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MOLECULAR PREVALENCE OF HAEMPATHOGENS INFECTING SYNANTHROPIC RODENTS IN SELANGOR AND KUALA LUMPUR, MALAYSIA

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MOLECULAR PREVALENCE OF HAEMPATHOGENS INFECTING SYNANTHROPIC RODENTS IN SELANGOR AND KUALA LUMPUR, MALAYSIA

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CERTIFICATION

It is hereby certified that I have read this project entitled "Molecular Prevalence of Haempathogens Infecting Synanthropic Rodents in Selangor, Malaysia", by Siti Nurliyana Binti Ab Aziz and in my opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirements for the course VPD 4999- Final Year Project,

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
=	Equal
-	Negative
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
IACUC	Institutional Animal Care and Use Committee
PCR	Polymerase Chain Reaction
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.

PREVALENS MOLEKULAR HEMOPATOGEN YANG MENGJANGKITI TIKUS SINANTROPIK DI SELANGOR DAN KUALA LUMPUR, MALAYSIA

Oleh

SITI NURLIYANA BINTI AB AZIZ

2022

Penyelia: Dr Reuben Sunil Kumar Sharma

Tikus sinantropik hidup berdekatan dengan manusia dan mempunyai adaptasi hidup yang tinggi terhadap persekitarannya. Mamalia kecil ini adalah vektor penting pelbagai patogen zoonosis yang dapat menggugat kesihatan awam. Patogen ini boleh disebarkan melalui pelbagai cara termasuklah vektor atropoda. Tikus terkenal dengan menanggung pelbagai patogen hemotropik yang mungkin menjadi kebimbangan zoonosis. Kajian ini bertujuan untuk menentukan kelaziman molekul haemopatogen terpilih iaitu *Bartonella* spp., *Hepatozoon* spp. and *Trypanosoma*

lewisi, dalam pelbagai lokaliti di Selangor dan Kuala Lumpur, Malaysia. Sebanyak 29 sampel darah tikus telah dikumpulkan dari pelbagai habitat termasuk kawasan perumahan, kawasan komersial (pasar basah, dan lot kedai) serta taman, dan pinggir hutan. Tikus yang terperangkap telah dibius dengan dietil eter dan dibius dengan tiletamine/zolazepam. Setiap tikus telah dikenal pasti, dan darah dikumpulkan untuk pengekstrakan DNA genomik. Serpihan separa gen *Bartonella* ITS, gen *Hepatozoon* 18SrRNA dan Trypanosoma lewisi ITS telah diamplifikasi menggunakan primer khusus genus. Fisher's Exact Test digunakan untuk menentukan perkaitan antara spesies, jantina dan habitat persekitaran dengan kehadiran hemopatogen. Prevalens tertinggi sebanyak 34.5% diperhatikan untuk *Bartonella* spp. yang merupakan patogen yang berpotensi zoonosis. *Hepatozoon* spp. diperhatikan dengan prevalens 27.6%. Walau bagaimanapun, prevalens yang lebih rendah sebanyak 3.4% diperhatikan untuk *Trypanosoma lewisi*. Tiada perkaitan yang signifikan ($p>0.05$) untuk jangkitan oleh hemopatogen ini dalam kalangan tikus berkenaan dengan jantina dan jenis habitat. Oleh itu, kajian semasa memberikan pengetahuan penting tentang kepelbagaian parasit dalam tikus sinantropik, yang akan memberi implikasi ke atas kesihatan tikus, serta kehadiran patogen yang berpotensi zoonosis yang menjadi kembimbangan kesihatan awam.

Kata kunci: *Bartonella; Hepatozoon; Trypanosoma;tikus; Selangor*

ABSTRACT

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

MOLECULAR PREVALENCE OF HAEMPATHOGENS INFECTING SYNANTHROPIC RODENTS IN SELANGOR AND KUALA LUMPUR, MALAYSIA

By

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2022

Supervisor: Dr Reuben Sunil Kumar Sharma

Synanthropic rodents live in proximity with humans and have high adaptability to their surroundings. These small mammals are important vectors of various zoonotic pathogens of public health concern. These pathogens may be transmitted via various routes including carriage by arthropod vectors. Rodents are known to harbour various haemotrophic pathogens that may potentially be of zoonotic concern. This study aimed to determine the molecular prevalence of selected haemopathogens namely

