Article

The relationship of ectoparasite prevalence to the capturing season, locality and species of the murine rodent hosts in Iran

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Abstract

The role of rodents in the transmission of different parasitic infections has been well documented. The objective of this study was to determine the relationship between capturing season and rodent habitat with ectoparasite prevalence in the murine rodents from Mashhad and vicinity, in northeast Iran. In addition, the host species-related effects on ectoparasitic prevalence were studied. A field survey of ectoparasites was carried out in 20 localities from April 2013 to April 2015. A total of 74 murine rodents were captured: 17.56% Apodemus witherbeyi, 35.13% Mus musculus, 21.62% Nesokia indica and 25.67% Rattus norvegicus. A total of 413 ectoparasites were collected as follows: 19.85% fleas, 24.45% ticks, 39.70% mites and 16.22% lice. The most infested rodent was R. norvegicus (P = 89.47%) and the most common ectoparasites were mites. The highest prevalence was observed in summer (P = 31.57%) and ticks were the most common ectoparasite for this season. Behesht Reza and Mashhad railway station were the highest infested areas with P = 12.28%. Prevalence of overall ectoparasites (P = 12.28%). 74.32%) was higher in Behest Reza cemetery and Mashhad railway station compared to the other localities. Seasonal changes in the prevalence of some ectoparasites paralleled those in the relative abundance of their hosts. Furthermore, the prevalence of some ectoparasites showed differences related to the locality of their hosts. There were significant differences between the prevalence of each of ectoparasitic group in all four species. These relationships are useful in understanding the role of arthropod vectors as well as their reservoirs in the transmission of diseases in humans and other animals.

Key words: Ectoparasite; Iran; rodents; sampling locality; seasonal variations.

Introduction

The order Rodentia has a worldwide distribution and the role of rodents as the most important reservoirs of zoonotic diseases has been well documented (Urceuhart *et al.* 1994; Hutchins *et al.* 2003; Nava *et al.* 2003). Various studies showed that rodents have a key role in transmission of many serious zoonotic diseases such as bubonic plague, leishmaniasis, murine typhus, and salmonellosis (e.g. Baker and Wharton 1952; Bell *et al.* 1988; Abel *et al.* 2000). Rodents, which inhabit residential areas and those which are

in close association with human's communities are of the main concern. Like most wild rodent species, these commensal rodents (mainly belong to subfamily Murinae) are hosts to a range of ectoparasite arthropods. The three most common genera of pest rodents are *Mus, Nesokia* and *Rattus*. Their small arthropods are important vectors of pathogens that cause diseases in human beings, and domestic and wild animals (Burgess 1990; Fasihi *et al.* 2000).

Anthropogenic habitat transformation and subsequent fragmentation of natural vegetation is regarded as one of the largest threats to biodiversity in the world. Increasing growth in agricultural activities in these regions has contributed to fragmentation of pristine natural vegetation which may affect rodents populations, including their density and subsequently their ectoparasitic fauna. Ectoparasites obtain some of their requirements, like oxygen, from the physical environment, and to some extent, are influenced by factors that affect their nonparasitic associates. However, ectoparasite-host association is the result of many factors, such as seasonal variations and ecological processes, interspecific variations of host may affect infestation parameters of its ectoparasites, too. So, ectoparasites are also dependent on their hosts for nutritional requirements and for developmental and maturation stimuli. The most important variations related to the host species are factors such as relative size and differences in the skin and its covering, differences in blood hormonal levels due to stress or other conditions, and behavioral factors such as differences in grooming, nesting and mobility (Marshall 1981).

In this issue, several studies have been carried out on the seasonal changes in the density of fleas infesting commensal rodents (Rifaat *et al.* 1982; Shoukry *et al.* 1986, 1987; Abdel-Gawad and Maher Ali 1987; Zeese *et al.* 1990; Soliman *et al.* 2001). Several studies have also been carried out on the seasonal changes in the density of mites infesting these kinds of rodents (Abdel-Gawad and Maher Ali 1987; Shoukry *et al.* 1987; Soliman *et al.* 2001). Abdel-Gawad and Maher Ali (1987), Shoukry *et al.* (1987) as well as Soliman *et al.* (2001) examined the seasonal changes in the density of lice infesting commensal rodents in Egypt. Studies on the relationship between the density of ectoparasite groups (fleas) and the locality of the host were conducted by Aboul-Ela *et al.* (1987), Soliman *et al.* (2001) as well as Benedek *et al.* (2011). Some researchers also have focused on the relationship between ectoparasites and wild rodents from the point of ectoparasite-host association view (e.g. Nava *et al.* 2003).

