Comparative Testing of Susceptibility Levels of Phlebotomus sergenti, the Main Vector of Anthroponotic Cutaneous Leishmaniasis, to Conventional Insecticides Using Two Capture Methods in Kerman City, Southeastern Iran

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Abstract
Background: Collecting live sand flies from indoor sites is a major challenge for researchers in large cities due to the reluctance of families to survey their homes. This study was conducted to assess the efficacy of two methods for collecting sand flies for use in susceptibility tests in the urban area of Kerman, southeastern Iran.

Methods: Sandflies were mainly collected using both baited traps and hand catch methods from outdoor and indoor sites. Susceptibility tests were separately done according to the standard World Health Organization testing protocol on Phlebotomus sergenti, including 60-minute exposure to DDT 4.0%, propoxur 0.1%, deltamethrin 0.05%, and malathion 5.0%.

Results: During this research, the natural habitats and suitable indoor sites were selected to predict the density of live Ph. sergenti with perfect accuracy. The number of live Ph. sergenti caught by hand catch and baited traps methods was 42 and 361 in indoor and outdoor sites, respectively. The mortality rate of Ph. sergenti exposed to DDT 4%, deltamethrin 0.05, malathion 5%, and propoxur was 100%.

Conclusion: The baited traps showed a significant efficiency compared to hand catch for collecting live Ph. sergenti for use in susceptibility tests in urban areas. The Ph. sergenti collected from both indoor and outdoor sites were susceptible to all insecticides.

Keywords: Phlebotomus sergenti; Susceptibility; Insecticides; Baited traps; Hand catch

Introduction

Anthroponotic cutaneous leishmaniasis (ACL) caused by Leishmania tropica is transmitted by Phlebotomus sergenti (1). In the ACL foci, the humans could act as a L. tropica reservoir for sand fly infection during the year. In this regard, patients with acute ulcers (2), chronic diffuse ulcers, lupoid or tuberculous ulcers (3), non-healing ulcers (4), recurrent ulcers (5) and ulcers resistant to pentavalent antimonial drugs could provide enough parasitic reservoirs for establishing an ACL transmission cycle by sandflies in the area (6). Dogs are considered as an accidental host (7) in which the lesions usually appear on the snout, interdigit, and sometimes on the corner of the eyelids (1, 8-9). The majority of ACL cases in the world occur in Morocco (10-11), Algeria (12), Libya (13), Tunisia (14-15), Afghanistan (16-21), Iran (22), Pakistan (23), Saudi Arabia (24), Syria (25), Jordan (26-27), Iraq (27), Israel (28), and Turkey (29). Anthroponotic cutaneous leishmaniasis is a well-known disease in densely popu-
lated cities in Iran and is predominantly endemic in urban and peri-urban areas including Tehran (30), Mashhad (31), Neishabour (32), Shiraz (33), Kerman (34-35), Bam (37-40), Isfahan (41), and Jiroft (42). *Phlebotomus sergenti* is a proven vector of ACL (43). Dissection of many specimens of this species in Tehran failed to show the *Leishmania* (1), while there are several reports of the leptomonad of *L. tropica* in dissected *Ph. sergenti* in Mashhad (44). Moreover, *Leishmania* parasites have been found in Kerman (45-46) and Shiraz using restriction fragment length polymorphism (RFLP) (47-49). Leptomonad infections of *Ph. sergenti* have also been reported from Afghanistan, Saudi Arabia (50) and Tunisia (51). In some urban foci, *Ph. caucasicus* is suspected as a secondary vector (1). In the ACL foci located in the plains, the behavior of *Ph. sergenti* is completely domestic with more density found at indoor sites versus the mountainous ACL foci. This species is found in moderate density in caves and crevices of mountains and foothills where the birds act as a host for blood feeding. The presence of light or other avian hosts attracts the sandflies to the houses located adjacent to the foothills or mountains (43). In southern ACL foci, the activity of *Ph. sergenti* begins in late June and ends in mid-September (52). In the central ACL foci, the activity starts in early April and ends in mid-November with two peaks at the end of April and in mid-September (53). Some ecological aspects and surveillance of *Ph. sergenti* have been studied in endemic foci of ACL (54-57). The ACL occurrence and emergence are characterized by several factors such as environmental changes, poor housing conditions, unprotected people from sandfly bites, resettlement of non-immune people, deterioration of the infrastructure and migration (58-62), natural disasters like the earthquake (63-65), displacement of people in borderlines (66), civil wars (67-69), and refugee camps (70-76). At present, spraying operations are limited to the residual foci of malaria in the country (79) and this method is not applied for controlling the ACL vectors. Based on the endophilic habits of *Ph. sergenti*, some attempts have been made to reduce the density of sandflies (78-81) using other insecticide-based vector control measures such as insecticide impregnation of the bed nets (ITNs) (82-86), curtains (ITCs) (87-88), tents (ITTs) (89). Moreover, integrated vector control methods (89-90) have also been applied to control *Ph. sergenti* population in epidemic conditions. In order to analyze the susceptibility tests on sand flies, testing of insecticides at discriminative doses has been used for ranking the susceptibility level and as a criterion for interpreting the results. It is difficult to collect live sandflies from indoor places in large cities. Therefore, the present study was conducted to determine the most convenient capture method for collecting live sandflies from indoor and outdoor sites and to use the collected sand flies for determining their susceptibility to conventional insecticides in the ACL foci in Kerman, southeastern Iran, where there is scarce data in this regard.

