

## Article

### Relationship between the sex and age of *Mus musculus* (Rodentia: Muridae) with ectoparasites prevalence in northeast of Iran

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#### Abstract

Rodents' significant role in transmission of serious zoonotic diseases is considered as an interesting subject in public attention. With respect to the importance of arthropod-infested rodents in zoonotic diseases transmission and extent distribution of *Mus musculus*, the present study aimed to analyze the interrelationship between hosts' sex and age of *M. musculus* with the ectoparasites prevalence in Mashhad and vicinity, northeast of Iran. Mites, ticks and lice were cleared in Nesbit and fleas were made transparent by 10% KOH and then individually mounted in Hoyer's medium. They were identified with the aid of valid identification keys. A total of 267 ectoparasites belonging to eight species were collected from 93 hosts (48.38% male vs. 51.62% female). Among parasite groups, mites, ticks, lice and fleas occurred in prevalence of 46.06%, 22.84%, 16.85% and 14.23%, respectively. Our results demonstrated significant higher ectoparasite burden on male hosts for *Haemolaelaps* sp., *Nosopsyllus fasciatus*, *Ctenophthalmus* sp. and *Laelaps algericus*. However, two tick species of *Haemaphysalis punctata* and *Haemaphysalis* sp. showed a tendency of higher prevalence in females and no significant difference in the prevalence of lice species of *Hoplopleura captiosa* were observed between the host sexes. Similarly, no significant differences in ectoparasite infestations were observed between the age categories.

**Key words:** Ectoparasite; infestation; gender; maturity; prevalence; rodents.

#### Introduction

Rodents are recognized as significant hosts in transmission of serious zoonotic pathogens including *Borrelia* ssp., *Babesia* ssp., *Trypanosoma* ssp., *Leishmania* ssp. (Baker and Wharton 1952; Karbowiak *et al.* 2005; Klimpel *et al.* 2007). The house mouse (*Mus musculus*) has been considered as a cosmopolitan species that lives in close association with humans, and transported frequently to all areas by human's civilization (Hutchins *et al.* 2003). Due to their importance in infection of humans and other animals, they are considered as an interesting subject in public attention (Klimpel *et al.* 2007; Dziemian *et al.* 2014). Some studies have reported infestation parameters and indices of some ectoparasite species on this host (Lareschi and Iori 1998; Lareschi *et al.*

2003; Nava *et al.* 2003). Some investigations have showed the relationship between ectoparasities and different sexes of wild rodents (Lareschi and Cicchino 2002; Solanki 2013; Kowalski *et al.* 2014). Furthermore, several studies have been done based on the occurrence of ectoparasites of rodents and their role in the transmission of zoonotic diseases in Iran (Hanafi-Bojd *et al.* 2007; Allymehr *et al.* 2012; Pakdad *et al.* 2012; Shirazi *et al.* 2013; Hamidi *et al.* 2015; Moravvej *et al.* 2015).

Numerous researches on the association of host sex and ectoparasites infestation demonstrated that male or female bias of parasitism was in direct relation to the biology and ecology of rodent hosts (Marshall 1981). Various responses to parasitism depend on differences between males and females hosts in their anatomy, physiology, behavior, and evolutionary roles which are related to their roles in population and community dynamics of parasites (Combes 2001). It is obvious that sex-biased in parasite infestation may occur in several host-parasite relationships, at numerous locations, and in some periods of time but not in all cases (Krasnov *et al.* 2012). Most reports of sex-biased infestations in rodents show that there is more male-biased (Matthee *et al.* 2010; Scantlebury *et al.* 2010; Harrison *et al.* 2010) than female-biased host-parasite relationships (Hillegass *et al.* 2008; Rossin *et al.* 2010). With respect to the importance of arthropod-infested rodents in zoonotic diseases and extent distribution of *M. musculus*, the aim of the present study was to analyze the interrelationship between the sex and the age of *M. musculus* with the ectoparasites prevalence in Mashhad and vicinity, Khorasan Razavi Province, Iran.

