tsianos E. 2003. Unusual causes of reactive arthritis: *Leptospira* and *Coxiella burnetii*. Clin Rheumatol. 22(4-5): 343-346.

Sehgal SC, Biswas D, Vijayachari P, Sugunan AP and Roy S. 2003. Molecular tools in leptospirosis diagnosis and characterization of isolates. Southeast Asian J Trop Med Public Health. 34 Suppl 2: 163-169.

Silva EF, Cerqueira GM, Seyffert N, Seixas FK, Hartwig DD, Athanazio DA, Pinto LS, Queiroz A, Ko AI, Brod CS and Dellagostin OA. 2009. *Leptospira noguchii* and human and animal leptospirosis, Southern Brazil. Emerg Infect Dis. 15(4): 621-623.

Slack AT, Symonds ML, Dohnt MF and Smythe LD. 2006. Identification of pathogenic *Leptospira* species by conventional or real-time PCR and sequencing of the DNA gyrase subunit B encoding gene. BMC Microbiol. 6: 95.

Suepaul SM, Carrington CV, Campbell M, Borde G and Adesiyun AA. 2010. Serovars of *Leptospira* isolated from dogs and rodents. Epidemiol Infect. 138(7): 1059-1070.

Suepaul SM, Carrington CV, Campbell M, Borde G and Adesiyun AA. 2011. Seroepidemiology of leptospirosis in livestock in Trinidad. Trop Anim Health Prod. 43(2): 367-375.

Suwancharoen D, Chaisakdanugull Y, Thanapongtharm W and Yoshida S. 2013. Serological survey of leptospirosis in livestock in Thailand. Epidemiol Infect. 141(11): 2269-2277.

Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA and Goldstein RE. 2011. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. J Vet Intern Med. 25(1): 1-13.

Tamura K, Dudley J, Nei M and Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 24(8): 1596-1599.

Thaipadungpanit J, Chierakul W, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Boonslip S, Smythe LD, Limpaiboon R, Hoffmaster AR, Day NP and Peacock SJ. 2011. Diagnostic accuracy of real-time PCR assays targeting *16S rRNA* and *lipL32* genes for human leptospirosis in Thailand: a case-control study. PLoS One. 6(1): e16236.

Victoriano AF, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, Limpakarnjanarat K, Ong BL, Gongal G, Hall J, Coulombe CA, Yanagihara Y, Yoshida S and Adler B. 2009. Leptospirosis in the Asia Pacific region. BMC Infect Dis. 9: 147.

Viera AJ and Garrett JM. 2005. Understanding interobserver agreement: the kappa statistic. Fam Med. 37(5): 360-363.

Villanueva SY, Ezoe H, Baterna RA, Yanagihara Y, Muto M, Koizumi N, Fukui T, Okamoto Y, Masuzawa T, Cavinta LL, Gloriani NG and Yoshida S. 2010. Serologic and molecular studies of *Leptospira* and leptospirosis among rats in the Philippines. Am J Trop Med Hyg. 82(5): 889-898.

Wangroongsarb P YS, Petkanjanapong W, Naigowit P, Hagiwara T, Kawabata H and Koizumi N 2014. Applicability of Polymerase Chain Reaction to Diagnosis of Leptospirosis. The Journal of Tropical Medicine and Parasitology. 28(2).

WHO. 2003. Isolation and Culture of Leptospires. Human leptospirosis : guidance for diagnosis, surveillance and control. 81-82.

Wuthiekanun V, Amornchai P, Langla S, Oyuchua M, Day NP and Limmathurotsakul D. 2014. Maintenance of leptospira species in leptospira Vanaporn Wuthiekanun agar. J Clin Microbiol. 52(12): 4350-4352.

Wuthiekanun V, Amornchai P, Paris DH, Langla S, Thaipadunpanit J, Chierakul W, Smythe LD, White NJ, Day NP, Limmathurotsakul D and Peacock SJ. 2013. Rapid isolation and susceptibility testing of *Leptospira* spp. using a new solid medium, LVW agar. Antimicrob Agents Chemother. 57(1): 297-302.

Wuthiekanun V, Chierakul W, Limmathurotsakul D, Smythe LD, Symonds ML, Dohnt MF, Slack AT, Limpaiboon R, Suputtamongkol Y, White NJ, Day NP and

Peacock SJ. 2007. Optimization of culture of *Leptospira* from humans with leptospirosis. J Clin Microbiol. 45(4): 1363-1365.

Yersin C, Bovet P, Merien F, Wong T, Panowsky J and Perolat P. 1998. Human leptospirosis in the Seychelles (Indian Ocean): a population-based study. Am J Trop Med Hyg. 59(6): 933-940.

APPENDIX

APPENDICES A

Ellinghausen-McCullough-Jonhson-Harris (EMJH) liquid media

Distill water	435 ml
Base EMJH	1.15 g
1% sodium pyruvate	0.5 ml
EMJH enrichment	50 ml
Rabbit serum	15 ml
Total	500 ml

Ellinghausen-McCullough-Jonhson-Harris (EMJH) semi-solid media

Distill water	435 ml
Base EMJH	1.15 g
1% sodium pyruvate	0.5 ml
Agar Noble	0.5 g
EMJH enrichment	50 ml
Rabbit serum	15 ml
Total	500 ml

Leptospira Vanaporn Wuthiekanun (LVW) agar

Distill water	435 ml
Base EMJH	1.15 g
1% sodium pyruvate	0.5 ml
Agar Noble	5 g
EMJH enrichment	50 ml
Rabbit serum	50 ml
Total	500 ml

Antimicrobial in EMJH semi-solid and broth.