**Materials and Methods**

**Study area**

The study area was located in Kerman, southeastern Iran. The city borders Khorasan and Yazd to the north, the Lut Desert to the east, Bam to the south, Sirjan to the west, and Rafsanjan to the northwest. Kerman is elevated 1793 meters above the sea level and the main coordinates of the study locations were between 30°06’32”N and 57°06’27.6”E. The study area was a wide plain surrounded by stony mountains with a poor vegetation. The studied localities for collecting the adult sand flies were Sarasiyab, Masjed-e-Sahebzaman, Shahzadeh-Mohammad, Safa Cave, Allahabad, Shahrak-Sanaati, Sarbaz Mountains and Shahrak-e-Almahdi (Fig. 1). The entomological operations were carried out from July to September 2019.

**Sand fly collection**

Live sand flies were collected using an as-
pirator from the homes located in the periphery of the mountains in the early morning. The collected sand flies were released into paper cups and transferred to the laboratory for testing. The baited traps were equipped with mini gas lamps, and the netted tents were installed close to the caves or crevices near the mountains around Kerman City (Fig. 2), where the presence of adult sand flies was already proven during pretest trials using sticky paper traps. The collected live sand flies were also released into paper cups and transferred in a box under a wet towel. Sucrose solution was provided on the top of cups as a cotton pad soaked in sucrose solution 10%.

Tested insecticides
The insecticide-impregnated papers were purchased from the WHO Representative in Penang, Malaysia. The papers were impregnated with DDT 4.0% (batch number DD 265, expire date: July 2022), deltamethrin 0.05% (batch number DE 527, expire date: August 2019), malathion 5.0% (batch number MA 234, expire date: July 2020), and propoxur 0.1%, (batch number PR, 123 expire date: August 2020) and tested using the WHO’s testing kit.

Susceptibility tests
Susceptibility tests were carried out on sand flies collected from both indoor and outdoor sites. The collected sand flies were tested separately according to the capture method using the World Health Organization (WHO) protocols (91-93). The mortality rates were determined at discriminative doses for 60 minutes using the WHO test tubes for the adult sand flies. At each replicate, 20–25 sand flies were tested. A 10% sucrose solution was provided during the recovery period. The mounted sandflies were identified using valid identification keys (94-96).

Data Analysis
The susceptibility data including the number of live, dead, and total sand flies were ranked according to sex (male and female) and physiological condition (blood-fed, unfed and gravid) separately in two treatment groups compared to the control group. The charts of mortality rate were drawn with standard errors to show any significant differences using the Microsoft Excel 2010 software. The independent t-test was applied to compare the abundance and sex ratio of Ph. sergenti between hand-catch and baited trap methods using the IBM SPSS software version (25).

Results

Efficiency of collection methods
The efficiency the baited traps for collecting live Ph. sergenti from outdoor was 370 compared to 45 sand flies collected using hand-catch from indoor sites (Table 1) indicating an significant difference (t= 7.214, df= 9, p= 0.001) (Fig. 3). The sex ratio of Ph. sergenti was 1.0 at indoor compared to 1.2 at outdoor places (Table 1) showing no significance difference (t= 2.574, df= 8, p= 0.07) (Fig. 4).

Susceptibility of Phlebotomus sergenti collected from indoors
The mortality rate of Ph. sergenti collected from indoor places using hand-catch method was 100% after exposure to DDT and malathion. The mortality rate was 0.0% in the control group (Fig. 5).

Susceptibility of Phlebotomus sergenti collected from outdoors
The mortality rate of Ph. sergenti collected from outdoor sites using baited traps was 100%. After exposed to DDT, propoxur, deltamethrin, and malathion. The mortality rate was 0.0% in the control group (Fig. 6).
Fig. 1. Map showing Kerman City and the locations studied the collecting methods of live sand flies used for susceptibility tests, Kerman Province, 2019

Fig. 2. Using the baited traps for collection of live Phlebotomus sergenti in the mountainous areas around Kerman City, southeastern Iran, Kerman Province, 2019
Table 1. Statistical comparison of efficiency of two collection methods used for live collection of *Phlebotomus sergenti*, Kerman City, southeastern Iran, July–Sep 2019

| Capture methods | Capture place | Sessions of collection | Total collected | Capture Rate (%) | No. male | No. female | Sex ratio | Physiologic conditions | Environmental condition |
|----------------|---------------|------------------------|-----------------|------------------|----------|------------|-----------|-----------------------|------------------------|
| Baited traps   | Outdoor       | 9                      | 370             | 89.2*            | 198      | 172        | 1.2†      | No. fed               | Temp °C                 |
|                |               |                        |                 |                  |          |            |           | No. unfed             | Relative humidity (%)   |
|                |               |                        |                 |                  |          |            |           | No. gravid            |                        |
|                |               |                        |                 |                  |          |            |           | 29                    | 28–30                  |
|                |               |                        |                 |                  |          |            |           | 112                   | <40                    |
|                |               |                        |                 |                  |          |            |           | 31                    |                        |
|                |               |                        |                 |                  |          |            |           | (16.8%)               |                        |
|                |               |                        |                 |                  |          |            |           | (65.1%)              |                        |
|                |               |                        |                 |                  |          |            |           | 31                    |                        |
|                |               |                        |                 |                  |          |            |           | (18.0%)              |                        |
|                |               |                        |                 |                  |          |            |           | 2                    |                        |
|                |               |                        |                 |                  |          |            |           | (8.7%)                |                        |
|                |               |                        |                 |                  |          |            |           | (17.4%)              |                        |
|    |               |                        |                 |                  |          |            |           | (73.9%)              |                        |
| Hand-catch     | Indoor        | 5                      | 45              | 10.8*           | 22       | 23         | 1.0†      | No. fed               | Temp °C                 |
|                |               |                        |                 |                  |          |            |           | No. unfed             | Relative humidity (%)   |
|                |               |                        |                 |                  |          |            |           | No. gravid            |                        |
|                |               |                        |                 |                  |          |            |           | 3                    | 26–28                  |
|                |               |                        |                 |                  |          |            |           | 17                   | <40                    |
|                |               |                        |                 |                  |          |            |           | 2                    |                        |
|                |               |                        |                 |                  |          |            |           | (16.8%)               |                        |
|                |               |                        |                 |                  |          |            |           | (65.1%)              |                        |
|                |               |                        |                 |                  |          |            |           | 3                    |                        |
|                |               |                        |                 |                  |          |            |           | (18.0%)              |                        |
|                |               |                        |                 |                  |          |            |           | 2                    |                        |
|                |               |                        |                 |                  |          |            |           | (8.7%)                |                        |