## Materials and Methods

### *Field study and parasitological collection*

The study was conducted in 11 locations from Mashhad and vicinity, Khorasan Razavi Province, northeast of Iran (From 35° 60' N, 59° 15' E to 36° 35' N, 60° 25' E) during the period between April 2013 and April 2015. These sites were selected based on the previous author's pilot field studies which had demonstrated the presence of house mice, so it leads to increase the potential rate of capturing. Old buildings, storerooms, factories, parks, caravansaries, railway stations and so on were considered as our sampling targets. The rodents were captured with live traps baited with cheese, cucumber and sunflower seeds. They were transported to the laboratory and euthanized with chloroform and then placed over a white cloth. Ectoparasites were collected by brushing, combing or using a fine-tipped forceps and stored in 70% ethanol for their preservation until their identification. More detection was done using a magnifier instrument around different parts of rodent's body such as anus, head, behind ears, face, thorax, abdomen, armpits and underneath the fur, especially near dermatological lesions (Xie and Zeng 2000). The use of the rodents for the study was approved by the Ethics Committee for Animal Experiments of Ferdowsi University of Mashhad, Iran. The collected ectoparasite specimens were categorized into four groups including Siphonaptera (fleas), Acarina (mites and ticks) and Anoplura (lice). For taxonomic identification, mites, ticks and lice were cleared in Nesbitt's fluid and fleas were made transparent by 10% KOH and then individually mounted in Hoyer's medium. The ectoparasite specimens were identified using the valid taxonomic keys provided for Siphonaptera (Lewis 1967; Acosta and Morrone 2003), Acarina (Tenorio and Goff 1980; Baker 1999) and Anoplura (Kim *et al.* 1986; Korytkowski 2002). Photos were taken for both hosts and ectoparasites.

### Data analysis

For age determination, we used many signs for grouping captured samples into two main age groups (mature and immature) as follows: 1. *Weight*: Mean weight of immatures and matures were  $14 \pm 2.5$  and  $18 \pm 1.3$  grams, respectively; 2. *Genital system*: In immatures it was hard to distinguish between male and female in first glance and it need more assessment. In matures, it was very easy to determine the sex based on the appearance of the testis in adult males and nipple glands in adult females; 3. *Tooth cusp pattern*: There are very significant differences in the tooth cusp pattern and also the amount of erosion of the molar teeth. In the other word, by increasing the age, the more erosion will be observed in molars.

The following parameters were calculated for each host sex and age category using SPSS. Mean Abundance (MA) = total number of individuals of a particular parasite species in a particular host sex or age category/total number of hosts of that sex or age category. Prevalence (P) = (number of hosts parasitized by a particular parasite species/total number of hosts examined for that parasite species)  $\times 100$  (Bush *et al.* 1997). The significance of prevalence differences between host sexes and age categories for each ectoparasite species were analyzed using Student's t-test ( $p < 0.05$ ) (Morales and Pino 1987).

### Results

A total of 93 individuals of *M. musculus* were trapped from the study sites. About 48.38% of captured rodents were male and the rest were female (45 male individuals vs. 48 female individuals). Among captured rodents, 28 individuals were immature and 65 individuals were mature (30.10% vs. 69.89%) (Table 1). Total number of collected ectoparasites was 267 in which mites (prevalence = 46.06%), ticks (22.84%), lice (16.85%) and fleas (14.23%) showed the highest to lowest prevalence rate, respectively. Among all the captured specimens, about 36.55% (34 individuals out of 93) showed no ectoparasites infestations.

**Table 1.** Total number of collected ectoparasites species for each host sex, age category, Student's t-test and p-value is detailed from *M. musculus* in Mashhad and vicinity, Iran during April 2013 to April 2015.

