



Invited article

Detection of rodent-borne parasitic pathogens of wild rats in Serdang, Selangor, Malaysia: A potential threat to human health

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ABSTRACT

Rodent species, such as *Rattus rattus diardii* and *Rattus norvegicus* are invasive species of wild rats that serve as potential reservoirs of important human's pathogens. Parasitic zoonosis accounts for over 60% of all human infectious diseases worldwide. This situation arises from the recent changes in the global climate and ecosystem composition, which led to the spread of rodents and rodent-borne pathogens globally. The aim of this study was to determine the occurrence of rodent's parasites and their zoonotic potentials in some selected areas in UPM. Rodents were captured using live-traps and euthanised for helminths and protozoan recovery. Intestinal parasites were detected and identified from stool samples using formalin ethyl-acetate concentration technique (FECT), while tissue parasites were identified by histopathological examination of selected tissue sections of the liver, brain, lungs, and muscle. In this study, a total of 89 wild rats were captured. Twelve species of intestinal and tissue parasites were recorded, of which, *Taenia taeniaeformis* accounts for the highest infection recorded (28%) followed by *Hymenolepis nana* (19.5%) and *Capillaria hepatica* (19.1%), while *Toxoplasma gondii* was the least parasite (6.7%) identified. Furthermore, other parasites species observed include, *Cryptosporidium* spp. (21.3%), *Entamoeba histolytica/Entamoeba dispar* and *Moniliformis moniliformis* (17.9%), *Angiostrongylus cantonensis* (16.8%), *Hymenolepis diminuta* (16.1%), *Giardia* spp. (14.6%), *Trichuris* spp. (12.3%), and *Sarcocystis* spp. (6.74). Based on the results obtained in the present study, 17.1% and 15.4% of the rodents captured were confirmed positive for at least one species of intestinal or tissue parasites, respectively. The presence of these zoonotic parasites in the wild rats suggests the potential risk of rodent-borne zoonotic disease transmission to humans. Hence, the need to improved rats control intervention and public health awareness among the populace.

1. Introduction

Most infectious diseases affecting humans are thought to have zoonotic potentials (WHO, 2018). Zoonotic diseases, especially those associated with rodents and other wildlife, pose a significant threats to human health and wellbeing (Cleaveland et al., 2001; Daszak et al., 2000).

Rodents constitute more than 42% of mammals, with over 1,700 species belonging to three families include, Muridae, Microtidae, and Sigmodontidae respectively. The members of the Muridae family are omnivorous and mostly found in Africa, Eurasia, and Australia (e.g., *Mus* sp., *Rattus rattus diardii*, and *Rattus norvegicus*), while the family

Microtidae are commonly found in Eurasia (e.g., *Microtus*), and Sigmodontidae are found in America (e.g., *Peromyscus*) (Steven, 2006).

An increasing number of cases associated to parasitic zoonosis were recorded in some parts of the world (Han et al., 2015; Hassell et al., 2017; WHO, 2019), in which factors believed to be responsible for this escalation include, habitat modification, overpopulation, and mass migration. All these occur as a result of natural or man-made disasters (Chomel et al., 2007). Residential areas, especially urban settings are of great concern considering the emergence of zoonotic diseases. These areas provides a favorable habitats to certain species of wild animals which, eventually led to regular and increased contact with humans (Luniak, 2004). Of all the animals found in urban areas, wild rats

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(*Rattus rattus* spp.) are the most dangerous due to their high number (high reproductive capacity), zoonotic potential, and propensity towards close association with humans (Battersby et al., 2002; Clinton, 1969).

Zoonotic diseases attributed to rodents are caused by protozoan (e.g., toxoplasmosis, leishmaniasis), helminths (e.g., hymenolepiasis, trichinellosis, echinococcosis, and capillariasis), viruses (e.g., Lassa fever, Hantavirus diseases, tick-borne encephalitis, as well as Argentine and Bolivian hemorrhagic fever), and bacteria (e.g., plague, leptospirosis, Lyme disease, and relapsing fevers) (Asante et al., 2019; Helmy et al., 2017; Steven, 2006). *Hymenolepis nana* and *Hymenolepis diminuta* for instance are parasites of rats that were reported to have infected over 175 million people worldwide (Crompton, 1999; Kim et al., 2014b; Macnish et al., 2003). In severe cases, infections with *Hymenolepis nana* and/or *Hymenolepis diminuta* may be life-threatening especially in immuno-compromised individuals (Muehlenbachs et al., 2015).

This study examines the distribution of rodent-borne zoonotic parasitic pathogens of wild rats in some selected areas of Universiti Putra Malaysia. The study aims to understand the possible risks associated with zoonotic parasitic pathogens of wild rats in the study area.

2. Materials and methods

2.1. Ethical statement

This study was approved by the Institutional Animal Care and Use Committee of the Universiti of Putra Malaysia (Ref. No: UPM/IACUC/AUP-R039/2018). All protocols involved in the handling of animals in this study are in accordance with the Malaysian Animal Welfare Act (2015).

2.2. Study area and sampling

The study was conducted at Universiti Putra, Serdang, Selangor, Malaysia, from September 2018 to March 2019. Universiti Putra Malaysia is situated (259°34. 19" N; 101 42'16.79" E) in central Peninsular Malaysia, Kuala Lumpur (Fig. 1). Rodents were trapped using a rectangular metal trap baited with fried fish as previously described (Christophe, 2011). Rodents captured were transported to the laboratory and humanely euthanised in line with the approved

guidelines, following which, age and sex were determined.