T=−7.214, df= 9, p= 0.001
† t=−2.574, df= 8, p= 0.07

Fig. 3. Statistical comparison of abundance of *Phlebotomus sergenti* collected using hand-catch and baited traps from indoor and outdoor places, Kerman City, southeastern Iran, 2019

Fig. 4. Statistical comparison of sex ratio of *Phlebotomus sergenti* collected using hand-catch and baited traps from indoor and outdoor places, Kerman City, southeastern Iran, 2019
Fig. 5. Susceptibility of *Phlebotomus sergenti* to DDT and malathion, collected using hand-catch from indoor places, Kerman City, southeastern Iran, 2019

Fig. 6. Susceptibility of *Phlebotomus sergenti* to DDT, deltamethrin, malathion and propoxur collected using baited traps from outdoor places, Kerman City, southeastern Iran, 2019
Discussion

Despite the implementation of the national leishmaniasis surveillance program and the efforts for adopting different control methods during past decades, ACL is still a major health challenge with increasing trends in many parts of Iran, particularly Kerman Province. This study was conducted to investigate the susceptibility level of Ph. sergenti to conventional insecticides according to the WHO instruction for the phlebotomine susceptibility test. Furthermore, there is an operational gap for employing a capture method for adequate yielding of live Ph. sergenti, which is necessary for the standard sample sizes used in susceptibility tests. In this study, efficiency of baited traps showed the more yield of live Ph. sergenti compared to hand-catch method from indoor places (t= 7.214, df= 9, p= 0.001) with no significant difference for the sex ratio. The most important obstacle for collecting sand flies from indoor places using the hand-catch method is the lack of cooperation and willingness of the households to check their houses for the presence of sand flies. In addition, in urban areas, due to the application of aerosol sprays or using cooling systems in the houses, the abundance of sand flies is usually lower than the suburbs. In the other study, different collecting methods were assessed for live sand flies with a total of 122 live Ph. sergenti collected at outdoor sites in the mountainous parts of Kashan District. The comparison of different traps showed the most efficiency (59.8%) for the black Shannon traps during the summer (97). In other attempts made in the northeastern parts of Iran as well as in Cukurova Plain, southern Anatolia, Turkey, the CO2 CDC light traps showed the most efficiency with 43.2% and 75.0 percent for collecting live Ph. sergenti with a total abundance of 2521 and 4 from outdoor sites (98-99). In another testing, different commercial traps were assessed for collecting live Ph. sergenti in the north of Aswan, southern Egypt which the BGS traps showed more efficacy (35.5%) (100). The results of susceptibility tests on Ph. sergenti collected from outdoor sites showed 100% mortality exposed to DDT 4.0%, deltamethrin 0.05%, malathion 5.0%, and propoxur 0.1%, indicating the complete susceptibility. The Ph. sergenti caught in the houses located adjacent to the mountains using hand-catch method revealed 100% mortality when exposed to DDT 4.0% and malathion 5.0%. Due to the obvious differences in the biology and behavior of Ph. sergenti with other species of sand flies, the finding of the present study was compared with other studies if the tests were only carried out on Ph. sergenti. The number of reports on the susceptibility level of Ph. sergenti to insecticides is limited in the world. During the years 1971, 1976, and 1998, three trials were carried out in the ACL foci in the large cities located in the northeast including Mashhad (31) and Neishabur (32) as well as Kerman (101) located in the southeast of Iran. The findings revealed the complete susceptibility of this species to the DDT. Moreover, there are three other published records for assessing the susceptibility status of Ph. sergenti in Mashhad, northeast of Iran (102) and Dehbakri County, southeast of Iran (103) in 2007 and 2011, which indicated the complete susceptibility of Ph. sergenti to DDT 4.0% and deltamethrin 0.05%. In a more recent study carried out in Maneh and Samalqan County, northeastern Iran, all sand flies were collected using CDC light traps from outdoor and tested with papers impregnated with DDT 4.0%, bendiocarb 0.1%, and permethrin 0.75%. The results showed mortality rates of 89.8±1.4, 93.6±1.4, and 95.6±1.7% respectively, indicating the resistance of Ph. sergenti to DDT (104). A study carried out in North Africa and the Middle East in 2007 found the high susceptibility of Ph. sergenti to malathion, DDT, cyfluthrin, bendiocarb, permethrin, and resmethrin (105). A susceptibility test conducted in Morocco showed the full susceptibility of wild populations of Ph. sergenti to DDT, lambdacyhalothrin, and malathion (106). The finding of the present research showed the susceptibility of Ph. sergenti...
to DDT, malathion, deltamethrin, and propoxur in indoor and outdoor populations collected in the ACL foci of Kerman. It is expected that application of the insecticide-based control strategies for sand flies, for example IRS, ITNs, ITC and ITT, could be an effective method for controlling Ph. sergenti in indoor and outdoor places if the measures are performed correctly.

Conclusion

Anthroponotic cutaneous leishmaniasis is still a major health problem in Kerman, southeast of Iran. This research showed the high susceptible status of Ph. sergenti in both the indoor and outdoor populations against all tested insecticides. The research-based evidence indicates the success of future insecticide-based vector control methods for controlling sand flies in the ACL foci.

