Original Research Article

Prevalence of ectoparasitic infection in rodents: Zoonotic implications on human health

ABSTRACT

Background and Aims: Rodents constitute more than 42% of the known mammalian species, with 1700 species which belongs to three different families, include Muridae, Microtidae, and Sigmodontidae. Rodents species such as *Rattus rattus diardii* and *Rattus norvegicus* play an important role as hosts for ectoparasites and reservoirs for various types of viruses, bacteria, rickettsia, protozoa and helminths which are responsible for causing zoonotic diseases to humans and other vertebrate animals. The aim of this work is to identify the species of mites, ticks, and fleas causing diseases to humans and determined the prevalence of infestation in relation to gender, age, and habitat of the rodents.
Place and Duration of the Study: Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, University Malaysia, Between September 2018 and March 2019
Methodology: Wild rats were captured using live traps from garbage areas, and places near the cafeteria in the student's residential colleges at University Putra Malaysia. The rats were humanely euthanised and identified. They were classified as adult or juveniles. Their sex was also determined. Ectoparasites were collected by combing the fur the rodents on to a white plan sheet paper. The ectoparasites collected were washed and mounted with Hoyer's media on a glass slide. Parasites were identified using a key morphological feature.
Results: A total of 89 wild rats were trapped and examined for ectoparasites. Eight different species of ectoparasites that comprised of *Laelaps echidnanus, Laelaps nuttalli, Ornthonyssus bacoti Ixodes granulatus, Heamaphysalis* spp*., Polyplex spinoluso, Hoplopleura pacifica*, *Xenopsylli cheopis*) were identified from the rodents examined. About 55% of the rodents trapped were positive for at least one species of ectoparasite parasites, and about 45.8% of the male rats and 30.8% of female were positive for ectoparasites. Meanwhile, in the adult, 42.9% are positive for at least one species of ectoparasites, whereas 32.2% of the juvenile rodent was also found positive for at least one species of ectoparasites.
Conclusion: The results of this study indicated that rodents trapped from the student's colleges in University Putra Malaysia are infected with various ectoparasites species that they may play an important role in the transmission of certain zoonotic diseases to humans. Therefore, we conclude that there is a potential risk of rodent-borne zoonotic diseases transmission to humans in the study area. Awareness of prevention and control of rodent-borne diseases should be introduced to educate the students on the importance of zoonotic diseases associated with rodents.

Keywords: Ectoparasites, Rodents, Zoonotic, Diseases

1. INTRODUCTION

Several numbers of devastating diseases in tropical and sub-tropical areas of the world are as the result of infection with parasites (32). A World Health Organization (W-H-O) report on the leading causes of death worldwide shows that one-third of all deaths are due to parasitic and infectious diseases (42). Rodents play an important role as hosts for

ectoparasites and reservoirs for various types of viruses, bacteria, rickettsia, protozoa and helminths which are responsible for causing zoonotic diseases to humans and other vertebrate animals (12). However, these zoonotic diseases from rodents can be transmitted to humans indirectly through ectoparasites such as mites, ticks, and fleas. It can also be transmitted directly through bite wounds, consuming food or water contaminated with rodent faeces or urine.

The etiological agents of many infectious diseases utilise invertebrate hosts during their life cycle. Most of these agents are adapted to hematophagous arthropods that share their vertebrate hosts. Therefore, the identification of these arthropod vectors and vertebrate reservoirs is usually a key to sustain an efficient control of vector-borne diseases (37).

Ectoparasites that include lice, fleas, mites, and ticks are commonly found in wild rats and other rodent species (16). They are classified into five main groups , namely; Mesostigmata (mites), Acarina (ticks), Prostigmata (chiggers), Phthiraptera (lice) and Siphonaptera (fleas) (37). Prevalence studies on ectoparasites infestation in rodent has been reported all over the world (19,17,12, 47, 11, 40, 15, 35, 43, 10, 9). But due to ecological differences in different areas of the country, the parasitic fauna of the rodents in each ecological setting might be different. This notion justifies new studies on parasitic infection of the rodents in other areas of the country.

The present study aimed to identified and determine the prevalence of ectoparasites species in rodents in relation to gender, age, and habitat of the host. The study discussed the effects of these parasite species in the context of broader public health importance of zoonotic species in student's hostels University Putra Malaysia.