2.3. Animal dissection

Animals were dissected according to the protocol previously described (Christophe, 2011). Briefly, animals were dorsoventrally placed in a dissecting tray, limbs fixed with dissecting pins and the skin incised, pinched and raised with dissecting forceps, while scissors were used to extend the cut from the posterior through to anterior regions, exposing the diaphragm. The diaphragm was later slit through the midline from throat to anus thereby, revealing the esophagus, stomach, intestine, liver, and urinary bladder. Blunt-end scissors were used to remove all internal organs. The intestine and stomach were cut and placed in a vessel containing 70% alcohol, while other organs; brain, kidney, lungs, liver, and muscle washed with Phosphate Buffered Saline (PBS) to remove bloodstains and preserved in small bijoux bottles containing 10% formalin for histopathological examination. A portions of stool samples from gastrointestinal tracts (GITs) were used to detect parasite's ova, cyst, and oocysts using the formalin ethyl-acetate concentration technique (FECT).

2.4. Formalin-ether concentration technique (FECT)

The portion of intestinal content was collected using an applicator stick and transferred into a beaker. Eight milliliters (8 mL) of normal saline was added and stirred. The mixture was then filtered using funnel and gauze into a 15 mL centrifuge tube. The filtrate was obtained for further studies while the lumpy residues were discarded. The filtrate was later centrifuged at 1500 rpm for 5 min following which, the supernatant was discarded. Seven milliliters (7 mL) of 10% formalin solution was added into the tube followed by 3 mL ethyl-acetate. The mixture was thoroughly mixed and centrifuged for 10 min at 1500 rpm. Three layers; ethyl-acetate, formalin, and sediment were formed. The two layers; formalin and ethyl-acetate were carefully discarded while the sediment was retained. This was gently mixed with a rubber pipette and a drop was placed onto a clean, grease-free glass slide, stained with iodine, and covered with a coverslip (Anne and Conboy, 2012).

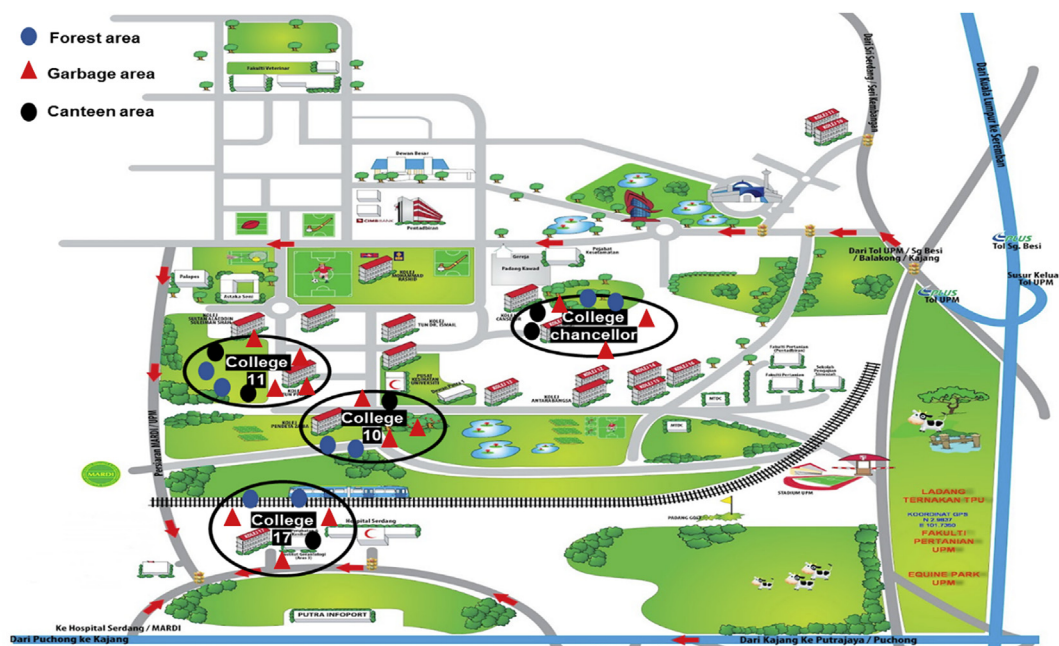


Fig. 1. The study area, Universiti Putra Malaysia showing the study sites circled in black.

Table 1
Prevalence of gastro-intestinal parasites in rodents captured from four locations.

Locations	College 17 (n = 32)	College Chancellor (n = 19)	College 10 (n = 18)	College 11 (n = 20)	
Parasites species	No. + ve (%)	No. + ve (%)	No. + ve (%)	No. + ve (%)	T.P (%)
<i>H. nana</i>	8 (25.0)	4 (21.0)	1 (5.5)	4 (20.0)	17 (19.5)
<i>H. diminuta</i>	6 (18.7)	5 (26.3)	2 (11.1)	2 (10.0)	15 (16.8)
<i>Trichuris</i> spp.	4 (12.5)	3 (15.7)	3 (16.6)	1 (5.0)	11 (12.3)
<i>Giardia</i> spp.	3 (9.3)	1 (5.2)	5 (27.7)	4 (20.0)	13 (14.6)
<i>E. histolytica</i>	8 (25.0)	3 (15.7)	2 (11.1)	3 (15.0)	16 (17.9)
<i>Cryptosporidium</i>	6 (18.7)	6 (31.5)	5 (27.7)	2 (10.0)	19 (21.3)
<i>M. moniliformis</i>	6 (18.7)	5 (26.3)	2 (11.1)	3 (15.0)	16 (17.9)
O. P	41 (18.3)	27 (20.3)	20 (15.8)	19 (38.3)	107 (17.1)

Note: No. +ve; Number of rodents positive, (%); Prevalence of infection, T.P: Total prevalence, O.P: Overall prevalence; n = : Total number of rodents captured in the study sites.