In Iran, prevalence rate of some ectoparasite species and their role in arthropod-borne diseases have been examined by several researchers (e.g. Shayan and Rafinejad 2006; Hanafi-Bojd *et al.* 2007; Paramasvaran *et al.* 2009; Rasouli *et al.* 2011; Allymehr *et al.* 2012; Pakdad *et al.* 2012; Shirazi *et al.* 2013; Moravvej *et al.* 2015). Few investigations have been conducted on the relationship between arthropods and wild mammals captured in different seasons (e.g. Jafari-Shoorijeh *et al.* 2008; Mosallanejad *et al.* 2012), nor have they considered seasonal and environmental conditions simultaneously. Furthermore, few researches focused on the relationship between the prevalence of ectoparasitic groups and the locality of rodent hosts (e.g. Dehghani *et al.* 2012).

With respect to the importance of arthropod-infested rodents in rodent-borne diseases, the present study aimed to determine the relationship between season and environmental condition with the ectoparasites prevalence in the murine rodents from Mashhad and its vicinity, northeast Iran. The interrelationship between the host species and ectoparasite prevalence was analyzed. This relationship will be useful in

understanding the role of arthropod vectors as well as mammalian reservoirs in the maintenance of various diseases in the study areas.

Materials and methods

Study area and field investigation

The study areas lie in Mashhad and its vicinity, Khorasan Razavi Province, northeast of Iran. This region is located in a generally cold and dry climatic condition. The average annual temperature is 14°C. The topography is generally flat with some mounds, well drained, barren, rocky habitats in the foothills of its vicinity. Furthermore lots of industrial and residential constructions have been built. Agriculture in rural areas is intensive and much land is under cultivation, and considerable pastureland and livestock grazing could also be observed.



Figure 1. Geographical position of the study area, Mashhad, Khorasan Razavi Province; Iran (A and B); Sampling locations shown by numbers in the vicinity of Mashhad (C): 1. Govareshk, 2. Sade Kardeh, 3. Goojgi, 4. Farokhad, 5. Mayamey, 6. Shoorak Maleki, 7. Abravan, 8. Tape Salam, 9. Torogh, 10. Moghan, 11. Hesar Golestan, 12. Zoshk, 13. Veyrani, 14. Kahoo, 15. Golmakan, 16. Khaje Morad, 17. Behesht Reza cemetery. Furthermore, Mashhad railway station, Ghadir camp and Koohestan park are sampling locations inside the Mashhad which have not been shown in the panel C.

Data on the infestation with ectoparasites were collected from rodents trapped monthly between April 2013 and April 2015, in 20 various locations (public areas, cemetery, Mashhad railway stations, farms and gardens, camps, parks, innovative areas e.g. woodland, meadows, sandy and rocky habitats) in Mashhad and its vicinity (Fig. 1).

Differences related to the standard of living, as indicated by the style of buildings, application of public health measures, and human activities, are significant in the selected sites. On this basis, Khaje Morad (as a holy public area) and Behest Reza cemetery (due to human traffic for the interment ceremony) are considered more populated sites than the others. Significant information regarding these locations is summarized in the Table 1.

Table 1. Significant information of sampling sites (public areas, cemetery, railway stations, farms, camps, parks, innovative areas e.g. woodland, meadows, sandy and rocky habitats) in Mashhad and its vicinity, northeast of Iran.

Sampling site	Locality*	Habitat type [¥]	Significant environmental parameter	
			Buildings structure	
Govareshk	V	I	-	
Sade Kardeh	V	F	simple	
Goojgi	V	I	-	
Farokhad	V	F	simple	
Mayamey	V	I	modern	
Shoorak Maleki	V	I	-	
Abravan	V	I	-	
Tape Salam	V	F	simple	
Torogh	V	F	simple	
Moghan	V	I	-	
Hesar Golestan	V	F	simple	
Zoshk	V	F	simple	
Veyrani	V	I	Simple	
Kahoo	V	F	simple	
Golmakan	V	F	simple	
Khaje Morad	V	P	modern	
Behesht Reza cemetery	V	C	modern	
Mashahd Railway station	I	R	modern	
Ghadir camp	I	CP	modern	
Koohestan park	I	PK	modern	

