Original Research Article

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

Comment [1]:
Ectoparasite infection

Comment [2]:
Rodents from a university community in Malaysia.

Comment [3]:
Delete. Zoonotic implication means impart on human health.
So you can say 'Implications on human health' or 'Zoonotic implications'

ABSTRACT

Background and Aims: Rodents constitute more than 42% of known mammalian species. The 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 of 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 study was to identify the ectoparasites on rats (mites, ticks, and fleas) capable of causing diseases in humans and determine the prevalence 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 of Malaysia, Between September 2018 and March 2019
Methodology: Wild rats were captured using live traps set in garbage areas, and near the cafeteria in the student's residential area of the University of 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 of the rodents on to a plain white paper. The ectoparasites collected were washed and mounted with Hoyer's media on a glass slide. Parasites were identified using a key based on morphology.
Results: A total of 89 wild rats were trapped and examined for ectoparasites. Eight different species of ectoparasites: *Laelaps echidnanus, Laelaps nuttalli, Ornthonyssus bacoti Ixodes granulatus, Haemaphysalis* 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 and about 45.8% of the male and 30.8% of female rats were positive for ectoparasites. Meanwhile, in the adult, 42.9% were positive for at least one species of ectoparasites, whereas 32.2% of the juvenile were 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 were infected with various ectoparasite species that may play an important role in the transmission of certain zoonotic diseases to humans. Therefore, we concluded that there was a potential risk of rodent-borne zoonotic disease 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, dDiseases

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 weare due to parasitic and infectious diseases (42). Rodents play an important role as hosts offor 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 like 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 haeematophagous 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)., bBut 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.

Comment [4]:
Place and duration should be written as a sentence.
Laelaps echidnanus or Echinolaelaps echidninus? Or l. echidnina?
Polyplax spinulosa not polyplex spinoluso
Xenopsylla cheopis -Rothschild, 1903

Comment [5]:
Add lice, mites, flea, tick

Comment [6]:
This should be [1]. Numbered in order of first citing.

Comment [7]:
Such that

Comment [8]:
Mites are not only in mesostigmata. Mites and ticks are Acarina. Parasitic Mites = Order parasitiformes and ticks O. Ixodida. Dust mites, fur mites belong to O. Sarcoptiformes. Sort out classification of mites and ticks and state source
The Prostigmata is a suborder of mites belonging to the order Trombidiformes, which contains the "sucking" members of the "true mites" (Acariformes). Many species are notorious pests on plants

The present study aimed of the present study was to identifyied 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 in the University Putra Malaysia.

2. MATERIALS AND METHODS

2.1 The study sites

The study was conducted between September 2018 - March 2019 in University Putra Malaysia (UPM), 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 with. mMaximums hover between 31 and 33 °C (88 and 91 °F) and have never exceeded 39.3 °C (102.7 °F), while minimums are 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.

2.2 Collection of rodents

Rats 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 traps baited with meat, as previously described by (4). The trapsped were set up in garbage collection areas, students hostel and students' cafeteria in the University campus. However, the traps were set late in the evenings, day and collected the next day's morning and brought back to the Parasitology laboratory of the Department of Medical Microbiology and Parasitology the following morning for investigation.

2.3 Animal euthaanaisiation

Trapped rodents were euthanised by introducing using carbon monoxide in to a sealed chamber with tThe animals were placed into a sealed chamber, and carbon monoxide was introduced. TAfter 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 classificationed as adult or juveniles based on their weight, length, and the degree of development of their reproductive organs and their gender was also determinationed (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-toothed comb. The pParasites that fell on the white paper from the fur were collected and transferred into a Bbijou bottle containing 70% alcohol for preservation. Separate containers were used for each animal. FA forceps wereas also used to dislodge the ticks and mites that weare difficult to be dislodged with the using a comb. The parasites collected were preserved before identification (4).

2.5 Mounting and identification of ectoparasites

The preserved ectoparasites were washed in using lactophenol and separatedorted 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 50i. Japan). The identification was based onperformed by determining the diagnostic characteristics of the ectoparasites. using However, the identification of the ectoparasites parasites was based on 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 Ggraph Pprism statistical software and Eexcels 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 at. $P < 0.05$.
was considered Significance.

Comment [9]:
Hostels or colleges. Be consistent with hostel because I think that's what it is

Comment [10]:
A description of the sanitary condition of the study site will be necessary. How do they dispose their refuse, do you have rats in the rooms or outside, do the rats have access to students food in the cafeteria etc

Comment [11]:
Hostel or colleges. Be consistent

Comment [12]:
Gave the mean night and day temperatures and the give the range ie lowest to highest.

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Now there are two typically, which one is the correct annual rainfall range and mean?

Comment [14]:
Rooms? Corridors? Kitchens? Where?