2.5. Parasites identification

Slides were placed in a microscope and magnified at 10X and 40X magnification for parasites identification. Cyst, ova, oocyst, and trophozoites of GIT parasites were detected by comparing their sizes and morphological features as previously described (Anne and Conboy, 2012; Shiba and Shaji Uga, 1996). Cyst, ova, oocyst, and trophozoites of different parasites species were counted separately. Prevalence and intensity of infection were calculated in line with the standard procedures for the quantification of parasites (Lajos and Jenó, 2000). However, some parasites were not identified up to species level, as microscopy only allows their identification to genus level.

2.6. Histopathological analysis of tissues

Sections of liver, heart, lungs, kidney, muscle, and brain were used for histopathological analysis in accordance with standard protocols (Slaoui and Fiette, 2011).

2.6.1. Tissue fixation

Tissues previously preserved in 10% formalin were cut to suitable sizes (1×2 cm, 3 mm thickness) for fixation. The cut tissues were placed into a container, fixed with formaldehyde solution and swirled to ensure complete immersion of all tissues in the fixative. These were then stored at 4 °C for 16 h to allow for total fixation. On the next day, samples were collected, trimmed, placed in a labeled cassette and kept in neutral buffered formalin until further analysis.

2.6.2. Paraffin embedding

The preserved tissues were dehydrated in ascending grades of

Table 2
Intensity of different species of parasites recovered in rodent from four locations.

Locations	College 17 (n = 32)	College Chancellor (n = 19)	College 10 (n = 18)	College 11 (n = 20)
Species	X ± SE	X ± SE	X ± SE	X ± SE
<i>H. nana</i>	1.88 ± 0.58	1.75 ± 0.17	7.00 ± 0.20	1.75 ± 0.17
<i>H. diminuta</i>	1.50 ± 0.29	2.20 ± 0.33	8.00 ± 0.00	3.00 ± 0.41
<i>Trichuris</i> spp.	2.25 ± 0.29	2.67 ± 0.17	10.00 ± 0.17	7.00 ± 1.17
<i>Giardia</i> spp.	3.00 ± 0.29	5.00 ± 0.17	17.00 ± 0.44	4.50 ± 0.58
<i>E. histolytica</i>	2.00 ± 0.33	3.33 ± 0.33	12.00 ± 0.41	3.00 ± 0.29
<i>Cryptosporidium</i> spp.	2.50 ± 0.29	3.50 ± 0.29	14.00 ± 0.29	6.50 ± 0.61
<i>M. morniliformis</i>	1.67 ± 0.17	3.40 ± 0.60	11.00 ± 0.20	4.67 ± 0.44

p = 0.0017*.

X ± SE: Mean abundance plus/minus standard error of the mean of parasites cyst, ova, oocyst, n : Total number of rodents captured in the study sites.

ethanol to absolute ethanol. The tissues were then cleaned with xylene for 1 h by gentle agitation, infiltrated with wax, and embedded in wax blocks.

2.6.3. Tissue sectioning

A water bath set at 35–37 °C was used to float the tissue block for 10 min, following which, a section of the tissue (s) was cut at 5 µm on a rotatory microtome. The sections were then floated on the surface of warm water (37 °C) and mounted onto the surface of albumenized glass slides. The glass slides were later placed on a hot plate set at 65 °C in oven for 20 min to melt the wax and to bind the tissue to the glass slide.

2.6.4. Tissue staining

The sections of tissues were dewaxed with xylene and hydrated in absolute ethanol, which were later washed with water. Tissues were initially stained in Harris and hematoxylin stain, differentiated in acid alcohol, and thereafter, stained with methylene blue. The tissues were dehydrated in 95% alcohol, stained in 10% alcohol eosin, dehydrated in absolute alcohol, cleared in xylene, and mounted in Canada balsam. The resulting slides were viewed under a light microscope (Nikon eclipse 50i. Japan).

2.7. Statistical analysis

All analyses were performed using Prism (GraphPad software) and Microsoft Excel 2010. Non-parametric tests (Kruskal Wallis and Mann Whitney tests) were carried out to compare the mean differences in prevalence and intensity of infection among habitat, species, sexes, and age of the rodents. Results obtained are presented as mean, standard error of the mean (Mean ± SEM) and percentage. P values ≤ 0.05 are considered significant.

