

# Detection and molecular identification of blood parasites in rodents captured from urban areas of southern Sarawak, Malaysian Borneo

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## Abstract

**Background:** Rodent species are well known for their potential as hosts and reservoirs for various zoonotic diseases. Studies on blood parasite infection in small mammals focused on urban cities in Peninsular Malaysia and have been conducted over the years. In contrast, there are information gaps related to molecular detection of blood parasites in urban areas of Sarawak that are associated with veterinary importance and zoonotic spillover potential. Increasing prevalence and transmission of blood parasite diseases is the most crucial public health issue, particularly in developing urban areas of Sarawak. Therefore, molecular identification studies were performed to determine and identify the blood parasites infecting rodents.

**Methods:** A total of 40 rodent blood samples were analysed for blood parasite infection and a combined approach using polymerase chain reaction-based technique, and traditional microscopic examination (blood smear test) was conducted. 18s rRNA (*Plasmodium* spp.) and cytochrome b (*Hepatocystis* spp.) gene marker were used to identify the blood parasites.

**Results:** Note that 67.5% ( $n = 27$ ) blood samples were tested negative for blood parasites, while 32.5% ( $n = 13$ ) blood samples collected were infected with at least one protozoan parasite. Out of 13 samples, 69.2% ( $n = 9$ ) were detected with *Hepatocystis* sp., while 15.4% ( $n = 2$ ) were positive with *Hepatozoon ophisauri*. Two individuals had multiple infections from both species. No *Plasmodium* spp. have been detected throughout this study using universal primer (targeted *Plasmodium* spp.); however, different parasite species which were *H. ophisauri* were detected.

**Conclusion:** Although there is no evidence of human infection from *H. ophisauri* and *Hepatocystis* sp. detected from the study, the data show the host species are heavily infected, and the information is essential for future prevention of zoonotic outbreaks and surveillance programmes. Therefore, it is suggested that the surveillance

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programmes should be incorporated in targeted areas with a high risk of disease emergence.

#### KEYWORDS

blood parasites, gene marker, molecular identification, rodent, zoonotic spillover

## 1 | INTRODUCTION

A parasite is a pathogen that injures and derives the host's sustenance and extracts metabolics from their host organisms to achieve its life cycle and transmit disease and act as vectors (Goltenboth et al., 2006). There are three main classes of parasites: helminths, ectoparasites, and blood protozoa (Smyth & Wakelin, 1994). *Plasmodium*, *Leishmania*, *Trypanosoma*, *Toxoplasma gondii*, and *Babesia* are the most common blood parasites in humans, animals, and vectors (Chen et al., 2016). Zoonosis is a disease in which the infectious agent is transmitted from animals to humans. Over 1 billion zoonotic disease cases are recorded every year, and zoonotic pathogens are known for the most outbreaks of novel pathogens worldwide (Han et al., 2015).

Rodents are recognised as a reservoir host of various zoonotic pathogens diseases and can also transmit diseases to humans (Raj et al., 2009). Rodents have high adaptability into a wide range of habitats worldwide. The adaptability is remarkable for their adjustment to human-induced environmental changes, resulting in adaptation to anthropic environments (Auffray et al., 2009; Mustapha et al., 2019). In Borneo Island including the state of Sarawak, 63 known species of rodents have been identified and documented (Phillipps & Phillipps, 2016). *Rattus norvegicus*, *Rattus rattus*, and *Mus musculus* are the most common rodent species in an urban area due to their remarkable acclimation to being in close proximity to people and also their rapid reproduction (Mustapha et al., 2019).

In parasitic diagnostic laboratories, conventional methods (optical microscopies) are used to identify parasitic species based on their morphological characteristics and life history, host symptoms, infected vertebrate taxa, and insect vectors (Perkins & Schall, 2002). There are challenges in the blood parasite identified through traditional methods of blood films, mainly for unknown species. Indeed, the morphological characteristics of blood parasite species can vary with infections, particularly parasites sampled from different hosts (Martinsen et al., 2006). Only experienced parasitologists can positively identify blood parasites. Therefore, as a result of this difficulty, molecular biology techniques have been used and introduced to help diagnose parasitic structures and identify species that are unprecedented and difficult to identify. Currently, parasitologists can use morphological characteristics or molecular recognition, as well as a combination of two techniques to describe and identify the parasite species being investigated (Martinsen et al., 2006).

Studies on blood parasite information of small mammals pertaining to urban cities in Peninsular Malaysia have been conducted over the years (Alias et al., 2014; Premaalatha et al., 2018a; Shafiyah et al.,

2012). However, not much published data are available for Malaysian Borneo, especially Sarawak. There are poor knowledge and information gaps regarding molecular detection of blood parasites in urban areas of Sarawak as well as the current status of the potential public health importance of parasitic diseases reported or documented, especially in Malaysia's tropical rainforest climate. Based on the previous study, the development and expansion of certain areas or regions can cause serious ecological changes that have irreversibly changed the composition and structure of terrestrial rodents, as well as including their parasites populations indirectly (Morand & Bordes, 2015). Conversely, this kind of study has not been reported throughout Sarawak. As Sarawak nowadays are facing rapid development and urbanisation with rapid intensification of agriculture, socioeconomic change, and ecological fragmentation, which can have profound impacts on the epidemiology of infectious diseases, the effect of this development needs to be considered and addressed.