Ectoparasite species	Male (n = 45)	Female (n = 48)	t	p-value	Mature (n = 65)	Immature (n = 28)	t	p-value
<i>Nosopsyllus fasciatus</i>	23	9	1.679	0.002*	19	13	0.809	0.107
<i>Ctenophthalmus</i> sp.	15	2	1.217	0.013*	13	2	-0.578	0.226
<i>Haemaphysalis punctata</i>	24	17	0.746	0.37	18	23	2.180	0.00
<i>Haemaphysalis</i> sp.	8	12	-0.492	0.494	7	13	2.289	0.00
<i>Haemolaelaps</i> sp.	71	27	2.281	0.007*	56	42	1.292	0.135
<i>Laelaps algericus</i>	3	0	1.399	0.005*	3	0	-0.883	0.73
<i>Microtrombicula</i> sp.	11	11	0.092	0.805	3	19	3.738	0.00
<i>Hoplopleura captiosa</i>	25	20	0.491	0.396	31	14	0.075	0.563
Total	179	88			150	126		

\*  $p < 0.05$

The identified species of the ectoparasites were as follows: Siphonaptera: *Ctenophthalmus* sp. (Pullicidae), *Nosopsyllus fasciatus* (Ceratophyllidae); Acari (ticks): *Haemaphysalis punctata* and *Haemaphysalis* sp. (Ixodidae); Acari (mites): *Laelaps algericus* and *Haemolaelaps* sp. (Laelapidae), *Microtrombicula* sp. (Trombiculidae); Anoplura: *Hoplopleura captiosa* (Hoplopleuridae).

#### *Siphonaptera (Fleas)*

A total of 38 fleas (Ceratophyllidae and Pullicidae) constituting of 14.23% of all captured ectoparasites were collected on the back of the rodent's body. From all the findings, 19 rodents had fleas of which 15 had *N. fasciatus* (16.12%) and 4 rodents had *Ctenophthalmus* sp. (4.30%). These results are shown in Table 1 and Fig. 1. Calculated values for mean abundance and prevalence rates of *N. fasciatus* ( $P_m = 22.22\%$ ,  $MA_m = 0.511$  and  $P_f = 10.41\%$ ,  $MA_f = 0.187$ ) and *Ctenophthalmus* sp. ( $P_m = 4.44\%$ ,  $MA_m = 0.333$  and  $P_f = 4.16\%$ ,  $MA_f = 0.041$ ) on *M. musculus*, showed differences between host sexes, and only *N. fasciatus* prevalence was significantly higher on male hosts. There was no difference between prevalence rate of immature hosts in comparison with mature hosts with *N. fasciatus* ( $P_I = 17\%$  and  $P_M = 15\%$ ) and *Ctenophthalmus* sp. ( $P_I = 7\%$  and  $P_M = 3\%$ ) as shown in Fig. 2 and Table 1.

