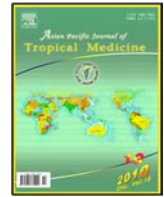




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Geographical distribution and ecological features of the great gerbil subspecies in the main zoonotic cutaneous leishmaniasis foci in Iran

Mohammad Reza Abai, Mohammad Ali Oshaghi*, Leila Tajedin, Yavar Rassi, Amir Ahmad Akhavan

Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Objective: To reveal subspecies composition of *Rhombomys opimus* (*R. opimus*) in Iran. **Methods:** In this study, field specimens of the gerbil were collected from all its geographical range in northeast and central parts of Iran and identified on basis of morphological characteristics. **Results:** Results revealed presence of two subspecies of *R. opimus sodalis* and *R. opimus sargadensis* in the country. The first subspecies with brown to hazel–nut color and a size bigger than a typical *R. opimus* was found only in Golestan province, an isolated colony which is located in plains of north slopes of Alborz Chain Mountains of the country, connected with the Turkmenistan. The subspecies of *R. opimus sargadensis* with a yellowish color and similar size as a typical *R. opimus* was found in all other areas of the great gerbil distribution in Iran. There was a distinct topographic difference but similar ectoparasites between colonies of the two subspecies in the study area. **Conclusions:** Further ecological and genetic investigations are required for more detailed description of the *R. opimus* subspecies range and structure. The *R. opimus*–*Phlebotomus papatasi*–*Leishmania major* association and the ZCL severity and outcome in hosts are discussed.

1. Introduction

Rhombomys opimus (*R. opimus*), the great gerbil, is the main animal reservoir of zoonotic cutaneous leishmaniasis (ZCL) in Iran, Afghanistan, and Central Asia^[1–3]. The rate of infection to *Leishmania major*, the cause of ZCL, is normally high and might raise more than 90% in endemic areas^[3–5]. Additionally, two species of *Leishmania*, *Leishmania gerbilli* (*L. gerbilli*) and *Leishmania turanica* (*L. turanica*) which are nonpathogenic to humans but can infect the great gerbil, have been found in Turkmenistan, Uzbekistan, China, and Iran^[1,3,6,7]. *Leishmania* parasites persist in great gerbils for long time up to 25 months^[1]. Great gerbils are also known to carry bubonic plague^[8] as well as being an agricultural pest^[9].

This species of gerbil is found in many parts of Central Asia such as Turkmenistan and Kazakhstan, Iran, Southern Mongolia, North–western China and Afghanistan. It is widespread in Iran^[3–5], Turkmenistan, Uzbekistan, Kazakhstan, and appears to have a smaller distribution in Kyrgyzstan and Tajikistan^[1,10]. In South Asia, it has been

recorded from northern Afghanistan^[2] and western Pakistan (Baluchistan)^[11]. In China the species is very widespread, and has been recorded from many areas^[12]. In Mongolia, it has been recorded from desert and semi–desert habitats across southern Mongolia^[13].

R. opimus is a diurnal, fossorial, colonial often seen associated with other gerbils. The social structure consists of family groups that occupy isolated systems of burrows consisting of one breeding male, from one to seven females, and juveniles^[14]. In China, the species occupies desert to semi–desert habitats, and is most successful in dry river beds dominated with shrubby vegetation^[12]. In South Asia, it has been recorded from apple orchards and clay–sandy embankments and has been found to occupy Steppe Mountains and upland deserts and sand dunes with scattered vegetation^[14]. It constructs large entrance holes to a very elaborate burrow system that consists of long deep tunnels; nest and food storage chambers^[12]. Settlements of the great gerbil are also associated with the borders of saline depressions with plant communities dominated by annual and perennial succulents in the family Chenopodiaceae (*Climacoptera* spp., *Salsola* spp., *Suaeda* spp.)^[14].

Lifespan varies from male to female. The males' maximum lifespan is 2 to 3 years and the female lives 3 to 4 years^[15]. *R. opimus* lives in large colonies composed of many subgroups. These subgroups are believed to be offspring of mother/father pairs within the colony. During the winter these large groups huddle together to keep warm when

*Corresponding author: MA Oshaghi, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran 14155–6446, Iran.