This study may serve as preliminary data for epidemiological parasitic infection from rodents in southern Sarawak, especially in Kota Samarahan. Therefore, the objectives of this research are to detect the blood parasite species found in rodents in southern Sarawak and to identify the blood parasite species present using molecular tools with 18s rRNA and cytochrome b gene as the gene markers.

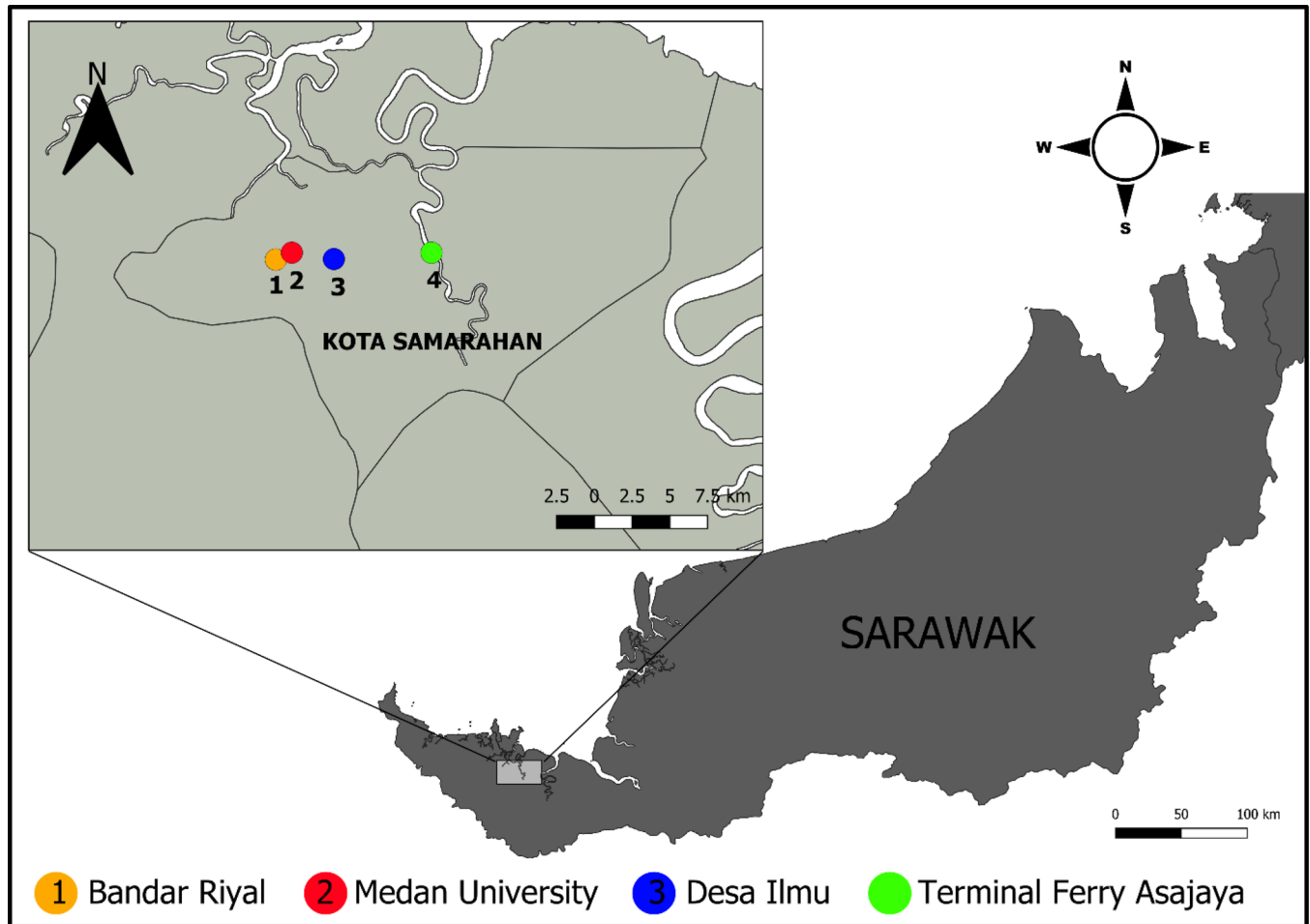
## 2 | MATERIALS AND METHODS

### 2.1 | Study sites

Samples were collected from June 2018 until March 2020 at four different urban areas close to human settlement in Kota Samarahan, Sarawak (1.4599° N, 110.4883° E), namely Desa Ilmu (1.4538° N, 110.4458° E), Medan University (1.4596° N, 110.4208° E), Terminal Ferry Asajaya (1.4596° N, 110.5039° E), and Bandar Riyal (1.4555° N, 110.4113° E) (Figure 1). All study sites are located in the southern part of Sarawak, Malaysian Borneo.