3. Results

3.1. Gastro-intestinal parasites

Seven species of intestinal parasites belonging to four phyla (Platyhelminthes, Nematodes; Protozoa, and Acanthocephalan) were identified in wild rats trapped from some selected residential colleges in Universiti Putra Malaysia. Parasites species identified include; *Hymenolepis nana*, *Hymenolepis diminuta*, *Trichuris* spp., *Giardia* spp., *Entamoeba histolytica/Entamoeba dispar*, *Cryptosporidium* spp., and *Moniliformis morniliformis*. Results obtained revealed that 17.1% of wild rats captured from all study sites were positive for at least one species of the intestinal parasite (Table 1). The most prevalent parasite species identified was *Cryptosporidium* spp. (21.3%), followed by *Hymenolepis nana* (19.1%), *Entamoeba histolytica/Entamoeba dispar* (17.9%), *Morniliformis morniliformis* (17.9%), *Hymenolepis diminuta* (16.8%), *Giardia* spp. (14.6%), and *Trichuris* spp. (12.3%). In terms of parasites intensity, a statistically significant difference (p = 0.0017*) was observed

Table 3
Prevalence and intensity of parasites according to the sex of the rodents.

Species	Prevalence		Intensity	
	Male (n = 36)	Female (n = 53)	Male	female
	No. + ve (%)	No. + ve (%)	X ± SE	X ± SE
<i>H. nana</i>	9 (25.0)	8 (15.0)	1.56 ± 0.71	2.50 ± 0.58
<i>H. diminuta</i>	6 (16.6)	9 (16.9)	1.67 ± 1.41	2.44 ± 1.17
<i>Trichuris</i> spp.	4 (11.1)	7 (13.2)	4.00 ± 0.35	2.71 ± 0.29
<i>Giardia</i> spp.	7 (19.4)	6 (11.3)	3.29 ± 0.71	2.83 ± 0.58
<i>E. histolytica</i>	7 (19.4)	9 (16.9)	3.57 ± 0.59	2.22 ± 0.49
<i>Cryptosporidium</i>	6 (16.6)	13 (24.5)	5.33 ± 0.71	2.00 ± 0.58
<i>M. morniliformis</i>	7 (1.7)	9 (16.9)	4.14 ± 0.94	2.33 ± 0.78
O.P	46 (18.2)	61 (16.4)	p = 0.01*	U = 28.00

Note: No. + ve; Number of rodents positive, (%): Prevalence of infection, T.P: Total prevalence, O.P: Overall prevalence; X ± SE; Mean, plus minus standard error of mean.

amongst rodents trapped from four locations (Table 2).

3.2. Distribution of gastro-intestinal parasites based on host sex

The prevalence and mean intensity of intestinal parasites in relation to host sex are summarized in Table 3. The overall prevalence of infection appeared to be slightly higher in male rats (18.2%) when compared to female rats (16.4%). However, a slightly high infection rate with *Cryptosporidium* spp. (24.5%), *Moniliformis moniliformis* (16.9%), and *Trichuris* spp. (13.2%) was observed in the female rodents, whereas males recorded high infections with *Hymenolepis nana* (25%), *Giardia* spp. (19.5%), and *Entamoeba histolytica/Entamoeba dispar* (19.5%) than females. For *Hymenolepis diminuta*, similar infection rate (16.9%) was observed across both sexes. Prevalence of infection in relation to sex was observed to be statistically significant ($p = 0.01^*$, $U = 28.00$) (Table 3).

3.3. Distribution of endoparasites based on host age

Out of the 89 rodents captured, 56 were adults and 39 were juveniles. Although, adults recorded a slightly high prevalence of infection with *Cryptosporidium* spp. (22.0%) and *Hymenolepis nana* (20.0%), infection rate with *Moniliformis morniliformis* (25.5%), *Cryptosporidium* spp. (20.5%), and *Hymenolepis diminuta* (20.5%) was higher in juveniles. *Trichuris* spp. (12.8%), *Giardia* spp. (12.8%), *Entamoeba histolytica/ Entamoeba dispar* (18.0%), and *Hymenolepis nana* (18%) were the least parasites recovered. The overall prevalence of infection was slightly higher in juveniles (18.3%), when compared to adults (15.3%). Statistically, significant difference was not observed ($p = 0.32$, $U = 54.50$) in terms of parasitic infection with respect to age (Table 4).

3.4. Tissue parasites

Histopathological examination of various organs (brain, liver, lungs, and muscle) revealed inflammations associated with parasitic infection. A cyst which appeared creamy white, and round, embedded in the liver was identified to be *Taenia taeniaeformis* (cyst), while fibrosis in liver tissue associated with *Capillaria hepatica* eggs were recorded. In the brain and lungs tissues, larvae of *Angiostrongylus cantonensis* was recorded, whereas *Toxoplasma gondii* and *Sarcocystis* spp. were identified in brain and skeletal muscle. The overall prevalence of infection showed that 15.5% of the rats examined were positive for at least one species of parasites in their tissues. The most predominant parasite species observed was *Taenia taeniaeformis* with a prevalence rate of 35%, followed by *Capillaria hepatica* (19%), *Angiostrongylus cantonensis* (15%), *Sarcocystis* spp. (7%), and *Toxoplasma gondii* (6.7%) (Table 5).

Table 4
Prevalence and intensity gastro-intestinal parasites according to age of the rodents.