Tel: 0098 21 88951393

Fax: 0098 21 88951393

E–mail: moshaghi@sina.tums.ac.ir

temperatures become critically low outside. They rely on the stable underground temperatures (20 to 25°C) which exist in their burrows. This species does not hibernate, although its activity is reduced during the winter months^[9].

According to the classification of Etemad^[16] and Goodwin^[17], four known subspecies named *Rhombomys opimus opimus* (*R. opimus opimus*), *Rhombomys opimus fumicolor* (*R. opimus fumicolor*), *Rhombomys opimus sargadensis* (*R. opimus sargadensis*), and *Rhombomys opimus sodalis* (*R. opimus sodalis*) has been described in its geographical rang. *R. opimus opimus* and *R. opimus fumicolor* were reported from central Asia, eastern region of Caspian Sea, including countries of Turkmenistan, Uzbekistan, Kazakhstan^[18]. Other two subspecies of *R. opimus sargadensis* and *R. opimus sodalis* were reported in Iran^[16]. *R. opimus sodalis* is bigger than *R. opimus opimus* but smaller than *R. opimus fumicolor*. This subspecies is brown to hazel–nut color. The subspecies of *R. opimus sargadensis* has a yellowish color and similar size as a typical *R. opimus*.

In this paper the distribution of the great gerbil subspecies in Iran was analyzed on the basis of morphological characteristics of the field specimens collected from the main areas of its habitats in the country constitute distinct populations.

2. Materials and Methods

2.1. Sample collection

Sample collection was performed in spring (April–June) 2008 in five provinces including Esfahan, Fars, Northern–Khorasan, Semnan, and Golestan Ps located in north, north–east, and central regions of the country (Figure 1). Colonies of the gerbils located about 1–1.5 km around the villages. All gerbil colonies, including occupied and unoccupied burrows were checked to select and to mark the active burrows. The active colonies of rodents were identified and rodents were captured by setting the Sherman® live traps (30 cm×15 cm×15 cm wire mesh live–traps) baited with a mixture of cucumbers, tomatoes, and peanut butter. Ecological and biological data such as topographic features, plantations, daily activities, and abundance of colonies was recorded in each region.

2.2. Species and subspecies identification

The genus and species of the rodents were determined by external characteristics including color, body measurements, ears, tail, feet, teeth, and skull^[19]. According to classification of Etemad and Goodwin^[16,17], the animal is one of the largest gerbil species up to 400 mm from head to tail. The upper body is yellowish–orange or dark grayish–yellow, matching the sandy deserts they inhabit. Great gerbil is significantly bigger than most gerbils weighing about 200 g, having a larger head and small ears than most gerbils. It has a relatively short tail with a distinctive crest of long hairs towards the end. Great gerbils have thick, dense, and soft fur and its body is stocky. The claws are long and sharp to help it dig its burrows^[9]. In addition to the general characteristic mentioned above, presence of two fine grooves on the incisor teeth is the most important characteristics for *R. opimus* identification. The specimens with hazel–nut color, body length ranging from 300 to 320 mm and a tail length of 135 to 140 mm, and back feet lengths of 43–45 mm were considered as *R. opimus sodalis* subspecies (Figure 2A). Specimens with brown to yellowish color and similar size as

R. opimus opimus were considered as *R. opimus sargadensis* subspecies (Figure 2B/C).



Figure 1. Details of geographical locations where *R. opimus* specimens were collected in Iran.

1: Kalaleh (Golestan P.); 2: Damghan (Semnan P.); 3: Ahmad Abad, Shahrood (Semnan P.); 4: Mayamay, Shahrood (Semnan P.); 5: Esfarayen (Northern Khorasan P.); 6: Shirvan (Khorasan Shomali P.); 7: Badrood (Esfahan P.); and 8: Habibabad (Esfahan P.).