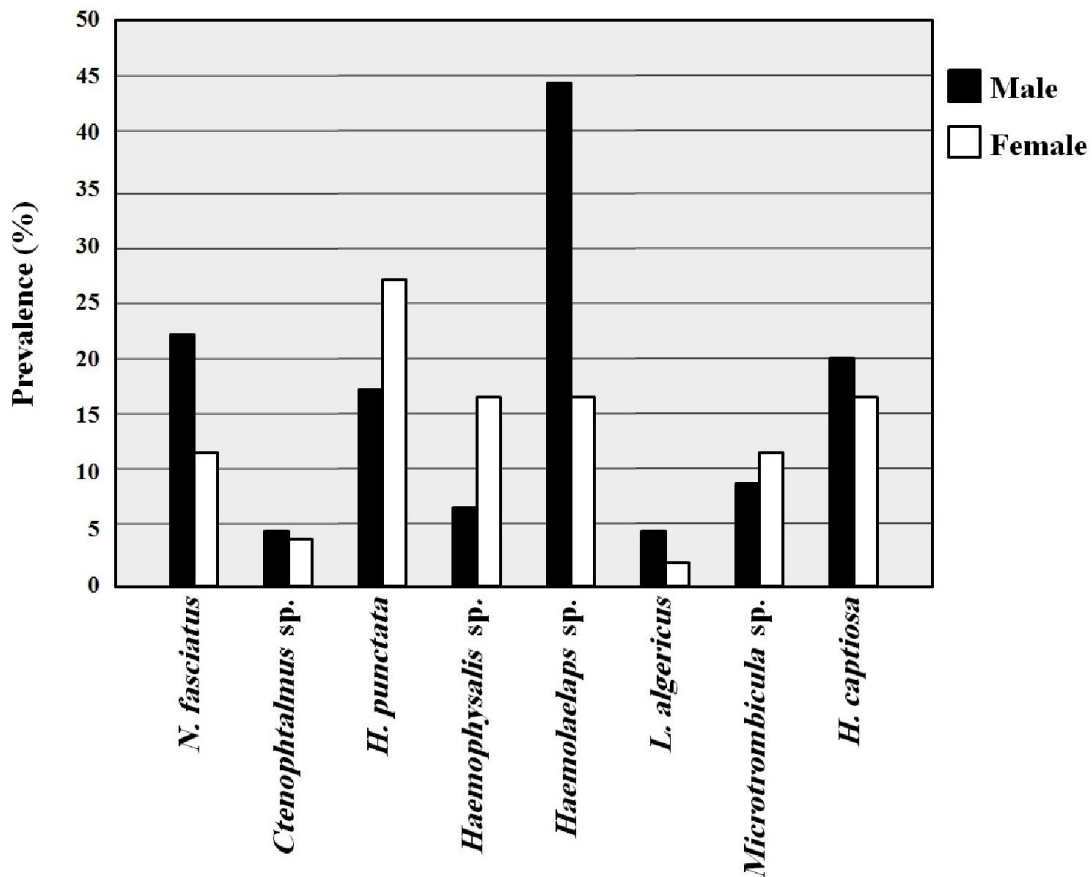
#### *Acari (Ticks)*

A total of 61 ticks (Ixodidae) were recovered from 26 rodents (27.95%) which were located mostly on ears, and some on the nose, tail and toes. Two species of *H. punctata* (22.58%) and *Haemaphysalis* sp. (11.82%) were found parasitizing *M. musculus* (Table 1, Fig. 1). Calculated values for mean abundance and prevalence rate of *H. punctata* ( $P_m = 17.77\%$ ,  $MA_m = 0.644$  and  $P_f = 27.08\%$ ,  $MA_f = 0.25$ ) and *Haemaphysalis* sp. ( $P_m = 6.66\%$ ,  $MA_m = 0.177$  and  $P_f = 16.66\%$ ,  $MA_f = 0.25$ ) on *M. musculus* in the investigated areas were higher in female hosts than male hosts. There was no significant difference in prevalence rates between immature and mature hosts in *H. punctata* ( $P_I = 25\%$  and  $P_M = 21\%$ ) and *Haemaphysalis* sp. infestations although immature rates were higher ( $P_I = 25\%$  and  $P_M = 6\%$ ) as seen in Fig. 2 and Table 1.

#### *Acari (Mites)*

A total of 123 mites (Laelapidae and Trombiculidae) constituting 46.06% of all the collected ectoparasites were collected either on the rear part of the back, abdomen and on hind legs of the hosts. Of all examined *M. Musculus*, 37 individuals were infested by *Haemolaelaps* sp. (30.10%), *L. algericus* (3.22%) and *Microtrombicula* sp. (9.67%) shown in Table 1 and Fig. 1. Calculated values for mean abundance and prevalence rate for *Haemolaelaps* sp. infestation in *M. musculus* in the investigated areas were significantly higher in males than in females ( $P_m = 44.44\%$ ,  $MA_m = 1.577$  and  $P_f = 16.66\%$ ,  $MA_f = 0.562$ ) but no significant differences host sex infestations for *L. algericus* ( $P_m = 4.44\%$ ,  $MA_m = 0.044$  and  $P_f = 2.08\%$ ,  $MA_f = 0.020$ ) and *Microtrombicula* sp. ( $P_m = 8.88\%$ ,  $MA_m = 0.244$  and  $P_f = 10.41\%$ ,  $MA_f = 0.229$ ).