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2. MATERIAL AND METHODS

2.1 The study sites

The study was conducted between September 2018 -March 2019 in University Putra Malaysia, which is located (2°59'34.19" N, 101°42'16.79"E) in central Peninsular Malaysia, Kuala Lumpur. The University has seventeen student's residential colleges out of which four colleges were selected randomly for the study. The climate of the study areas is tropical rainforest climate which is warm and sunny, along with abundant rainfall, especially during the northeast monsoon season from October to March. Temperatures tend to remain constant. Maximums hover between 31 and 33 °C (88 and 91 °F) and have never exceeded 39.3 °C (102.7 °F), while minimums hover between 22 and 23.5 °C (71.6 and 74.3 °F) and have never fallen below 14.4 °C (57.9 °F). Typically receives minimum 2,600 mm (100 in) of rain annually; June and July are relatively dry, but even then, rainfall typically exceeds 133 mm (5.2 in) per month.

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2.2 Collection of rodents

Rodents were trapped from the study sites for the period of seven months from September 2018 to March 2019. The rodents were trapped using rectangular metal trap baited with meat, as previously described (4). The trapsped were set in the late evening closer to up in garbage stogege compiles areas, students hostel and students' canteen areas feteria in the University campus. However, the traps were set late evening day and collected and The traps were brought back to the Parasitology laboratory of the Department of Medical Microbiology and Parasitology the following morning for investigation.

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2.3 Animal euthanisation

Trapped rodents were euthanised using carbon monoxide. The animals were placed into a sealed chamber, and carbon monoxide was introduced. After the successful euthanisation of the animal, the rodents were removed and place on a clean dissecting board for identification and dissection (24). After the euthanisation, the rats were classified as adult or juveniles based on their weight, length, and the degree of development of their reproductive organs and their gender was also determined (4).

2.4 Collection of ectoparasites

The fur of the animal was combed thoroughly on to a white A4 plan sheet paper using a fine-tooth comb. The Parasites that fell on the white paper from the fur were collected and transferred into a bijou bottle containing 70% alcohol for preservation. Separate containers were used for each animal. A forcep was also used to dislodge the ticks and mites that are difficult to be dislodged detached using thea comb. The parasites insects collected were preserved before identification (4).

2.5 Mounting and identification of ectoparasites

The preserved ectoparasites were washed using lactophenol and sorted based on their morphology. Preliminary identification of preserved ectoparasites was made under a dissecting microscope. The identification of the ectoparasites was carried out by mounting the parasites on slides with Hoyer's mounting media and observed under a microscope (Nikon eclipse 50ί. Japan). The identification was performed by determining the diagnostic characteristics of the ectoparasites. However, the identification of the ectoparasites ~~parasites~~ was based on morphological characteristics using taxonomic keys ~~ectoparasites identification keys~~ (28, 44). Identified ectoparasites specimens were classified into four groups, including fleas, mites, ticks, and lice.

2.6 Statistical analysis

All analysis was carried out using graph prism statistical software and excels spreadsheet. Data were presented in percentage. Non-parametric test such as Mann Whitney and Kruskal Wallis test were used to compare the mean differences in parasitic infection between the gender, age, and habitat of the host. $P < 0.05$ was considered Significance.

3. RESULTS

A total of 89 rodents that comprised of three different species (*Rattus rattus diardii*, *Rattus norvegicus,* and *Rattus tiomanicus*) were examined for ectoparasites. However, eight genera of ectoparasites consisting of seven species that belong to four different groups were identified in the present study. Three species of mites (*Laelaps echidnanus, Laelaps nuttalli, Ornthonyssus bacoti*), two species of ticks (*Ixodes granulatus*, *Heamaphysalis spp*.), two species lice (*Polyplex spinoluso, Hoplopleura pacifica*), and one species of flea (*Xenopsylli cheopis*) were identified. The overall prevalence of infestation shows that 55% of the rodents captured were positive for at least one species of parasites.

The results in Table 2 show the prevalence of ectoparasites in relation to the habitat of rodents. All the rodents captured from the four habitats were found infested with similar ectoparasites species. However, rodent trapped from college 11 showed high ectoparasites infestation rate with 45.8% are positive for at least one species of ectoparasites, followed by college 10 with 41%, college chancellor 38%, and college 17 which has the less prevalence rate of 29.6%. A Kruskal-Wallis H test showed that there was a statistically significant difference in the prevalence of ectoparasites infestation between rodents from the four colleges, H value =13.55, *P* =.0036.