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References

1. Javadian E (2008) Epidemiology of cutaneous leishmaniasis in Iran. In: Nadim A, Javadian E, Momeni A (Eds) Leishmania and leishmaniasis. Iran University Press 1295, Tehran, pp. 191–211.
2. Seyedi-Rashti MA, Keighobadi K, Nadim A (1984) Urban cutaneous leishmaniasis in Kerman, southeast Iran. Bull Soc Pathol Exot. 77: 312–319.
3. Jafari S, Hajjabdolbaghi M, Mohebali M, Hajjaran H, Hashemian H (2010) Disseminated leishmaniasis caused by Leishmania tropica in HIV-positive patients in the Islamic Republic of Iran. East Mediterr Health J. 16: 340–343.
4. Bamorovat M, Sharifi I, Mohammadi MA, Eybpoosh S, Nasibi S, Aflatoonian MR, Khosravi A (2018) Leishmania tropica isolates from non-healed and healed patients in Iran: A molecular typing and phylogenetic analysis. Microb Pathog. 1 (116): 124–129.
5. Sharifi I, Fekri AR, Aflatoonian MR, Khamsehpour A, Mahboudi F, Dowlati Y, Nadim A, Modabber F (2010) Leishmaniasis recidivans among school children in Bam, Southeast Iran, 1994–2006. Intern J Dermatol. 49: 557–561.
6. Bamorovat M, Sharifi I, Aflatoonian MR, Sharifi H, Karamoozian A, Sharifi F, Karamoozian A, Sharifi F, Khosravi A, Hassanzadeh S (2018) Risk factors for anthroponotic cutaneous leishmaniasis in unresponsive and responsive patients in a major focus, southeast of Iran. PloS One. 13: e0192236.
7. Bamorovat M, Sharifi I, Dabiri S, Mohammadi MA, Fasihi Harandi M, Mohebali M, Aflatoonian MR, Keyhani A (2015) Leishmania tropica in stray dogs in southeast Iran. Iran J Public Health. 44(10): 1359–1366.
8. Mohebali M, Malmasi A, Hajjaran H, Jamsheid S, Akhoudi B, Rezaei M, Janitabar S, Zarei H, Charehdar S (2011) Disseminated leishmaniasis caused by Leishmania tropica in a puppy from Karaj, central Iran. Iran J Parasitol. 6: 69–73.
9. Dereure J, Rioux JA, Gallego M, Perieres J, Pratlong F, Mahjour J, Saddiki H (1991) Leishmania tropica in Morocco: infection in dogs. Trans R Soc Trop Med Hyg. 85(5): 595–599.
10. Guilvard E, Rioux JA, Gallego M, Pratlong F, Mahjour J, Martinez-Ortega E, Dereure J, Saddiki A, Martini A (1991) Leishman-
nia tropica in Morocco. III identification of 89 isolates from the vector Phlebotomus sergenti. Ann Parasitol Hum Comp. 66: 96–99.
11. Yahia H, Ready PD, Hamdani A, Testa JM, Guessous N (2004) Regional differentiation of Phlebotomus sergenti in three Moroccan foci of cutaneous leishmaniasis cause by Leishmania tropica. Parasite. 11: 189–199.
12. Izri A, Bendjaballah A, Andriantsoanirina V, Durand R (2014) Cutaneous leishmaniasis caused by Leishmania killicki, Algeria. Emerg Infect Dis. 20(3): 502–504.
13. Amro A, Gashout A, Al-Dwibe H, Zannahir Alam M, Annajar B, Hamarshen O (2012) First Molecular Epidemiological Study of Cutaneous Leishmaniasis in Libya. PLoS Negl Trop Dis. 6(6): e1700.
14. Tabbabi A (2019) Review of Leishmaniasis in the Middle East and North Africa. Afr Health Sci. 19(1): 1329–1337.
15. Tabbabi A, Ghrab J, Aoun K, Ready PD, Bourbatine A (2011) Habitats of the sandfly vectors of Leishmania tropica and L. major in a mixed focus of cutaneous leishmaniasis in South East Tunisia. Acta Trop. 119: 131–137.
16. Nadim A, Rostami GS (1974) Epidemiology of cutaneous leishmaniasis in Kabul, Afghanistan. Bull World Health Org. 51: 45–49.
17. Nadim A, Javadian E, Noushin MK, Nayil AK (1979) Epidemiology of cutaneous leishmaniasis in Afghanistan. Part II. Anthroponotic cutaneous leishmaniasis. Bull Soc Pathol Exot Filiales. 72: 461–466.
18. Javadian E, Nadim A, Nayil AK (1982) Epidemiology of cutaneous leishmaniasis in Afghanistan. Part III. Notes on sandflies of Afghanistan. Bull Soc Pathol Exot Filiales. 75: 284–290.
19. Reithinger R, Mohsen M, Aadil K, Sidiqi M, Erasmus P, Coleman PG (2003) Anthroponotic cutaneous leishmaniasis, Kabul, Afghanistan. Emerg Infect Dis. 9: 727–729.
20. Hewitt S, Reyburn H, Ashford R, Rowland M (1998) Anthroponotic cutaneous leishmaniasis in Kabul, Afghanistan: vertical distribution of cases in apartment blocks. Trans R Soc Trop Med Hyg. 92: 273–274.
21. Reyburn H, Rowland M, Mohsen M, Khan B, Davies C (2003) The prolonged epidemic of anthropoontic leishmaniasis in Kabul, Afghanistan: ‘brining down the neighborhood’. Trans R Soc Trop Med Hyg. 97: 170–176.
22. Shirzadi MR, Gouya MM (2010) National Guidelines for Cutaneous Leishmaniasis Surveillance in Iran. Ministry of Health and Medical Education, Zoonoses Control Department, Tehran, Iran.
23. Hussain M, Munir S, Jamal MA, Ayaz S, Akhoundi M, Mohamed K (2017) Epidemic outbreak of anthropoontic cutaneous leishmaniasis in Kohat District, Khyber Pakhtunkhwa, Pakistan. Acta Trop. 172: 147–155.
24. El-Beshbishy HA, Al-Ali KH, El-Badry AA (2013) Molecular characterization of Leishmania infection in sand flies from Al-Madinah Al-Munawarah Province, Western Saudi Arabia. Exp Parasitol. 134: 211–215.
25. Muhjazi G, Gabrielli AF, Ruiz-Postigo JA, Atta H, Osman M, Bashour H, Al Tawil A, Hussein A, Allahham R, Allan R (2019) Cutaneous leishmaniasis in Syria: A review of available data during the war years: 2011–2018. PLoS Negl Trop Dis. 13(12): e0007827.
26. Kamhawi S, Abdel-Hafez SK, Arbaki A (1995) A new focus of cutaneous leishmaniasis caused by Leishmania tropica in northern Jordan. Trans R Soc Trop Med Hyg. 89(3): 255–257.
27. Salam N, Al-Shaqha WM, Azzi A (2014) Leishmaniasis in the Middle East: incidence and epidemiology. PLoS Negl Trop Dis. 8(10): e3208.
28. Svobodova M, Votycka J, Peckova J, Dvorak V, Nasereddin A, Baneth G, Sztern J,
Kravchenko V, Orr A, Meir D, Schnur LF, Volf P, Warburg A (2006) Distinct transmission cycles of *Leishmania tropica* in 2 adjacent foci. Emerg Infect Dis. 12: 1860–1868.