### 2.2 | Host and blood collections

One hundred cage traps were set for 2 weeks consecutively at each study site and placed on the ground, drain, and garbage study areas. Shrimp paste, salted fish, banana, and bread were used as baits in the traps. Cage traps were checked twice daily in the morning and evening, and baits were replenished every morning. The trapped rodents were



**FIGURE 1** Maps of study sites in Kota Samarahan, Sarawak

placed in cloth bags and transported to the laboratory for further processing.

In the laboratory, all trapped rodents were measured, such as the total length, head-body length, tail length, and hindfoot, and the weight was also recorded and their species were identified following Phillipps and Phillipps (2016). The rodents were anaesthetised in a container holding pads soaked with excess isoflurane and once the animals were dead, blood was collected from the heart using a 5 ml syringe through the intracardiac route. Two drops of blood were immediately put on a clean slide for thin and thick blood film preparation, while the remaining blood in the syringe was transferred into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) to avoid blood clotting. The collected blood samples were stored and preserved in EDTA vacutainer tubes with proper labels and sent to the molecular ecology laboratory for further molecular detection process.

Two types of blood smear tests are thick and thin smear. A thick blood smear test is conducted by applying a drop of blood sample on a clean slide to identify the existence of parasites, whether it is positive or negative as it assesses a larger blood sample. Meanwhile, a thin blood smear test is performed by applying a drop of blood sample that is dispersed across a large area of a slide with the target of identify-

ing blood parasites. In this study, a thin blood smear was fixed onto a glass slide by immersing in 75% methanol for 1 min, whereas for the thick blood smear test, a drop of blood sample was dripped onto a clean slide and left to dry. Then, the samples for both blood smear tests were immersed in a quick Giemsa stain for 1 min, drained of water, and left to air-dry (Alias et al., 2014).

### 2.3 | Molecular diagnostics

The DNA was extracted according to QIAamp DNA Blood Mini Kit protocols following the manufacturing protocols (Qiagen, Hilden, Germany). The genomic DNA was extracted from the EDTA-treated blood samples, and a 200  $\mu$ l blood sample was used for a single reaction.

Two separate polymerase chain reaction (PCR) protocols were used to target and amplify different regions of 18S rRNA of *Plasmodium* spp. and cytochrome b of *Hepatozoon* spp. (Table 1). *Plasmodium* spp. detection used the forward and reverse primers of Plasmodium F (5'-TTA AAA TTG TTG CAG TTA AAA CG-3') and Plasmodium R (5'-CCT GTT GTT GCC TTA AAC TTC-3') following Schaer et al. (2013). Meanwhile, for *Hepatozoon* spp. detection, the primer followed

**TABLE 1** List of genes and sequences of the primers used for polymerase chain reaction (PCR) protocol

Genes	Organism detection	Primer's name	Primer sequence	PCR product length (bp)	References
18S rRNA	<i>Plasmodium</i> spp. (Universal)	Plasmodium F	F: 5'-TTA AAA TTG TTG CAG TTA AAA CG -3'	900	Schaer et al. (2013)
		Plasmodium R	R: 5'- CCT GTT GTT GCC TTA AAC TTC-3'		
Cytochrome b	<i>Hepatoctystis</i> spp.	HepF3	F: 5'- CTT ACC TTG GGG ACA AAT GAG TTA TT-3'	600	Krishna et al. (2015)
		HepR3	R: 5'- CTC TAG CAC CAA ATG TCA TTT TAA ATT G-3'		

Abbreviations: F, forward; R, reverse.

Krishna et al. (2015) with the forward primer of HepF3 (5'-CTT ACC TTG GGG ACA AAT GAG TTA TT-3') and the reverse primer of HepR3 (5'-CTC TAG CAC CAA ATG TCA TTT TAA ATT G-3') (Table 1).

The PCR tube held a total volume of 25  $\mu$ L master mix consisting of 5  $\times$  Green GoTaq® Flexi Buffer, 10 mM dNTP Mix, 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ M of each primer, and 1.0 units of GoTaq Flexi DNA polymerase (Promega, Madison, USA). A negative control was used to detect contamination for every reaction. The PCR condition for detecting *Plasmodium* spp. underwent 35 cycles of initial denaturation process at 94°C for 30 s, annealing at 50°C for 1.3 min, and finally the elongation process at 72°C for 1 min. Meanwhile, *Hepatoctystis* spp. detection needed 35 cycles of initial denaturation process at 94°C for 30 s, annealing in the range of 47–50°C for 1.3 min, and the elongation process at 72°C for 1 min. The PCR products were visualised by electrophoresis on 1% agarose gel stained with ethidium bromide. Then, the unpurified DNA was sent to Apical Scientific Sequencing for DNA sequencing.

BLAST version 5.0 software was used for data analysis. Nucleotide sequences were compared with the sequence database, and the statistical significance of matches was calculated in percentage of query cover and percentage of identity. Species identification for each sample followed the high percentage match.