Table 3 shows the distribution of ectoparasites infestation in relation to the gender of the host. The results showed that more male rodents 45.8% were infested with ectoparasites compare to the female that has a prevalence rate of 30.8%. Furthermore, Mann Whitney U test indicated that the differences in terms of parasitic infection between male and female rodent were not statistically significant (P>0.05, *P*=.87). However, in Male rodents, the most prevalent species of ectoparasites species identified were *Xenopsylla cheopis* (64.5%), *Hoplopleura pacifica* (55.5%), *Haemaphysalis spp.* (50%), *Polyplax spinulosa* (50%). Whereas *Ixodes granulates* (33.8%), *Liponyssoides sanguineus* (33.3%), *Laelaps echidnina* (27.7%), *Ornthonyssus bacoti* (27.7%), were found to be less prevalent species. Meanwhile in female *Xenopsylla cheopis* (37.7%), *Polyplax spinulosa* (35.8%), *Ornthonyssus bacoti* (35.8%), and *Hoplopleura pacifica* (30.1%) showed the highest prevalent rate, whereas were *Laelaps echidnina* (24.1%), *Liponyssoides sanguineus* (26.4%), and *Ixodes granulatus* (28.3 %) *Haemaphysalis* spp. (28.3 %) shows a low infestation rate.

The rodents population was composed of more adult (n=50/56%) than juvenile (n=39/44%). However, both the adult and juvenile wild rats were found positive for ectoparasites parasites. The results show that ectoparasitic infestation was slightly higher among the adult rodents with 42.9% of the adult are infested with at least one species of ectoparasites whereas 32.2% of the male rodent was also found infested with at least one species of ectoparasites, But however the difference was not statistically significant (*P* = .91, Mann-Whitney *U*=30.50, Mean rank for adult = 66.50, Mean rank for juvenile=6950) (Table 4). Among the adult, high infestation rate with *Xenopsylla cheopis* (79.9%), *Polyplax spinulosa* (48.7%), *Haemaphysalis* spp. (46.1%), was observed compared to *Liponyssoides sanguineus* (38.4%), *Laelaps echidnina* (30.7%), *Ornthonyssus bacoti* (38.4)%, *Ixodes granulatus* (33.3%), *Hoplopleura pacifica* (30.7%). Whereas in juvenile *Hoplopleura pacifica* 48%, *Polyplax spinulosa* 36%, *Haemaphysalis* spp. 36%, *Laelaps echidnina* (32%), *Ixodes granulatus* (32%), *Xenopsylla cheopis* (30%), were the most encountered ectoparasites among the juvenile rodents compared to *Liponyssoides sanguineus* (22%), *Ornthonyssus bacoti* (28%) which are rarely observed.

Table 1: Prevalence of ectoparasites on rodents according to the group of parasites

Ectoparasites group

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Rodent spp.	Examined	Infested	Mites	Ticks	Lice	Fleas
R. rattus diardii	44	21	14 (31.8%)	20 (45.4%)	14.5 (33%)	15 (34%)
R. norvegicus	28	14	10 (35.7%)	6 (19.6%)	12 (43%)	12 (71%)
R. tiomaticus	17	10	4 (21.6%)	9 (50%)	5.5 (42.3%)	11 (64.7%)
Total	89	45	28 (62%)	35 (77.7%)	32 (71.1)	38 (84.4%)

Table 2: Prevalence of ectoparasites infection in college 17, College Chancellor, College 10, and 11

Habitat	College 17	College Chancellor	College 10	College 11
No examined	(n=32)	(n=19)	(n=21)	(n=18)
No. Positive	10 (29.6%)	7 (38%)	8.6 (41%)	8.2 (45.8%)
Laelaps echidnanus	9 (28.1%)	3 (15.7%)	7 (33.3%)	7 (38%)
Laelaps nuttalli	9 (28.1%)	9 (47.3%)	8 (38%)	2 (11.1%)
Ornthonyssus bacoti	8 (25%)	5 (26.3%)	5 (23.8%)	11 (61.1%)
Ixodes granulatus	7 (21.8%)	6 (31.5%)	11 (52.3%)	11 (61.1%)
Heamaphysalis spp.	13 (40.6%)	7 (36.8%)	9 (42.8%)	4 (22.2%)
Polyplex spinoluso	12 (37.5%)	7 (36.8%)	8(38%)	10 (55.5%)
Hoplopleura pacifica	7 (21.8%)	10 (52.6%)	9 (42.8%)	10 (55.5%)
Xenopsylli cheopis	11(34.3%)	11 (57.8%)	12 (57.1%)	11 (61.1%)
Total	**76 (29.6%)**	**58 (38%)**	**69 (41%)**	**66 (45.8%)**