Bartonella spp., *Hepatozoon* spp. *and Trypanosoma lewisi*, in various localities in Selangor and Kuala Lumpur, Malaysia. A total of 29 rodent blood samples were collected from various habitats including residential areas, commercial areas (wet markets, and shop lots) as well as parks, and forest fringes. Trapped rodents were sedated with diethyl ether and anesthetized with tiletamine/zolazepam. The rodents were identified, and blood was collected for genomic DNA extraction. A partial fragment of the *Bartonella* ITS gene, *Hepatozoon* 18SrRNA gene and *Trypanosoma lewisi* ITS were amplified using genus-specific primers. Fisher's exact test was used to determine the association between the species, gender and environmental habitat to the presence of the haemopathogens. A high prevalence of 34.5% was observed for *Bartonella* spp. which is a potentially zoonotic pathogen. *Hepatozoon spp.* was observed with the prevalence of 27.6%. However, a lower prevalence of 3.4% is observed for *Trypanosoma lewisi.* There was no significant ($p>0.05$) association for infection with this haemopathogen among the rodents with regards to gender and habitat type. Thus, current study provides significant knowledge on the diversity of parasites in synanthropic rodents, which will have implications on the health of the rodents, as well as the presence of potentially zoonotic pathogens of public health concern.

Keywords: *Bartonella; Hepatozoon; Trypanosoma;* rodents; Selangor

1.0 INTRODUCTION

1.1 Background

Synanthrops include undomesticated animals and plants that inhabit the human living environment and its approximate surroundings. Synanthropic rodents are of primary importance since they act as efficient reservoirs and host many zoonotic pathogens of public health concern (Torres-Castro, 2017). These small mammals inhabit a diverse array of habitats, and with their high adaptability, are able to thrive in urban environments worldwide. The rapid destruction of forested habitats, the conversion of land use for urban development, and the lack of proper garbage disposal and sewerage, has escalated the population of urban rodents.

Haemopathogens of rodents including haemotropic bacteria, rickettsias and protozoa, consists of important zoonotic pathogens that are efficiently transmitted via arthropod vectors to other animal species as well as humans. *Bartonella* spp. are notable zoonotic bacteria that have been reported to infect rodents since the mid-twentieth century (Gutiérrez *et al*., 2015). The infection is associated with biting arthropods, namely fleas which are capable to act as carriage of the highest diversity of *Bartonella* with high transmission efficiency (Gutiérrez *et al*., 2015). *Hepatozoon* spp. are known to infect rodents and various other vertebrate species worldwide. These apicomplexan alveolates

belong to a group collectively known as haemogregarines, all of which have a heteroxenous cycle involving an intermediate vertebrate host and a blood feeding definitive invertebrate host (Smith, 1996). The infection of rodents with *Trypanosoma* spp. is rampant worldwide and these include rodent-specific as well as potentially zoonotic species (Pumhom *et al*., 2015). To date there remains a dearth of published information on rodent-borne haemopathogens in Malaysia. The present study aims to determine the molecular prevalence of selected rodent haemopathogens, namely *Bartonella, Hepatozoon* and *Trypanosoma*, in the peridomestic environment in Selangor and Kuala Lumpur, Malaysia.

1.2 Justification

Molecular detection of haemopathogen species in synanthropic rodents will provide fundamental information on the potential for zoonotic disease carriage in the peridomestic environment. In addition, an evaluation of rodent hosts and environmental risk factors will provide a basis for formulation of control and prevention measures to safeguard public health.

1.3 Study Objectives

1. To determine the prevalence of *Bartonella*, *Hepatozoon* and *Trypanosoma* in synanthropic rodents in Selangor, Malaysia using molecular methods.

2. To ascertain the diversity of these haemopathogens in relation to intrinsic rodent factors (species, gender) and environmental habitat type.

1.4 Study Hypothesis

H0: Synanthropic rodents will harbour a diversity of haemopathogens which are related to host species and environmental habitat type.