Species	Prevalence		Intensity	
	Adult (n = 56)	Juvenile (n = 39)	Adult	Juvenile
	No. + ve (%)	No. + ve (%)	X ± SE	X ± SE
<i>H. nana</i>	10 (20.0)	7 (18.0)	1.90 ± 0.40	2.14 ± 0.54
<i>H. diminuta</i>	7 (14.0)	8 (20.5)	2.86 ± 0.80	1.50 ± 0.91
<i>Trichuris</i> spp.	6 (12.0)	5 (12.8)	2.67 ± 0.30	3.80 ± 0.34
<i>Giardia</i> spp.	8 (16.0)	5 (12.8)	2.25 ± 0.40	4.40 ± 0.45
<i>E. histolytica</i>	9 (18.0)	7 (18.0)	3.33 ± 1.50	2.14 ± 1.70
<i>Cryptosporidium</i>	11(22.0)	8 (20.5)	2.09 ± 1.20	4.38 ± 1.36
<i>M. morniliformis</i>	9 (18.0)	10 (20.6)	3.11 ± 0.60	2.20 ± 0.68
O. P	60 (15.3)	50 (18.3)	p = 0.322	U = 54.50

Note: No. + ve; Number positive, O.P: Overall prevalence; (%): Prevalence; X ± SE; Mean, plus minus standard error of mean.

The prevalence of parasite infection in tissues was slightly higher in male rats (22.7%) when compared to female rats (14.3%) Table 6. With regard to host age, results obtained revealed that 18.9% of adult rats were positive for at least one species of tissue parasite, while 16.6% of juvenile rats were positive for parasites (see Table 7).

4. Discussion

Parasitic zoonosis accounts for most outbreaks of novel pathogens worldwide, with helminths and protozoan parasites accounting for most cases of zoonosis recorded (Han et al., 2015; Robinson and Dalton, 2009; Garcia et al., 2007). In most cases, rodents constitute the major reservoir for various species of parasites with zoonotic potential (Seifollahi et al., 2016). In this study, 17.1% of rodents examined were found to harbor at least one parasites of zoonotic importance or the other. Parasites of zoonotic importance identified include; Platyhelminthes (Cestodes), protozoan, nematodes, and acanthocephalan.

The presence of helminths and protozoan parasites in rodents, particularly *Rattus rattus* spp., had been widely reported in the literature. Although parasites community structures were revealed to vary according to regional characteristics (e.g. pH of the soil, and temperature). The parasite species richness observed in this study, is similar with previous studies conducted in Indonesia, Netherlands, Serbia, and Iran, which reported approximately 3–13 different species of parasites infecting wild rats (Claveria et al., 2005; Franssen et al., 2016; Gholipour et al., 2016; Kataranovski et al., 2011). On the other hand, a low parasites load was observed in this study. Contrarily, other previous studies in Malaysia revealed a high parasites load (Paramasvaran et al., 2009; Nursyazana et al., 2013). However, this variation in the parasites load observed, in this study, may be attributed to the locations environmental conditions of the study area in which rodents were caught (UPM campus) as against the geographical areas (rice field, coastal area, forest reserved, and urban area) reported previously (Paramasvaran et al., 2009).

The occurrence of cestodes, particularly members of the genus *Hymenolepis* in urban areas are known to pose a serious health threat. More than 21 million people worldwide have been reported to suffer from Hymenolepiasis infection, especially those from tropical and subtropical regions (Parija, 1990). The prevalence of *Hymenolepis* spp. in urban rodents is of particular interest due to auto-infection. The ova of *Hymenolepis* spp. hatches in the intestine of the host without being passed outside and grows into an adult worm (Miyazaki, 1991). This increases the number of adult worms in the hosts' intestine, thereby increasing the chances of environmental contamination with parasite eggs/ova in the stool. Of the two species of *Hymenolepis* identified, in the present study, high prevalence rate; *Hymenolepis nana* (19.1%) and

Table 5
Distribution of tissue parasites in wild rats captured from four different locations.

Locations	College 17 (n = 32)	College Chancellor (n = 19)	College 10 (n = 18)	College 11 (n = 20)	T. P (%)
Parasites species	No. + ve (%)	No. + ve (%)	No. + ve (%)	No. + ve (%)	
<i>Taenia taeniaeformis</i>	9 (28.1)	3 (15.7)	12 (66.6)	1 (5.0)	25 (28.0)
<i>Capillaria hepatica</i>	7 (21.8)	5 (26.3)	1 (5.55)	4 (20.0)	17 (19.1)
<i>Toxoplasma gondii</i>	3 (9.3)	1 (5.26)	–	2 (10.0)	6 (6.7)
<i>Angiostrongylus cantonensis</i>	4 (12.5)	5 (26.3)	5 (27.7)	1 (5.0)	15 (16.8)
<i>Sarcocystis</i> spp.	2 (6.2)	2 (10.5)	–	2 (10.0)	6 (6.7)
O. P	25 (15.6)	16 (16.8)	18 (20.0)	10 (10.0)	69 (15.5)

Note: No. + ve: Number positive; O.P: Overall prevalence; T.P; Total prevalence; (%): Prevalence.

Table 6
Prevalence of tissue parasites in rodents according to sex of the host.

Parasites species	Male (n = 36)	Female (n = 53)	
	No. + ve (%)	No. + ve (%)	T. P (%)
<i>Taenia taeniaeformis</i>	14 (38.8)	11 (20.7)	25 (28.0)
<i>Capillaria hepatica</i>	12 (33.3)	7 (13.2)	19 (21.2)
<i>Toxoplasma gondii</i>	2 (5.5)	4 (7.5)	6 (6.7)
<i>Angiostrongylus cantonensis</i>	9 (25.0)	6 (11.3)	15 (16.8)
<i>Sarcocystis</i> spp.	3 (8.3)	3 (5.6)	6 (6.7)
O. P	40 (22.7)	31 (14.3)	69 (15.5)

Note: No. + ve: Number positive, O.P: Overall prevalence, T.P; Total prevalence, P%: Prevalence.