29. Zeyrek FY, Korkmaz M, Ozbel Y (2007) Serodiagnosis of anthropotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* in Sanliurfa Province, Turkey, where ACL is highly endemic. Clin Vaccine Immunol. 14: 1409–14015.

30. Nadim A, Javadian E, Seyedi-Rashti MA (1994) Epidemiology of cutaneous leishmaniasis in Iran. In: Ardehali S, Rezaei R, Nadim A (Eds) *Leishmania Parasite and leishmaniasis*. University Publishing Center, Tehran, pp. 176–208.

31. Nadim A, Seyedi-Rashti MA, Mesghali A (1971) Epidemiology of cutaneous leishmaniasis in Iran. B. Khorassan. Part IV. Distribution of sand flies. Bull Soc Path Exot. 64(6): 865–870.

32. Nadim A, Tahvildari-Bidruni GH (1977) Epidemiology of cutaneous leishmaniasis in Iran: B. Khorassan, Part VI: Cutaneous leishmaniasis in Neishabur, Iran. Bull Soc Pathol Exot. 70: 171–177.

33. Ghaee MA, Mirhendi H, Marashifard M, Kanannejad Z, Taylor WR, Sharifi I (2018) Population structure of *Leishmania tropica* causing anthropotic cutaneous leishmaniasis in southern Iran by PCR-RFLP of kinetoplastid DNA. BioMed Res Int. 2018: 6049198.

34. Yaghoobi-Ershadi MR (1977) Studies on Cutaneous Leishmaniasis in the City of Kerman. [MSPH Thesis]. University of Tehran, Iran.

35. Sharifi I, Aflatoonian MR, Fekri AR, Hakimi Parizi M, Aghaei-Afshar A, Khosravi A (2015) A comprehensive review of cutaneous leishmaniasis in Kerman Province, southeastern Iran. Narrative review article. Iran J Public Health. 44: 299–307.

36. Yaghoobi-Ershadi MR, Hanafi-Bojd AA, Javadian E, Jafari R, Zahraei-Ramazani AR, Mohebali M (2002) A new focus of cutaneous leishmaniasis caused by *Leishmania tropica*. Saudi Med J. 23: 291–294.

37. Nadim A, Aflatoonian MR (1995) Anthropotic cutaneous leishmaniasis in Bam, southeast Iran. Iran J Public Health. 24: 15–24.

38. Aflatoonian MR, Sharifi I, Nadim A, Aflatoonian B (2014) Comparison of the period prevalence of urban cutaneous leishmaniasis (CL) in Bam in two time periods of 1990–1992 and 2010–2012. Iran J Public Health. 9: 32–39.

39. Sharifi I, Fekri AR, Aflatoonian MR, Nadim A, Nikian Y, Khamesipour A (1998) Cutaneous leishmaniasis in primary school children in the southeastern Iranian city of Bam, 1994–1995. Bull World Health Org. 76: 289–293.

40. Sharifi I, Nakhaei N, Aflatoonian MR, Hakimi Parizi M, Fekri AR, Safizadeh H, Shirzadi MR, Goya MM, Khamesipour A (2011) Cutaneous leishmaniasis in Bam: A comparative evaluation of pre- and post-earthquake years (1999–2008). Iran J Public Health. 40: 49–56.

41. Zahraei-Ramazani A, Yaghoobi-Ershadi MR, Mokhtari A, Akhavan AA, Abdoli H, Arandian M (2007) Anthropoonyct cutaneous leishmaniasis in nonendemic quarters of a central city in Iran. Iran J Public Health. 36(2): 7–11.

42. Mirzaei M, Sharifi I, Poursmaelian S (2012) A new focus of anthropoonyct cutaneous leishmaniasis and identification of parasite species by nested PCR in Jiroft, Iran. Comp Clin Pathol. 21(5): 1071–1075.

43. Yaghoobi-Ershadi MR (2012) Phlebotomine sand flies (Diptera: Psychodidae) in Iran and their role on *Leishmania* transmission. J Arthropod Borne Dis. 6(1): 1–17.

44. Mesghali A, Seyedi-Rashti MA, Nadim A (1967) Epidemiology of cutaneous leishmaniasis in Iran. II. Natural leptomonom infection of sand flies in the Mashhad and
Lotfabad areas. Bull Soc Pathol Exot. 60: 514–517.