^{*} V: Mashhad vicinity, I: Inside the Mashhad city

After capturing rodents in the field, targeted species (murine rodents) were homed in a separate cage and transferred into the animal house (in the laboratory). Other trapped rodent's species were released in their habitat. Collected rodents were euthanized using chloroform. All experimental procedures were performed in compliance with the Ferdowsi University of Mashhad guidelines on the care and use of

^{*} P: Public areas, C: Cemetery, R: Mashhad railway station, F: Farms and gardens, CP: Camps, PK: Parks, I: Innovative areas such as woodland, meadows, sandy and rocky habitats.

laboratory and experimental animals. Dead samples were kept in appropriate fixative solutions for further studies. The rodents were identified using taxonomic keys based on morphological traits followed Corbet (1978), Etemad (1984) and Ziaei (2008).

Parasitological examination

The fur and skin of rodents were precisely examined for the presence of ectoparasites. All external parasites were removed by tissue forceps. The parasites were sorted into four taxonomic groups: fleas, mites, ticks and lice. Any fleas captured were fixed in 70% ethanol and cleared in 10% KOH, and subsequently examined under a light microscope. All the collected ticks, mites and lice were preserved in 70% ethanol and cleared in Nesbitt's solution. For small and bright specimens transparent process was omitted. After the transparency step, they were mounted on permanent glass slides by using the Hoyer's solution separately. The specimens were identified under a stereomicroscope by comparing their characteristics to those presented in the taxonomic keys provided for Siphonaptera (Lewis 1967; Gonzalez 1987; Acosta and Morrone 2003), Acari (Keegan 1956; Tipton 1960; Fain 1974; Tenorio and Goff 1980; Baker 1999) and sucking lice (Johnson 1960; Kim *et al.* 1986; Gonzalez 1987; Korytkowski 2002) for further analyses (unpublished data). Photos were taken for both host and ectoparasites.

Data analysis

Prevalence of parasite infestation was calculated. Prevalence (P) = (number of hosts parasited with a particular parasite group / total number of hosts examined for that parasite group) × 100 (Bush *et al.* 1997). Prevalence was calculated for each group of ectoparasite, overall ectoparasites combined, and in some cases for each species of rodents separately. Data analysis was performed with SPSS v.11.5 for Windows (SPSS Inc, Chicago, IL, USA).

Results

A total of 197 individuals of rodents were trapped representing 11 species belonging to the families of Muridae (*Apodemus witherbeyi*, *Mus musculus*, *Nesokia indica*, *Rattus norvegicus*, *Meriones libycus*, *Meriones persicus*, *Tatera indica*), Cricetidae (*Cricetulus migratorius*, *Ellobius fuscocapillus*, *Microtus transcaspicus*) and Sciuridae (*Spermophilus fulvus*). As mentioned before, only the first four species which are regarded as murine rodents (Muridae; Murinae) were included in the study. From 197 captured rodents, 74 specimens belonged to the Murinae. Numbers of murine rodents collected from different study sites were presented in the Table 2.

Out of 74 murine rodent's specimens that were examined, 54.05% were found to be parasitized (prevalence). *Rattus norvegicus* and *Apodemus witherbeyi* were the most (17 infested individuals out of 19 examined individuals) and the least (7 infested individuals out of 13 examined individuals) infested murine species, respectively (Table 2 and Fig. 2A). A total of 413 ectoparasites that consisted of 82 fleas, 163 mites, 101 ticks and 67 lice species were recorded. Among the parasite groups, the highest occurrence was recorded for mites, found on about one third (31.08%) of the examined specimens, while lice were found on 20.27% of the examined rodents (Fig. 2B).

Table 2. Collected murine rodents species captured from different sites in Mashhad and its vicinity during April 2013 till April 2015. Numbers in brackets represent the number of infested individuals.