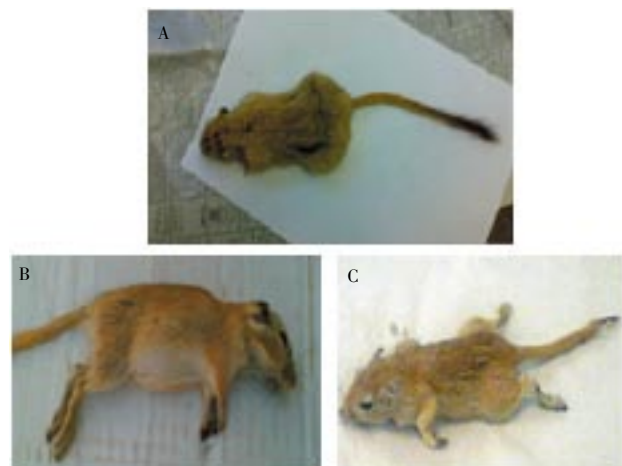


Figure 2. Photos of two *R. opimus* subspecies found in Iran. A/B: *R. opimus sargadensis* and C: *R. opimus sodalis*.

3. Results

We caught 50 adult specimens including 31 females and 19 males. Details of the captured specimens were shown in Table 1. The most active colonies were found in Habibabad, Esfahan province (P.). In this area, the burrows are dug in plain area at sandy–loamy soil. The development of desert control projects resulted in suitable habitats for increasing and flourishes of great gerbils’ population. In general, observations on ecotype of this animal in the country showed that great gerbils preferred sandy soils, desert, and semi–desert areas and subtropical steppes. Their diet mainly consisted of desert bushes and local agricultural crops. In hot seasons, they were more active at dawn and dusk, whereas in winter, they were more active during the middle of the day.

Large parts of the animal territory with a high number of populations were found both in the south (Esfahan P.) and

Table 1Details of *R. opimus* subspecies specimens collected in different locations in Iran.

Subspecies	No.	Male/female	Province	District	Village
<i>R. opimus sodalis</i>	5	2/3	Golestan	Kalaleh	Ghareh Gol Gharbi, Aziz-Abad, Allah nor
<i>R. opimus sargadensis</i>	4	2/2	Northern-Khorasan	Esfarayen	Kalateh-Shor, Emarat, Toy
<i>R. opimus sargadensis</i>	1	1/0	Northern-Khorasan	Shirvan	Hosseini-abad, Shorak
<i>R. opimus sargadensis</i>	5	2/3	Semnan	Shahrood	Ahmad-Abad, Mayamey
<i>R. opimus sargadensis</i>	11	5/6	Semnan	Damghan	Hasan-Abad, Soltanieh Ghasem-Abad
<i>R. opimus sargadensis</i>	7	2/5	Esfahan	Badrood	Badrood
<i>R. opimus sargadensis</i>	17	5/12	Esfahan	Habibabad	Habibabad
--	0	0/0	Fars	Neyriz	Neyriz, Lar
Total	50	19/31	5	9	17

north (Semnan P.) of Dashte-Kavir desert in center of Iran. Observations on the site of colonies showed that the great gerbils dug their burrows near the agricultural lands e.g. sugar beet, cotton, corn, clover, barley, grape, pistachio, alfalfa, wheat farms and causes damage to crops. These territories showed small differences in burrow density but the number of gerbils was more stable in the south (Esfahan P.), in comparison to the north. Less density was found in Northern-Khorasan P., near the north-eastern limit of its distribution in Iran. There was no trace of *R. opimus* in Fars P. in southern limit of distribution of this species in Iran. In northern slope of Alborz Mountain Chain (AMC), Golestan P., also less number of great gerbils was captured. In this area, the burrows of great gerbils were mostly located at foothills. This kind of habitat is a new record on the ecological habitats used by this animal. In this area, small part of populations was seen near wheat and barley farms.