45. Mozafary M, Dayer MS, Aghaei-Afshar A, Mollaie HR (2016) Epidemiology of cutaneous leishmaniasis and molecular characterization of its causative agents in naturally infected sand flies in endemic foci of Kerman City, southeastern Iran. Asian Pac J Trop Dis. 6(3): 188–192.

46. Aghaei Afshar A, Rassi Y, Sharifi I, Vatandoost H, Mollaie HR, Oshaghi MA, Abai MR, Rafizadeh S (2014) First report on natural Leishmania infection of Phlebotomus sergenti due Leishmania tropica by high resolution melting curve method in southeastern Iran. Asian Pac J Trop Med. 7(2): 93–96.

47. Azizi K, Askari MB, Kalantari M, Moemenbeighi Fard MD (2016) Molecular detection of Leishmania parasites and host blood meal identification in wild sand flies from a new endemic rural region, south of Iran. Pathog Glob Health. 110: 303–309.

48. Moemenbellah-Fard MD, Ahmadyousefi-Sarhadi M, Azizi K, Fakoorziba MR, Kalantari M, Amin M (2015) Faunal identification and frequency distribution of wild sand flies infected with Leishmania tropica. Asian Pac J Trop Dis. 5(10): 792–797.

49. Oshaghi MA, Rasolian M, Shirzadi MR, Mohtarami F, Doosti S (2010) First report on isolation of Leishmania tropica from sand flies of a classical urban cutaneous leishmaniasis focus in southern Iran. Exp Parasitol. 126: 445–450.

50. Al-Zahrani MA, Peters W, Evans DA, Ching C, Smith IV, Lane RP (1988) Phlebotomus sergenti, a vector of Leishmania tropica in Saudi Arabia. Trans R Soc Trop Med Hyg. 82: 416.

51. Tabbabi A, Bousslimi N, Rhim A, Aoun K, Bouratbine A (2011) First report on natural infection of Phlebotomus sergenti with Leishmania promastigotes in the cutaneous leishmaniasis focus in southeastern Tunisia. Am J Trop Med Hyg. 85: 646–647.

52. Aghasi M, Sharifi I (2003) Survey of the fauna and monthly activity of the sand fly as the vectors of the cutaneous leishmaniasis in the city of Bam. J Kerman Univ Med Sci. 10(2): 85–91.

53. Mirhoseini M, Salehzadeh A, Ramazan Jaamaat S, Zahirnia A H, Rahmanzadeh N (2017) Distribution and seasonal activity of Phlebotominae sand flies in Yazd and its outskirts, center of Iran. Sci World J. 1486845.

54. Nadim A, Seyedi-Rashti MA (1991) Some aspects to the ecology of Phlebotomus sergenti in Iran. First International Symposium on Phlebotomine Sand flies Rome, Italy, p. 79.

55. Fakoorziba MR, Nazari M (2006) Entomological studies of Phlebotomus papatasii and Ph. sergenti (Diptera: Psychodidae) as vectors of cutaneous leishmaniasis in Shiraz, Iran. Southeast Asian J Trop Med Public Health. 37 Suppl 3: 115–117.

56. Yaghoobi-Ershadi MR, Hakimiparizi M, Zahravi-Ramazani AR, Abdoli H, Akhavan AA, Aghasi M, Arandian MH, Ranjbar AA (2010) Sand fly surveillance within an emerging epidemic focus of cutaneous leishmaniasis in southeastern Iran. Iran J Arthropod Borne Dis. 4(1): 17–23.

57. Zahraei-Ramazani AR, Akhavan AA, Abdoli H, Jafari R, Jalalizand AR, Arandian MH, Shareghi N, Ghanei M (2008) Some ecological aspects of phlebotominae sandflies (Diptera: Psychodidae) in an endemic focus of anthroponotic cutaneous leishmaniasis of Iran. J Entomol. 5 (1): 17–23.

58. Hmamouch A, El Alem MM, Hakkour M, Amarir F, Daghbach H, Habbari K, Fellah H, Bekhti K, Sebti F (2017) Circulating species of Leishmania at microclimate area of Boulemane Province, Morocco: impact of environmental and hu-
55. Yassini A, Khan NH, Sepilveda N, Munir A, Wahid S (2016) Assessing incidence patterns and risk factors for cutaneous leishmaniasis in Peshawar region, Khyber Pakhtunkhwa, Pakistan. J Parasitol. 102: 501–506.

56. Aflatoonian MR, Sharifi I, Parizi MH, Fekri AR, Aflatoonian B, Sharifi M, Khosravi A, Khamesipour A, Sharifi H (2014) A prospective cohort study of cutaneous leishmaniasis risk and opium addiction in south eastern Iran. PLoS One. PMID: 24586494.

57. Desjeux P (2001) The increase in risk factors of leishmaniasis worldwide. Trans R Soc Trop Med Hyg. 95: 239–245.

58. Dujardin JC (2006) Risk factors in the spread of leishmaniases: towards integrated monitoring? Trends Parasitol. 22: 4–6.

59. Aflatoonian MR, Sharifi I, Poursmaelian S, Hakimi-Parizi M, Ziaali N (2013) The emergence of anthropoontic cutaneous leishmaniasis following the earthquake in southern villages of Bam District, southeastern Iran, 2010. J Arthropod Borne Dis. 7(1): 8–14.

60. Aflatoonian MR, Montazeri F, Jalali M, Nadim A, Sharifi F (2006) The status of the disease in Bam in 2005 years and the comparison with the last five years (before and after the earthquake). J Kerman Uni Med Sci. 13: 37–43.

