UNIVERSITI PUTRA MALAYSIA

MOLECULAR PREVALENCE AND CLINICOPATHOLOGICAL ASSOCIATION OF BARTONELLA INFECTION IN CATS PRESENTED TO THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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By

UMMUKULTHUM LAWAL HASSAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Master of Science

June 2015

DEDICATION

Dedicated to my husband Dr. Yusuf Yakubu and kids; Ilham and Ayman

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Master of Science

MOLECULAR PREVALENCE AND CLINICOPATHOLOGICAL ASSOCIATION OF *BARTONELLA* INFECTION IN CATS PRESENTED TO THE UNIVERSITY VETERINARY HOSPITAL, UNIVERSITI PUTRA MALAYSIA

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Chairperson: Assoc. Prof. Gurmeet Kaur Dhaliwal, PhD
Faculty: Veterinary Medicine

Feline bartonellosis is a bacterial infection caused by *Bartonella* species transmitted by the cat flea (*Ctenocephalides felis*). The disease is asymptomatic in a majority of cats but can be transmitted from infected cats to humans via inoculation or ingestion of flea excrement through scratches or bites. Human *Bartonella* infection has a variety of clinical manifestations such as bacillary angiomatosis, endocarditis, neuroretinitis and cat scratch fever. Cat owners and veterinarians who are in direct contact with cats are at risk of infection. However, despite the zoonotic potential of the disease, there is paucity of information on feline and human bartonellosis in Peninsular Malaysia. Hence, this study was conducted to determine the molecular prevalence of *Bartonella* and if the presence of this bacteria is associated with any clinicopathological findings in cats presented to the University Veterinary Hospital (UVH), University Putra Malaysia (UPM).

Of 284 blood samples collected from healthy and ill cats with the median age of 2 years 10 months, presented to the UVH, 48 were PCR positive for the internal transcribed spacer region (ITS) in *Bartonella* species with an overall prevalence of 16.9% (n=48) (95% CI: 12.8-21.9). Univariate analysis of demographic data showed the detection rate to be significantly associated with younger cats, below 2 years of age [OR= 1.690 (95% CI: 0.989-2.889), *p*-value = 0.051]. Ocular discharge was the only clinical sign observed to be associated with the presence of *Bartonella* [OR= 3.211 (95% CI: 1.422-7.248), *p*-value= 0.003], while laboratory results revealed significant association of bartonellosis with neutrophilia [OR: 2.24 (95% CI: 1.131-4.452, *p*-value = 0.019] and monocytosis [OR: 2.476 (95% CI: 1.154-5.312), *p*-value = 0.017]. While these findings are statistically associated, a causal relationship cannot be implied as other concurrent diseases could not be ruled out. Other hematological findings such as anemia, reticulocytosis, lymphocytosis, eosinophilia and azotemia, were not significant.

This study reveals a significant presence of *Bartonella* infection in pet cats with various medical conditions presented to the University Veterinary Hospital, Faculty of Veterinary Medicine, UPM. In view of the potential public health risk of feline bartonellosis especially amongst children, the elderly and immunosuppressed individuals, there is the need to educate cat owners on the latent infection of *Bartonella* and its zoonotic risk and the importance of flea control in their pet cats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGESANAN MOLEKULAR, PREVALENS DAN PENEMUAN KLINIKOPATOLOGi BARTONELLA PADA KUCING DI HOSPITAL UNIVERSITI VETERINAR, UNIVERSITI PUTRA MALAYSIA

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Bartonellosis kucing adalah jangkitan bakteria yang disebabkan oleh spesies *Bartonella* dan disebarkan oleh pinjal kucing (*Ctenocephalides felis*). Penyakit ini adalah asimptomatik dalam majoriti kucing tetapi boleh berjangkit dari kucing yang telah dijangkiti, kepada manusia melalui inokulasi dan pengingesan najis pinjal. Jangkitan *Bartonella* pada manusia mempunyai pelbagai manifestasi klinikal seperti angiomatosis basilari, endokarditis, neuroretinitis dan *cat scratch fever.* Pemilik kucing dan veterinawan yang berhubung secara langsung dengan kucing yang telah dijangkiti, berisiko mendapat jangkitan ini. Walaupun penyakit ini berpotensi zoonotik, terdapat kekurangan maklumat tentang bartonellosis pada kucing dan manusia di Semenanjung Malaysia. Justeru, kajian ini dijalankan untuk menentukan prevalens molekular Bartonella dan sekiranya kewujudan bakteria ini berkaitan dengan penemuan klinikopatologi pada kucing di Hospital Veterinar Universiti (UVH), Universiti Putra Malaysia (UPM).