### 3 | RESULTS

A total of 40 rodents were captured during this study, and two species of host were identified namely *Rattus tanezumi* ( $n = 39$ ) and *Mus* sp. ( $n = 1$ ). Thirteen out of 40 blood samples (32.5%) collected from the captured rodents were infected with at least one protozoan parasite detected, while 27 out of 40 (67.5%) blood samples were tested negative for blood parasite presence through the blood smear test. All blood sample that tested positive for the presence of blood parasites were from the host, *R. tanezumi*. From 13 blood samples, rodents trapped at Desa Ilmu recorded the highest number of blood samples (46%) infected with blood parasites, whereas the lowest infected samples were from Medan University (7.7%). Most of the infected blood samples were from female rodents (61.5%) compared to male rodents (38.5%). Based on the age of infected rodents, the juvenile stage (46.2%) had the most blood samples infected with blood parasites, whereas the sub-adult stage (15.4%) had the lowest number of blood samples infected with blood parasites (Table 2).

Two species of blood parasites, namely *Hepatoctystis* sp. and *Hepatoctystis ophisauri*, were identified from the genetic analysis using molecular detection. The cytochrome b sequences from *Hepatoctystis* sp. showed the maximum score of 835 from a total score of 835 of the alignments (Table 3). The percentage of the query cover of the sequence aligned with the GenBank is 99%, while the identification percentage is 95.27%. The accession number of the sequences is DQ396529.1 from Kuching, Sarawak localities. Meanwhile, by using 18s rRNA gene, *H. ophisauri* sequences obtained the total score of 1419 from a total score of 1419. The percentage query cover matches 97% of the sequences from the GenBank, while the percentage of identification is 98.87%. The sequences were previously deposited in the GenBank of MN723845.1 from the Czech Republic (Table 3).

*Hepatoctystis* sp. is the most detected blood parasite species infecting the rodents examined, with a total of nine individuals being infected (69.2%), while the remaining two individuals were (15.4%) infected by *H. ophisauri*. In addition, two out of 13 positive samples were found with mix-infected blood parasites in which both species of blood parasites were detected in the sample. *Hepatoctystis* sp. was detected infecting rodents in all study areas, with the highest number of infected individuals recorded were from Desa Ilmu ( $n = 5$ ) compared to other study areas (Table 2). Meanwhile, *H. ophisauri* infection was only recorded from Bandar Riyal and Terminal Ferry Asajaya, with each area recording only an infected individual. Moreover, each blood sample from Bandar Riyal and Desa Ilmu was determined to be infected with a mixed infection of *Hepatoctystis* sp. and *H. ophisauri* (Table 2).

### 4 | DISCUSSION

To the best of our knowledge, this is the first documented evidence regarding molecular detection of blood parasites of urban rodents in Kota Samarahan, Sarawak. The result can be useful references for future investigation on epidemiological parasitic infection of urban rodents in Kota Samarahan, Sarawak, and can also be helpful for future control of parasitic disease mortality or other zoonotic outbreaks in the area.

In this study, dominant rodent species (*R. tanezumi*) was successfully captured and studied for the presence of blood parasites in the urban areas of Kota Samarahan, Sarawak. This finding is appropriate and similar to previous studies, where this rodent species is often reported in human settlements and urban areas (Hamdan et al., 2017;

**TABLE 2** Summary of a total number of host species, host sex, and host-age of rodents infected with blood parasites based on location of sample collected

Location	Host species	Host-sex		Host-age			Blood parasites species		
	<i>Rattus tanezumi</i> (%)	Male (%)	Female (%)	Juvenile (%)	Sub-adult (%)	Adult (%)	<i>Hepatozoon sp. n</i> (%)	<i>Hepatozoon ophisauri n</i> (%)	Mixed infection n (%)
Bandar Riyal	4 (30.8)	1 (20.0)	3 (37.5)	1 (16.7)	1 (50.0)	2 (40.0)	2 (22.2)	1 (50.0)	1 (50.0)
Medan University	1 (7.7)	1 (20.0)	0	1 (16.7)	0	0	1 (11.1)	0	0
Desa Ilmu	6 (46.1)	2 (40.0)	4 (50.0)	3 (50)	1 (50.0)	2 (40.0)	5 (55.6)	0	1 (50.0)
Terminal Ferry Asajaya	2 (15.4)	1 (20.0)	1 (12.5)	1 (16.7)	0	1 (20.0)	1 (11.1)	1 (50.0)	0
Total number	13	5	8	6	2	5	9	2	2

**TABLE 3** Species identification with BLAST analysis

Field number	Location	BLAST analysis result	Maximum identity score	Total score	Query coverage (%)	E-value	Identification percentage	GenBank accession number
AM03	Medan University	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
BR02	Bandar Riyal	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
BR06	Bandar Riyal	<i>Hepatozoon ophisauri</i>	1419	1419	97	0.00	98.87	MN723845.1
BR07	Bandar Riyal	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
		<i>Hepatozoon ophisauri</i>	1419	1419	97	0.00	98.87	MN723845.1
BR09	Bandar Riyal	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
PF04	Terminal Ferry Asajaya	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
PF07	Terminal Ferry Asajaya	<i>Hepatozoon ophisauri</i>	1419	1419	97	0.00	98.87	MN723845.1
WN01	Desa Ilmu	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
WN02	Desa Ilmu	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
WN07	Desa Ilmu	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
WN10	Desa Ilmu	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
WN13	Desa Ilmu	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
WN14	Desa Ilmu	<i>Hepatozoon sp.</i>	835	835	99	0.00	95.27	DQ396529.1
		<i>Hepatozoon ophisauri</i>	1419	1419	97	0.00	98.87	MN723845.1