Table 7
Prevalence of tissue parasites in rodents according to age of the host.

Parasites species	Adult (n = 39)	Juvenile (n = 50)	T. P (%)
	No. + ve (%)	No. + ve (%)	
<i>Taenia taeniaeformis</i>	9 (23.0)	16 (32.0)	25 (28.0)
<i>Capillaria hepatica</i>	11 (28.2)	8 (16.0)	19 (21.2)
<i>Toxoplasma gondii</i>	2 (5.1%)	4 (8.0)	6 (6.7)
<i>Angiostrongylus cantonensis</i>	7 (17.9)	9 (18.0)	15 (16.8)
<i>Sarcocystis</i> spp.	4 (10.2)	2 (4.0)	7 (7.8)
O. P	37 (18.9)	42 (16.8)	69 (15.5)

Note: No. + ve: Number positive, O.P: Overall prevalence, T.P; Total prevalence, P%: Prevalence.

Hymenolepis diminuta (16.8%), respectively, were recorded. The presence of these parasites in rodents in the study area poses a serious health threat to the inhabitants of the study sites, especially in the latter case in which, insect intermediate host is not required for parasite development, thus increasing the chances of contaminating food for human consumption.

Furthermore, parasitic helminth such as the nematode, *Trichuris* spp. (whipworm), the etiological agent of trichuriasis was identified in wild rats in this study, accounting for 12.8% prevalence rate. Globally, over 500 million cases of human Trichuriases were reported (Pullan et al., 2014). These cases are attributed to infections with *Trichuris trichiura*. Although, the *Trichuris* spp. identified in this study was not identified up to species, but there is likelihood that the *Trichuris* species was *Trichuris muris*, due to reasons associated with the host specificity of *Trichuris muris*, known to infect mice (Cheng, 1986), and the morphological features of the ova previously described (Koyama, 2013). This result is consistent with earlier reports from Iran that also reported *Trichuris muris* in wild rats (Arzamani et al., 2017; Kia et al., 2010).

The intestinal protozoan parasite; *Cryptosporidium* spp. was also detected in this study. Although this parasite was not classified to species level, but there is a high possibility that the *Cryptosporidium* spp.

could be *Cryptosporidium muris*. This assertion was based on the morphological features observed in addition to the host specificity of the parasite as previously described (Tyzzer, 1910; Xiao et al., 2004). In this study, an overall prevalence (21.3%) of infection with *Cryptosporidium* spp. was recorded in wild rats. This finding fell within the range of 9%–70% as previously reported in similar studies (Chaochao et al., 2009; Elwin et al., 2010; Josephine et al., 2013; Kimura et al., 2007; Torres, 2000; Wei et al., 2019). *Cryptosporidium muris* is predominantly a rodent species of *Cryptosporidium* and is not normally considered a human pathogen. Based on molecular characterization, previous studies have demonstrated that infection with *Cryptosporidium muris* is also common among humans (Palmer et al., 2003; Sarfati et al., 2001; Tatsuna et al., 2014; Tiangtip and Jongwutiwes, 2002). Hence, the detection of *Cryptosporidium muris* in rodents as observed in the study area may present a potential health risk to the students.

Humans and animals can acquire *Cryptosporidium* infection through direct contact with infected individuals or contaminated fomites or by consuming food or water contaminated with oocyst (Baines et al., 2017; Glaberman et al., 2002). Cryptosporidiosis in humans is usually manifested as self-limiting watery diarrhea, which usually goes away within a week or two, but may turn into a life-threatening infection with severe consequences in immuno-compromised individuals.

Another important parasite of public health importance observed in this study is *Entamoeba* spp. Rodents are believed to serve as reservoirs of infection, thus playing important roles in the transmission pattern. In this study, about 17.9% prevalence of *Entamoeba histolytica/Entamoeba dispar* was recorded. However, the two species of *Entamoeba*; *Entamoeba histolytica* and *Entamoeba dispar* could hardly be differentiated microscopically (Fotedar et al., 2007), microscopic examinations of stool samples revealed the presence of cysts. In this study, *Entamoeba histolytica/Entamoeba dispar* was differentiated from non-pathogenic *Entamoeba muris* based on the number of nuclei and cyst size. The mature cyst of *Entamoeba histolytica* has 4 nuclei, while immature cyst has 1 or 2 nuclei as opposed to *Entamoeba muris* which has up to 8 nuclei, and the cyst size of *Entamoeba histolytica/Entamoeba dispar* ranges between 10 and 15 μ m in size, whereas in *Entamoeba muris* the cyst size ranges between 9 and 20 μ m (David, 2006; Fotedar et al., 2007). However, this method is insufficient to differentiate *Entamoeba histolytica* from the non-pathogenic form, *Entamoeba dispar* due to their morphological identity, a more sensitive method such as the antigen detection technique and polymerase chain reaction (PCR), was proposed for consideration in future studies.

The *Entamoeba histolytica* is the etiological agent of amoebic dysentery in humans and animals. The disease can be asymptomatic in light infections, mild, or may present severe symptoms, such as abdominal pain, diarrhea, and dysentery (Jeremy et al., 2013). In more severe cases, trophozoites *Entamoeba* spp. invade the intestinal mucosa and find their way to blood circulation, thus increasing their chances of spread to other parts of the body where they develop to cause extra-intestinal abscesses. In most cases, trophozoites end up in liver tissues, thus causing amoebic liver abscesses. In rare occasions, however, other

tissues, such as the brain and lungs, may also be infected. According to the WHO (1997), over 50 million cases and 40,000-100,000 deaths due to amoebiasis were reported annually.