According to the classification mentioned in material and method, two *R. opimus* subspecies, *R. opimus sodalis* and *R. opimus sargadensis* were found in the great gerbil territories studied. *R. opimus sargadensis*, with brown to yellowish (Figure 2A/B) was the dominant subspecies and found in all territories including Esfahan, Semnan, and Northern-Khorasan Provinces around the Dashte-Kavir desert, located in southern slope of AMC. Interestingly, *R. opimus sodalis* with hazel-nut color was found only in Golestan P. in northern slope of AMC. This province is connected to the northern neighboring country Turkmenistan, and seems to be the southern limit of this subspecies in the region.

4. Discussion

In this study we found two *R. opimus* subspecies in two distinct geographical regions of Iran. *R. opimus sodalis* was present in northern slope of AMC whereas *R. opimus sargadensis* has occupied all territories in the southern slope of AMC positioned around the central desert of the country. It seems the AMC acts as a natural barrier between these two subspecies preventing gene flow between them. These two subspecies serve as the natural animal reservoir hosts of ZCL in their territories^[4,5].

Presence of two subspecies in the country with noticeable variations in morphology and ecology suggests potential genetic variation between them which might affect on ZCL disease severity and outcome. Identification of genetic variation particularly the genes that determine various outcomes such as developing asymptomatic, symptomatic or severe disease upon infection with *Leishmania major* (*L. major*) is highly recommended. Some researchers indicated that a number of genes of hosts (human or animals) including

TNF- α , TNF- β , IL-4, IL-10 and IFN- γ might play role in severity and outcome of the disease^[20–22]. There is also a correlation or specificity between *L. major* strains and their local natural animal hosts; hence, specific *L. major* strain can cause infection only in their proven regional hosts^[23]. It also correlated with the apparent specific resistance of local natural animal reservoir hosts to cross-infection with foreign strains of *L. major* from other regions. This information should be determined by comparison studies on the *L. major* strains present in each great gerbil subspecies. Further genetic studies on these two subspecies could provide valuable information in epidemiology of the disease and can be applied to human leishmaniasis and could lead to the development of novel and specifically targeted therapeutic approaches.

This study demonstrated difference in density of the great gerbil in different regions. This different density depends on many ecological features such as food abundance, distribution and environmental factors and human interventions. It shows that climate forcing synchronizes the dynamics of gerbils over large geographical areas^[24]. They suggested that based upon predicted climate changes during the next century the densities of *R. opimus* will be increased and diseases such plague epizootics may become more frequent in central Asia.

Sand flies which are vectors of the leishmaniasis use burrows of great gerbil as the main resting and breeding sites. Great gerbil is the favorite host for blood-feeding of *Phlebotomus* sand flies. In all old World regions where *L. major* exists, it is transmitted by sand flies of the genus *Phlebotomus*. *R. opimus*-*P. papatasi*-*L. major* association in Central Asia, Iran and Afghanistan are stable well-described zoonotic systems^[25–28]. The *Leishmania* parasite can live in some tissues of *R. opimus* for a long time, practically, thorough the lifetime of great gerbil without losing their virulence and availability for vectors. Substantial part of the great gerbil population lives for more than 1 year, which is of great importance in epizootiology of cutaneous leishmaniasis (the agent is preserved safely in the animals' populations during the 7–8 month-long absence of active vectors). Great gerbil is a diurnal herbivorous animal, while most of the other species of this subfamily are nocturnal seed-eating forms. As a diurnal animal the great gerbil is guided by sight and has small thick conchae, while other gerbils are nocturnal species that are guided by hearing and have large thin conchae. That is important because sand flies usually pierce skin of the upper edge of the conchae while feeding on gerbils, where short and sparse hairs make the skin more available for the short proboscis of sand flies. Apparently thick ears of great gerbils contain more blood and interstitial fluid, compared to the thin ears

of other gerbils, and sand flies prefer to feed on the first. In general, most of the ecological features of *R. opimus* favor the existence of sand flies and the leishmanias in direct or indirect way. That is evidenced by complete coincidence of the area of *L. major* in the old world with the area of great gerbils, the latter being the only principal carrier of the leishmanias.