Daripada 284 sampel darah yang diambil daripada kucing yang sihat dan sakit, berusia di antara 1 bulan hingga 20 tahun (median 2 tahun 10 bulan), di UVH, terdapat 48 sampel yang positif terhadap *internal transcribed spacer region* (ITS) dalam spesies *Bartonella* dengan mengunakan PCR, dan terdapat prevalens sebanyak 16.9 % (n = 48) (95 % sela keyakinan: 12.8-21.9). Analisis univariat data demografi menunjukkan kadar pengesanan yang ketara dengan kucing yang muda, berumur bawah 2 tahun [nisbah kemungkinan = 1.690 (95 % sela keyakinan: 0.989-2.889), nilai-p = 0.051]. Hanya petanda klinikal lelehan okular diperhatikan berkaitan dengan kewujudan *Bartonella* [nisbah kemungkinan = 3.211 (sela keyakinan 95%: 1.422-7.248), nilai-p = 0.003], manakala keputusan makmal menunjukkan hubungan yang signifikan di antara bartonellosis dengan neutrofilia [nisbah kemungkinan: 2.24 (sela keyakinan 95%:1.131-4.45, nilai-p = 0.019] dan monositosis [nisbah kemungkinan: 2.476 (sela keyakinan 95%: 1.154-5.312), nilai-p =0.017]. Walaupun penemuan ini adalah signifikan secara statistik, perhubungan penyebab tidak boleh disangka kerana penyakit serentak tidak boleh disingkirkan. Penemuan hematologi yang

lain seperti anemia, retikulositosis, limfositosis, eosinofilia dan azotemia, didapati tidak signifikan.

Kajian ini menunjukkan jangkitan *Bartonella* yang ketara pada kucing kesayangan yang mengalami pelbagai masalah perubatan di Hospital Veterinar Universiti, Fakulti Perubatan Veterinar, UPM. Memandangkan potensi risiko bartonellosis kucing terhadap kesihatan awam, terutamanya di kalangan kanak-kanak, warga tua dan individu yang mengalami imunosupres, maka terdapat keperluan untuk mendidik pemilik kucing terhadap jangkitan terpendam *Bartonella*, risiko zoonotik dan kepentingan kawalan pinjal pada kucing peliharaan mereka.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory committee were as follows:

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LIST OF ABBREVIATIONS

µg	Micro gram(s)
µl	Micro liter(s)
µm	Micrometer
^{0}C	Degree(s) Celsius
AAFP	American Association of Feline Practitioners
AIDS	Acquired Immuno Deficiency Syndrome
B	Bacteremia
B. spp	*Bartonella* species
b.p	Base pair(s)
BLAST	Basic Local Alignment Search Tool
$C0_2$	Carbon (IV) Oxide
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CDC	Centre for Disease Control
CI	Confidence Interval
CSD	Cat Scratch Disease
d	desired absolute precision (*P value*)
DLH	Domestic Long Hair
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DSH	Domestic Short Hair
EDT	Ethylenediamine tetra acetic acid
ELISA	Enzyme- Linked Immunosorbent Assay

FUO	Fever of Unknown Origin
g	Gram(s)
HIV	Human Immunodeficiency Virus
IACUC	Institutional Animal Care and Use Committee
IFA	Immuno fluorescent Assay
Ig G	Immunoglobulin G
Ig M	Immunoglobulin M
ITS	Internal Transcribe Spacer
Kg	Kilogram(s)
L	Liter(s)
m	Meter(s)
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mg	Milligram
min	Minutes(s)
ml	Milliliter(s)
mm	Millimeter(s)
ng	Nano gram(s)
nm	Nanometer(s)
OR	Odd Ratio
PCR	Polymerase chain reaction
PCV	Packed Cell Volume
P^{exp}	expected prevalence
RBC	Red Blood Cells
Rpm	Round(s) per minutes

rRNA	ribosomal Ribonucleic acid
S	Serology
SD	Standard Deviation
TBE	Tris-Borate-EDTA
UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United States of America
UV	Ultraviolet
UVH	University Veterinary Hospital
VO	Veterinary Officer
WBC	White Blood Cells

