UNIVERSITI PUTRA MALAYSIA

HISTOPATHOLOGICAL FINDINGS OF ZOONOTIC PARASITES IN *RATTUS NORVEGICUS* CAPTURED IN KLANG VALLEY

NUR AFIFAH AFNIE BINTI MOHD NOR SHOKRI

Ip
FPV 2023 93

HISTOPATHOLOGICAL FINDINGS OF ZOONOTIC PARASITES IN *RATTUS NORVEGICUS* CAPTURED IN KLANG VALLEY

NUR AFIFAH AFNIE BINTI MOHD NOR SHOKRI

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

2023

@COPYRIGHT UPM

CERTIFICATION

It is hereby certified that we have read this project paper entitled “Histopathological Findings of Zoonotic Parasites in *Rattus norvegicus* Captured in Klang Valley”, by Nur Afifah Afnie Binti Mohd Nor Shokri and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 - Final Year Project.

@COPYRIGHT UPM

DR. NUR FAZILA SAULOL HAMID
DVM (UPM), PHD (LIVERPOOL)
Lecturer,
Faculty of Veterinary Medicine
University Putra Malaysia
(Supervisor)

DR. NOR YASMIN ABD RAHAMAN
DVM (UPM), PHD (UPM)
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

DR. NUR MAHIZA MD ISA
DVM (UPM), PHD (UNIVERSITY OF GLASGOW)
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

DR. MAZLINA MAZLAN
DVM (UPM), PHD (UPM)
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

@COPYRIGHT UPM

ACKNOWLEDGEMENT

First of all, I would like to express my gratitude to Allah because with His permission, I was able to complete this project with patience and strength.

I also would like to thank those involved in providing support and encouragement whether physically, emotionally and mentally. Especially to my parents for understanding my time to complete this project. They were my source of strength not only for the final year project, but throughout my DVM journey. Thank you for handing out food whenever I am too busy with my study.

I would like to express my gratitude to my supervisor and co-supervisors. This project would not have been completed without the guidance and advice from my supervisor, Dr Nur Fazila. Thank you for being patient with all the mistakes that I have made. In addition, I am also thankful to my co-supervisors, Dr Yasmin for guiding me during mock presentations, and also not forgetting Dr Mahiza and Dr Mazlina for guiding me to understand more about parasitology and histopathology.

Not forgetting my project partner, Adlina for being a great partner. I am very grateful to her for helping me a lot, especially when collecting results and analyzing data. Thank you for giving me a ride everytime we go to the lab and sharing references with me.

Besides that, I also would like to thank other DVM friends, who were involved either directly or indirectly in completing this project.

@COPYRIGHT UPM

CONTENTS

@COPYRIGHT UPM

@COPYRIGHT UPM

LIST OF TABLES ***Page***

LIST OF FIGURES

@COPYRIGHT UPM

LIST OF ABBREVIATION

%	Percent
IACUC	Institutional Animal Care & Use Committee
cm	Centimeter
kg	Kilogram
spp.	Species
°C	Degree celcius
mm	Milimeter
µm	Micrometer

@COPYRIGHT UPM

ABSTRAK

PENEMUAN HISTOPATOLOGI PARASIT ZOONOTIK DALAM *RATTUS NORVEGICUS* YANG DITANGKAP DI LEMBAH KLANG

Oleh

Nur Afifah Afnie Mohd Nor Shokri

2023

Penyelia: Dr. Nur Fazila Saulol Hamid

Penyelia Bersama: Dr. Nor Yasmin Abd Rahaman, Dr. Nur Mahiza Md Isa & Dr. Mazlina Mazlan

Rattus norvegicus ialah spesies tikus biasa yang meluas. Ia biasa ditemui di pasar basah kerana sumber makanan yang banyak. Kawasan ini juga menunjukkan jarak dekat antara tikus dan aktiviti sosial manusia di kawasan bandar. Mereka menjadi pembawa banyak penyakit zoonosis termasuk penyakit cacing paru-paru tikus, kapilariasis hepatik, toksoplasmosis, sarcocystis dan strongyloidosis yang menjadi kebimbangan kesihatan awam utama. Objektif kajian ini adalah untuk mengenal pasti parasit dan meneliti lesi patologi parasit zoonosis di *R. norvegicus* di sekitar kawasan Lembah Klang. Sebanyak 25 sampel arkib *R. norvegicus* telah diproses untuk pemeriksaan histopatologi organ terpilih buah pinggang, paru-paru, hati, otak, limpa, jantung, otot, dan usus. Sampel tetap diwarnakan menggunakan kaedah pewarnaan Hematoxylin dan Eosin dan spesimen dilihat di bawah mikroskop cahaya pada pembesaran 4x, 10x dan 40x. Kesemua 25 sampel adalah positif untuk parasit tisu berdasarkan histomorfologi

©COPYRIGHT UPM

mereka. *Sarcocystis* spp. berada pada jangkitan tertinggi (83.3%:20/24) dalam otot, diikuti oleh *Capillaria hepatica* (52%:13/25) dan *Cysticercus* spp. (24%:6/25) diperhatikan dalam hati dan *Toxoplasma* spp. dalam buah pinggang (8%:1/25). Parasit yang diperhatikan dalam usus menunjukkan *Strongyloides* spp. (48%:12/25) dan *Hymenolepis* spp. (4%:1/25). Lesi histopatologi yang ditunjukkan oleh parasit boleh dilihat dalam kebanyakan sampel termasuk infiltrat radang, keradangan eosinofilik, nekrosis tisu, fibrosis tisu dan pembentukan sista dan larva parasit. Walau bagaimanapun, tiada parasit dengan perubahan yang tidak ketara ditemui di otak, limpa, paru-paru dan jantung. Secara keseluruhannya, kami mendapati sekurang-kurangnya satu parasit tisu zoonosis hadir dalam semua sampel yang menjadi ancaman zoonosis di kalangan orang yang lemah imun seperti bayi, warga emas dan orang yang mempunyai penyakit yang mengancam nyawa. Kajian ini mengetengahkan perubahan histopatologi yang disebabkan oleh jangkitan parasit tisu zoonosis pada tikus Lembah Klang. Oleh itu, adalah penting untuk mengawal populasi tikus di kawasan kediaman manusia bagi mengelakkan penularan penyakit parasit kepada populasi manusia.

Kata kunci: ***Rattus norvegicus, parasit zoonotik, histopatologi, organ***

@COPYRIGHT UPM

ABSTRACT

HISTOPATHOLOGICAL FINDINGS OF ZOONOTIC PARASITES IN *RATTUS NORVEGICUS* CAPTURED IN KLANG VALLEY

By

Nur Afifah Afnie Mohd Nor Shokri

2023

Supervisor: Dr Nur Fazila Saulol Hamid

Co-Supervisors: Dr. Nor Yasmin Abd Rahaman, Dr. Nur Mahiza Md Isa & Dr. Mazlina Mazlan

Rattus norvegicus is a widespread species of common rats. It is commonly found in the wet market due to the abundance of food sources. This area also shows close proximity between the rats and human social activity in urban areas. They become carriers of many zoonotic diseases including rat lungworm disease, hepatic capillariasis, toxoplasmosis, sarcocystis and strongyloidosis that become major public health concerns. The objective of this study is to identify the parasites and examine the pathological lesions of zoonotic parasites in *R. norvegicus* around the Klang Valley areas. A total of 25 archived samples of *R. norvegicus* were processed for histopathological examination of selected organs of kidney, lung, liver, brain, spleen, heart, muscle, and intestine. The fixed samples were stained using Hematoxylin and Eosin staining