Ng et al., 2017; Phillipps & Phillipps, 2016; Prompiram et al., 2020). However, compared with a similar study by Alias et al. (2014) in Peninsular Malaysia, urban rats dominantly caught were *Rattus rattus diardii*, followed by *R. norvegicus*, *Rattus exulans*, *Rhinobatos annandalei*, and *Rattus argentiventer*. Therefore, the differences in rodent species caught are influenced by the distribution of rodent species in an area or a habitat.

Rodents are well known as a repository for numerous zoonotic disorders, including toxoplasmosis, babesiosis, and leishmaniasis (Dahmana et al., 2020). *Toxoplasma gondii* and *Leishmania* sp. are commonly found as blood protozoa in rodents, where in Iran, a significant number of rodents species were identified and reported as a cutaneous leishmaniasis reservoir (Seifollahi et al., 2016). Compared to previous studies in Malaysia, *Trypanosoma lewisi* and *Plasmodium* sp. were known as predominantly blood protozoa found in infected rodents (Alias et al.,

2014; Sahimin et al., 2014; Shafiyah et al., 2012). In contrast, in this study, the rodent blood samples were predominantly infected by *Hepatozoon sp.* and *H. ophisauri*. Multiple infections by both blood parasites were also recorded. Therefore, both species of blood parasites recorded in this study are the first record of these blood parasites, especially for the state of Sarawak where rodents are known as their hosts. This research is also a pioneer for further studies in Sarawak.

Two species of blood parasites, namely *Hepatozoon sp.* and *H. ophisauri*, were identified in this study, with the former infecting most of the hosts examined. However, *Hepatozoon* spp. can only be identified until genus level through BLAST analysis because there is a lack of sequence data related to phylogenetic relationships between the *Hepatozoon* species recorded in the GenBank for comparison purposes. Therefore, there are difficulties in identifying positive blood samples with this genus up to the species level, and this is a gap for this study.

The genomic information from common and distantly related species and also the phylogenetic relationship between organisms are important to identify the specific species through BLAST analysis (Cummings et al., 2002). The genus *Hepaticystis* is a parasite in the order Haemospororida, which is the most studied order and known as a malaria agent in humans, and has also been identified to infect the groups of mammals, birds, and reptiles (Boundenga et al., 2017). Mammals of the order Cetartiodactyla, Primates, Rodentia, and Chiroptera are animals commonly infected by blood parasites of the genus *Hepaticystis* (Schaer et al., 2013). Therefore, this genus is relevant to be detected in this study because the host (Rodentia) is one of the groups of mammals known as carriers for these blood parasites.

Moreover, *Hepaticystis* is closely related to the *Plasmodium* species, which is known to cause malaria, and this was evidenced by the phylogenetic analysis showing that *Plasmodium* sp. and *Hepaticystis* sp. are a close relative (Schaer et al., 2018). The life cycle of *Hepaticystis* parasites tends to be incomplete in asexual erythrocytic replication cycles that contradict the *Plasmodium* species. The arthropod vector of *Hepaticystis* is *Culicoides adersi*, also known as biting midges (Schaer et al., 2017). Research by Schaer et al. (2013) also indicates that *Hepaticystis*, a bat malaria parasite, is closely associated with rodent *Plasmodium*.

*Hepatozoon ophisauri* was detected in this study, and this finding is consistent with previous studies, where the genus *Hepatozoon* was also detected in other rodent species in Brazil, such as *Akodon* sp., *Calomys callosus*, *Oligoryzomys flavescens*, and *O. nigripes* (Demoner et al., 2016; Wolf et al., 2016). Compared with studies in Malaysia, *Hepatozoon* sp. has mainly been detected in dogs and cats, and the species detected was *Hepatozoon canis* (Masrin et al., 2019; Prakash et al., 2018; Premaalatha et al., 2010; Premaalatha et al., 2018b). Therefore, rodents and other animal species are possible carriers for this genus because this blood parasite is known to infect domestic and wild animals, including rodents, birds, reptiles, and amphibians (Perles et al., 2019).

Infection from *Hepatozoon* sp. occurred when the intermediate host ingested the definitive host containing oocytes (Sousa et al., 2017). Blood-sucking invertebrates such as ticks, mites, sand flies, tsetse flies, mosquitoes, fleas, lice, reduviid bugs, and leeches are the definitive hosts for *Hepatozoon* spp. (Baneth, 2011). Meanwhile, the intermediate hosts for *Hepatozoon* spp. are mostly vertebrate hosts, such as birds, reptiles, amphibians, and marsupials (Perles et al., 2019). Prey-predator interactions can also act as an essential pathway for transmitting *Hepatozoon* sp. to vertebrate animals (Baneth, 2011; Perles et al., 2019). *Hepatozoon canis* and *Hericium americanum* commonly infect canine species, while *Helicobacter felis* infects feline species (Baneth, 2011). Hepatozoonosis can cause anaemia or even mortality for a severe case in canine species (Baneth, 2011). In contrast, no evidence of *Hepatozoon* sp. infection in humans has been reported. However, the infection with haemoprotezoan in humans can occur through zoonotic spillover events in which the pathogens can move and infect from animals to humans (Plowright et al., 2017).