H1: Synanthropic rodents will harbour a diversity of haemopathogens which are not related to host species and environmental habitat

2.0 LITERATURE REVIEW

2.1 Parasites of rodents in Malaysia

Rodents mainly to those belonging to the family Muridae form the largest group of mammals in Malaysia (Ow-Yang, 1971). In a previous study against the synanthropic wild rodents in Malaysia, the first prevalence of parasites populated in wild rodents was reported by Adams in 1933 (Adams, 1933). In 1971, 999 wild rats were examined and reported for nematodes (Singh & Chee-Hock, 1971). Later in 1977, Lim *et al.* (1977) reported about the prevalence of Capillaria infection in wild rodents, covering the study area from the States of Kelantan, Selangor and Johor in Peninsular Malaysia.

2.2 Haemoparasite in rodents in Malaysia

Paramasvaran *et al.* (2009) stated that rodents act as reservoir hosts for many zoonotic pathogens including parasites that can constitute a great risk to human health. Few studies that were done previously show scarce information regarding haemoparasite in wild rodents' population in Malaysia. Twenty-seven rats were examined in an Orang Asli village in Bukit Kemandol, Selangor back in 2003 by Paramasvaran *et al.* but the presence of blood parasite was failed to be detected (Paramasvaran *et al*., 2003). Correspondingly in 2010, blood screening results from 10 rats captured from the surroundings of Veterinary Research Institute (VRI) Ipoh revealed negative in the presence of blood parasites (Premaalatha *et al.*, 2010). However, Siti Shafiyyah *et al.* (2012) reported the presence of *T. lewisi* at low prevalence (1.5%) from 137 wild rats trapped in Kuala Lumpur.

2.2.1 *Bartonella* spp.

The genus *Bartonella* contains a diversity of emerging, notably zoonotic, gram-negative, facultative intracellular bacterium that have been reported to mostly infect rodents as it serves as the most common wildlife host of *Bartonella* since the mid-twentieth century (Buffet *et al.*, 2013; Gutierrez *et al.*, 2015; Regier *et al.*, 2016). Even though the transmission of *Bartonella* between the animal host is known to be primarily *via* ectoparasite vectors such as fleas, mites and ticks, the association between the diversity of *Bartonella* spp. to the expanding range of mammalian hosts and potential arthropod vectors, providing that the ecology of these particular bacteria is complex and poorly understood (Lei & Olival, 2014; Regier *et al.*, 2016; Kosoy *et al.*, 2017; Blasdell *et al.*, 2019)

2.2.2 *Hepatozoon* spp.

Hepatozoon are blood parasite under phylum Apicomplexa that are known to use rodents and diverse range of other vertebrate species worldwide as the member of this genus display heteroxenous life cycle of oocysts form in invertebrate definitive hosts (Smith, 1996; Baneth *et al.*, 2003). Laakkonen *et al.* (2001) described that the sexual stage of *Hepatozoon* (sporogony) take places in different blood-sucking arthropods while the asexual phase mainly resides in the endothelial cells of various organs or parenchymal cells of liver specifically to the species of the parasite and its intermediate hosts. However, the intracellular gametocytes often reside in erythrocytes or leukocytes. (Laakkonen *et al.*, 2001)

2.2.3 *Trypanosoma lewisi*

Trypanosomes are a type of flagellated blood protozoa that are infecting both warm-blooded and cold-blooded mammals. The infection of rodents with *Trypanosoma* is rampant worldwide and these include rodent-specific as well as potentially zoonotic species (Pumhom *et al.*, 2015). *Trypanosoma lewisi* is a rodents-specific blood parasite that is commonly transmitted by various species of rat flea via direct infestation, direct contact with feces of infected fleas or ingestion of the infected fleas (Kamaruzaman *et al.*, 2021). This rodent-specific blood parasite can be found widely in urban and wild rats, mainly in Asia (Rayat *et al.*, 2014; Pumhom *et al.*, 2015)

3.0 MATERIALS AND METHODS

3.1 Ethical approval

This study was conducted after approval by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia with the reference no: UPM/IACUC/AUP-U045/2022

3.2 Sampling method

Twenty-nine rodents were captured around the study areas mentioned using standard wire-mesh traps and transported back to the Parasitology Laboratory, Faculty of Veterinary Medicine, UPM Serdang. Two types of baits were used in the trapping process which are salted fish and burnt coconut.