©COPYRIGHT UPM

methods and specimens were viewed under a light microscope at 4x, 10x and 40x magnifications. All 25 samples are positive for tissue parasites based on their histomorphology. *Sarcocystis* spp. was at the highest infection (83.3%:20/24) in muscle, followed by *Capillaria hepatica* (52%:13/25) and *Cysticercus* spp. (24%:6/25) observed in liver and *Toxoplasma* spp. in kidney (8%:1/25). Parasites observed in the intestine were suggestive of *Strongyloides* spp. (48%:12/25) and *Hymenolepis* spp. (4%:1/25). Histopathological lesions exhibited by the parasites can be seen in most samples including inflammatory infiltrates, eosinophilic inflammation, tissue necrosis, tissue fibrosis and the formation of parasites cyst and larvae. However, no parasites with insignificant changes were found in the brain, spleen, lung and heart. Overall, we found at least one zoonotic tissue parasite present in all samples that become a zoonotic threat among immunocompromised people such as infants, elderly and people with life-threatening disease. This study highlights the histopathological changes caused by zoonotic tissue parasites infection in rats of Klang Valley. Hence, it is important to control the population of rats in human living areas to avoid parasitic disease transmission to the human population.

Keywords: ***Rattus norvegicus, zoonotic parasites, histopathology, organ***

@COPYRIGHT UPM

1. INTRODUCTION

1.1. Background

Rattus norvegicus originated from northern China and is known as brown rat. They are the largest member of the mouse family, which is the family of *Muridae*. They are omnivorous and mostly found in Africa, Eurasia, and Australia. More than 1700 species of rodents were identified (Steven, 2006). Two common rats in Malaysia are *R. rattus diardii, R. tiomanicus, R. exulans* and *R. norvegicus. R. norvegicus* are a widespread species of common rats where they can easily be found everywhere, especially around human living areas due to the abundance of food sources. They are invasive animals that can transmit many zoonotic diseases (Gage, 1999)

Some studies have been conducted to detect the endoparasites in rodents from various countries which are Malaysia (Mohd-Qaqiem et al., 2022; Tijjani et al., 2020), Thailand (Ribas et al., 2016), Iran (Meshkekar et al., 2014) and India (Coomansingh-Springer et al., 2019). From those studies, a lot of parasite species can be found in different tissue samples and some of them are potentially zoonotic. Those studies used different methods to identify the parasites, for instance fecal floatation for intestinal parasites, blood smear to detect blood parasites and histopathological analysis to observe tissue parasites.

In this study, it was mainly focused on tissue histopathology. There were 7 wet markets in Klang Valley that were chosen to capture *R. norvegicus*. Those wet markets were actively selling vegetables, meats, fishes and groceries to the local community nearby. However, due to the lack of discipline among the wet market traders, there are a lot of unmanaged food remnants, poor sanitation and improper waste disposal. These conditions will attract the rats and provide suitable place for rodents to breed and increase their population at the wet market area (Syahrul

©COPYRIGHT UPM

& Ibrahim, 2020). Once the population is increasing and uncontrolled, there is a high possibility that rats with parasitic disease will contaminate the food with their urine, saliva and feces then transmit the disease to humans through ingestion. Hence, high density of rats in human living areas increases the risk of disease transmission. It is important to understand the histopathological changes caused by tissue parasites and their risk on public health. This study highlights the histopathological changes caused by zoonotic tissue parasites infection in organs. Pathological lesions and parasite cysts can be accessed in the organs either macroscopically or microscopically. Hence, it is important to control the population of rats in human living areas to avoid parasitic disease transmission to the human population.

1.2 Justifications

1. Parasitic detection studies in rodents have been conducted in some states in Malaysia. However, it only focused on a few organs and did not involve some of the vital organs.
2. *Rattus norvegicus* may harbor potential zoonotic parasites in different vital organs.

1.3 Objectives

This study aims to detect the parasites and their histopathological findings in kidney, liver, lung, brain, intestine, spleen, heart and muscle tissue of *Rattus norvegicus* in Klang Valley.

@COPYRIGHT UPM

1.4 Hypothesis

H_0: There are absence of parasites and their histopathological findings in *Rattus norvegicus* captured in Klang Valley.

H_A: There are presence of parasites and their histopathological findings in *Rattus norvegicus* captured in Klang Valley.

@COPYRIGHT UPM

2. LITERATURE REVIEW

2.1 Reported parasitic infection in human from rodents

There are a few cases reported about Angiostrongyliasis or known as Rat Lungworm disease that infect humans. This parasite is prevalent in Southeast Asia and tropical Pacific Islands. In addition, it was also identified in Africa, the Caribbean and United States (CDC - *Angiostrongylus*, 2019). In 2019, the disease was confirmed in three Hawaii visitors (Scutti, 2019). Adult stage of *A. cantonensis* can only be found in rodents and they pass their larvae through feces. Intermediate hosts such as snails and slugs can be infected by ingesting the larvae. This parasitic infection can be transmitted to humans by ingesting infected intermediate host.

In addition, news from the USA also reported that Rat Lungworm was found in rodents and it can invade the human brain. It can cause severe effects on a human's brain and spinal cord. The most common symptoms are headache and neck stiffness. This infection also can cause eosinophilic meningitis which is a very rare type of meningitis.

2.2 Worldwide parasitic detection studies

Table 1 shows parasitic detection studies have been done worldwide to identify the presence of parasites and their prevalence in rodents. One study was done in Malang, East Jawa and the result shows that 100% of wild rats were positive for endoparasites (Kusumarini et al., 2021). In Malaysia, detection of rodent-borne parasites was done in Serdang, Selangor and 15.4% of the rats were positive for tissue parasites (Tijjani et al., 2020). The most infected parasites found are *Taenia taeniaeformis* (28%), followed by *Hymenolepis nana* (19.5%) and *Capillaria hepatica* (19.1%).

@COPYRIGHT UPM

Meanwhile in India, intestinal parasitic burden in *R. norvegicus* were identified and the most prevalent parasites found are *Nippostrongylus brasiliensis* (50%), followed by *Strongyloides ratti* (42.4%) and *Angiostrongylus spp.* (35.2%) (Coomansingh-Springer et al., 2019). Helminth detection studies in Thailand also show *Capillaria hepatica* (63%) at the highest infection, followed by *Trichostrongylidae* (35%) and *Taenia taeniaeformis* (28%) (Ribas et al., 2016).

Table 1. Parasites detection studies worldwide

Country	Method(s) of examination	Parasites detected	Percentage (%)
Iran (Meshkekar, 2014)	1. Impression smear (liver and spleen) 2. Intestinal content examination	*H. nana*	35.8
		Heterakis spumosa	18.3
		C. fasciolaris	18.2
		H. diminuta	7.5
		C. annulosa	1.7
Malaysia (Tijjani et al., 2020)	Tissue Histopathology	*Taenia taeniaeformis*	35
		C. hepatica	19
		Angiostrongylus cantonensis	15
		Sarcocystis spp.	7
		Toxoplasma gondii	6.7
India (Coomansingh-Springer et al., 2019)	Centrifugation floatation of intestinal content	*Angiostrongylus* spp.	35.2
		Nippostrongylus brasiliensis	50.6
		Heterakis spumosa	15.4

©COPYRIGHT UPM

		Strongyloides ratti	43.2
		Aspiculus tetraptera	2.5
		Syphacia spp.	1.9
		Protospirura spp.	1.2
		Hymenolepis diminuta	7.4
		Eimeria spp.	4.7
		Moniliformis moniliformis	3.1

2.3 Common parasites found in organs histopathology

Some organs are manifested by different parasite species depending on their life cycle and mechanism of action. Table 2 shows the list of observed organs infected by parasites that have been reported previously. All parasites in the table below are potentially zoonotic and humans can get infected from the rats in their living area.

