



UNIVERSITI PUTRA MALAYSIA

***DETECTION OF ANTIBODIES AGAINST LEPTOSPIRA SEROVARS
IN DOGS AND CATS IN AN INDIGENOUS VILLAGE, BELUM,
PERAK***

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**DETECTION OF ANTIBODIES AGAINST *LEPTOSPIRA* SEROVARS IN
DOGS AND CATS IN AN INDIGENOUS VILLAGE, BELUM, PERAK**

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**A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia**

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It is hereby certified that we have read this project paper entitled “Detection of Antibodies Against Leptospira Serovars in Dogs and Cats in an Indigenous Village, Belum, Perak” by Choong Jia Jie and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 Final Year Project.

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CONTENTS

	Page number
TITLE	1
CERTIFICATION	2-3
ACKNOWLEDGEMENTS	4
CONTENTS	5-6
LIST OF TABLES	7
LIST OF FIGURES	8
LIST OF ABBREVIATIONS	9
ABSTRAK	10-11
ABSTRACT	12-13
1.0 INTRODUCTION	14-16
2.0 LITERATURE REVIEW	
2.1 Epidemiology of Leptospirosis	16-18
2.2 Pathogenesis and Clinical Features of Leptospirosis	18-19
2.3 Seroprevalence of Canine and Feline Leptospirosis in Worldwide	20-22
2.4 Leptospirosis status in Malaysia	22-24
2.5 Diagnosis of Leptospirosis	24-26
2.6 Potential Risk of Leptospirosis among Indigenous People	26-27
3.0 MATERIALS AND METHODS	
3.1 Sample Collection	28
3.2 Transportation and Storage of Samples	28
3.3 Microscopic Agglutination Test (MAT)	28-30

4.0 RESULTS	31-36
5.0 DISCUSSION	37-41
6.0 CONCLUSION	42
7.0 RECOMMENDATIONS	42
8.0 REFERENCES	43-50
9.0 APPENDICES	51



LIST OF TABLES

	Page
Table 1 : Seroprevalence of Canine Leptospirosis in Various Countries	19
Table 2 : Seroprevalence of Feline Leptospirosis in Various Countries	20
Table 3 : Prevalence of Leptospirosis in Malaysia	22
Table 4 : Data of Animals (dogs and cats) Collected	29



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LIST OF FIGURES

	Page
Figure 1 : Sterile 96-Wells Microtiter Plate Containing Serum Sample, Positive Control and Negative Control	28
Figure 2 : Species of sampled animals (dogs and cats)	30
Figure 3 : Age group of sampled animals	31
Figure 4 : Gender group of sampled animals	31
Figure 5 : Seroprevalence of Leptospirosis of dog's serum samples collected	32
Figure 6 : Percentage of Seropositive Dogs against Gender	32
Figure 7 : Seroprevalence of Leptospirosis of cat's serum samples collected	33
Figure 8 : Percentage of Seropositive Cats against Gender	33
Figure 9 : Map of Royal Belum State Perak	35
Figure 10 : Sungai Tiang, Royal Belum	36
Figure 11 : MAT positive under 200x dark field microscopy	46
Figure 12 : MAT negative under 200x dark field microscopy	46

LIST OF ABBREVIATIONS

μL	microliter
°C	degree Celsius
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked immunosorbent assay
EMJH	Ellinhausen-McCullough-Johnson-Harris
IACUC	Institutional Animal Care and Use Committee
LPHS	Leptospirosis Pulmonary Haemorrhage Syndrome
LPS	Lipopolysaccharide
MAT	Microscopic Agglutination Test
mL	millilitre
n	sample size
OIE	World Organisation for Animal Health
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
pH	Potential Chain Reaction
rpm	round per minute
spp	species
UPM	Universiti Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 Projek Ilmiah Tahun Akhir

PENGESAHAN ANTIBODI TERHADAP SEROVAR *LEPTOSPIRA* DALAM KALANGAN ANJING DAN KUCING DI PERKAMPUNGAN ORANG ASLI, BELUM, PERAK