3.3 Rodent Identification

Morphometric measurements (weight, tail length, head and body length) were recorded and the gender was determined. The rodents were identified to species using taxonomic references (Pimsai *et al.*, 2014).

3.4 Anaesthetisation and Blood Collection

The rodents were sedated subsequently anaesthetized with zolazepam/tiletamine intramuscularly. Blood was drawn using 3mL syringes and 23-gauge needles via cardiac

puncture. The collected blood wasl be placed in EDTA tubes and transferred into individually labelled 2mL microcentrifuge tubes for storage at -80°C.

3.6 DNA extraction and PCR amplification

Genomic DNA was extracted from the blood samples using a conventional extraction kit (DNeasy Blood & Tissue Kit, Qiagen) according to the manufacturer's protocol. Molecular detection of *Bartonella, Hepatozoon* and *Trypanosoma* DNA was performed by amplification of a partial fragment of the ITS and 18SrRNA using genus-specific primers. (Table 1). The amplification by the PCR for each species was performed with total of 25µL reaction mixture consisting of 8.4µL sterile distilled water, 10.6µL of GoTaq® G2 Hot Start Master Mixes, 1µL of forward primer, 1µL of reverse primer and 4µL of DNA template. The amplification was done using Bio-Rad C1000 Touch Thermal Cycler. The PCR products were run on 1.5% agarose for electrophoresis and later visualised in a gel documented system UV transillumination.

3.9 Statistical Analysis

To determine the prevalence of *Bartonella, Hepatozoon* and *Trypanosoma lewisi*, collected data was analysed using XLSTAT Cloud where percentage of each prevalence was calculated. Other than that, the prevalence of each haemopathogens to differences of gender, rodent species and locations were also evaluated. The association of each haemopathogens to respective gender, location and breed were also analysed using Fisher's Exact Test. The difference was considered significant if $p<$**0.05**.

Table 1: Primers used for PCR detection of *Bartonella, Hepatozoon* and *Trypanosoma lewisi* from the blood samples collected from rodents.

Parasite	Primers	Genes	Size	References
Bartonella	321s F (5' – AGATGATGATCCCAAG CCTTCTGG - 3') 983as R (5' – TGTTCTYACAACAATG ATGATG - 3')	ITS	453bp - 704bp	Maggi and Breitschwerdt, 2005 Hou *et al*., 2018
Hepatozoon	HepF300 (5' - GTTTCTGACCTATCAGC TTTCGACG – 3') Hep900 (5' - CAAATCTAAGAATTTC ACCTCTGAC – 3')	18SrRNA	600bp	Ujvari *et al*., 2004

Trypanosoma lewisi	LEW1S (5' - ACCACCACACGCTCTCT TCT – 3') LEW1R (5' - TGTATGTGCGTGCTTGT TCA – 3')	ITS	220bp	Marc *et al*., 2011

4.0 RESULTS

Overall, from the total sample of 29 rodents captured, 10 out of 29 rodents were tested positive for *Bartonella*. Eight out of 29 rodents were tested positive for *Hepatozoon* while only one out of 29 rodents was tested positive for *Trypanosoma lewisi*. Thus, the result shows high prevalence of *Bartonella*, among other haemopathogens tested, at 34.5% as compared to Hepatozoon and *Trypanosoma lewisi* which at 27.6% and 3.4%, respectively.