In this study, 18S rRNA gene was chosen as a genetic marker to detect *Plasmodium* spp. as it has a high level of conservation and also due to the presence of multiple specific variable regions (Leonard et al., 2016). The primer chosen is categorised as a universal primer. There-

fore, the probability through BLAST analysis shows that the detection and presence of *H. ophisauri* are relevant. This is because genus *Hepatozoon* is known as a tick-borne protozoan parasite, and the gene is closely related to *Plasmodium* sp. (Amoli et al., 2012). The fragments in the universal primer may bind with the samples, and *H. ophisauri* can be detected. Moreover, assay sensitivity is the most critical parameter in the detection of blood protozoan (Chen et al., 2016).

This research was conducted in four different localities in Kota Samarahan, Sarawak, close to the human settlement and restaurant area. Even though there is no evidence of zoonotic infections from *Hepaticystis* sp. and *Hepatozoon* sp. in this study, the detection of *Hepatozoon* sp. will aid in infection prevention and treatment control in medical veterinary in Kota Samarahan, Sarawak. The close contact between humans and potential hosts (i.e., rodents) might lead to a significantly high risk of zoonotic infection in humans (Sahimin et al., 2014; Shafiyah et al., 2012). Therefore, in order to reduce the risk of infection among the community, it is suggested that the City Council conducts a systematic garbage disposal system to prevent the transmission of rodent-borne diseases. In addition, the Ministry of Health must also constantly visit public dining premises to ensure the cleanliness of the premises and food safety is guaranteed for the community. Moreover, anthropological research will identify the contributing social and cultural factors. In another aspect, this research will be helpful for the Ministry of Health to control zoonotic spillover and conduct surveillance programmes in case of a future outbreak caused by *H. ophisauri* and *Hepaticystis* sp. as medical or veterinary importance from rodents.

## 5 | CONCLUSION

In conclusion, 13 blood samples from *R. tanezumi* from the urban areas of Kota Samarahan, Sarawak, were detected to be infected with *H. ophisauri* and heavily infected with *Hepaticystis* spp. This research may serve as preliminary data for blood parasite detection and identification from rodents in Kota Samarahan, Sarawak, and the data are essential for future prevention of zoonotic outbreaks and surveillance programmes. Consequently, agencies like the Department of Health and the City Council can play a critical part in the diagnosis for blocking and managing illnesses from rats to humans and they need to be prepared for the incidence of environmental hygiene of emerging zoonotic diseases. It is critical for public health to have a better understanding of numerous complex elements that underpin the potential zoonotic spillover and zoonosis.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### ETHIC STATEMENTS

All procedures in this study have been permitted and approved by the Universiti Malaysia Sarawak Animal Ethic Committee along with permit number UNIMAS/AEC/R/F07/046.

### AUTHOR CONTRIBUTIONS

*Data curation, investigation, visualisation and writing-original draft:* Putri Wulan Dari Perison. *Data curation, investigation, resources, and visualisation:* Nurul-Shafiqah Amran. *Conceptualisation, funding acquisition, project administration, supervision, validation, and writing-review and editing:* Madinah Adrus. *Formal analysis, methodology, validation, and writing-review and editing:* Faisal Ali Anwarali Khan.

### DATA AVAILABILITY STATEMENT

Data that support the findings of this study are openly available in this article

### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.849>.