Table 2. Common parasites found in each organ

Organs	Parasites Found	Reference(s)
Brain	*Toxoplasma* spp.	Tijjani et al., 2020
	Angiostrongylus cantonensis	Thiengo et al., 2013
Lung	*Angiostrongylus cantonensis*	Thiengo et al., 2013
Liver	*Capillaria hepatica*	Quilla & Paller, 2020 Berentsen et al., 2015 Rothenburger et al., 2014

@COPYRIGHT UPM

	Cysticercus fasciolaris	Sharma et al., 2017 Lee et al., 2016
Intestine	*Moniliformis* spp.	Teimoori et al., 2011
	Strongyloides spp.	Mahmuda et al., 2017
	Nippostrongylus spp.	Fransen et al., 2016
	Hymenolepis spp.	Tijjani et al., 2020 Franssen et al., 2016
Heart	*Sarcocystis* spp.	Kim et al., 2011
Spleen	*Leishmania infantum*	Hermida et al., 2018
Kidney	*Toxoplasma* spp.	Tijjani et al., 2020
	Babesia microti	Jasik et al., 2023
Muscle	*Sarcocystis* spp.	Kim et al., 2011

2.4 Effect of parasitic infestation to the host

Based on previous study, tissue parasites can cause various histopathological changes in tissue structure. For instance, *C. hepatica* infection in the liver can cause hepatic necrosis, hypertrophy, fibrosis and cholestasis (Quilla & Paller, 2020). In humans, it can cause acute hepatitis, hypereosinophilia, anemia, and chronic fever. Without proper treatment, it can cause fatal diseases especially to immunocompromised people (Aghdam et al., 2015). Heavy infection of Hepatic Capillariasis may cause fatal liver dysfunction (CDC, 2019). Other studies also reported that Hymenolepiasis is life-threatening to immunocompromised patients (Ahmad et al., 2020).

Another important parasitic infection from rodents is Toxoplasmosis. It may not have a serious effect on healthy people. However, in immunocompromised people, it is severe and can cause abortion for pregnant mothers. Besides that, it also can cause eyes and brain damage (Robert-Gangneux & Darde, 2012).

©COPYRIGHT UPM

3. MATERIALS AND METHOD

3.1 *Rattus norvegicus* identification method

Identification of *Rattus norvegicus* relies on distinct physical features. Notably, their body coloration is a prominent characteristic, with dark grey or dark brown on the dorsal part and light grey or light brown on the ventral side. Additionally, these rats exhibit blunt noses, small closely spaced bald ears and hairless tail with a length shorter than their bodies, reaching up to 40 cm in length and weighing up to 0.5 kg. *R. norvegicus* were recognised based on Armitage (2019). From 25 captured rats, 4% (1/25) are juvenile and 96% (24/25) are adults. In terms of sex, 32% (8/25) are male and 68% (17/25) are female.

3.2 Archived tissue processing

Under ethical clearance of UPM/AUP/R021/2021, a total of twenty-five (25) archived *R. norvegicus* from wet markets in Klang Valley were obtained. The selected organs of liver, lung, brain, kidney, intestine, spleen, heart and muscle were fixed in 10% buffered formalin before tissue processing was done.

3.2.1 Tissue cutting and fixation

The fixed tissues were trimmed to suitable sizes (1×2 cm, 3 mm thickness) for fixation. The tissues were placed into a cassette, fixed with formaldehyde solution and swirled to ensure complete immersion. These tissues were stored at 4 °C for 16 hours to allow for total fixation.

3.2.2 Tissue dehydration

Fixed tissues were immersed in ascending grades of ethanol to absolute ethanol to remove the water and formalin from the tissue. The sequence of dehydration process are;

@COPYRIGHT UPM

1. 70% ethanol for 15 minutes
2. 90% ethanol for 15 minutes
3. 100% ethanol for 15 minutes
4. 100% ethanol for 15 minutes
5. 100% ethanol for 30 minutes
6. 100% ethanol for 45 minutes

After that, the tissue was cleaned with xylene for 1 hour by gentle agitation to remove excess ethanol.

3.2.3 Paraffin embedding

The specimens were infiltrated with paraffin wax. The tissues were embedded in the wax block until the paraffin wax was fully hardened.

3.2.4 Tissue sectioning

Wax block that contained tissue was sectioned with 5 µm thickness using a rotary microtome. The tissue section was floated on a water bath with temperature 35-37 °C. Then, the sections were mounted onto the glass slides and let dry for a few hours.

3.3 Tissue staining

The glass slides were submerged in xylene for 5 minutes and hydrated in absolute ethanol for 5 minutes followed by 70% ethanol for another 5 minutes then rinsed with water. Then, those glass slides were submerged in Haematoxylin for 5 minutes and rinsed with water for 3-5 minutes. The glass slides were dipped in 1% Acid alcohol for 3 seconds before submerged in running tap water. After 5 minutes, the glass slides were submerged in eosin for 1 minute and then sprayed with 95% alcohol. Glass slides were rinsed again in running tap water for 5-10 seconds and sprayed again with 95% alcohol. All specimens were cleaned and left to dry before cleared with xylene and mounted with DPX. The slides will be examined under a light microscope at 40X, 100X

©COPYRIGHT UPM

and 400X magnifications. The pathological findings and histomorphological changes were described and assessed based on Suttie et al. (2017).

@COPYRIGHT UPM

4. RESULTS

As seen in table 3, six parasites were recognized. *Sarcocystis* spp. (83.3%) shows the highest infection, followed by *Capillaria hepatica (52%), Cysticercus* spp*. (20%), Strongyloides* spp*. (48%), Hymenolepis* spp. (4%) and *Toxoplasma* spp*. (4%).*

In muscle samples, only one parasite species can be detected, which is *Sarcocystis* spp. Sarcocystis is a group of protozoans that are commonly found in muscle. Even though no gross lesions were seen in all *R. norvegicus* muscle samples, different stages of the cyst can be seen clearly under histology. *Sarcocyst* spp. shows the highest infection as compared to other parasites found in *R. norvegicus* (Figure 1 & Figure 2). 83.33% (20/24) of the muscle samples show the presence of *Sarcocystis* spp. cyst.

In the liver, 2 parasite species were identified which were *Capillaria hepatica* (52%) and *Cysticercus* spp*.* (20%). These findings were categorized into single infection and co-infection as seen in table 4. *C. hepatica* is a parasite from the nematode group meanwhile *Cysticercus* spp. is a parasite from cestode group. From the findings, 40% of the liver samples were only infected with *C. hepatica* and 8% of them only infected with *Cysticercus* spp. Meanwhile, 12% of the liver samples were infected with both parasites. For *C. hepatica*, eggs (Figure 3) and larvae form (Figure 4) can be seen under histology. Meanwhile for *Cysticercus* spp, only the larvae stage can be seen (Figure 5). The mature larvae were seen located in the cyst and surrounded by the capsule with inflammatory cells.