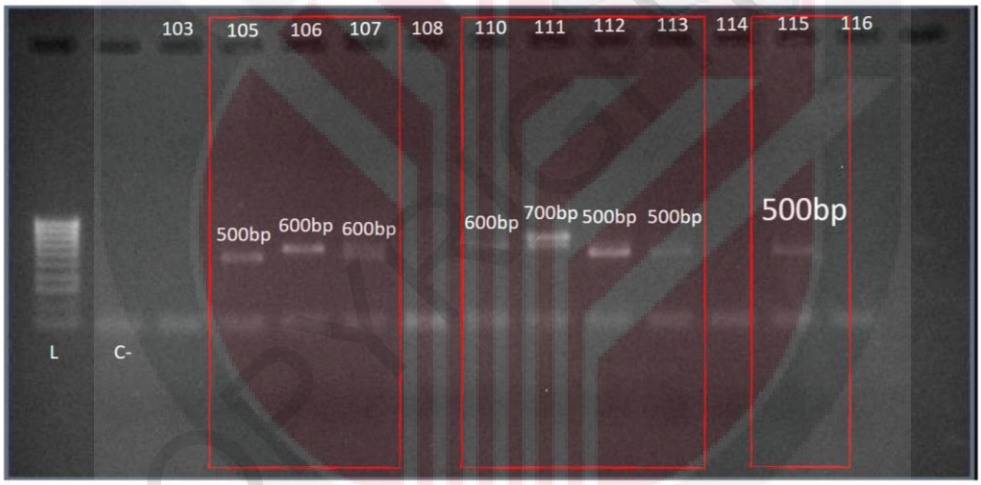

Figure 1: Agarose gel electrophoresis showing the amplification of *Bartonella* ITS gene with 321s F and 983as R primers. Approximately a range of 500 bp to 700 bp was obtained from the positive sample of *Bartonella*. Lane L = DNA ladder and lane C- = negative control

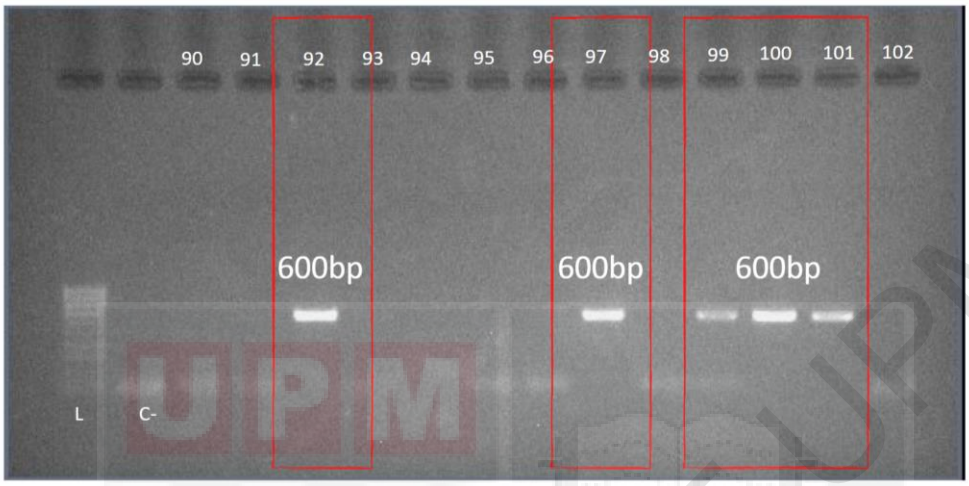

Figure 2: Agarose gel electrophoresis showing amplification of *Hepatozoon* 18SrRNA gene with HepF300 and Hep900 primers. Approximately 600 bp was obtained from the positive sample of *Hepatozoon*. Lane L = DNA ladder and lane C- = negative control

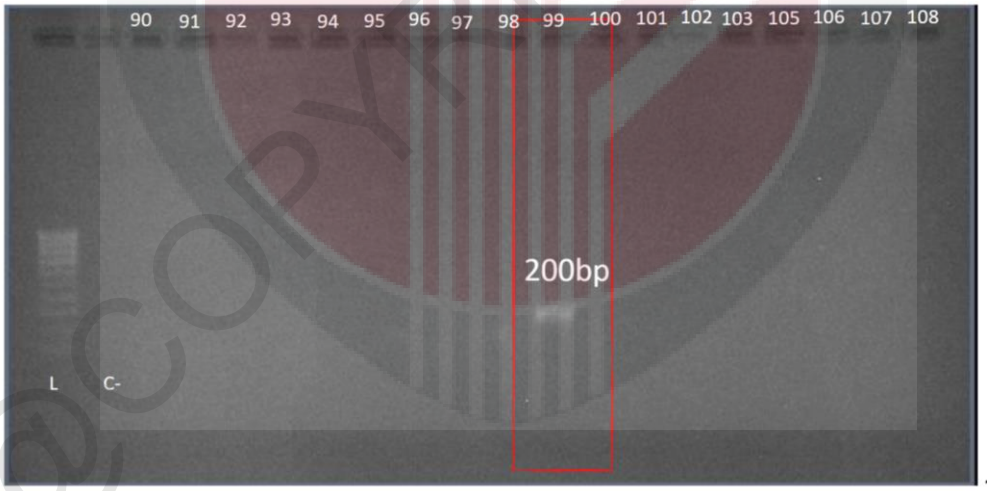

Figure 3: Agarose gel electrophoresis showing amplification of Trypanosoma lewisi ITS gene with LEW1S and LEW1R primers. Approximately 200 bp was obtained from the positive sample of Hepatozoon. Lane L = DNA ladder and lane C- = negative control