Similarly, the presence of *Giardia* species, an important protozoan parasite of public health importance was recorded in this study. *Giardia* is an important gastro-intestinal parasite and the causative agent of giardiasis in humans and animals (Feng and Xiao, 2011), nonetheless, not all species of *Giardia* are pathogenic. *Giardia duodenalis* is responsible for giardiasis in humans. Based on morphological features and host specificity, as previously described (David, 2006), the *Giardia* spp. observed in the present study was suspected to be *Giardia muris*, with 14.6% prevalence recorded. This result confirms those of similar studies (Harry et al., 2002; Traub et al., 2003) who reported the presence of *Giardia muris* in rodents. In similar studies, the genotypes of human-pathogenic forms; *Giardia duodenalis* were reported in rodents, dogs, cats, and some other mammals (Sprong et al., 2009; Thompson et al., 2010). This finding suggests the likelihood that rodents may serve as important reservoirs of *Giardia* spp.

The only parasite species belonging to the acanthocephalan group identified in this study is *Moniliformis moniliformis*. Basically, *Moniliformis moniliformis* is a parasite of rats, hamsters, cats, and dogs but not human. However, the parasite has some of zoonotic significance as similar studies reported human infections with *Moniliformis moniliformis* in Italy, British Honduras, and Sudan (Faust and Carroll, 1964). *Moniliformis moniliformis* require at least two hosts to complete their life cycle, with beetles or cockroaches serving as an intermediate host. A 17.9% prevalence of infection was recorded in this study. Humans may acquire this infection via accidental consumption of the intermediate host (beetles or cockroaches) containing the infective stage of the parasites or by eating food infested with arthropods and eating raw or undercooked insects. The above finding is in agreement with those reported in the literature (Kumarasingha and Premajith, 2006).

Similar parasites species composition was noted in rodents caught from all the study sites, in this research. However, a possible explanation for this finding may be the similarities in the topographical nature of the four study sites across UPM campus. As previously reported, the similarity in the geographical structure of an area influences the similarity in endo and ectoparasites species composition in animals colonizing the same or different areas (Brown et al., 1994). The significant difference ($p = 0.0017^*$) in the prevalence and intensity of infection recorded among the rodents trapped from the four locations observed in this study may be attributed to the varying number of rodents trapped from each college (study sites).

The overall prevalence of endoparasite infection based on host gender was slightly higher in males (18.6%) than females (16.4%) rats. This variation in prevalence of infection may not be unconnected to the immunosuppressive effect male sex hormones has on male rats, thus accounting for a greater tendency for parasitic infection (Folstad and Karter, 1992; Grossman, 1989; Nicola et al., 2004; Kia et al., 2001; Mafiana et al., 1997; Zain, 2008). Other possible reasons for variation of infection include host factors, such as intrinsic (sex, age, and species) as well as extrinsic (habitat, season, and density), that are known to influence parasite prevalence and intensity in rodents (Easterbrook et al., 2007).

Based on the host age, more juvenile rats (18.3%) were infected than adult rats (15.3%). This outcome is attributed to the fact that juvenile rats are more active in foraging for food, thus increasing their exposure to infections. This finding is in line with that reported in Malaysia (Nursheena et al., 2015).

Rodents are frequently infected with tissue parasites that cause human diseases. Most of the pathogenic parasites in blood and tissue of rodents can either be transmitted directly to humans through the ingestion of food/water contaminated with the parasites or via arthropod vector, which serves as an intermediary host (Paramasvaran et al., 2009). The zoonotic tissue parasites observed in the present study included *Taenia taeniaeformis*, *Capillaria hepatica*, *Toxoplasma gondii*,

Angiostrongylus cantonensis, and *Sarcocystis* spp.

Taenia taeniaeformis, particularly the larval stage, has been reported previously from different regions of the world (McInnes et al., 2014; Zhang et al., 2012). Rodents, birds, insectivores, and even humans have been reported as the intermediate hosts (Bowman et al., 2002; Mino et al., 2013; Sterba and Barus, 1976). This parasite occurs as an adult tapeworm in the small intestine of carnivores (cats) which serve as definitive hosts. Infection is, however, acquired via carnivory when cats predate on rodents (intermediate hosts) or by ingesting food or soil contaminated with cat feces. In this study, nevertheless, the presence of *Taenia taeniaeformis* cyst observed in the liver was not a coincidence. The presence of creamy white, round in color embedded in the liver tissue of the various rodents trapped confirms infection. The study outcomes showed that *Taenia taeniaeformis* was the most predominant parasite species encountered with 35% of the rodents found to be positive for this parasite. This finding may be attributed to environmental and climate conditions, such as suitable relative humidity and moderate temperatures, presence of cats as definitive hosts, and sanitary status in the study area, which are essential to maintain the parasites' cycle (Barthold and Griffey, 2016; Yi et al., 2010). Despite the sporadic cases of human infection with *Taenia taeniaeformis* as previously reported in Argentina, Czechoslovakia, Denmark, and Taiwan (Ekanayake, 1999; Nichol, 1981), *Taenia taeniaeformis* remains a pathogen of public health significance, thus demanding effective prevention and control measures.