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### REFERENCES

- Alias, S. N., Sahimin, N., Edah, M. A., & Mohd-Zain, S. N. (2014). Epidemiology of blood parasitic infections in the urban rat population in Peninsular Malaysia. *Tropical Biomedicine*, 31(2), 230–240.
- Amoli, A. R., Khoshnegah, J., & Razmi, G. (2012). A preliminary parasitological survey of *Hepatozoon* spp. infection in dogs in Mashhad, Iran. *Iranian Journal of Parasitology*, 7(4), 99.
- Auffray, J. C., Renaud, S., & Claude, J. (2009). Rodent biodiversity in changing environments. *Agriculture and Natural Resources*, 43(1), 83–93.
- Baneth, G. (2011). Perspectives on canine and feline hepatozoonosis. *Veterinary Parasitology*, 181(1), 3–11.
- Boundenga, L., Perkins, S. L., Ollomo, B., Rougeron, V., Leroy, E. M., Renaud, F., & Prugnolle, F. (2017). Haemosporidian parasites of reptiles and birds from Gabon, Central Africa. *Journal of Parasitology*, 103(4), 330–337.
- Chen, M. X., Ai, L., Chen, J. H., Feng, X. Y., Chen, S. H., Cai, Y. C., Lu, Y., Zhou, X.-N., Chen, J.-X., & Hu, W. (2016). DNA microarray detection of 18 important human blood protozoan species. *PLoS Neglected Tropical Diseases*, 10(12), e0005160.
- Cummings, L., Riley, L., Black, L., Souvorov, A., Resenchuk, S., Dondoshansky, I., & Tatusova, T. (2002). Genomic BLAST: Custom-defined virtual databases for complete and unfinished genomes. *FEMS Microbiology Letters*, 216(2), 133–138.
- Dahmana, H., Granjon, L., Diagne, C., Davoust, B., Fenollar, F., & Mediannikov, O. (2020). Rodents as hosts of pathogens and related zoonotic disease risk. *Pathogens*, 9(3), 202.
- Demoner, L., Magro, N. M., da Silva, M. R. L., de Paula Antunes, J. M. A., Calabuig, C. I. P., & O'Dwyer, L. H. (2016). *Hepatozoon* spp. infections in wild rodents in an area of endemic canine hepatozoonosis in southeastern Brazil. *Ticks and Tick-borne Diseases*, 7(5), 859–864.
- Goltenboth, F., Timotius, K. H., Milan, P. P., & Margraf, J. (Eds.). (2006). *Ecology of insular southeast Asia: The Indonesian archipelago*. Elsevier.
- Hamdan, N. E. S., Ng, Y. L., Lee, W. B., Tan, C. S., Khan, F. A. A., & Chong, Y. L. (2017). Rodent species distribution and hantavirus seroprevalence in residential and forested areas of Sarawak, Malaysia. *Tropical Life Sciences Research*, 28(1), 151–159.
- Han, B. A., Schmidt, J. P., Bowden, S. E., & Drake, J. M. (2015). Rodent reservoirs of future zoonotic diseases. *Proceedings of the National Academy of Sciences*, 112(22), 7039–7044.
- Krishna, S., Bharti, P. K., Chandel, H. S., Ahmad, A., Kumar, R., Singh, P. P., Singh, M. P., & Singh, N. (2015). Detection of mixed infections with *Plasmodium* spp. by PCR, India, 2014. *Emerging Infectious Diseases*, 21(10), 1853–1857.
- Leonard, C. A., Meli, M. L., Novacco, M., & Borel, N. (2016). 18S Ribosomal RNA evaluation as preanalytical quality control for animal DNA. *BioMed Research International*, 2016, 6104323.
- Martinsen, E. S., Paperna, I., & Schall, J. J. (2006). Morphological versus molecular identification of avian Haemosporidia: An exploration of three species concepts. *Parasitology*, 133(3), 279–288.
- Masrin, A., Fazly, A., Aisyah Munira, M. A., Premaalatha, B., Erwanas, A. I., Aisyah Naama, T., Sharil, A. M. Z., Fakhurulisham, R., Zaini, C. M., & Chandrawathani, P. (2019). Common parasitic infections in cats and dogs diagnosed in veterinary research institute, Malaysia from 2014 to 2018. *Malaysian Journal of Veterinary Research*, 10(2), 125–130.
- Morand, S., & Bordes, F. (2015). Parasite diversity of disease-bearing rodents of Southeast Asia: Habitat determinants and effects on sexual size dimorphism and life-traits. *Frontiers in Ecology and Evolution*, 3, 110.
- Mustapha, T., Daskum, A. M., Abd Majid, R., & Unyah, N. Z. (2019). A review on rodent-borne parasitic zoonosis: Public health risk to humans. *South Asian Journal of Parasitology*, 3, 1–15.
- Ng, Y., Hamdan, N., Tuen, A., Mohd-Azlan, J., & Chong, Y. L. (2017). Short communication co-infections of ectoparasite species in synanthropic rodents of western Sarawak, Malaysian Borneo. *Tropical Biomedicine*, 34(3), 723–731.
- Perkins, S. L., & Schall, J. (2002). A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. *Journal of Parasitology*, 88(5), 972–978.
- Perles, L., Roque, A. L. R., D'Andrea, P. S., Lemos, E. R. S., Santos, A. F., Morales, A. C., Machado, R. Z., & André, M. R. (2019). Genetic diversity of *Hepatozoon* spp. in rodents from Brazil. *Scientific Reports*, 9(1), 1–9.
- Phillipps, Q., & Phillipps, K. (2016). *Phillipps' field guide to the mammals of Borneo and their ecology: Sabah, Sarawak, Brunei, and Kalimantan*. John Beaufoy Publishing Ltb.
- Plowright, R. K., Parrish, C. R., McCallum, H., Hudson, P. J., Ko, A. I., Graham, A. L., & Lloyd-Smith, J. O. (2017). Pathways to zoonotic spillover. *Nature Reviews Microbiology*, 15(8), 502–510.
- Prakash, B. K., Low, V. L., Tan, T. K., Vinnie-Siow, W. Y., Lim, Y. A. L., Morvarid, A. R., Azman, A. S., Yeong, Y. S., AbuBakar, S., & Sofian-Azirun, M. (2018). Detection of *Hepatozoon canis* in the brown dog tick and domestic dogs in Peninsular Malaysia. *Journal of Medical Entomology*, 55(5), 1346–1348.
- Premaalatha, B., Chandrawathani, P., Jamnah, O., Farah Haziqah, M. T., Tan, P. S., Tharshini, J., Nor Ikmal, S., Ramlan, M., Norhafizah, H., Khadijah, S.,

