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Original Article

# Epidemiological Study and Reservoir Identification of Cutaneous Leishmaniasis From Ardestan in Isfahan, Iran (2015-2016)

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## Abstract

**Background and aims:** Cutaneous leishmaniasis (CL) has been considered one of the most common serious parasitic diseases. Some cities in Iran are known as the center of this important parasitic disease. The World Health Organization (WHO) defines CL as an infectious parasitic disease in the tropics, which can be challenging. The aim of this study was to investigate the epidemiological situation of CL (the identification of parasite, vector, and reservoir) in Ardestan.

**Methods:** This descriptive-analytical cross-sectional study was performed in 2015-2016. Overall, 121 patients with CL who referred to Ardestan Dermatology and Leishmaniasis Center were sampled, and the *Leishmania* species were determined in the samples using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, following the amplification of the internal transcribed spacer 1 (ITS1) region in the parasite genome. Finally, the vector and reservoir species were detected by zoology experts according to identification keys.

**Results:** The incidence of the disease had the highest (57.8%) and lowest (4.1%) levels in summer and spring, respectively. The disease was prevalent in both women and men but was more common in men (51.2%). The majority of patients (27.3%) were in the age group of 21-30 years, and most of the wounds (71.1%) were nodules. More than one wound on the body was observed in 54.5% of patients, and the disease was prevalent in 13.2% of patients and their family members. Occupationally, students showed the highest disease frequency (32%). The response to treatment with meglumine antimoniate (glucantime) was more effective than the other treatments. The species of the *Leishmania* vector in the Ardestan region was identified as *Phlebotomus papatasi*, and the species of the reservoirs in this region were *Rhombomys opimus* and *Meriones libycus*.

**Conclusion:** Further research is needed to determine the carriers and reservoirs of the disease in other regions in order to reach a constructive decision for appropriate strategies to control the disease.

**Keywords:** Cutaneous leishmaniasis, Epidemiology, Carrier, Reservoir, ITS1, PCR-RFLP

## Introduction

Leishmaniasis is one of the most common parasitic skin diseases caused by a unicellular parasite belonging to trypanosomatids and the genus *Leishmania*.[1,2] *Leishmania* is an obligate intracellular protozoan parasite, and sandfly is a vector that transmits infectious forms of the parasite or metacyclic promastigotes to its vertebrate host, including humans by biting.[3-5] Leishmaniasis is a skin disease caused by a single-cell parasite called *Leishmania*, which has two types of leishmaniasis caused by *Leishmania major* and *Leishmania tropica* in Iran. More than 70% of the cases of leishmaniasis in Iran are due to *Leishmania major*.[6,7] The disease is transmitted by sandfly bites from infected animals (rodents and dogs) or humans, and the symptoms usually appear 3 months to a year after the bite. Cutaneous leishmaniasis (CL) is a common disease that exists in the New World (South and Central America, Mexico City) and the Old World (Europe, Africa, Central Asia, and the Indian subcontinent). So far, many studies and activities have been performed in the field of identification, control, and prevention of CL in Iran. Socio-political and climatic factors are effective in increasing the number of cases of the disease.[3,8-10] According to the World Health Organization reports, leishmaniasis is a common skin disease that is threatening in different parts of Iran; therefore, Iran is considered one of the endemic countries for CL in the world. In Iran, both types of CL, including wet or rural type (one-third of the provinces involved), were caused by *L.* major, and dry or urban type (all provinces involved) were caused by *L. tropica*.[10-12] The causes of the spread of CL are divided into five sections, including environmental, carrier-related, and reservoir-related factors and the lack of health education or community awareness. Currently, 10 million people in the world suffer

from this disease. Factors such as reservoirs, carriers, and climatic conditions play important roles in the endemic establishment of the disease so that the disease is existed in hot and semi-arid climates in most of the known centers, which are usually located in the plains and the vicinity of desert areas.[13,14] Malformed lesions and complications of social, psychological, and psychological factors are the unfortunate consequences of the disease.[15] The key objective of this study was to accurately investigate the epidemiological status of CL in Ardestan, Isfahan, Iran. For this purpose, the relative frequency of the CL infection, along with the relative frequency of vectors and reservoirs, was determined in this city. The obtained results will be a basis for further studies on disease control planning.