The relative effects of host subspecies and locality on ectoparasite assemblages harboured by the *R. opimus* populations were assessed by Tajedin *et al*[29]. They found only one flea species of *Xenopsylla nuttalli* and one mite species of *Ornithonussus bacoti* across the area study. The flea was found on both subspecies in all parts of the study area whereas the mite was found only on southern *R. opimus sodalis* populations. This suggests that locality has particular effect on the mite species but flea subspecies.

As a conclusion, because *R. opimus* is the major principal carrier of the leishmanias and plays an important role in the existence of the parasitic system of cutaneous leishmaniasis in the Old World such as Iran, additional field observations plus genetic structure studies are required for more detailed description of the great gerbil range, subspecies, rate of leishmaniasis infection, its collaboration with specific *L. major* strain over its large geographical range.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Strelkova MV, Eliseev LN, Ponirovsky EN, Dergacheva TI, Annacharyeva DK, Erokhin PI, et al. Mixed leishmanial infections in *Rhombomys opimus*: a key to the persistence of *Leishmania major* from one transmission season to the next. *Ann Trop Med Parasitol* 2001; **95**: 811–9.

[2] Faulde M, Schrader J, Heyl G, Amirih M, Hoerauf A. Zoonotic cutaneous leishmaniasis outbreak in Mazar-e Sharif, northern Afghanistan: an epidemiological evaluation. *Int J Med Microbiol* 2008; **298**: 543–50.

[3] Akhavan AA, Mirhendi H, Khamesipour A, Alimohammadian MH, Rassi Y, Bates P, et al. Leishmania species: Detection and identification by nested PCR assay from skin samples of rodent reservoirs. *Exp Parasitol* 2010a; **126**(4): 552–6.

[4] Rassi Y, Abai MR, Javadian E, Rafizadeh S, Imamian H, Mohebbali M, et al. Molecular data on vectors and reservoir hosts of zoonotic cutaneous leishmaniasis in central Iran. *Bull Soc Pathol Exot* 2008a; **101**: 425–8.

[5] Rassi Y, Sofizadeh A, Abai MR, Oshaghi MA, Rafizadeh S, Mohebbali M, et al. Molecular detection of *Leishmania major* in the vectors and reservoir hosts of cutaneous leishmaniasis in Kalaleh District, Golestan Province, Iran. *Iranian J Arthropod-Borne Dis* 2008b; **2**: 21–7.

[6] Mohebbali M, Javadian E, Yaghoobi-Ershadi MR, Akhavan AA, Hajjarian H, Abai MR. Characterization of leishmania infection in rodents from endemic areas of the Islamic Republic of Iran. *East Mediterr Health J* 2004; **10**: 591–9.

[7] Zhou XN, Lv S, Yang GJ, Kristensen TK, Bergquist R, Utzinger

J, et al. Spatial epidemiology in zoonotic parasitic diseases: insights gained at the 1st International Symposium on Geospatial Health in Lijiang, China, 2007. *Parasites Vectors* 2009; **2**:10.

[8] Zhang YJ, Dai X. Study on the situation of plague in Junggar Basin of China study. *Zhonghua Liu Xing Bing Xue Za Zhi* 2008; **29**: 136–44.

[9] Nowak R. *Walkers mammals of the world*. 6th ed. Baltimore: Johns Hopkins University Press; 1999.

[10] Shar S, Lkhagvasuren D, Molur S. *Rhombomys opimus*. IUCN red list of threatened species version 2, 2009. [Online]. Available from: www.iucnredlist.org. [Accessed on 29 September 2010].

[11] Molur S, Srinivasulu C, Srinivasulu B, Walker S, Nameer PO, Ravikumar L. *Status of non-volant small mammals. Conservation Assessment and Management Plan (CAMP) workshop report*. India: Coimbatore; 2005.

[12] Smith A, Xie Y. *The mammals of China*. Princeton, New Jersey: Princeton University Press; 2008.

[13] Mallon DP. The mammals of the Mongolian People's Republic. *Mammal Rev* 1985; **15**: 71–102.