The prevalence rates amongst the immature hosts in comparison with mature hosts for *Haemolaelaps* sp. ( $P_I = 42\%$ ,  $P_M = 26\%$ ), *Microtrombicula* sp. ( $P_I = 25\%$ ,  $P_M = 3\%$ ) and *L. algericus* ( $P_I = 0\%$ ,  $P_M = 3\%$ ) showed higher prevalence rates in immature hosts than mature hosts but there was no significant differences in infestations between host age categories for any mite species (Fig. 2, Table 1).



**Figure 1.** The prevalence rate of every ectoparasitic species for each host's sex from *M. musculus* in Mashhad and vicinity during April 2013 to April 2015.

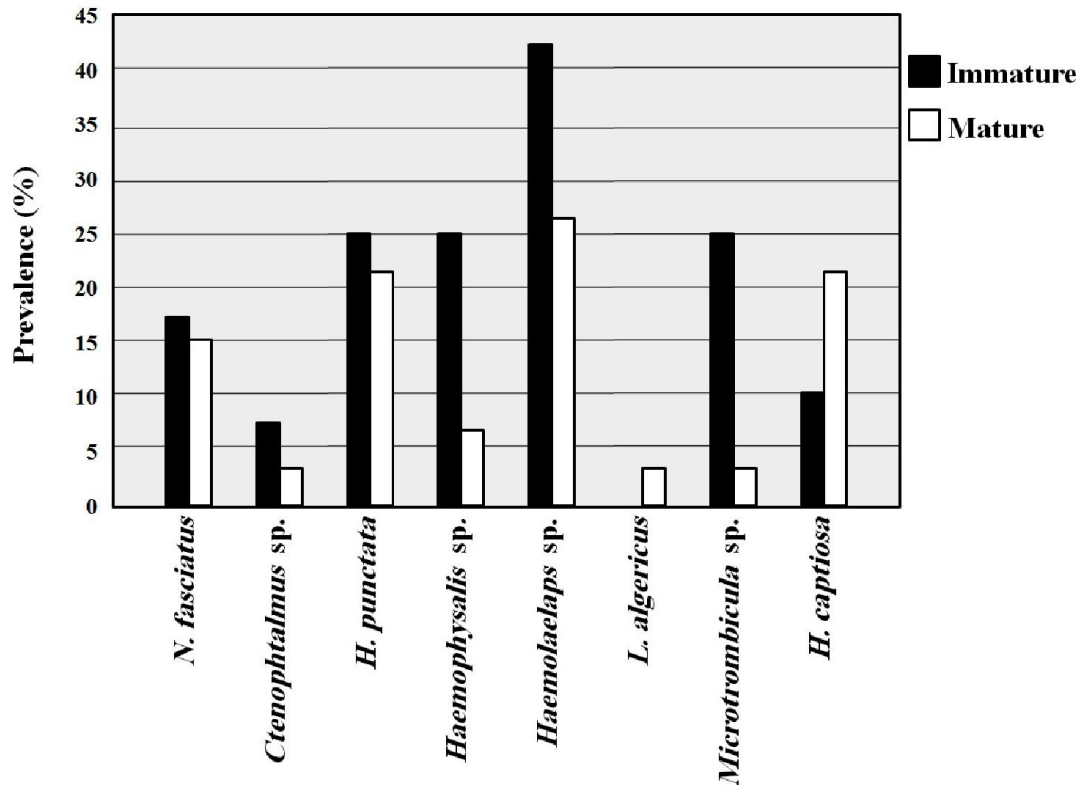
#### *Anoplura* (Lice)

Among all examined rodent specimens, 17 individual (18.28%) were infested by *H. captiosa* which was the only founded lice species in our study (Table 1, Fig. 1). In case of Anoplura, the prevalence rate appears to be independent of host sex, although males showed a higher insignificant rate ( $P_m = 20\%$ ,  $MA_m = 0.555$  and  $P_f = 16.67\%$ ,  $MA_f = 0.416$ ). Similarly, the prevalence rate in immature hosts showed lower degree in comparison to matures, but this was not significant ( $P_I = 10\%$  and  $P_M = 21\%$ ) (Fig. 2, Table 1).