## Materials and Methods

In general, 121 patients with CL who referred to Ardestan Dermatology and Leishmaniasis Center during 2015-2016 were included in this descriptive-analytical cross-sectional study.

### *Study Area*

The study was conducted in Ardestan, which is a city located on the slopes of Mount Karkas in 110 km northeast of Isfahan province, central Iran with a population of about 47113 people and a hot-dry climate. The area of this city is 14240 $km^2$, and it consists of 2 districts, 7 villages, and 306 inhabited villages (Figure 1).

### *Collection and Microscopic Examination of Samples*

Sampling was performed in the laboratory of the Ardestan Dermatology and Leishmaniasis Center, which is a CL admission center. Samples were taken from the protruding edge of the skin lesions of 121 patients with suspected CL with a disposable razor blade. A questionnaire about each patient's demographic information (e.g., incidence and type of disease, geographical location, age, gender, nationality, occupation, and the clinical spectrum of the disease) was filled out before sample collection. Then, the lesion site was disinfected with 70% ethanol and 0.9% saline, and the xylocaine solution was used for local anesthesia. Next, the infected tissue (serosite) was removed by scratching, and two slides were prepared by using them. The prepared slides were dried at room temperature and fixed with 96% methanol for several minutes. Subsequently, one of the slides was stained with 10% Giemsa dye, and after 20 minutes, it was washed with water and kept for parasitological analysis. The other slide was kept without staining for molecular testing. The stained slides were observed under a light microscope with 100X magnification in order to find parasite amastigotes. If only one amastigote was observed extra- or intra-cellular in the total area of the smear, the sample was considered positive; otherwise, a negative test result was recorded in this regard.[16,17] Unstained smears were used for DNA extraction and polymerase chain reaction (PCR) amplification.

### *DNA Extraction From the Slides and PCR Amplification*

First, 300 µL of lubricating phosphate buffered saline was poured on the unstained smear, gently shaved using a sampler tip, and then transferred to pre-autoclaved 1.5 mL microtubes. DNA extraction was performed using a DNA extraction Kit (DN8115C, CinnaGen, Iran) according to the kit protocol. The extracted DNA was qualitatively and quantitatively evaluated by electrophoresis and spectrophotometry, respectively. The PCR protocol was implemented using forward (LITSR) and reverse (L5.8S) primer pairs for the amplification of a 350 bp nucleotide sequence among ribosomal internal transcribed spacer 1 (ITS1). The length and sequence primers are presented in Table 1. The PCR master mix involving 0.5 µM primers, 1.5 mM $MgCl_2$, 0.5 mM dNTP mix, 1 unit Taq DNA polymerase, and 100 ng genomic DNA was applied in a thermal cycler (Eppendorf, Germany). DNA samples from standard *Leishmania* strains, including *L. major* (MHOM/IR/75/ER), *L. tropica* (MHOM/IR/99/YAZ1), and *L. infantum* (MCAN/IR/97/LON49) were used as the positive control, and distilled water, instead of template DNA, was employed as negative control. Following the optimization of the PCR protocol, a thermal cycle was used, including an initial denaturation stage at 95˚C for 5 minutes, followed by 30 cycles, including denaturation at 95˚C for 20 seconds, primer annealing at 50˚C for 30 seconds, and extension at 72˚C for 1 minute, and a final extension at 72˚C for 6 minutes. The PCR products, along with a 50 bp size of the weight marker, and positive and negative controls were evaluated on 1.5% agarose gel electrophoresis. At this stage, if a 350 bp PCR product was detected in the genus *Leishmania* parasite, and if a band profile other than 350 bp was observed, it indicated the lack of *Leishmania* parasite.[18-21]