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Table 3: Distribution of the ectoparasites infection according to the gender of the host

Ectoparasite species	Male (n=36)	Female (n=53)
Mites		
Liponyssoides nuttalli	12 (33.3%)	14 (26.4)
Laelaps echidnina	15 (41.6%)	13 (24.5)
Ornthonyssus bacoti	10 (27.7%)	19 (35.8)
Ticks		
Ixodes granulatus	14 (38.8%)	15 (28.3)
Haemaphysalis	18 (50%)	15 (28.3)
Lice		
Polyplax spinulosa	18 (50%)	19 (35.8)
Hoplopleura pacifica	20 (55.5%)	16 (30.1)
Flea		
Xenopsylla cheopis	25 (69.4%)	20 (37.7)
Overall prevalence	**45.8%**	**30.8%**

4. DISCUSSION

The ectoparasites of rodents play an important role as vectors of pathogenic microorganisms that transmit different diseases to humans (20,29). However, several studies on ectoparasites of rodents were reported in Malaysia (25,33,39), and other neighbouring countries, including Indonesia, Viet~~ }~~n~~ N~~am, Bangladesh, and Singapore. The ectoparasites recovered from rodents in the present study belong to four main groups. They are Mites (Mesostigmata), ticks (Acarina), louse (Phthiraptera) and fleas (Siphonaptera). Fleas are known to transmit bubonic plague, essentially a zoonotic disease caused by a bacteria *Yersinia pestis*, from rodents to humans. However, in South and Southeast Asia, plague remains endemic in several regions (e.g., India and Vietnam), with regular outbreaks among humans (11). The flea species *Xenopsylla cheopis* and *Ctenocephalides canis* serve as intermediate hosts for species of tapeworms that occasionally infect humans (38).

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Furthermore, it has been reported that fleas serve as vectors of several diseases that include Salmonellosis, Tularemia, Leishmaniasis, Trypanosomiasis and relapsing fever infections. In the current study, we recorded the presence of *Xenopsylla cheopis* among wild rat captured at the prevalence rate of 84.4%. However, the high prevalence of *Xenopsylla cheopis* in the present study indicates a potential risk of transmission of diseases (Rickettsia typhi and plague,) associated with this parasite to humans. Other previous studies have reported a high prevalence of *Xenopsylla cheopis* in rodents (25, 33,9,34). According to ~~World health organisation (~~W~~.~~H~~.~~O~~)~~ in 2015, the *Xenopsylla cheopis* on wild rats represents a potentially dangerous situation with regard to increased plague risk for human beings in the event of an outbreak of plague (WHO Plague Manual). Although, there have not been outbreaks in recent years in Peninsular Malaysia. But, It's endemic in other Southeast Asian countries, including Indonesia (13,45). Thailand (8), Vietnam (36) and Myanmar (3). The first case of plague in Malaysia occurred in Penang in 1896, and the most recent case was in Perak in 1928 (30). The mite species *Laelaps nuttali, Laelaps echidinus* and *Orthonyssus bacoti* are also ectoparasites

that are often found in rodents. However, in the present study, all three species of mites were found in the trapped rodents. The *Laelaps nuttali, Laelaps echidinus* and *Ornithonyssus bacoti* are medically importance ectoparasites species, and they are known to transmit diseases to humans. The mite species *Ornithonyssus bacoti* does not only parasitizes the wild and domestic rats but also bites the human as the accidental host, consequently leading to transmission of filariasis to humans since that the *Ornithonyssus bacoti* is the intermediate host of the filarial worm (40).

Furthermore, *Ornithonyssus bacoti* also caused mite dermatitis in humans. The first case report of *Ornithonyssu bacoti* causing dermatitis in humans was reported from Australia, followed by other cases reported in the USA and Germany (2). It has been estimated that approximately 80% of the wild rodents in Germany are infested by this parasite (2). In Malaysia, the first authentic case of dermatitis caused by *Ornithonyssu bacoti* was reported in 1974 (31). Therefore, the presence of *Laelaps nuttali, Laelaps echidinus*, *Ornithonyssus bacoti* in the rodent's population in the student's residential colleges in UPM may have a potential risk to students living in the respective colleges.