From the *R. norvegicus* sample, 2 parasites can be identified in intestinal walls which were *Strongyloides* spp. from the nematode group and *Hymenolepis* spp. from cestode group. The parasite species were determined based on histomorphology and the location where they were found. 48% of the intestinal samples were positive with *Strongyloides* spp*.* The larvae (Figure 6)

©COPYRIGHT UPM

and the eggs (Figure 7) were found embedded in the intestinal wall, specifically in the Crypts of Lieberkuhn. Besides that, 4% were positive with *Hymenolepis* spp.

In the kidney, only one protozoan parasite, *Toxoplasma* spp. was found based on the histomorphology that accounts for. 4% of the kidney samples were positive for *Toxoplasma* spp. The morphology of the cyst containing hundreds of bradyzoites (Figure 9).

Unfortunately, no parasites can be found in the spleen, lung, brain and heart (Figure 10). In these organs, no infiltration of inflammatory cells and insignificant tissue changes of parasite infection could be seen.

Table 3: Parasites finding in *R. norvegicus*

ORGAN	PARASITES FOUND	GROUP	POSITIVE NUMBER	PERCENTAGE (%)
Muscle	*Sarcocystis* spp.	Protozoa	20/24	83.3
Liver	*Capillaria hepatica*	Nematode	13/25	52
	Cysticercus spp.	Cestode	5/25	20
Intestine	*Strongyloides* spp.	Nematode	12/25	48
	Hymenolepis spp.	Cestode	1/25	4
Kidney	*Toxoplasma* spp.	Protozoa	1/25	4
Lung	-	-	0/25	0
Spleen	-	-	0/25	0
Heart	-	-	0/25	0
Brain	-	-	0/25	0

©COPYRIGHT UPM

Table 4: Category of parasites infection in liver

Single infection	*C. hepatica*	10/25
	Cysticercus spp.	2/25
Co-infection	*C. hepatica* + *Cysticercus* spp.	3/25

@COPYRIGHT UPM

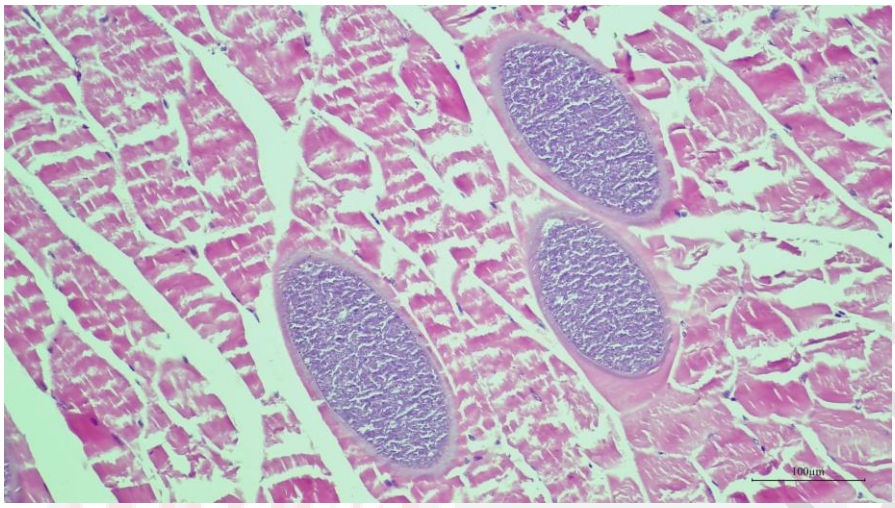

Figure 1. Photomicrograph showing numerous *Sarcocystis* spp. cyst. The cysts are oval shaped with smooth and thin striated walls. (H&E, 200X)

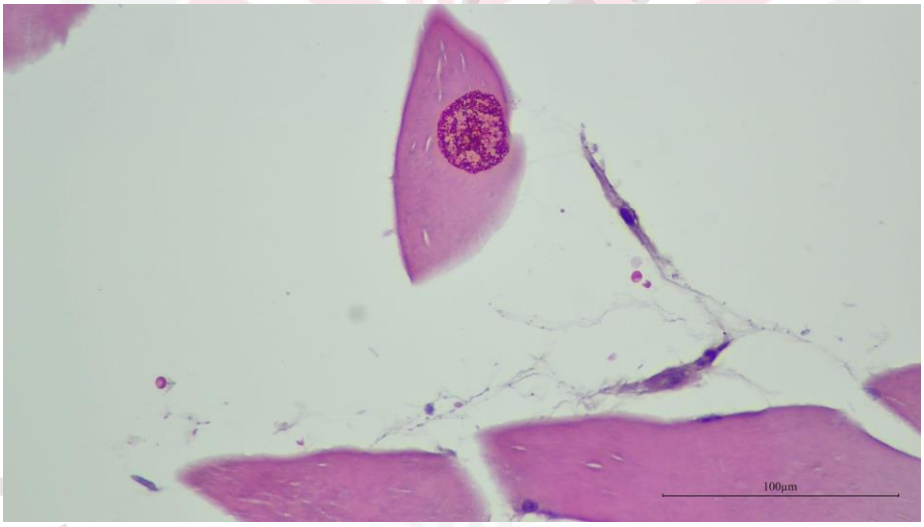

Figure 2. Photomicrograph showing early stage of *Sarcocystis* spp. characterized by the thin-walled cyst with numerous cystozoites embedded in the muscle cells. (H&E, 400X).

@C UPM

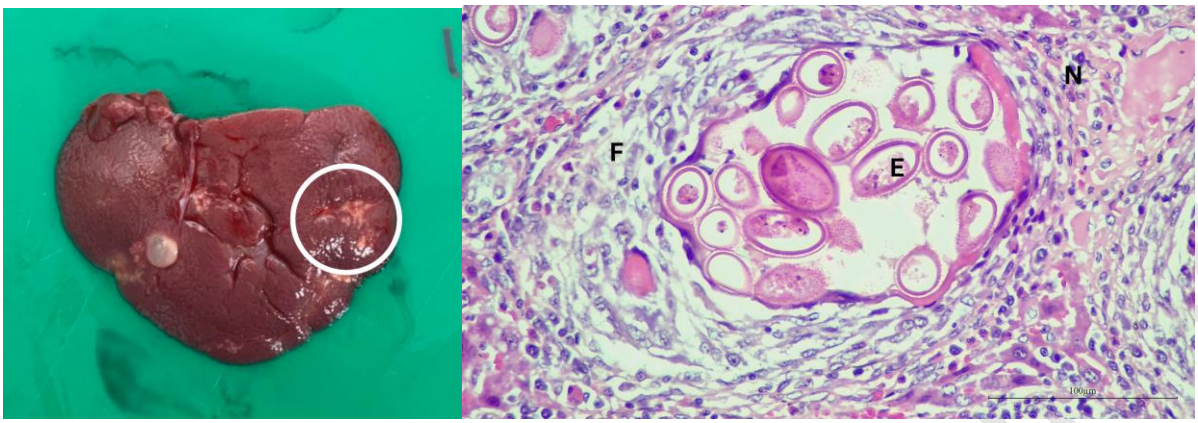

Figure 3. Gross lesion of the liver infected with *C. hepatica.* There are multifocal white tracks in the liver parenchyma (white circle). Photomicrograph showing *C. hepatica* eggs. The eggs were barrel shaped with an opercula plug on each end and double-layered shells. (H&E, 400X).