### *Digestion by Restriction Endonucleases and Restriction Fragment Length Polymorphism Study*

To identify different species of *Leishmania*, the PCR products were examined by the Restriction Fragment Length Polymorphism (RFLP) method. In this study, type II restriction endonucleases were used for the cleavage of the amplified region in the parasite genome. The resulting fragments were then observed on agarose gel electrophoresis, and the patterns obtained by different isolates underwent comparison. The restriction enzyme *Hae III (BsuR I)* was employed; this enzyme, which is derived from the bacterium *Haemophilus aegyptius*, identifies *3'…CC|GG…5'* sequence and forms blunt-ended fragments. It was incubated with the PCR products at 37˚C. In addition to the samples, the standard parasites, including *L. major* (MHOM/IR/75/ER) and *L. tropica*

**Table 1.** The Sequence and Length of Primers

| Primer | Name | Sequence (5´→ 3´) | Length |
|---|---|---|---|
| Forward (F) | LITSr | CTGGATCATTTTCCGATG | 18 |
| Reverse (R) | L5.8s | TGATACCACTTATCGCACTT | 20 |

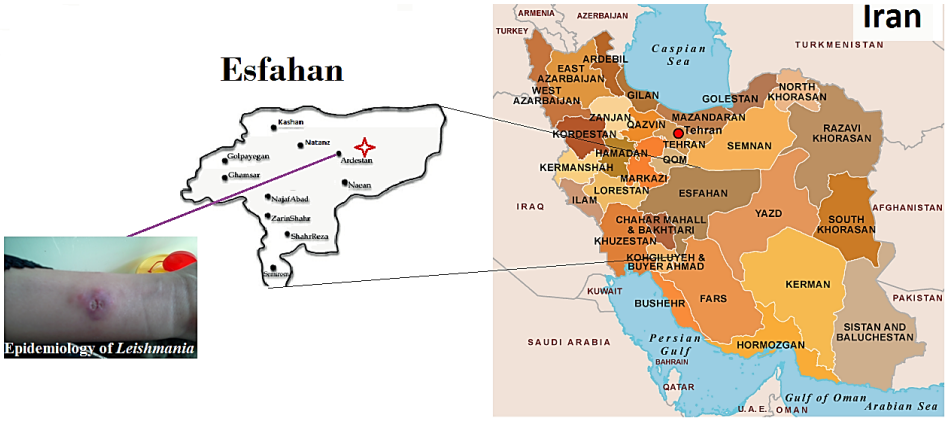


**Figure 1.** Geographical Location of Ardestan, Isfahan, Iran .

(MHOM/IR/99/YAZ1), along with a 50 bp DNA size marker, were applied in all steps. Following the digestion of PCR products with the *Hae III* enzyme, the observation of 220 and 140 bp, as well as 200 bp and 60 bp bands on agarose gel electrophoresis showed that the isolates belonged to *L. major* and *L. tropica*, respectively. If three bands of 200 bp, 80 bp, and 60 bp were observed, the isolate was considered as an *L. infantum* strain.[21,22]

### *Carrier Identification*

With the help of the Ardestan Health Network Administration, sandflies were caught in the area of the study from the beginning of (2015-2016). We used Mosquito catcher to trap the mosquitoes (e.g., catching with an aspirator and trapping with a trap). Twice a week, sandflies from inside buildings (bedrooms, covered warehouses, and corridors) and the opened areas (rodent nests, cracks in walls, and bird nests) were caught using 30 glue traps (white papers measuring 15 × 20 cm impregnated with the castor oil).[23] In all cases, the next morning after trapping, adhesive traps were collected before sunrise and transferred to the laboratory for the identification of the mosquitoes species. For this purpose, first, they were identified by entomological experts using organological tests, and then the sandflies were carefully collected from the traps with a dissection needle. To remove the castor oil, the sandflies were placed inside a crucible containing acetone. Next, the acetone was pulled out with a syringe, and the sandflies were taken after the process was repeated several times. Subsequently, the mosquitoes were transferred to a storage tube containing 70% alcohol. Afterward, microscopic slides were prepared for sandfly identification. To this end, for some samples, the permanent mounting method was used with the Puri-media, and the remaining samples were analyzed by fast Monte. Accordingly, the sandflies were boiled in 10% KOH for one minute and then were mounted between a microscope slide and a lamella using lactophenol cotton blue.[24]