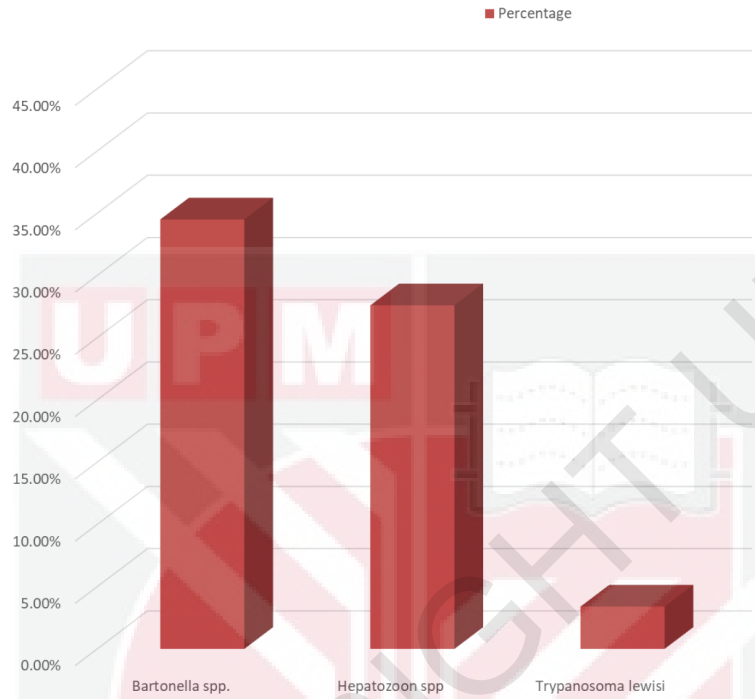

Figure 4: Prevalence of *Bartonella*, *Hepatozoon* and *Trypanosoma lewisi* among rodents in Selangor and Kuala Lumpur, Malaysia. (n=29).

Rodents with single infection were identified where 12 out of 29 rodents were tested positive for only *Bartonella*, six others were tested for only *Hepatozoon* and no rodents were tested positive for only *Trypanosoma lewisi*. Different types of infection can also be observed in this study. Dual infections of *Bartonella* and *Hepatozoon* were only managed to be obtained where three out of 29 rodents were tested positive. However, one out of 29 rodents were tested positive for all haemopathogens (Table 2).

Table 2: Co-infection of *Bartonella*, *Hepatozoon* and *Trypanosoma lewisi* among rdents from Selangor and Kuala Lumpur.

Type of infection		Number of rodents
Single infection	*Bartonella*	6
	Hepatozoon	4
	Trypanosoma lewisi	0
Dual infection	*Bartonella & Hepatozoon*	3
	Bartonella & Trypanosoma	0
	Hepatozoon & Trypanosoma	0
Triple infection with *Hepatozoon, Hepatozoon & Trypanosoma*		1

The association of each heamopathogens prevalence to the rodents' gender, species and location was assessed by contingency table analysis using Fisher's Exact Test. The results showed that there was no significant association between the rodent's gender, breed and location ($p>0.05$) to the prevalence of respective haemopathogens. (Table 3, Table 4 and Table 5).

Table 3: Prevalence and association of *Bartonella* with the gender, species and location of rodents sampled from Selangor and Kuala Lumpur.

Parameters		n	Positive *Bartonella*	P-value
Gender	Male	13	4 (30.7%)	1
	Female	16	6 (37.5%)	
	Total	29	10 (34.5%)	
Species	*Rattus rattus*	17	6 (35.3%)	1
	Rattus norvegicus	10	3 (30%)	
	Rattus tiomanicus	2	1 (50%)	
	Total	29	10 (34.5%)	
Location	Market	22	9 (40.9%)	0.626
	Shoplot	1	0 (0%)	
	Forest	3	0 (0%)	
	Park	3	1 (33.3%)	
	Total	29	10 (34.5%)	

Table 4: Prevalence and association of *Hepatozoon* with the gender, species and location of rodents sampled from Selangor and Kuala Lumpur.