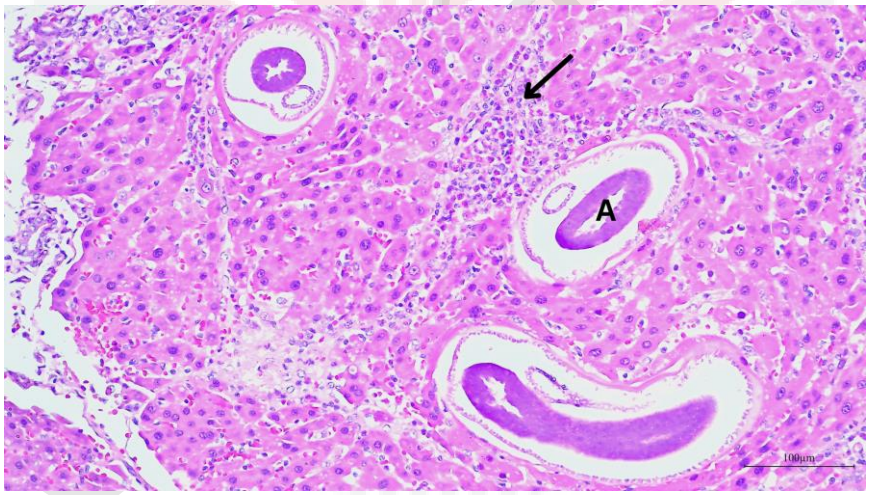

Figure 4. Photomicrograph showing the cross section of *C. hepatica* adult worms (A). Infiltration of inflammatory cells (black arrow) can be seen adjacent to the adult worms. (H&E, 200X).

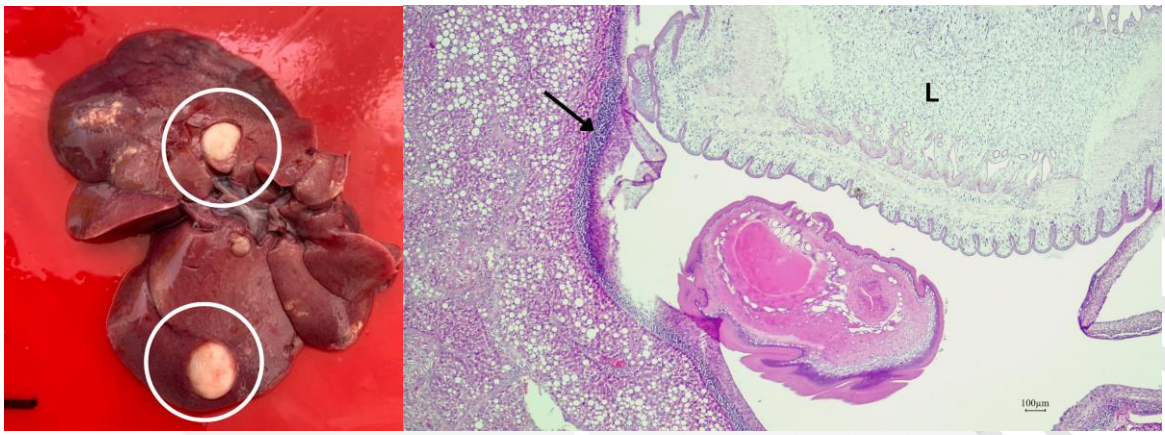

Figure 5. This is the gross lesion of *Cysticercus* spp. in the liver (white circle). Photomicrograph shows folds of mature *Cysticercus* spp. larvae (L) surrounded by the thin cyst wall. Inflammatory reaction surrounding the cyst with infiltration of eosinophils (black arrow). (H&E, 40X).

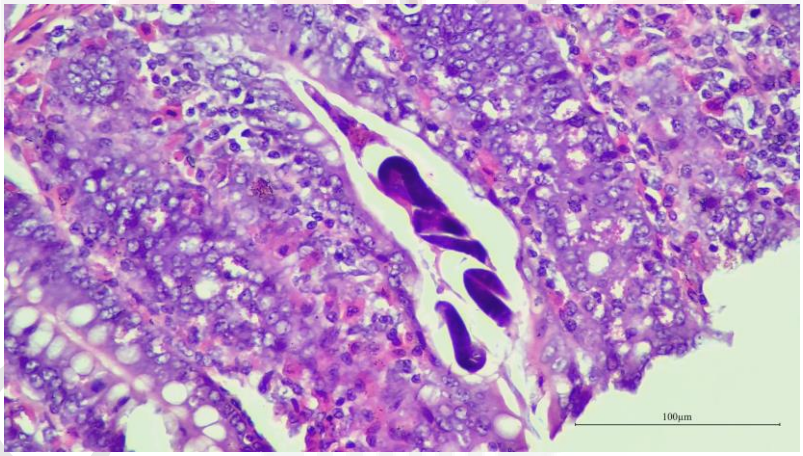

Figure 6. Photomicrograph showing the larvae of *Strongyloides* spp. surrounded by the vacuole. (H&E, 400X)

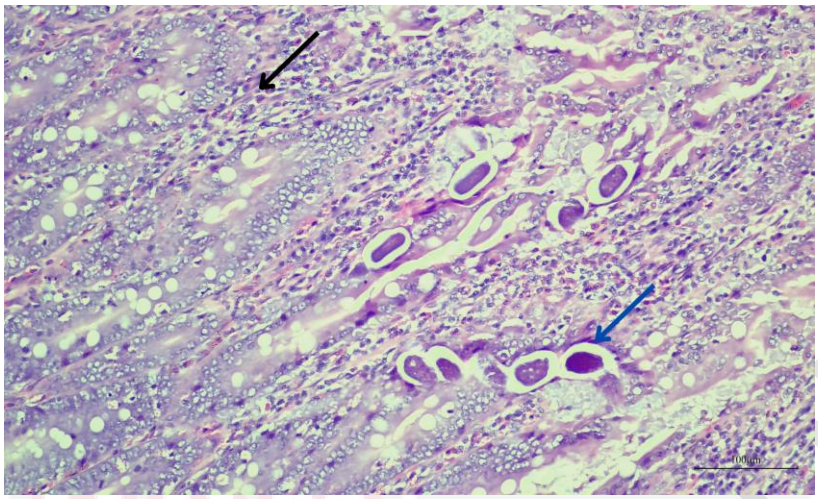

Figure 7. Photomicrograph showing *Strongyloides* spp. eggs (blue arrow) surrounded by the vacuole. Note the infiltration of inflammatory cells (black arrow). (H&E, 200X).

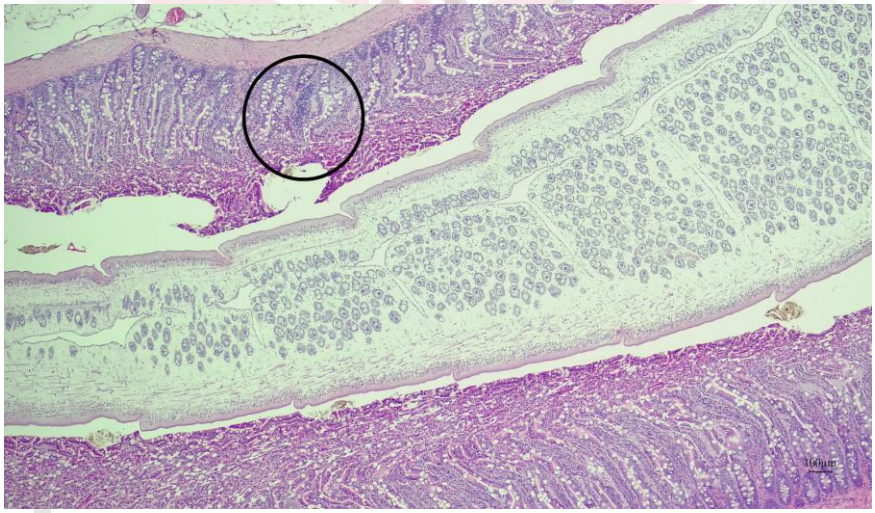

Figure 8. Photomicrograph showing *Hymenolepis* spp. in the intestinal lumen and infiltration of inflammatory cells in the intestinal wall (black circle). (H&E, 40X).