Capillaria hepatica was the second most predominant species detected in tissues (liver) of wild rats studied. *Capillaria hepatica*, was previously reported to infect rodents population in different countries across the globe (Isaac et al., 2018; Seguel et al., 2017; Sinniah et al., 2014; Tung et al., 2013). This parasite causes a disease called hepatic capillariasis; a zoonotic disease mostly related to rats. Although rare in humans, *Capillaria hepatica* is responsible for hepatic capillariasis and spurious. In fact, 163 human cases (72 reports of hepatic capillariasis, 13 serologically confirmed infections, and 78 observations of spurious infections) were reported in humans from different parts of the world (Fuehrer, 2014; Fuehrer et al., 2011). Apart from rodents and humans, this parasite was reported to parasitize the liver tissue of more than 30 mammalian species, including dogs, cats, and monkeys (Hiroshi et al., 1995).

A 19% prevalence of *Capillaria hepatica* was observed in this study. The infected liver of the rodents exhibited diffuse and irregular small yellowish-white patches that appeared in streaks in the liver. The deposits of the parasite eggs surrounded by fibrosis were also observed.

Histological sections of brain tissues of rodents caught reveal the presence of the *Toxoplasma gondii*, a parasite of zoonotic importance, with a prevalence rate of 6.7%. This finding is in line with those of similar studies in Nigeria, which also reported low prevalence (0.43%) of *Toxoplasma gondii* in rodents population (Isaac et al., 2018). The presence of *Toxoplasma gondii* in rodents can be attributed to poor hygienic and sanitary conditions practiced in the study area, where stray cats are also frequent. Another possible explanation for this outcome is environmental and climatic conditions in the area (humid and warm) that is suitable for the spread and persistence of *Toxoplasma gondii* oocysts. Although rats may not be a direct source of toxoplasmosis infection to humans, they can be an intermediate host for the parasites, which later serves as a source of infection for cats and other animals, thus turning into sources of infection to humans (Vujanic et al., 2010; Webster, 1994). Humans get infected with this parasite after ingesting oocysts from contaminated water/food, or by consuming poorly cooked or undercooked meat harboring the tissue cysts, or via a congenital transmission (Duey, 2010; Tenter et al., 2000). Findings of previous studies showed that pregnant women and immune-compromised persons, such as HIV/AIDS positive persons, are most susceptible as they could suffer complications arising from *Toxoplasma gondii* infection (Busari et al., 2011).

In this research, a 16% prevalence of *Angiostrongylus cantonensis* was

observed. *Angiostrongylus cantonensis*, commonly known as rat lung-worm, is a major cause of eosinophilic meningitis in Malaysia and Southeast Asia (Punyagupta and Juttijudata, 1975). Adult parasites of *Angiostrongylus cantonensis* inhabit the pulmonary arteries of several genera of rodents as definitive hosts, mostly in the species of *Rattus* and *Bundicota*. The symptoms of *Angiostrongylus cantonensis* infection in humans range from mild (flu-like) to severe (paralysis, coma, and eosinophilic meningitis) forms. This parasite is typically described as a parasite of the tropical but can be found in gastropod (snail) host found in a more temperate climate (Walden et al., 2017). The presence of *Angiostrongylus cantonensis* in rodents observed in this study can be attributed to the distribution of the intermediate host in the study area and conducive weather (moist soil) for their survival (Kim et al., 2014a). The intermediate host of this parasite has a significant role in maintaining the lifecycle of this parasite among the rodent population. Hence, uninfected rodents may be infected by ingesting an infected intermediate host. The appearance of *Angiostrongylus cantonensis* in rats and humans causes neurologic disorders commonly termed neurologic angiostrongyliasis, which can be an indicator of instability within the human community and the consequent general failure of both direct and indirect disease control measures.

Histopathological examination of skeletal muscle revealed the presence of cysts suspected to be those of *Sarcocystis* spp. at a prevalence rate of 9%. Cysts observed had a striated cyst wall, but it was difficult to differentiate the species under microscopy. Hence, a more extensive study is required to confirm the identity of this parasite. The molecular technique is the alternative means required to support the differentiation of this parasite to species level in future studies. Previous studies have reported the cysts of *Sarcocystis* spp. in skeletal muscles of rodents (Thomas et al., 1997).

Infection with *Sarcocystis* species results in a disease called Sarcocystosis in humans as well as wild and domestic animals (Dubey et al., 2015). Generally, cats and dogs have been recognized as competent definitive hosts, while rodents, cattle, pigs, horses, sheep, goats, birds, camelids, reptiles, and humans are intermediate hosts (Ambu et al., 2011; Dubey et al., 2015; Fayer, 2004). Sarcocystiasis in human is characterized by diarrhea, nausea, bloating, dyspnea, tachycardia, and loss of appetite (Fayer, 2004; Morera et al., 1979).

5. Conclusion

This is the first report on endoparasites of wild rats in Serdang, Selangor, Malaysia. The findings reported in this study showed that wild rats captured in the study area were infected with various species of parasites of zoonotic importance. This poses a potential risk of rodent-borne disease transmission to humans. Therefore, there is a need to promote awareness on prevention and control of rodents population, not only in the study area but also in other places, where most populations live in close contact to rodents and other animals. Further studies on other rodents associated zoonosis such as viruses, bacteria, and fungi are recommended.

Declaration of competing interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2020.01.008>.

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