Oleh

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2018

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Leptospirosis adalah penyakit muncul semula yang menjejaskan manusia dan haiwan. Walaupun dengan insiden yang tinggi, kajian telah dijalankan di Malaysia adalah kurang terutamanya di kawasan pedalaman seperti kampung orang asli di Belum. Wabak jangkitan herpangina virus pada tahun 2015 yang mengorbankan hampir separuh bilangan daripada anak-anak orang asli di kampung itu telah membangkitkan kebimbangan kesihatan awam. Bidang kajian yang terletak di Tiang River, Belum, Perak. Hakikat badan air (tasik buatan manusia), hidupan liar seperti tikus dan tingkah laku orang aslimeletakkan mereka dalam keadaanberisiko tinggi daripada berdedah dengan wabak leptospirosis di kawasan itu. Dalam kajian ini, 40 sampel (37 anjing dan 3 kucing) dikumpul untuk menentukan kewujudan jangkitan leptospiral dari anjing dan status leptospirosis kucing di kawasan tersebut dan mengenal pasti serovar yang dominan. Ujian aglutinasi mikroskopik (MAT) digunakan untuk menentukan kewujudan antibodi anti-leptospiral dalam sampel serum yang dikumpul. Panel ujian termasuk 12 serovar leptospira: *Canicola*, *Pomona*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Australis*, *Pyrogenes*, *Lai*, *Celledoni*, *Bataviae*, *Javanica*, *Hardjo* dan *Copenhageni*. Tiga daripada 37 anjing (8.1%) dan satu daripada 3 kucing (33%) menunjukkan seropositive untuk *Leptospira* pada titisan penentuan 1: 100 dengan mengikuti standard World Organisation for Animal Health (OIE). *Celledoni* 5.4% (n = 2/37) didapati sebagai serovar yang paling dominan, diikuti oleh *Australis* 2.7% (n = 1/37) dan *Lai* 2.7% (n = 1/37). 33% (n = 1/3) sebagai satu-satunya serovar utama bagi kucing yang dkaji. Penemuan ini memaklumkan bahawa risiko zoonosis yang berpotensi tinggi untuk menyebarkan leptospirosis dari haiwan kepada orang asli. dan juga kewujudan *leptospira* dalam persekitaran hidup mereka. Kajian dan penyiasatan yang melanjutkan diperlukan untuk menilai epidemiologi leptospirosis di kawasan tersebut.

Kata kunci: Leptospirosis, seroprevalensi, MAT, asli, terpencil, epidemiologi

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 Final Year Project

DETECTION OF ANTIBODIES AGAINST *LEPTOSPIRA* SEROVARS IN DOGS AND CATS IN AN INDIGENOUS VILLAGE, BELUM, PERAK

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2018

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Leptospirosis is a re-emerging disease that affecting both human and animals. Despite the high incidence, limited study has been conducted in Malaysia especially in indigenous population. A recent outbreak of herpangina viralinfection in year 2015 that wiped out almost half of the population of the indigenous children in the village has raised the public health concern. The targeted area of this study located at Tiang River, Belum state, Perak. Presence of large water bodies (man-made lake), exposure to the wildlife such as rats and living in vicinity with ownerless dogs and cats predispose this indigenous people to leptospirosis in the area. In this study, 40 samples (37 dogs and 3 cats) were collected to detect the presence of leptospiral infection of canine and feline leptospirosis status in the area and identify the predominant serovars. Microscopic agglutination test (MAT) was used to determine the presence of anti-leptospiral antibodies in the serum samples collected. The testing panel included 12 common serovars of *leptospira*: *Canicola*, *Pomona*, *Icterohaemorrhagiae*, *Grippotyphosa*, *Australis*, *Pyrogenes*, *Lai*, *Celledoni*, *Bataviae*, *Javanica*, *Hardjo* and *Copenhageni*. Three out of 37 dogs (8.1%) and one out of three cats (33%) were seropositive for *Leptospira* at the cut-off titer of 1:100 by following World Organisation for Animal Health (OIE) standard. *Celledoni* 5.4% (n=2/37) was found to be the most predominant serovar, followed by *Australis* 2.7% (n=1/37) and *Lai* 2.7% (n=1/37) among the dogs studied while *Lai* 33% (n=1/3) was found in cat as the only predominant serovar. These findings alerted that potential zoonosis risk of spreading leptospirosis from the animals to the indigenous people and also presence of *leptospira* in their living environment. Further study and investigation are crucial to evaluate the epidemiology of leptospirosis in the area.