### *Reservoir Identification*

To identify the reservoirs of parasites, mice were caught using hand traps containing baits such as walnut or cucumber. The traps were placed near the place where the mice lived, and after trapping them, the mice were transferred to the health center. Then, the sex and species of the reservoirs were identified with the help of zoologists.[25]

### *Statistical Analysis*

The chi-square test was used to investigate the frequency distribution analysis, and $P<0.05$ was considered as the significant level.

## Results

### *Overall Assessment of the Study Population*

Overall, 140 patients with suspected CL referring to Ardestan Dermatology and Leishmaniasis Center were sampled in this study. Out of 140 patients with suspected CL, 121 patients had positive smear samples.

### *Investigating the Relationship Between CL and Demographic Characteristics of Patients*

Table 2 presents the frequency distribution of CL infection according to the gender of the studied patients. The highest prevalence of CL skin lesions was related to men (51.2%), and women showed a lower prevalence (48.8%) than men.

Based on the results, among CL patients, the highest rate of infection (23.9%) belonged to the age group of 21-30, while the lowest rate of infection (10.7%) was related to the age group of 31-40 years.

As shown, the highest and lowest rates of CL skin lesions were observed in the hands (45.5%) and the neck (2.4%), respectively.

Table 2 showed the frequency distribution of CL prevalence by seasons in the studied area. According to the findings, the highest and lowest prevalence of skin lesions due to CL were related to autumn (57.9%) and spring (4.1%), respectively.

Table 2 explained the frequency of CL distribution in different patients' occupations. The most and least affected people were students (32%) and drivers (0.9%), respectively.

TThe frequency distribution of CL patients according to the geographic area of living was revealed (Table 2).

**Table 2.** Demographic Characteristics of the Frequency Distribution of Patients With CL

| Characteristics | No. | Percent |
|---|---|---|
| Gender | | |
| Male | 62 | 51.2 |
| Female | 59 | 48.8 |
| Age of patients | | |
| 10-0 | 14 | 11.6 |
| 20-11 | 19 | 15.7 |
| 30-21 | 29 | 24 |
| 40-31 | 13 | 10.7 |
| 50-41 | 17 | 14.1 |
| 60-51 | 14 | 11.6 |
| 60< | 14 | 11.6 |
| Location of patients' lesions | | |
| Hands | 55 | 45.5 |
| Legs | 46 | 38.1 |
| Body | 10 | 8.2 |
| Neck | 3 | 2.4 |
| Face | 7 | 5.8 |
| Seasons of infection | | |
| Spring | 5 | 4.1 |
| Summer | 37 | 30.6 |
| Autumn | 70 | 57.9 |
| Winter | 9 | 4.7 |
| Occupation of patients | | |
| Teacher | 6 | 5.9 |
| Employee | 21 | 20.3 |
| Housewife | 16 | 15.6 |
| Student | 33 | 32 |
| Driver | 1 | 0.9 |
| Farmer and Worker | 6 | 5.9 |
| Others | 20 | 19.4 |
| Disappearance time in terms of (days) | | |
| 7-1 | 10 | 8.3 |
| 14-7 | 21 | 17.3 |
| 21-14 | 33 | 27.3 |
| 28-21 | 16 | 13.2 |
| 35-28 | 20 | 16.5 |
| 42-35 | 3 | 2.5 |
| 42 < | 18 | 14.9 |
| Appearance of lesions | | |
| Ulcerative form | 10 | 8.2 |
| Nodular | 86 | 71.1 |
| Wounded yellow | 4 | 3.3 |
| Tumoral form | 8 | 6.6 |
| Sporotrichoid form | 2 | 1.7 |
| Zosteriform | 5 | 4.1 |
| Red wind | 1 | 0.9 |
| Plaque like | 3 | 2.4 |
| Leishmaniasis lesions | 2 | 1.7 |
| Patients' spatial area | | |
| Zavareh area | 6 | 4.9 |
| Mahabad region | 3 | 2.4 |
| Moghar area | 3 | 2.4 |
| Surrounding villages | 14 | 11.5 |
| Our neighborhood | 10 | 8.2 |
| Fahra neighborhood | 3 | 2.4 |
| Mahal neighborhood | 10 | 8.2 |
| Rahmian neighborhood | 7 | 5.7 |
| Bazaar neighborhood | 6 | 4.9 |
| Kushk neighborhood | 3 | 2.4 |
| Kaboudan neighborhood | 2 | 1.6 |
| Haft-Tir town | 6 | 4.9 |
| Kaveh town | 30 | 24.8 |
| Beheshti town | 6 | 4.9 |
| Imam Hussein town | 8 | 6.7 |
| Tabatabai Nejad town | 5 | 4.1 |