Two species of ticks recovered in the present study include; *Haemaphysalis* spp, and *Ixodes granulatus*. The *Haemaphysalis spp.* is medical importance tick species which transmits different groups of pathogens including protozoa (Babesia), bacteria (Tularemia), Richettsia spp and arboviruses. Their bites can also cause stress and blood loss to the animal and human hosts (1). *Ixodes granulatus* is also one of the medical importance ectoparasites because it is the main vector of Langat Virus (42). Apart from Langat virus, *Ixodes* spp. is also known to transmit other pathogens such as babesiosis, human granulocytic anaplasmosis, Lyme disease (28). In Malaysia, *Ixodes* spp is involved in the spread of tick typhus and Q fever to humans in the climax forest of Peninsular Malaysia (26). The high prevalence of tick species (77.7%) infesting rodents seen in the present study may be due to the suitable environment for tick survival, because ticks are likely to be found in habitats such as shrubs, forest, and plantation and some of the students hostels in UPM where the trapping of rodents conducted was very close to forest plantations. Previous similar studies have also reported these tick species infesting wild rats and other forest rodents (5,12,18,27).

The *Polypolax spinulosa* and *Hoplopleura pacifica* were two common lice species encountered in the present study. They were found infecting rodent population at the prevalence rate of 37%, and 36 % respectively. Lice are medically importance ectoparasites in both human and rodents; they are known to harbour and transmit plague bacilli and transmit tularemia bartonellosis to humans. There bites in human causes a condition called pediculosis. Furthermore, *Polypolax spinulosa* can also transmit *Trypanosoma lewisi*, *Hemobartonella muris*, and Rickettsia typhi and the Clinical manifestation associated with *Polyplax* spp. comprised of anaemia and general unthriftiness, leading to debilitation (5). In Malaysia, infestation with *Polypolax* spp and *Hoplopleura pacifica* has been previously reported in urban rats (37,33). *Hoplopleura pescinata, Polyplax spinulosa* and *Hoplopleura pacifica* have also been previously reported in rodents from Kuala Selangor Nature Park (5). Similar findings on rodents ectoparasites infestation with louse species *Polyplax spinulosa* and *Hoplopleura pacifica* has been reported worldwide (10, 47, 41, 15).

Age-related differences in term of ectoparasitic infestations among the wild rats observed in the present study could be attributed to the larger body of male hosts, this demonstrates higher parasite infestation levels than smaller individuals (female), as they constitute a better nutritional resource for parasites and provide them with a greater variety of niches. In addition, the home range of males tends to overlap, (in search for food, reproductive partner, courtship) which could also increase their exposure to infection, whereas the reproductive female shows a stronger site-specific organisation which could explain the low rate of transmission (21,6). Other previous studies have reported a similar finding (21,22). Furthermore, these finding may be as results of the fact that older rats have a longer exposure time to potential infection compared to juvenile (7). On the other hand, the low prevalence of infestation observed in the juvenile rats is probably biased due to the low number of juvenile rats captured. The similarities in species recovered from four different locations may be as a result of similarity in the geographical structure of the samplings sites since the trapping was carried out within similar geographical areas that have similar vegetation cover. It has been previously reported Similarity in geographical structure can bring the similarity in the fauna of the ectoparasites in the different regions (22).

4. CONCLUSION

The finding of this study showed that wild rats captured from four colleges (College 17, College Chancellor, College 10 and 11) in UPM are infected with different ectoparasites of zoonotic importance. This suggests a potential risk of arbo-borne disease transmission to humans. Therefore, there is a need for further studies to investigate the distribution of diseases which are transmitted by ectoparasites to humans in places, where most of the population live in close contact to rodents, livestock, and dogs. Awareness of prevention and control of rodent-borne diseases should also be introduced to educate the students on the importance of zoonotic diseases associated with rodents.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as with guidelines of the animal care and use committee (ACUC), University Putra Malaysia. All experiments have been examined and approved by the Animal ethics committee of the University of Putra Malaysia (Ref. No: UPM/IACUC/AUP-R039/2018).

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