Sampling site	Nu	Prevalence (%) in			
	A. witherbeyi	M. musculus	N. indica	R. norvegicus	each locality
Govareshk	3 (1)	-	1 (1)	-	3.5%
Sade Kardeh	-	-	3 (2)	1(1)	5.26%
Goojgi	4 (2)	-	-	-	3.5%
Farokhad	-	1(1)	-	2 (2)	5.26%
Mayamey	-	-	-	2 (2)	3.5%
Shoorak Maleki	-	-	-	-	-
Abravan	-	-	-	-	-
Tape Salam	-	3 (3)	-	1(1)	7.01%
Torogh	-	3 (3)	-	1 (1)	7.01%
Moghan	-	1(1)	-	-	1.75%
Hesar Golestan	2 (2)	2 (2)	-	1(1)	8.77%
Zoshk	4 (2)	-	-	1 (1)	5.26%
Veyrani	-	1(1)	-	3 (2)	5.26%
Kahoo	-	-	-	-	-
Golmakan	-	-	1(1)	-	1.75%
Khaje Morad	-	1(1)	-	3 (3)	7.01%
Behesht Reza		2 (2)	6 (5)	1 (0)	12.28%
cemetery	-				
Mashahd railway		2 (2)	1 (1)	2 (2)	12.200/
station	-	3 (3)	1 (1)	3 (3)	12.28%
Ghadir camp	-	8 (3)	4 (2)	-	8.77%
Koohestan park	-	1(1)	-	-	1.75%
Total	13 (7)	26 (21)	16 (12)	19 (17)	-

Seasonal changes in ectoparasite prevalence

During the study period, a general season-based pattern of change in ectoparasite prevalence was seen. There was a significant seasonal decrease in the prevalence rate from summer (31.57%) to winter (12.28%) (Fig. 3A). These data also indicated a negative relationship with the abundance of the host species. Ticks and lice were found to be more abundant on murine species during cool wet months, whereas ticks and the mites were more abundant during the hot dry months of the year (Fig. 3B).

Relation of ectoparasites to host locality

The total prevalence rate among the researched areas, ranging between zero and 12.28%, was found to be significantly influenced by sampling sites. Prevalence of overall ectoparasites combined (P = 74.32%) (Table 2) and separately for fleas (0 to 5.40%), ticks, mites and lice (0 to 4.05%) were higher in Behest Reza cemetery and Mashhad railway station compared to the other localities.

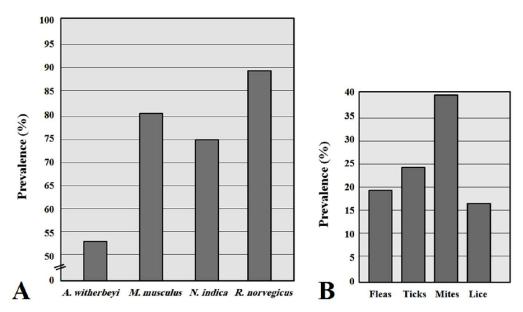


Figure 2. Total prevalence of all four ectoparasites groups on each murine rodents species (A); Prevalence of each ectoparasites groups on all murine rodents host (B).

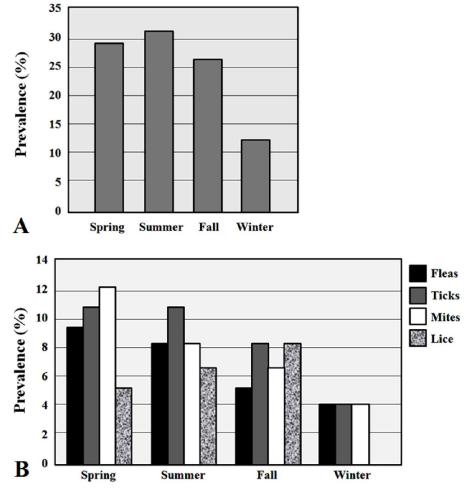


Figure 3. Seasonal variation of overall ectoparasites combined prevalence (A); Prevalence of each ectoparasites group on all murine rodents host in each season (B).

Relation of ectoparasites to host species

The host species have a different influence on the parasite groups. Based on the overall prevalence, *Rattus norvegicus* (P = 89.47%) was the dominant murine rodent that was infested by all ectoparasite groups (Fig. 2A).

Prevalence rates of fleas were found to be independent from the variable considered. Prevalence of lice was higher in *Nesokia indica* (P = 8.10%). For mites and ticks, *Mus musculus* (P = 13.50 and 16.21%, respectively) and *R. norvegicus* (10.81% and 8.10%, respectively) have a higher rate than other species. The other ectoparasite group (fleas), showed the highest prevalence for *N. indica* and *R. norvegicus* (P = 10.81% and 8.10%, respectively (Fig. 4).