61. Sharifi I, Poursmaelian S, Aflatoonian MR, Fotouhi Ardakani R, Mirzaei M, Fekri AR, Khamesipour A, Hakimi Parizi M, Fashi Harandi M (2011) Emergence of a new focus of anthropoontic cutaneous leishmaniasis due to *Leishmania tropica* in rural communities of Bam District after the earthquake, Iran. Trop Med Intern Health. 16(4): 510–513.

62. Fazaeli A, Fouladi B, Sharifi I (2009) Emergence of cutaneous leishmaniasis in a border area at south-east of Iran: an epidemiological survey. J Vector Borne Dis. 46: 36–42.

63. Alawieh A, Musharrafieh U, Jaber A, Berry A, Ghosn N, Bizri AR (2014) Revisiting leishmaniasis in the time of war: the Syrian conflict and the Lebanese outbreak. Int J Infect Dis. 29: 115–119.

64. Alasaad S (2013) War diseases revealed by the social media: massive leishmaniasis outbreak in the Syrian Spring. Parasite Vectors. 6: 94.

65. Inci R, Ozturk P, Mulayim MK, Ozyurt K, Alatas ET, Inci MF (2015) Effect of the Syrian civil war on prevalence of cutaneous leishmaniasis in southeastern Anatolia, Turkey. Medical science monitor. Intl Med J Exp Clin Res. 21: 2100–2104.

66. Kolaczinski J, Brooker S, Reyburn H, Rowland M (2004) Epidemiology of anthropoontic cutaneous leishmaniasis in Afghan refugee camps in northwest Pakistan. Tran Roy Soc Trop Med Hyg. 98: 373–378.

67. Rowland M, Munir A, Durrani N, Noyes H, Reyburn H (1999) An outbreak of cutaneous leishmaniasis in an Afghan refugee settlement in north-west Pakistan. Trans R Soc Trop Med Hyg. 93: 133–136.

68. Saroufim M, Charafeddine K, Issa G (2014) Ongoing epidemic of cutaneous leishmaniasis among Syrian refugees, Lebanon. Emerg Infect Dis. 20(10): 1712–1715.

69. Brooker S, Mohammed N, Adil K, Agha S, Reithinger R, Rowland M, Ali I, Kolaczinski J (2004) Leishmaniasis in refugee and local Pakistani populations. Emerg Infect Dis. 10(9): 1681–1684.

70. His Excellency, Murshidi MM, Hijjawi MQ, Jeriesat S, Eltom A (2013) Syrian refugees and Jordan’s health sector. Lancet. 382(9888): 206–207.

71. Du R, Hotez PJ, Al-Salem WS, Acosta-Serrano A (2016) Old world cutaneous leishmaniasis and refugee crises in the Middle East and North Africa. PLoS Negl Trop Dis. 10(5): e0004545.

72. Saroufim M, Charafeddine K, Issa G, Kha-
lifeh H, Habib RH, Berry A, Ghosn N, Rady A, Khalifeh I (2014) Ongoing epidemic of cutaneous leishmaniasis among Syrian refugees, Lebanon. Emerg Infect Dis. 20(10): 1712–1715.

77. Seyedi-Rashti MA, Nadim A (1975) Re-establishment of cutaneous leishmaniasis after cessation of anti-malaria spraying. Trop Geogr Med. 27(1): 79–82.

78. World Health Organization (2010) Control of the Leishmaniasis. Report of a meeting of the WHO export committee on the control of leishmaniasis, WHO Technical Report Series 949, Geneva.

79. Yaghoobi-Ershadi MR (2016) Control of phlebotomine sand flies in Iran: a review article. J Arthropod Borne Dis. 10(4): 429–444.

80. González U, Pinart M, Sinclair D (2015) Vector and reservoir control for preventing leishmaniasis. Cochrane Database Syst Rev. published by John Wiley and Sons, Ltd. on behalf of The Cochrane Collaboration. p. 80.

81. Maroli M, Khoury C (2004) Prevention and control of leishmaniasis vectors: current approaches. Parasitologia. 46: 211–215.

82. Nadim A, Motabar M, Houshmand B, Keyghobadi K, Aflatoonian MR (1995) Evaluation of Pyrethroid Impregnated Bed Nets for Control of Anthroponotic Cutaneous Leishmaniasis in Bam (Islamic Re-public of Iran). WHO, Geneva, Switzerland. Available at: https://apps.who.int/iris/handle/10665/61138.

83. Yaghoobi-Ershadi MR, Moosa-Kazemi SH, Zahraei-Ramazani AR, Jalalzand AR, Akhavan AA, Ardandian MH (2006) Evaluation of deltamethrin impregnated bed nets and curtains for control of zoonotic cutaneous leishmaniasis in hyperendemic area of Iran. Bull Soc Pathol Exot. 99(1): 43–48.

84. Kolaczinski JH, Muhammad N, Khan QS, Jan Z, Rehman N (2004) Subsidized sales of insecticide-treated nets in Afghan refugee camps demonstrate the feasibility of a transition from humanitarian aid towards sustainability. Malar J. 3: 15.

85. Courtenay O, Gillingwater K, Gomes PA, Garcez LM, Davies CR (2007) Deltamethrin-impregnated bednets reduce human landing rates of sandfly vector Lutzomyia longipalpis in Amazon households. Med Vet Entomol. 21(2): 168–176.

86. Jalouk L, Al Ahmed M, Gradoni L, Maroli M (2007) Insecticide-treated bed nets to prevent anthroponotic cutaneous leishmaniasis in Aleppo Governorate, Syria: results from two trials. Trans R Soc Trop Med Hyg. 101(4): 360–367.

87. Kroeger A, Avila EV, Morison L (2002) Insecticide impregnated curtains to control domestic transmission of cutaneous leishmaniasis in Venezuela: cluster randomized trial. BMJ. 325(7368): 810–813.