©COPYRIGHT UPM

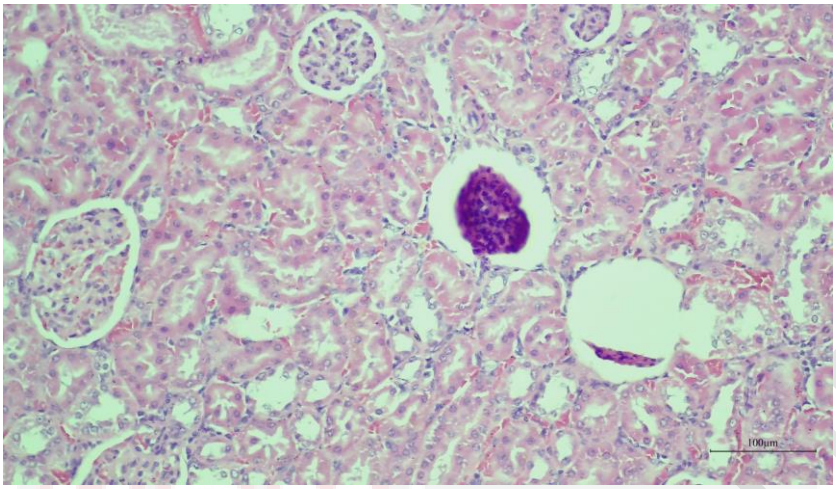

Figure 9. Photomicrograph showing the *Toxoplasma* spp. cyst in the kidney. (H&E, 200X)

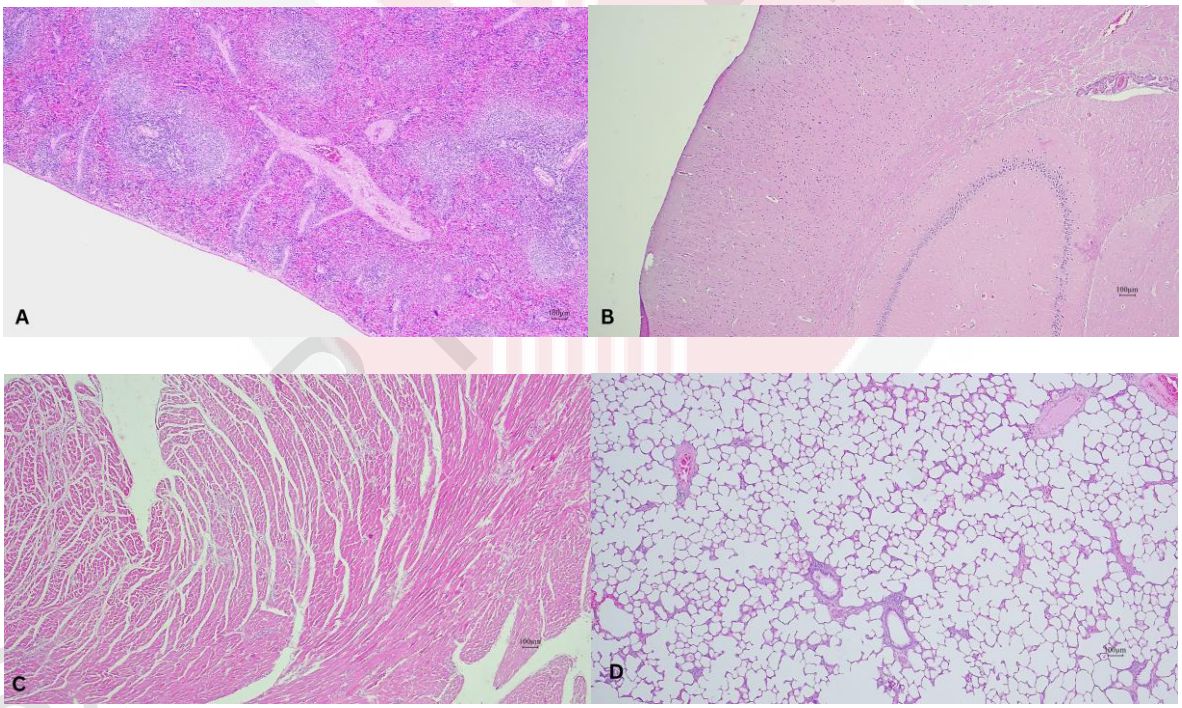

Figure 10. Photomicrograph showing no parasites were found in the spleen (A), brain (B), heart C and lung (D). (H&E, 40X).

5. DISCUSSION

In this study, all 25 rats harbor at least one parasite in their tissue sample. 16% of the *R. norvegicus* harbor only one parasite and 84% of them harbor more than one parasite. All of them are potentially zoonotic and have the potential risk to transmit the parasitic infection to humans. The parasites can be characterized based on their histomorphology.

Rodents is an intermediate host for *Sarcocystis* spp. and prevalence rate of *Sarcocystis* spp. infection is high in Southeast Asia (Ambu, 2011). *Sarcocystis* spp. has zoonotic potential that can be transmitted to humans that can cause myalgia, erythematous subcutaneous nodules, cough, fever, loss of appetite and headache. Among all *Sarcocystis* spp., only *S. hominis*, *S. heydorni* and *S. suihominis* use humans as definite hosts. One of the histopathological changes that can be observed in the infected tissue is eosinophilic myositis. However, the occurrences are very rare (Rosenthal, 2021). Hence, no histopathological changes in the muscle cells structure were seen in this study. Based on histomorphology, *Sarcocystis* spp. only can be identified until species level. The species can be identified by looking at the sizes and wall thickness. These findings show that rodents can harbor more than one species of *Sarcocystis* spp.

A parasitic detection study in rodents has been done in Thailand and the results show that *C. hepatica* is the most prevalent (63%) (Ribas et al., 2016). However, in this study, *C. hepatica* was the second most predominant parasite found in the liver tissue. Globally, it was reported that *R. norvegicus* is the most important host species for *C. hepatica* and the prevalence was more than 50% in several studies (Fuehrer, 2013). However, human infection is very rare. Gross lesions can be seen in the liver that was infected with *C. hepatica*. There were multifocal white tracks in the liver parenchyma. Based on histomorphology, *C. hepatica* eggs were confirmed. The eggs were barrel shaped with opercula plug on each end and double-layered shells. In the infected liver, the histopathological lesion appears due to the host immune response towards the

©COPYRIGHT UPM

parasites. In this study, fibrosis can be seen adjacent to the *C. hepatica* eggs in the liver. Host immune response had caused necrosis and fibrosis in the liver (Quilla & Paller, 2020).

C. fasciolaris is an adult stage of tapeworm *Taenia taeniaeformis* which is mainly found in the liver. In this study, *Cysticercus* spp. Infection was not so high compared to other parasites whereby only 20% of the liver was positive with *Cysticercus* spp. However, another parasitic detection study was done in Serdang, Malaysia and it was recorded that *Taenia taeniaeformis* was at the highest infection (28%) from 89 captured wild rats. Gross lesions could be seen in the liver infected with *Cysticercus* spp. There was a cystic structure raised above the parietal and visceral surface of the liver. Inflammatory reactions can be seen surrounding the *Cysticercus* spp. larvae. A capsule was formed surrounding the larvae to protect the host liver parenchyma from further infection. At the same time, this mechanism also protects the larvae from the host inflammatory response and prepares a suitable environment for the parasites to mature. The capsule consisted of inflammatory cells and collagen fibers secreted by hepatic stellate cells (HSC) via exocytosis (Lee et al., 2016). *Cysticercus* spp. that found in the liver is highly suggestive of *Cysticercus fasciolaris* and it is zoonotic parasite. However, it rarely can infect humans and only a few cases were reported. Humans usually get infected after ingesting food, water or soil that was contaminated with feces contaminated with embryonated eggs.