Keywords: Leptospirosis, seroprevalence, MAT, indigenous, isolated, epidemiology

1.0 INTRODUCTION

Leptospirosis is recognized as a re-emerging zoonotic disease and raised public health concern at recent due to its increasing incidence in worldwide distribution particularly in developing tropical countries like Thailand, India and Malaysia (Tangkanakul *et al*, 2005; Vijayachari *et al*, 2008; Suut *et al*, 2016). This incidence is related to the climatic conditions and geographical topography in the countries that maintain the reservoir vectors in the environment which eventually lead to higher risk of exposure to human and domestic animals (Pappas *et al*, 2008). The exposure is higher when raining season comes into effect. The disease is caused by infection with pathogenic spirochaete bacteria of the genus *Leptospira* and can survive for months in water and moist soil (Alexander, 1975).

Clinical leptospirosis in dogs is more common than in cats (André-Fontaine, 2006; Arbour *et al.*, 2012). Due to this reason, studies on canine leptospirosis is more advanced than feline leptospirosis in both local and global perspective. However, both dogs and cats can shed leptospirosis in their urine without having any clinical presentations of the disease (Rojas *et al.*, 2010; Fenimore *et al.*, 2012; Llewellyn *et al.*, 2013; Rodriguez *et al.*, 2014). Based on the literature, the most common serovars that affecting dogs include Canicola, Icterohaemorrhagiae, Pomona, Grippityphosa and Australia (Koteeswaran, 2006). In cats, the most common serovars include Canicola, Griptyphosa and Pomona (Larsson *et al*, n.d; Jamshidi *et al*, 2009). Despite the development of histological lesions in the kidneys and liver had been reported in experimental infected cats, clinical signs are rarely showed up in nautral infection (Jamshidi *et al*, 2009). It is also stress that cats can excrete potential zoonotic leptospire in their urine up to 3 months following the experimental infection (Willoughby *et al*, 2004).

There was no publication regarding the prevalence of Leptospirosis among isolated group of dogs and cats in indigenous village at the moment. The contribution towards the lack of study on the disease prevalence in the area probably due to unreachable rural area, lack of diagnostic tools and lack of public awareness (Suut *et al*, 2016). The targeted area (Belum indigenous village) for this study

located at the border of Thailand on the north, the state of Kelantan to the east, and Sungai Gadong in the west. The landscape of the area consists of mainly reserved forest, small areas of grassland, and abandoned agricultural lands, and a large man-made lake, Tasik Temengor (World Wildlife Fund - Malaysia). The only human population living in Belum are indigenous people and mostly consists of Jahai community. The presence of wildlife, domestic animals and water bodies have placed high risk factors of harbouring Leptospirosis in the area and hence pose a potential spread of the disease to the indigenous communities. The recent study among rural communities in East Malaysia reported that a seroprevalence of human leptospirosis was 37.4% (n=508) (Suut *et al*, 2016).

The need of studying zoonotic diseases in Belum indigenous village was further supported by the incidence of Jahai community's children were succumbed to a mysterious illness in the year 2015 (R.AGE, 2015; The Star Online, 2015). The final diagnosis was herpangina viral infection which weaken the immune system of the children (The Star Online, 2015). Poor sanitation, unbalanced diets and lack of medical facilities were believed to be the contributing factors to the incidence. Public health concern was raised and study on both human and domestic leptospirosis was initiated by Penang Medical College (PMC) and Universiti Putra Malaysia (UPM) supported by Malaysia One Health University Network (MyOHUN) in the year 2016.

According to Bahaman (1988), a total of 37 serovars had been isolated from both human and animals while rats had been described as the major reservoir of leptospires in Malaysia. The recent studies showed that the seroprevalence of Leptospirosis in non-human primates from Sarawak, Malaysia was 66% (Robertson *et al*, 2014). In addition, another seroprevalence study of Leptospirosis was done on wildlife in Sarawak showed that 80% of monkeys, 44% of rats, 20.8% of bats, 100% of squirrels and 100% of mongoose reacted against one or more serovars of *Leptospira* (Thayaparan, 2014).

The studies on both canine and feline leptospirosis among domestic population in Malaysia is still not sufficient to date, not to mention the isolated population in indigenous village. The objective of this study was to detect canine and feline

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