Cyclohexamide (actidione)	1 g
5-fluorouracil	2.5 g
Bacitacin	0.4 g
Rifampicin	0.1031 g
Polymixin B (0.5 mg/ml)	4 ml
Neomycin	0.02 g
Add water to final volume	10 ml

APPENDICES B

>Seq1 [organism=*Leptospira weilii*] [isolate=CUB4] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854349

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq2 [organism=Leptospira weilii] [isolate=CUB5] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854350

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq3 [organism=Leptospira weilii] [isolate=CUB6] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854351

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq4 [organism=Leptospira weilii] [isolate=CUB7] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854352

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq5 [organism=Leptospira weilii] [isolate=CUB8] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854353

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq6 [organism=Leptospira weilii] [isolate=CUB12] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854354

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq7 [organism=Leptospira weilii] [isolate=CUB13] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854355

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq8 [organism=Leptospira weilii] [isolate=CUB14] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854356

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq9 [organism=Leptospira weilii] [isolate=CUB16] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854357

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq10 [organism=Leptospira weilii] [isolate=CUB18] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854358

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq11 [organism=Leptospira weilii] [isolate=CUB19] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854359

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq12 [organism=Leptospira weilii] [isolate=CUP5] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854360

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq13 [organism=Leptospira weilii] [isolate=CUP40] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854361

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq14 [organism=Leptospira weilii] [isolate=CUP37] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854362

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq15 [organism=Leptospira weilii] [isolate=CUD13] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854363

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq16 [organism=Leptospira weilii] [isolate=CUDO6] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854364

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq17 [organism=Leptospira weilii] [isolate=CUP39] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854365

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq18 [organism=Leptospira weilii] [isolate=CUP11] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854366

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq19 [organism=Leptospira weilii] [isolate=CUP13] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854368

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq20 [organism=Leptospira weilii] [isolate=CUP14] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854369

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGCCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq21 [organism=Leptospira weilii] [isolate=CUP2] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854370

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq22 [organism=Leptospira weilii] [isolate=CUP9] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854373

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAGAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGCTGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCGATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq23 [organism=Leptospira interrogans] [isolate=CUP1] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854374

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq24 [organism=Leptospira interrogans] [isolate=CUD1] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854375

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq25 [organism=Leptospira interrogans] [isolate=CUDO5] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854376

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq26 [organism=Leptospira interrogans] [isolate=CUDO8] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854377

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq27 [organism=Leptospira interrogans] [isolate=CUD3] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854380

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAACACGTATGGTGCAAGCGTTGTTCGGAA

>Seq28 [organism=Leptospira interrogans] [isolate=CUB20] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854381

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAGCGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq29 [organism=Leptospira interrogans] [isolate=CUB10] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854382

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGGGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq30 [organism=Leptospira interrogans] [isolate=CUP7] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854383

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGGGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq31 [organism=Leptospira interrogans] [isolate=CUP38] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854384

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGGGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGGGATCATAAGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAGTAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq32 [organism=Uncultured Leptospira sp.] [isolate=CUB11] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854385

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGAAGC
TAATACTGGATGGTCCCGAGAGATCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGTCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq33 [organism=Uncultured Leptospira sp.] [isolate=CUB9] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854386

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCTGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGATCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

>Seq34 [organism=Uncultured Leptospira sp.] [isolate=CUB15] 16S ribosomal RNA gene, partial sequence (443 bp), Accession number KU854387

GAACGGGTGAGTAACACGTGGGTAATCTTCCTCCGAGTCTGGGATAACTTTCCGAAAGGGGAGC
TAATACTGGATGGTCCCGAGAGGTCATATGATTTTTCGGGTAAAGATTTATTGCTCGGAGATGA
GCCCGCGCCCGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCGGTAGCCGGC
CTGAGAGGGTGTTCGGCCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAG
TTAAGAATCTTGCTCAATGGGGGGAACCCTGAAGCAGCGACGCCGCGTGAACGATGAAGGTCTT
CGGATTGTAAAGTTCAATAAGCAGGGAAAAATAAGCAGCAATGTGATGATGGTACCTGCCTAAA
GCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTATGGTGCAAGCGTTGTTCGGAA

VITA

AUTHOR Mr. Alongkorn Kurilung

DATE OF BIRTH 26th December 1987

EDUCATIONAL CREDENTIALS

Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Khon kaen University

ACADEMIC PRESENTATION

1. A. Kurilung, P. Chanchaithong, K. Lugsomya, N. Phumthanakorn, W. Niyomthum, A. Jaijan, N. Prapasarakul. Detection and isolation of Leptospira sp. in Domestic Animals, Nan Province, Thailand. The 14th Chulalongkorn University Veterinary Conference (CUVC) 2015, Bangkok, Thailand, April 20-22.

2. A. Kurilung, P. Chanchaithong, K. Lugsomya, W. Niyomthum, R. Tantilertcharoen, N. Prapasarakul. Comparison of microscopic agglutination, culture and molecular tools for diagnosis of pathogenic Leptospira in asymptomatic dogs. VPAT Regional Veterinary Congress 2015 (VRVC 2015), Bangkok, Thailand, July 26-29.

3. A. Kurilung, P. Chanchaithong, K. Lugsomya, W. Niyomthum, R. Tantilertcharoen, N. Prapasarakul. Comparison of microscopic agglutination, culture and molecular tools for diagnosis Leptospira in domestic animals, Nan province, Thailand. Emerging Infectious Diseases in Animals Conferences 2015, Chulalongkorn University,Bangkok, Thailand, August 20.

AWARD

1. Research Excellent award : VPAT Regional Veterinary Congress 2015 (VRVC 2015) , Bangkok, Thailand. Comparison of microscopic agglutination, culture and molecular tools for diagnosis of pathogenic Leptospira in asymptomatic dogs.