Parameters		n	Positive *Hepatozoon*	P-value
Gender	Male	13	5 (38.5%)	0.46
	Female	16	3 (18.8%)	
	Total	29	8 (27.6%)	
Species	*Rattus rattus*	17	5 (29.4%)	0.694
	Rattus norvegicus	10	2 (20%)	
	Rattus tiomanicus	2	1 (50%)	
	Total	29	8 (27.6%)	
Location	Market	22	5 (22.7%)	0.856
	Shoplot	1	0 (0%)	
	Forest	3	2 (66.7%)	
	Park	3	1 (33.3%)	
	Total	29	8 (27.6%)	

Table 5: Prevalence and association of *Trypanosoma lewisi* with the gender, species and location of rodents sampled from Selangor and Kuala Lumpur.

Parameters		n	Positive *Trypanosoma lewisi*	P-value
Gender	Male	13	0 (0%)	1
	Female	16	1 (6.25%)	
	Total	29	1 (3.4%)	
Species	*Rattus rattus*	17	0 (0%)	0.069
	Rattus norvegicus	10	0 (0%)	
	Rattus tiomanicus	2	1 (50%)	
	Total	29	1 (3.4%)	
Location	Market	22	0 (0%)	0.241
	Shoplot	1	0 (0%)	
	Forest	3	0 (0%)	
	Park	3	1 (50%)	
	Total	29	1 (3.4%)	

5.0 DISCUSSION

This current study had managed to provide current insight of the possible infection of haemopathogens of Bartonella, Hepatozoon and *Trypanosoma lewisi* among rodents in Selangor and Kuala Lumpur, Malaysia where these haemopathogens have high potential of causing zoonotic infection. In this study, the results revealed the highest overall prevalence of 34.5% was for *Bartonella*. This particular result may indicate that *Bartonella* haa the highest infection activity in rodents in Selangor and Kuala Lumpur, Malaysia, as compared to *Hepatozoon* and *Trypanosoma lewisi*. The high prevalence of *Bartonella* among the rodents is similar to that reported by Blasdell *et al.* (2019) where highest prevalence of *Bartonella* spp. was also detected in the urban area in Sarawak.

This recent study also observed the lowest prevalence of *Trypanosoma lewisi,* similarly to the previous study done by Alias *et al.* in 2013 in the urban area in Peninsular Malaysia. Another study by Shafiyyah *et al*. (2012) also recorded the low prevalence of *Trypanosoma lewisi* at 1.5% from 137 wild rodents caught in Kuala Lumpur.

This current study revealed that prevalence of *Trypanosoma lewisi* in female rodents was higher (6.25%) and the prevalence was also higher in *Rattus tiomanicus* (50%). These findings were contraindicated with a previous study by Alias *et al*. (2019), who presented that infections of *Trypanosoma lewisi* were slightly higher in males (28.9%) compared to females (21.7%), and the infection was the highest in *Rattus rattus diardii* (62.2%).

However, the result of the current study may be due to the small sample size that was obtained from the study, in which only a single rodent was infected with *Trypanosoma lewisi*.

6.0 CONCLUSION

The present study has presented the molecular prevalence and diversity of haemopathogens infecting synanthropic rodents in Selangor and Kuala Lumpur, Malaysia. *Bartonella* was the most prevalent haemopathogen compared to *Hepatozoon* and *Trypanosoma* among the rodents sampled. However, there is no association ($p>0.05$) between the intrinsic factors of the rodents (species and gender) and the environmental habitat of the rodent to the diversity of the haemopathogens observe in this study.

7.0 RECOMMENDATIONS

Future studies should consider extending the sampling habitats to other peridomestic habitats as well as increasing the diversity of rodent species sampled. The increase in habitats and sample size will facilitate more rigorous statistical analyses to be carried out. This will enable epidemiological risk factor analysis to be performed to ascertain the relative risk of exposure to humans as these haemopathogens especially *Bartonella* may potentially be zoonotic and is of public health concern.

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