3. RESULTS

TA total of 89 rodents that comprised of three different rat species (*Rattus rattus diardii*, *Rattus norvegicus,* and *Rattus tiomanicus*) were collected examined for ectoparasites. Of the 89 rodents 55% was infected with at least one ectoparasite. However, Eeight speciesgenera fromof ectoparasites consisting of seven genera 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 of 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. Table 1

The results in Table 2 shows 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, rodents 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 hads the leasst 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 compared to the females that hads 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 mMale 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 females *Xenopsylla cheopis* (37.7%), *Polyplax spinulosa* (35.8%), *Ornthonyssus bacoti* (35.8%), and *Hoplopleura pacifica* (30.1%) showed the highest prevalent rates, whereas were *Laelaps echidnina* (24.1%), *Liponyssoides sanguineus* (26.4%), and *Ixodes granulatus* (28.3 %) *Haemaphysalis* spp. (28.3 %) showeds a low infestation rate.

The rodents population was composed of more adults (n=50/56%) than juveniles (n=39/44%). However, both the adult and juvenile wild rats were found positive for ectoparasites parasites. The results showed 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 rodents whereas 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			
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%)

Comment [15]: Echinolaelaps echidninus?

Comment [16]: Polyplax spinulosa?

Comment [17]: Xenopsylla cheopis?

Comment [18]: There is no mention of Table 1?How come Table 2

Comment [19]: Because you lumped all together

Comment [20]: Appearing for the first time here. Not in table Not in abstract Makes the 9th spp

Comment [21]: Is 28.3 % infestation really low? That is a 1 in 5.

Comment [22]: What does this mean? Range or male female ratio?

Comment [23]: Not presented

Comment [24]: After first mention of parasite, subsequent mention should be for eg L. echidninus.

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%)

Comment [25]:
Rattus tiomanicus?

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

Comment [26]:
This table 2 has information that can be brought out if it is presented a bit differently.
1. No Infested = total prevalence
2. The 8 species should show percentage prevalence in the infected ones.
3. Looks like what you have is the counts ie total number found in infested rats. This is intensity and is not prevalence.
4. Indicate multiple infestations. When you have more than one parasite per rat
5. Intensity = total count / number infested
% Prevalence= no infested / no examined x 100.
6.. then you can compare prevalence, intensity and multi parasitism along stations

Habitat	**College 17**	**College Chancellor**	**College 10**	**College 11**
No examined	(n=32)	(n=19)	(n=21)	(n=18)
No infested	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%)**

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)

Comment [27]:
Arrange Table 2 like this but include observations in the notes
This is for cummulative of all rats. But you had 3 types of rats. Show this table to show which rat type had gender differences in the ectoparasite infestation

Comment [28]:
Liponyssoides sanguineus?

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 have beenwere reported in Malaysia (25,33,39), and other neighbouring countries, including Indonesia, Viet Nam, Bangladesh, and Singapore. The ectoparasites recovered from rodents in the present study belong to four main groups [. They are Mites (Mesostigmata), ticks (Ixodoidae, or Argasidae orAcarina), 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(ref). 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).

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 rats 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) 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, b. 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 *L. aelaps nuttali, L. aelaps echidinus* and *O. rnithonyssus bacoti* are medically importantce 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 as to humans since that the *O. rnithonyssus bacoti* is the intermediate host of the filarial worm (40).

Furthermore, *O. rnithonyssus 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 *O. rnithonyssu bacoti* was reported in 1974 (31). Therefore, the presence of *L. aelaps nuttali, L. aelaps echidinus*, *O. rnithonyssus 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 of medical importance as it tick species which transmits different groups of pathogens including protozoa (*Babesia*), bacteria (Tularemia), *Richettsia* spp and arbovirusees. Their bites can also cause stress and blood loss to the animal and human hosts (1). *I. xodes granulatus* is also one of the medical importance ectoparasites because it is the main vector of Langat vVirus (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 wais 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 importantce ectoparasites in both human and rodents; they are known to harbour and transmit plague bacilli and

Comment [29]:
Mites are also acarina

Comment [30]:
Are all the rats wild rats?
R. tiomanicus is a field rat, what of the ones you got from the hostels like R. rattus?

Comment [31]:
Number?

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Not in Table 3

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transmit tularemia bartonellosis to humans. ~~There~~Their bites in human causes a condition called pediculosis. ~~Furthermore, Polypolax spinulosa~~ can also transmit *Trypanosoma lewisi*, *Hemobartonella muris*, and *Rickettsia typhi* and the ~~c~~Clinical manifestation are ~~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 ectoparasite~~s~~ 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 tend~~s~~ 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 infestation~~transmission~~ (21,6). Other previous studies have reported ~~a~~ similar finding~~s~~ (21,22). T~~Furthermore, t~~he similarity~~se finding~~ may be because ~~as results of the fact that~~ older rats have a longer exposure time to potential infection compared to juvenile~~s~~ (7). On the other hand, the low prevalence of infestation observed in the juvenile rats wa~~i~~s 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 that s~~S~~imilarity in geographical structure can bring about ~~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 wea~~r~~e 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 more~~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).

R~~EFERENCES~~EFERENCES

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Comment [34]:
Do you mean overindulgence, folly?

Comment [35]:
Age related is not the same as gender difference. If you want to relate to size then say size not age. A small female and a large male could be same age with different intensity because of size with more surface area.

Comment [36]:
Numbering is according to first citing and not alphabetical. So renumber from 1 to the end and rearrange references accordingly.
Journal names should be italicised

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