### Discussion

The Overall prevalence of ectoparasites in rodent hosts was 63.44%, in which mites (46.06%) and fleas (14.23%) constituted the highest and lowest prevalence in comparison to other ectoparasite groups. Similar to inferences by Soliman *et al.* (2001), our result could be attributed to the burrowing habit the host that made its mites to be influenced less by climatic changes and insecticides used for agricultural protection. Due to the fact that mites mainly live in the base parts of the hair- near the skin- they may be less affected by factors such as air disturbance, rain or using insecticides, in comparison with fleas which have flying ability and are distributed mainly on the body surface of their hosts. Furthermore, *M. musculus* live in colonies and so, transmission of

ectoparasite from one host to the other host in the colony is very common. Some ecological and behavioral features including relative size and differences in the skin and anti-parasitic defenses, in addition to physiological aspects such as hormonal levels, might also affect ectoparasite infestation parameters and indices (Marshall 1981).



**Figure 2.** The prevalence rate of every ectoparasitic species for age categories from *M. musculus* in Mashhad and vicinity during April 2013 to April 2015.

#### *Relation of host's sex and ectoparasites species*

Our results demonstrated a significant higher ectoparasite burden on male host for *Haemolaelaps* sp., *N. fasciatus*, *Ctenophthalmus* sp. and *L. algericus* which were similar to some finding of previous studies (Hillegass *et al.* 2008; Matthee *et al.* 2010; Kowalski *et al.* 2014). Greater size of male hosts might be the reason for higher load of ectoparasites because of its extensive ability of movements, home range size, and a superior chance of contact with individuals of the same and other species in comparison to the females (Sánchez López 1998). These behavioral features of males may provide better potentials of them being colonized by ectoparasites. Some earlier studies also indicated that male hosts are more seriously infested by fleas (Marshall 1981), while others specified that females have higher infestation (Soliman *et al.* 2001). In *R. pumilio*, the increase in male-biased parasitism of ectoparasites was affected by host density that might be related to increased male dispersal in a growing population of small mammals (Matthee *et al.* 2010). The other famous hypotheses to describe sex-biased parasitism in males is associated to their higher degree of androgens which can affect their immune system (Zuk 1996; Zuk and McKean 1996). This hormone is related

to reproductive system and aggressive actions during mate competition (Razzoli *et al.* 2003).

In the present study, two tick species of *H. punctata* and *Haemaphysalis* sp. showed a tendency of higher prevalence in females than males but this were not significant. Similarly, in some studies on ixodid ticks of rodents, no significant difference between infestations in host sexes was detected (Matthee *et al.* 2010; Kiffner *et al.* 2011) but in other research, male mice showed greater tick loads than females and analyses suggested that this sex-bias was related to body mass as opposed to sex category (Harrison *et al.* 2010).

Our results demonstrated that there was no significant difference in the prevalence of lice species of *H. captiosa* in different host sex which could be related to the fact that lice spend their whole life on the individual host (Kim *et al.* 1986). Similar rate of the prevalence and mean abundance of ectoparasites in male and female rodents was recorded for *Oxymycterus rufus* with the exclusion of those unintentionally connected (Lareschi 2006). Another investigation recorded that *Hoplopleura* species presented similarities of infestations on female and male hosts, whereas others reported male-bias due to larger body size (Marshall 1981). Moreover, no significant differences between both host sexes have been documented for *H. scapteromydis* and the spatial distribution of its eggs on the host body has been found to be similar (Liljeström and Lareschi 1998). Furthermore, there are documented studies of no sex-biased parasitism for rodents such as *Apodemus flavicollis*, *Peromyscus leucopus* and *Myodes glareolus* (Ferrari *et al.* 2004; Luong *et al.* 2009; Ribas *et al.* 2009). In our result, there was no difference between hosts sex for the mite, *Microtrombicula* sp. Previous results on Laelapidae from other host species and areas displayed substantial higher prevalence rate on males (Soliman *et al.* 2001).