CHAPTER 1

INTRODUCTION

Feline bartonellosis is a disease of cats caused by the bacteria *Bartonella*. It was first reported in 1992 following the isolation of the organism in clinical samples of cats (Regnery et al., 1992). The disease is transmitted by blood-feeding arthropods such as the cat flea (*Ctenocephalides felis*), sand flies (*Lutzomyia verrucarum*), human lice (*Pediculus humanus corporis*) and rodent fleas (*Ctenophthalmus nobilis*) (Bown et al., 2004; Chomel et al., 1996). The disease is often asymptomatic with infected cats appearing apparently healthy. However clinical signs such as uveitis, lymphadenomegaly, gingivitis, stomatitis, urinary tract infection and fever have been reported to be associated with severe infection (Breitschwerdt and Kordick, 2000). In experimental studies, cats infected with *Bartonella* developed relapsing bacteremia, granulomatous inflammation of the lymph nodes, liver or spleen, endocarditis and chronic intra-erythrocytic and vascular endothelial infections. Hematological anomalies such as thrombocytopenia, lymphocytosis, neutropenia, and eosinophilia have also been reported (Breitschwerdt, 2008).

Bartonella has a worldwide distribution with high prevalence being reported in environments with conditions favorable for the arthropod vectors to thrive (wet and humid). The disease in animals and humans has been reported in the United States (Nutter et al., 2004), France (Gurfield et al., 2001), Switzerland (Glaus et al., 1997), Netherlands (Bergmans et al., 1997), Denmark (Chomel et al., 2001), Australia (Flexman et al., 1995), Singapore (Nasirudeen and Thong, 1999), Japan (Ueno et al., 1995), Thailand (Assarasakorn et al., 2012), Indonesia (Marston et al., 1999) and the Philippines (Chomel et al., 1999).

Feline bartonellosis is a disease of public health concern, particularly so as infected cats are asymptomatic. Humans in close contact with infected cats are at risk of infection following scratches or bites. Furthermore, immunocompromised individuals such as HIV/AIDs victims, geriatrics, children and pregnant women are at higher risk of infection and could develop an array of clinical conditions such as bacillary angiomatosis, parenchymal bacillary peliosis, relapsing fever with bacteremia, endocarditis, optic neuritis, pulmonary, hepatic, or splenic granulomas, and osteomyelitis (De La Rosa et al., 2001; Fournier et al., 2001; Wong et al., 1995; Relman et al., 1990; Slater et al., 1990). However, the most common *Bartonella* infection in humans is known as cat scratch disease (CSD) which, in immunocompetent individuals, causes localized nodular lesions at the bite or scratch site. The disease is more severe in children with CSD, as encephalopathy and associated neurological signs in addition to rheumatic manifestations have been reported (Al-Matar et al., 2002).

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To date, there are fourteen (14) *Bartonella* species that are considered zoonotic, of which few have been reported to be transmitted to humans from companion animals, particularly cats (Guptill, 2010). The zoonotic species

which are reported to be harbored by domestic cats include *B. henselae, B. clarridgeiae, B. koehlerae, and B. bovis (weissii)*. However, *B. henselae* is the most commonly isolated species from cats and has two genotypes - *B. henselae* I and *B. henselae* II, previously known as *B. henselae* Houston I strain and *B. henselae* Marseilles strain respectively (La Scola et al., 2002; Breitschwerdt and Kordick, 2000). Despite worldwide distribution of human bartonellosis, the disease has always been under reported due to misdiagnosis for other ailments. Antibodies against the pathogen in humans have been reported in western Europe, Sweden (Holmberg et al., 1999), Greece (Karpathios et al., 1998), India (Jacob et al., 2006), Japan (Yoshida et al., 1996) and Australia (Flexman et al., 1995).

In Peninsular Malaysia, cats are popular pets and in many households, have very close attachment with their owners. The cats often live indoors, but also have outdoor access, thus increasing their risk of exposure to the stray cat population, which may be potential carriers of feline bartonellosis. Despite the risk of *Bartonella* infection in pet cats and its public health significance, there is a paucity of information on feline bartonellosis and cat scratch fever in Peninsular Malaysia. Hence, this study was conducted to determine the prevalence, risk factors and clinicopathological findings associated with bartonella infection in cats presented to the University Veterinary Hospital, UPM.

1.1 Hypotheses

The hypotheses of this study are:

i. Cats presented to the UVH-UPM with various disease conditions are infected with *Bartonella* species
ii. Feline bartonellosis is associated with some clinical pathology and hematological anomalies.

1.2 Study Objectives

The objectives of this study were:

i. To determine the prevalence of *Bartonella species* in cats using molecular techniques.
ii. To determine if a statistical association exists between the presence of *Bartonella* spp and clinicopathological findings in cats.

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