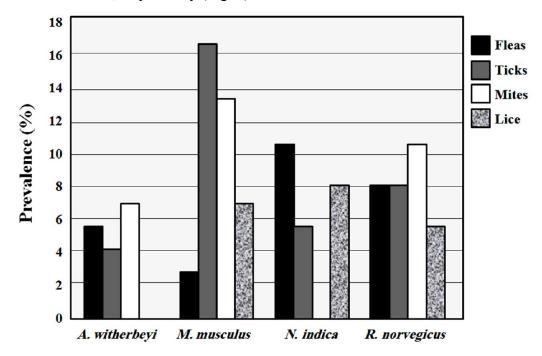


Figure 4. Prevalence of each ectoparasites group on each murine rodent's host.

Discussion

Rodentia has a worldwide distribution and many species of them threaten human health, especially in densely populated areas. The suitable conditions of many rural and urban places promote rodent infestation. This makes it crucial to focus on rodent host of arthropods and their diversity to prevent zoonotic disease which may threaten humans (Stanko *et al.* 2002; Krasnov *et al.* 2004).

Climatological conditions can affect the survival and multiplication of ectoparasites. For example, the development of flea larvae occurs in protected microhabitats with moderate temperatures and high relative humidity (Dryden and Rust 1994). In the present study we noted a significant difference in the mean number of murine rodents infested with ectoparasites in different seasons (from P = 31.57% in summer to P = 12.28% in winter). Average annual temperature and precipitation in each study site were noted in Table 1. Lower autumn/winter temperatures, when compared to spring/summer, may have contributed to the reduced level of ectoparasite infestation observed in autumn/winter. The increased availability of food during spring and summer, might allow an increase in pregnancy rates of rodents. This, in turn, might result in increased abundance of them during that season (Brooks and Rowe 1987).

Moreover, habitat fragments within agricultural landscapes can facilitate higher parasite burdens and prevalence in rodent populations. This can lead to an increase in disease risk given that several of the parasite species are important vectors of pathogens that can cause disease in domestic or wild animals and also humans. In our study we noted that Behesht Reza cemetery in Mashhad vicinity has considered as transmissible areas for parasitic infections. With regards to the high human traffic for the interment ceremony in this area, there is lots of garbage which provide great amount of food for rodents, too. This promotes their survival and reproduction. Consequently, it may lead to high rate of ectoparasitic infestation. Differences related to the standard of living in the study sites affected the number of rodents in each of them (Soliman et al. 2001), e.g. the number of murine rodents in Koohestan park within Mashhad was low as compared to those numbers collected from Mashhad vicinities. During the study period a total of 54 murine rodents (infested by 494 ectoparasites) were collected from Mashhad vicinity compared with 20 murine species (with 68 ectoparasites) collected from Mashhad, respectively. This difference in the relative abundance of rodents between Mashhad and its vicinity partly be attributed to the tendency of these rodents for burrowing, ages of buildings, main structure material of buildings and awareness of owners from the rate of rodent damage to their houses. Houses in Mashhad vicinity, mainly with walls and floors of soft damp soil, provided suitable habitats for the burrowing rodents. Houses inside the Mashhad, on the other hand, were mostly built of hard materials such as concrete and bricks that were more impenetratable than soft damp soil. Moreover the difference may be due to the intensive control measures applied to rodents by public health authorities in urban areas. Furthermore rodents in rural areas have larger average movements, and home range size, with a greater chance of contact with individuals of the same and other species, than urban rodents do. Hence, these behavioral characteristics of rural murine rodents may give them better possibilities of being colonized by ectoparasites.

In addition to the above mentioned, ecological and behavioral characteristics related to each host species, morphological features such as relative size and differences in the skin and its covering, as well as physiological factors such as difference in blood hormonal levels due to stress, also cause variations in ectoparasite infestation parameters. For example, big murine rodents with dense fur (such as *Nesokia* and *Rattus*) are assumed to have high level of ectoparasitic infestation in compare with small rodents which have thin and sparse fur (such as *Apodemus* and *Mus*). In addition, stress exposure may weaken the rodent's immune system and cause more infection. Climatological and other environmental conditions greatly affect rodent hosts as well as their ectoparasites.