In the intestine, the ellipsoid eggs and the larvae found in intestinal mucosa are highly suggestive of *Strongyloides* spp. (Viney & Kikuchi, 2016). This is because *Strongyloides* spp. is a tissue dwelling and commonly found in the Crypts of Lieberkuhn. The eggs and larvae were surrounded by the vacuole and peripheral blood eosinophilia usually could be observed. Two species of *Strongyloides* that can infect *R. norvegicus* were *S. ratti* and *S. venezuelensis*. Both of them are not zoonotic and only *S. stercoralis* were reported that can cause serious health effects to humans, especially immunocompromised individuals (Mahmuda et al., 2017). In addition, *Hymenolepis* spp. were found in the intestinal lumen with characteristics of mature proglottids that contain oval-shaped eggs (Roberts & Janovy, 2000). Humans can get infected by accidentally

©COPYRIGHT UPM

ingesting food contaminated with the infected feces or ingesting the insect as an intermediate host (CDC, 2019). For immunocompromised individuals, Hymenolepiasis may be life-threatening (Ahmed et al., 2020). The prevalence of Hymenolepis spp was slightly different with other places. Helminth detection in Iran shows that *H. nana* was at the highest infection (35.8%) (Meshkehar et al., 2014) meanwhile in Serdang, Selangor, *H. nana* infection was 25% from the captured rats (Tijjani et al., 2020).

Furthermore, *Toxoplasma* spp. in the kidney was characterized by the presence of the cysts consisting of hundreds of bradyzoites between the renal cells. This finding is highly suggestive of the protozoan group of *Toxoplasma* spp. based on the morphology and the size. The cyst is usually in the range of 20-70 micrometers. However, *Toxoplasma spp*. rarely can be found in the kidney. It was more significant to be found in the liver, heart, lung, brain, lymphoid tissue and small intestine (Denk at al., 2022).

Last but not least, no identified parasites were found in the 4 other organs, which were spleen, heart, lung and brain. Two potential contributing factors to these findings are species specific preference and low incidence of reported infection. Different parasite species prefer a particular texture for establishment and formation of cyst (Shahraki et al., 2018).

6. CONCLUSION

In conclusion, there was presence of parasites in all *R. norvegicus* samples and their histopathological changes observed in muscle, liver, intestine, and kidney. All rats were positive with at least one parasitic infection whereby more than 80% harbor multiple species of parasites in their tissue histology. Furthermore, the histopathological changes observed were the result of host immune response to the invading pathogen as a mechanism to protect the host organ.

©COPYRIGHT UPM

7. RECOMMENDATIONS

It is recommended to perform further molecular identification to confirm the species of the parasites because some of them are difficult to identify based on histomorphology only. It is also recommended to increase the sample size to get better results. Other than that, it is also advised to equalize the number of juvenile and adult rats in the sample size. The unbalanced age distribution age can affect the parasitic burden in the rat populations.

@COPYRIGHT UPM

REFERENCES

Aghdam MK, Karimi A, Amanati A, Ghoroubi J, Khoddami M, Shamsian BS, Far SZ. (2005) *Capillaria hepatica*, A case report and review of the literatures. *Archives Pediatric Infectious Disease*. https://doi.org/10.5812/pedinfect.19398

Ahmed, Hanan Taher Hamza, & Mashael Alhumaidi Alotabi. (2020). Histopathological and biochemical studies on immunocompetent and immunocompromised Hymenolepis nana infected mice treated with Commiphora molmol (Mirazid). *Journal of Parasitic Diseases*, *44*(4), 837–849. https://doi.org/10.1007/s12639-020-01263-z

Ambu, S. (2011). Prevalence of Sarcocystis spp. in rodents in Peninsular Malaysia. *International E-Journal of Science, Medicine & Education*, *5*(2), 29–38. https://doi.org/10.56026/imu.5.2.29

Armitage, D. (2019). *Rattus norvegicus (brown rat)*. Animal Diversity Web. https://animaldiversity.org/accounts/Rattus_norvegicus/

Berentsen, A. R., Vogt, S., Guzman, A. N., Vice, D. S., Pitt, W. C., Shiels, A. B., & Spraker, T. R. (2015). Capillaria hepatica infection in black rats (Rattus rattus) on Diego Garcia, British Indian Ocean Territory. *Journal of Veterinary Diagnostic Investigation*, *27*(2), 241–244. https://doi.org/10.1177/1040638715573298

CDC - DPDx - *Angiostrongyliasis cantonensis*. (2019). Www.cdc.gov. https://www.cdc.gov/dpdx/angiostrongyliasis_can/index.html

CDC - DPDx - Hepatic Capillariasis. (2019). Www.cdc.gov. https://www.cdc.gov/dpdx/hepaticcapillariasis/index.html

CDC. (2019). *CDC - DPDx - Hymenolepiasis*. Centers for Disease Control and Prevention. https://www.cdc.gov/dpdx/hymenolepiasis/index.html

Coomansingh-Springer, C., Vishakha, V., Acuna, A. M., Armstrong, E., & Sharma, R. N. (2019). Internal parasitic burdens in brown rats (Rattus norvegicus) from Grenada, West Indies. *Heliyon*, *5*(8), e02382. https://doi.org/10.1016/j.heliyon.2019.e02382

Denk, D., De Neck, S., Khaliq, S., & Stidworthy, M. F. (2022). Toxoplasmosis in Zoo Animals: A Retrospective Pathology Review of 126 Cases. *Animals*, *12*(5), 619. https://doi.org/10.3390/ani12050619

Franssen, F., Swart, A., van Knapen, F., & van der Giessen, J. (2016). Helminth parasites in black rats (Rattus rattus) and brown rats (Rattus norvegicus) from different environments in the Netherlands. *Infection Ecology & Epidemiology*, *6*(1), 31413. https://doi.org/10.3402/iee.v6.31413

Fuehrer, H.-P. (2013). An overview of the host spectrum and distribution of Calodium hepaticum (syn. Capillaria hepatica): part 1—Muroidea. *Parasitology Research*, *113*(2), 619–640. https://doi.org/10.1007/s00436-013-3691-x

Gage, K. L. (1999). Plague Surveillance. In Plague Manual Epidemiology, Distribution, Surveillance and Control. World Health Organisation.