#### *Relation of host's age and ectoparasites species*

A total of 267 ectoparasites were collected in which 56.17% infested mature hosts and the rest of infested immature hosts. However, there was no significant difference between prevalence rates of immature hosts in comparison to mature hosts in eight ectoparasite species collected from *M. Musculus*. In another study on 339 *Apodemus flavicollis*, immature hosts demonstrated a higher prevalence of ectoparasites than matures, but the differences related to the ectoparasite prevalence, were not significant between the host sex and also the age categories (Benedek *et al.* 2006). A study examining relationship of endoparasites infection and ectoparasite infestation with sex, age and sampling site on *Rattus* demonstrated that several parasites infection and infestation increased with age and depended on habitat but none of them was related to sex (Stojcevic *et al.* 2004).

### **Conclusion**

The results attained in the present research support the observation that the sex of the *M. musculus* may affect its ectoparasite load and specific richness which is related to the prevalence and abundance of some parasite species. Regarding the worldwide distribution of house mice and their habitat overlapping with mankind, this information will provide more knowledge of the interrelationship between ectoparasites and *M. musculus* sex and age as a cosmopolitan and high mobility species in Mashhad and vicinity. Understanding the role of ectoparasite vectors and rodents reservoirs in the preservation of diseases in the study area, and the possibilities of distribution of

pathogens will help in strategies to control zoonotic diseases which threaten human and other animals.

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## ارتباط میزان آلودگی به انگل های خارجی با جنسیت و سن در *Mus musculus* (Rodentia: Muridae) شمال شرق ایران

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### چکیده

اهمیت جونندگان در انتقال بیماری‌های زئونوتیک به عنوان یکی از موضوعات مورد توجه عموم است. با توجه به اهمیت جونندگان آلوده به بندپایان در بیماری‌های زئونوتیک و توزیع گسترده گونه موش خانگی (*Mus musculus*)، هدف این مطالعه بررسی رابطه بین جنسیت و سن میزبان‌های *M. musculus* با میزان شیوع انگل‌های خارجی در نواحی مشهد و حومه، شمال شرق ایران است. شفاف‌سازی نمونه‌های مایت، کنه و شپش در محلول نسبیّت و نمونه‌های کک با استفاده از هیدروکسید پتاسیم ۱۰٪ انجام و هر نمونه به صورت جداگانه در محلول هویر نصب شد. در مجموع ۲۶۷ انگل خارجی متعلق به ۸ گونه از ۹۳ میزبان جمع‌آوری شد. در بین گروه‌های انگلی به ترتیب مایت‌ها (۴۶/۰۶٪)، کنه‌ها (۲۲/۸۴٪)، شپش‌ها (۱۶/۸۵٪) و کک‌ها (۱۴/۲۳٪) بیشترین و کمترین درصد فراوانی را نشان دادند. نتایج نشان داد که بیشترین بار انگلی معنی‌دار در جنس نر متعلق به *Ctenophthalmus* sp.، *Nosopsyllus fasciatus* و *Haemolaelaps* sp. بودند. دو گونه کنه *Haemaphysalis punctata* و *Haemaphysalis* sp. بیشترین آلودگی را در جنس ماده نشان دادند و هیچ گونه بار انگلی معنی‌داری در شپش *Hoplopleura captiosa* دیده نشد.

واژگان کلیدی: انگل های خارجی؛ آلودگی؛ جنسیت؛ بلوغ؛ میزان شیوع؛ جونندگان.

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