- & Mariappan, C. (2018a). A survey of parasitic infections in wild rats from urban areas in Kuala Lumpur, Malaysia. *Malaysian Journal of Veterinary Research*, 9(1), 31–38.
- Premaalatha, B., Chandrawathani, P., Omar, J., Mamat, Z. C., Mohamad, R., S. L., & G. B. (2018b). A survey of parasite pathogens in stray dogs from Pudu Pasar and Chow Kit area of Kuala Lumpur. *Malaysian Journal of Veterinary Research*, 9(2), 130–135.
- Premaalatha, B., Nurulaini, R., Zawida, Z., Norakmar, I., Imelda Lynn, V., Adnan, M., Zaini, C. M., Jamnah, O., Tan, L. J., Zainab, Z., Khoo, L. L., Chandrawathani, P., & Ramlan, M. (2010). A survey of bacterial and parasitic infections of rats caught in the Veterinary Research Institute (VRI), Ipoh. *Malaysian Journal of Veterinary Research*, 1(1), 45–50.
- Prompiram, P., Poltep, K., Pamonsupornvichit, S., Wongwadhunyo, W., Chamsai, T., & Rodkvamtook, W. (2020). Rickettsiae exposure related to habitats of the oriental house rat (*Rattus tanezumi*, Temminck, 1844) in Salaya suburb, Thailand. *International Journal for Parasitology: Parasites and Wildlife*, 13, 22–26.
- Raj, S., Ghazali, S. M., & Hock, L. K. (2009). Endo-parasite fauna of rodents caught in five wet markets in Kuala Lumpur and its potential zoonotic implications. *Tropical Biomedicine*, 26, 67–72.
- Sahimin, N., Alias, S. N., Woh, P. Y., Edah, M. A., & Mohd Zain, S. N. (2014). Comparison between Quantitative Buffy Coat (QBC) and Giemsa-stained Thin Film (GTF) technique for blood protozoan infections in wild rats. *Tropical Biomedicine*, 31(3), 422–431.
- Schaer, J., Perkins, S. L., Decher, J., Leendertz, F. H., Fahr, J., Weber, N., & Matuschewski, K. (2013). High diversity of West African bat malaria parasites and a tight link with rodent *Plasmodium* taxa. *Proceedings of the National Academy of Sciences*, 110(43), 17415–17419.
- Schaer, J., Perkins, S. L., Ejotre, I., Vodzak, M. E., Matuschewski, K., & Reeder, D. M. (2017). Epauletted fruit bats display exceptionally high infections with a Hepatocystis species complex in South Sudan. *Scientific Reports*, 7(1), 1–11.
- Schaer, J., McMichael, L., Gordon, A. N., Russell, D., Matuschewski, K., Perkins, S. L., Field, H., & Power, M. (2018). Phylogeny of *Hepatocystis* parasites of Australian flying foxes reveals distinct parasite clade. *International Journal for Parasitology: Parasites and Wildlife*, 7(2), 207–212.
- Seifollahi, Z., Sarkari, B., Motazedian, M. H., Asgari, Q., Ranjbar, M. J., & Abdolahi Khabisi, S. (2016). Protozoan parasites of rodents and their zoonotic significance in Boyer-Ahmad District, Southwestern Iran. *Veterinary Medicine International*, 2016, 1–5.
- Shafiyah, C. S., Jamaiah, I., Rohela, M., Lau, Y. L., & Aminah, F. S. (2012). Prevalence of intestinal and blood parasites among wild rats in Kuala Lumpur, Malaysia. *Tropical Biomedicine*, 29(4), 544–550.
- Smyth, J. D., & Wakelin, D. (1994). *Introduction to Animal Parasitology*. Cambridge University Press.
- Sousa, K. C. M., Fernandes, M. P., Herrera, H. M., Benevenuto, J. L., Santos, F. M., Rocha, F. L., Barreto, W. T. G., Macedo, G. C., Campos, J. B., Martins, T. F., de Andrade Pinto, P. C., Battesti, D. B., Piranda, E. M., Cançado, P. H. D., Machado, R. Z., & André, M. R. (2017). Molecular detection of *Hepatozoon* spp. in domestic dogs and wild mammals in southern Pantanal, Brazil with implications in the transmission route. *Veterinary Parasitology*, 237, 37–46.
- Wolf, R. W., Aragona, M., Muñoz-Leal, S., Pinto, L. B., Melo, A. L. T., Braga, I. A., Costa, J. d. S., Martins, T. F., Marcili, A., Pacheco, R. d. C., Labruna, M. B., & Aguiar, D. M. (2016). Novel *Babesia* and *Hepatozoon* sp. agents infecting non-volant small mammals in the Brazilian Pantanal, with the first record of the tick *Ornithodoros guaporensis* in Brazil. *Ticks and Tick-borne Diseases*, 7(3), 449–456.

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