The total prevalence rate of ectoparasites was significantly higher in *Mus* and *Rattus* than in *Apodemus* and *Nesokia*. This result could be referred to the limited activity and the burrowing habit of *Apodemus* and *Nesokia* that made their ectoparasites fauna less affected by climatic changes, however insecticides used for agricultural purposes could greatly affected these farm habitants (Ryckman 1971). The habitat of the host is also a determining factor in the distribution of arthropod ectoparasites. Geographic and habitat-based differences in the prevalence and general index of ectoparasites infesting either *R. rattus* or *R. norvegicus* have been repeatedly documented in Egypt (Aboul-Ela *et al.* 1987). Increased prevalence and general index of ectoparasites are positively correlated to the increased densities of their hosts (Anderson 1982; Soliman *et al.* 2001). Generally there is a relationship between

prevalence of ectoparasites and their rodent hosts. Seasonal changes in the general index of ectoparasites paralleled seasonal changes in the relative abundance of their host.

Although a single species of ectoparasite may infest several hosts, there is, however, a tendency to host specificity. For example differences in the prevalence and/or general index of mites between the two rat species hosts (*R. rattus* and *R. norvegicus*) indicated the presence of such a tendency (Soliman *et al.* 2001). Similar differences were recorded by Hadi *et al.* (1976). On the other hand, results of Walton and Tun (1978) study indicated that there was an equal chance of contact of flea species with both rat hosts. Differences in the general index of these arthropods between the two rat hosts might be related to differences in size, skin, and hair characters of the host, as well as differences in the habit of fleas themselves. Similar differences were also reported by Hadi *et al.* (1976) in Indonesia and Walton and Tun (1978) in Myanmar.

Conclusion

This information, which contributes to a better knowledge of the interrelationship between ectoparasites and rodents from northeast of Iran, is important to understand the role of ectoparasite vectors and mammalian reservoirs in the maintenance of diseases in the study area, and the possibilities of dissemination of pathogens. The results obtained in the present study are important from an epidemiological point of view. With respect to this fact that environmental conditions, such as topography, vegetation and many other factors can affect rodent hosts and their ectoparasites (Soliman *et al.* 2001), thus further epidemiological investigations needs to be conducted in order to ascertain the role of rodents and their ectoparasite affinity - especially in poorly studied host species in the lifecycle of emerging new infections.

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جكىدە

مطالعات بی شماری نشان دادهاند که جوندگان در انتقال انواع بیماریهای انگلی نقش عمدهای دارند. هدف این مطالعه، تعیین رابطهٔ بین فصل نمونهبرداری و زیستگاه جوندگان خانوادهٔ میزبان شمال شرق ایران (مشهد و حومه) با بار انگلی آنها است. افزون بر این، اثرات وابسته به گونهٔ میزبان شمال شرق ایران (مشهد و حومه) با بار انگلی نیز مورد بررسی قرار گرفته است. مطالعات میدانی از بهار ۱۳۹۲ تا بهار ۱۳۹۴ در ۲۰ ایستگاه مشخص انجام گرفت. در مجموع، ۷۴ جونده صید شد و ۴۱۳ انگل خارجی (متعلق به چهار گروه انگلیِ کک ها، کنهها، مایتها و شپشها) از سطح بدن آن ها جمع-آوری شد. آلوده ترین جونده رت نروژی بود و فراوان ترین گروه انگلی مایتها بودند. بیشترین بار انگلی در تابستان گزارش شده و کنهها متداول ترین نواحی مشخص شدند. بار انگلی کلی در بهشت رضا و ایستگاه راه آهن مشهد به عنوان آلوده ترین نواحی مشخص شدند. بار انگلی کلی در این دو ناحیه نسبت به سایر ایستگاههای مورد بررسی بیشتر گزارش شد. تغییرات فصلی در بار انگلی تعدادی از انگلهای خارجی با فراوانی میزبانشان در ارتباط است. افزون بر این، بار انگلی در تعدادی از انگلهای خارجی اختلافاتی را با توجه به زیستگاه میزبان نشان می دهد. در این مطالعه تعدادی از انگلهای خارجی اختلافاتی را با توجه به زیستگاه میزبان نشان می دهد. در این مطالعه شد. مطالعه و درک این گونه روابط بین انگل و میزبان در درک نقش این بندپایان ناقل در انتقال شد. مطالعه و درک این گونه روابط بین انگل و میزبان در درک نقش این بندپایان ناقل در انتقال شد. مطالعه و درک این گونه روابط بین انگل و میزبان در درک نقش این بندپایان ناقل در انتقال

واژگان کلیدی: انگلهای خارجی؛ ایران؛ جوندگان؛ نواحی نمونه برداری (زیستگاه جونده)؛ تغییرات فصلی.

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