88. Moosa-Kazemi SH, Yaghoobi-Ershadir MR, Akhavan AA, Abdoli H, Zahraei-Ramazani AR (2007) Deltamethrin-impregnated bed nets and curtains in an anthroponotic cutaneous leishmaniasis control program in northeastern Iran. Ann Saudi Med. 27: 6–12.

89. Reyburn H, Ashford R, Mohsen M, Hewitt S, Rowland M (2000) A randomized controlled trial of insecticide-treated bednets and chaddars or top sheets, and residual spraying of interior rooms for the prevention of cutaneous leishmaniasis in Kabul, Afghanistan. Trans R Soc Trop Med Hyg. 94: 361–366.

90. Aghaei-Afshar A, Vatandoost H, Sharifi I, Mollaie H, Oshaghi MA (2013) First determination of impact and outcome indicators following indoor residual spraying (IRS) with deltamethrin in a new focus of Anthroponotic Cutaneous Leishmaniasis (ACL) in Iran. Asian Pac J Trop Dis. 3: 5–9.

91. World Health Organization (1981) Instructions for determining the susceptibility
or resistance of blackflies, sand flies and biting midges to insecticides. WHO/VBC/81.810.

92. World Health Organization (1986) Resistance of vectors and reservoirs of disease to pesticides. 10th report of the WHO Expert committee. Vector Biology and Control. Tech Rep Ser, p. 737.

93. World Health Organization (2016) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, II, WHO, Geneva. Available at: http://apps.who.int/iris/bitstream/handle/10665/250677/9789241511575-eng.pdf?sequence=1

94. Nadim A, Javadian E (1976) Key for species identification of sandflies (Phlebotominae: Diptera) of Iran. Iran J Public Health. 5: 33–44.

95. Javadian E, Mesghali A (1975) Checklist of phlebotomine sand flies (Diptera: Psychodidae) of Iran. Bull Soc Pathol Exot. 68: 207–209.

96. Rassi Y, Hanafi-Bojd AA (2006) Phlebotomine Sand Flies, Vectors of Leishmaniasis: Morphology, biology, ecology, and field and laboratory methods with pictorial key of Iranian sand flies. Noavaran-Elm Publication, Tehran, Iran.

97. Hesam-Mohammadi M, Rassi Y, Abai MR, Akhavan AA, Karimi F, Rafizadeh S, Sanei-Dehkordi A, Sharafkhah M (2014) Efficacy of different sampling methods of sand flies (Diptera: Psychodidae) in endemic focus of cutaneous leishmaniasis in Kashan District, Isfahan Province, Iran. J Arthropod Borne Dis. 8(2): 156–162.

98. Arzamani K, Rassi Y, Vatandoost H, Akhavan AA, Abai MR, Alavinia M, Akbarzadeh K, Mohebali M, Rafizadeh S, Karimian F, Badakhshan M, Absavaran A (2019) Comparative Performance of different traps for collection of phlebotomine sand flies and estimation of biodiversity indices in three endemic leishmaniasis foci in North Khorasan Province, Northeast of Iran. J Arthropod Borne Dis. 13(4): 399–406.

99. Kasap ÖE, Belen A, Kaynas S, Simsek FM, Biler L, Ata N, Alten B (2009) Activity patterns of sand fly (Diptera: Psychodidae) species and comparative performance of different traps in an endemic cutaneous leishmaniasis focus in Cukurova Plain, Southern Anatolia, Turkey. Acta Vector Borne. 78(2): 327–335.

100. Hoel DF, Kline DL, Hogsette JA, Bernier UR, El-Hossary SS, Hanafi HA, Watany N, Fawaz EY, Furman BD, Obenauer PJ, Szumlas DE (2010) Efficacy of commercial mosquito traps in capturing Phlebotomine sand Flies (Diptera: Psychodidae) in Egypt. J Med Entomol. 47(6): 1179–1184.

101. Aghasi M (1996) Present Status of Anthropootic Cutaneous Leishmaniasis in Kerman, Southeast Iran. [MS Ph thesis]. School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

102. Moosa-Kazemi SH, Yaghoobi-Ershadi MR, Akhavan AA, Abdoli H, Zahraei-Rama­zani AR, Jafari R, Houshmand B, Nadim A, Hosseini M (2007) Deltamethrin-imregnated bednets and curtains in an anthropootic cutaneous leishmaniasis control program in northeastern Iran. Ann Saudi Med. 27(1): 6–12.

103. Afshar AA, Rassi Y, Sharifi I, Abai MR, Oshaghi M, Yaghoobi-Ershadi MR, Vatandoost H (2011) Susceptibility status of Phlebotomus papatasi and Ph. sergenti (Diptera: Psychodidae) to DDT and deltamethrin in a focus of cutaneous leishmaniasis after earth­quake strike in Bam, Iran. J Arthropod Borne Dis. 5(2): 32–41.

104. Arzamani K, Vatandoost H, Rassi Y, Abai MR, Akhavan AA, Alavinia M, Akbar­zadeh K, Mohebali M, Rafizadeh S (2017) Susceptibility status of wild pop­
ulation of *Phlebotomus sergenti* (Diptera: Psychodidae) to different insecticides in an endemic focus of cutaneous leishmaniasis in northeast of Iran. J Vector Borne Dis. 54: 282–286.

105. Tetreault GE, Zayed AEBB, Hanafi-Bojd AA, Beavers GM, Zeichner BC (2001) Susceptibility of sandflies to selected insecticides in North Africa and the Middle East. J Am Mosq Control Assoc. 17 (1): 23–27.

106. Faraj C, Ouahabi S, Adlaoui EB, El Elkhohli M, Lakraa L, El Rhazi M, Ameur B (2012) Insecticide susceptibility status of *Phlebotomus (Paraphlebotomus) sergenti* and *Phlebotomus (Phlebotomus) papatasi* in endemic foci of cutaneous leishmaniasis in Morocco. Parasite Vectors. 5: 51.