© COPYRIGHT UPM

Hermida, M. d'El-Rei, de Melo, C. V. B., Lima, I. dos S., Oliveira, G. G. de S., & dos-Santos, W. L. C. (2018). Histological Disorganization of Spleen Compartments and Severe Visceral Leishmaniasis. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00394

Jasik, K., Kleczka, A., & Filipowska, S. (2023). Histopathological Analysis of Selected Organs of Rats with Congenital Babesiosis Caused by Babesia microti. *Veterinary Sciences*, *10*(4), 291–291. https://doi.org/10.3390/vetsci10040291

Kim, T., Han, J., Chang, S., Kim, D., Tamer Said Abdelkader, Seung Hyeok Seok, Park, J., Oh, H., Kim, J.-T., Lee, B.-H., Shin, J.-H., Kim, J.-H., Jong Min Kim, & Park, J. (2011). Detection of sarcocystic infection in a wild rodent (Apodemus agrarius chejuensis) captured on Jeju island. *Laboratory Animal Research*, *27*(4), 357–357. https://doi.org/10.5625/lar.2011.27.4.357

Kusumarini, S., Yesica, R., Bagus, I., Rama Wisesa, G., Hermanto, J., Nurholizah, Y., & Trinastuti, M. (2021). *ACTA VETERINARIA INDONESIANA Special Issues: 95-101, Preliminary Study: Detection of Ecto and Endoparasites Among Wild Rats From Urban Area in Blimbing, Malang, East Java*. https://journal.ipb.ac.id/index.php/actavetindones/article/download/35071/21656/

Lee, B.-W., Jeon, B.-S., Kim, H.-S., Kim, H.-C., & Yoon, B.-I. (2016). *Cysticercus fasciolaris* infection in wild rats (*Rattus norvegicus*) in Korea and formation of cysts by remodeling of collagen fibers. *Journal of Veterinary Diagnostic Investigation*, *28*(3), 263–270. https://doi.org/10.1177/1040638716643129

Mahmuda, A., Al-Zihiry, K., Roslaini, A., Rukman, A., Abdulhaleem, N., Bande, F., Mohammed, A., Alayande, M., Abdullah, W., & Zasmy, U. (2017). Histopathological confirmation of disseminated larvae (iL3) of Strongyloides ratti in an immunosuppressed Wistar rat. *Tropical Biomedicine*, *34*(1), 212–223. https://msptm.org/files/Vol34No1/212-223-Zasmy-U.pdf

Meshkekar, M., Sadraei, J., Mahmoodzadeh, A., & Mobedi, I. (2014). Helminth Infections in Rattus ratus and Rattus norvigicus in Tehran, Iran. *Iranian Journal of Parasitology*, *9*(4), 548–552. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4345094/

Mohd-Qawiem, F., Nur-Fazila, S. H., Ain-Fatin, R., Yong, Q. H., Nur-Mahiza, M. I., & Yasmin, A. R. (2022). Detection of zoonotic-borne parasites in Rattus spp. in Klang Valley, Malaysia. *Veterinary World*, *15*(4), 1006–1014. https://doi.org/10.14202/vetworld.2022.1006-1014

Nazari, N., Saeedeh Shojaee, Mehdi Mohebali, Aref Teimouri, Keyghobad Ghadiri, Saber Raeghi, Mohammad Reza Shiee, Yousef Azarakhsh, & Arezoo Bozorgomid. (2019). Toxoplasma gondii And Neospora caninum In Brain Tissue Of Rodents In North-West Iran. *Veterinary Medicine : Research and Reports*. https://doi.org/10.2147/vmrr.s224664

Quilla, M. H. D. P., & Paller, V. G. V. (2020). Histopathological features and prevalence of Capillaria hepatica infection in Rattus spp. in Philippine Mount Makiling forest reserve and its adjacent areas. *Journal of Parasitic Diseases*, *44*(2), 338–348. https://doi.org/10.1007/s12639-019-01189-1

© COPYRIGHT UPM

Ribas, A., Saijuntha, W., Agatsuma, T., Thongjun, C., Lamsan, K., & Poonlaphdecha, S. (2016). Helminths in rodents from Wet Markets in Thailand. *Helminthologia*, *53*(4), 326–330. https://doi.org/10.1515/helmin-2016-0036

Roberts, L., J. Janovy Jr (2000). *Foundations of Parasitology, Sixth Edition*. MA: Mcgraw-Hill Higher Education.

Robert-Gangneux, F., & Darde, M.-L (2012). Epidemiology of and Diagnostic Strategies for Toxoplasmosis. *Clinical Microbiology Reviews*, *25*(2), 264–296. https://doi.org/10.1128/cmr.05013-11

Rosenthal, B. M. (2021). Zoonotic Sarcocystis. *Research in Veterinary Science*, *136*, 151–157. https://doi.org/10.1016/j.rvsc.2021.02.008

Rothenburger, J. L., Himsworth, C. G., Krista, Leighton, F. A., Nemeth, N. M., J. Steven Alexander, & Jardine, C. M. (2019). Pathology of wild Norway rats in Vancouver, Canada. 31(2), 184–199. https://doi.org/10.1177/1040638719833436

Scutti, S. (2019). *Parasite in paradise: Rat lungworm disease confirmed in three Hawaii visitors*. CNN. https://edition.cnn.com/2019/05/27/health/rat-lungworm-hawaii-cdc-trnd/index.html

Sharma, R., Tiwari, K., Birmingham, K., Armstrong, E., Montanez, A., Guy, R., Sepulveda, Y., Mapp-Alexander, V., & DeAllie, C. (2017). Cysticercus fasciolaris in Brown Rats (Rattus norvegicus) in Grenada, West Indies. *Journal of Parasitology Research*, *2017*, e1723406. https://doi.org/10.1155/2017/1723406

Shahraki, M., Ghanbarzehi, A., & Dabirzadeh, M. (2018). Prevalence and histopathology of Sarcocystosis in slaughtered carcasses in southeast Iran. *Journal of Advanced Veterinary and Animal Research*, *5*(4), 381. https://doi.org/10.5455/javar.2018.e288

Steven R. B. (2006). Rats and Human Health in Africa: Proceedings of an international workshop on rodent-borne diseases and the RatZooMan research project. Vol 23. http://projects.nri.org/ratzooman/docs/workshop_proceedings.pdf

Suttie, A. W., Boorman, G. A., Leininger, J. R., Eustis, S. L., Elwell, M. R., MacKenzie, W. F., & Bradley, A. (2017). Boorman's Pathology of the Rat:

Syahrul, M., & Ibrahim, N. (2020). A Study on Parasite Fauna of Rats and Shrews Caught at Three Wet Markets in Kuala Terengganu, Malaysia. *International Journal of Nature and Life Sciences (IJNLS)*, *4*(1), 1–13. https://dergipark.org.tr/tr/download/article-file/1116956

Teimoori, S., Gharaguzlu, M., Makki, M., Shahbazi, F., Mobedi, I., Saboor Yaraghi, A., Hasanpour, G., Rokni, M., & Mowlavi, G. (2011). Heavy Worm Burden of Moniliformis moniliformis in Urban Rats with Histopathological Description. *Iranian Journal of Parasitology*, *6*(3), 107–112. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279893/

Thiengo, S. C., de Oliveira Simões, R., Fernandez, M. A., & Júnior, A. M. (2013). Angiostrongylus cantonensis and Rat Lungworm Disease in Brazil. *Hawai'i Journal of Medicine & Public Health*, *72*(6 Suppl 2), 18–22.

©COPYRIGHT UPM

Tijjani, M., Majid, R. A., Abdullahi, S. A., & Unyah, N. Z. (2020). Detection of rodent-borne parasitic pathogens of wild rats in Serdang, Selangor, Malaysia: A potential threat to human health. *International Journal for Parasitology: Parasites and Wildlife*, *11*, 174–182. https://doi.org/10.1016/j.ijppaw.2020.01.008

Viney, M., & Kikuchi, T. (2016). Strongyloides ratti and S. venezuelensis – rodent models of Strongyloides infection. *Parasitology*, *144*(3), 285–294. https://doi.org/10.1017/s0031182016000020

@COPYRIGHT UPM