[14] Rogovin K, Randall JA, Kolosova I, Moshkin M. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. *Horm Behav* 2003; **43**: 132–9.

[15] Wilson DE, Reeder DM. *Mammal species of the world: A taxonomic and geographic reference*. 2nd ed. Washington and London: Smithsonian Institution Press; 1993.

[16] Etemad A. Iranian mammals: *Rodents and their identification keys*. 1st ed. Publication of National Association of natural resources and human environment protection. Tehran: Tehran University Publication; 1978.

[17] Goodwin GG. Mammals collected by the Legendre 1938 Iran expedition. *American Museum Novitates* 1940; **1082**: 1–17.

[18] Vinogradov BS, Argiropulo AI. *Key to rodents. Fauna of the USSR*. Jerusalem: Israel Program for Scientific Translations; 1968, p. 230.

[19] Ziaei H. *A field guide for identifying of Iranian desert mammalians*. Tehran: Iranian Environment Organization; 1996.

[20] Kamali-Sarvestani E, Rasouli M, Mortazavi H, Gharesi-Fard B. Cytokine gene polymorphisms and susceptibility to cutaneous leishmaniasis in Iranian patients. *Cytokine* 2006; **35**: 159–65.

[21] Salhi A, Rodrigues VJr, Santoro F, Dessein H, Romano A, Castellano LR, et al. Immunological and genetic evidence for a crucial role of IL-10 in cutaneous lesions in humans infected with *Leishmania braziliensis*. *J Immunol* 2008; **180**: 6139–48.

[22] Sakthianandeswaren A, Foote SJ, Handman E. The role of host genetics in leishmaniasis. *Trends Parasitol* 2009; **25**: 383–91.

[23] Elfari M, Schnur LF, Strelkova MV, Eisenberger CL, Jacobson RL, Greenblatt CL, et al. Genetic and biological diversity among populations of *Leishmania major* from Central Asia, the Middle East and Africa. *Microbes Infect* 2005; **7**: 93–103.

[24] Kausrud KL, Viljugrein H, Frigessi A, Begon M, Leirs H, DUBYANSKIY V, et al. Climatically driven synchrony of gerbil populations allows large-scale plague outbreaks. *Proc Biol Sci* 2007; **274**: 1963–9.

[25] Oshaghi MA, Rasolian M, Shirzadi MR, Mohtarami F, Doosti S. First report on isolation of *Leishmania tropica* from sandflies of a classical urban cutaneous leishmaniasis focus in southern Iran. *Exp Parasitol* 2010; **126**(4): 445–50.

[26] Oshaghi MA, Yaghoobi-Ershadi MR, Abbassi M, Parvizi P, Akhavan AA, Rahimi Foroshani A, et al. Detection of *Leishmania major* in naturally infected sand flies using semi nested-PCR. *Iranian J Pub Health* 2008; **37**: 59–64.

[27] Rassi Y, Oshaghi MA, Azani SM, Abaie MR, Rafizadeh S, Mohebbali M, et al. Molecular detection of Leishmania infection due to *Leishmania major* and *Leishmania turanica* in the vectors and reservoir host in Iran. *Vector Borne Zoonotic Dis* 2010. [Epub ahead of print]

[28] Akhavan AA, Yaghoobi-Ershadi MR, Khamesipour A, Mirhendi H, Alimohammadian MH, Rassi Y, et al. Dynamics of *Leishmania* infection rates in *Rhombomys opimus* (Rodentia: Gerbillinae) population of an endemic focus of zoonotic cutaneous leishmaniasis in Iran. *Bull Soc Pathol Exot* 2010b; **103**: 84–9.

[29] Tajedin L, Rassi Y, Oshaghi MA, Telmadarraiy Z, Akhavan AA, Abai MR, et al. Study on ectoparasites of *Rhombomys opimus*, the main reservoir of zoonotic cutaneous Leishmaniasis in endemic foci in Iran. *Iranian J Arthropod-Borne Dis* 2009; **3**: 41–5.