*Note*. CL: Cutaneous Leishmaniasis. * Chi-square test was used to calculate the percentage of all patients with CL.

According to the results, the highest number of people with CL lived in Kaveh town (24.8%) whereas the lowest counts were in the Kaboudan neighborhood (1.6%).

Based on the obtained data, in patients with CL, the highest distribution was related to the lesions' onset time of 14-21 days (27.3%), while the lowest frequency belonged to the time of 35-42 days (2.5%).

The results demonstrated that the highest and lowest rates of lesions appearance were related to the nodular (71.1%) and high inflammation (0.9%) forms, respectively.

### *Microscopic Examination of Direct Slides Obtained From CL Lesions*

A few examples of the form of amastigotes inside the cells by the microscopic investigation are shown in Figure 2.

### *Results From Clinical Manifestations of CL*

The studied patients had different forms in terms of appearance and clinical manifestations of wounds. These clinical manifestations were different not only in terms of wet or dry wounds but also with regard to the lesion shape, approximate size of wounds, and lesion time duration. Examples of the appearance of the lesions are illustrated in Figure 3.

### *Identification of Parasite According to ITS1 Amplification by PCR*

The results are depicted in Figure 4. *L. infantum* was not observed in the isolates. DNA fragments were amplified.

### *Results of Determining the Sex and Species of CL Disease in the Ardestan Region*

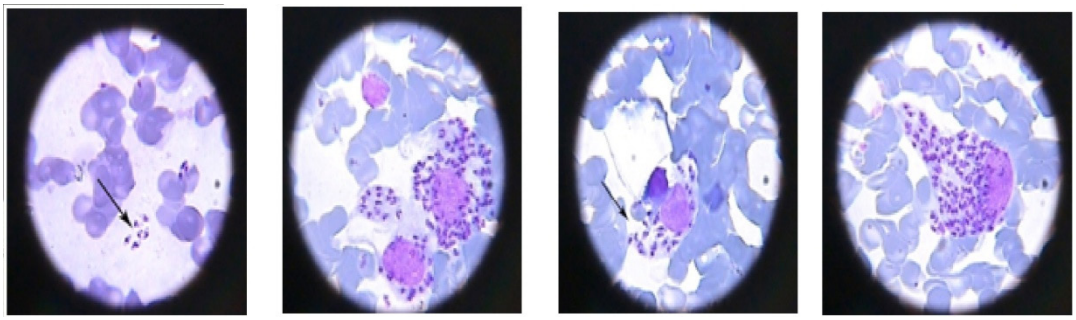

**Figure 2.** Images of Microscopic Examination of a Number of Patients Under Study and Observation of Amastigotic Forms of the Parasite

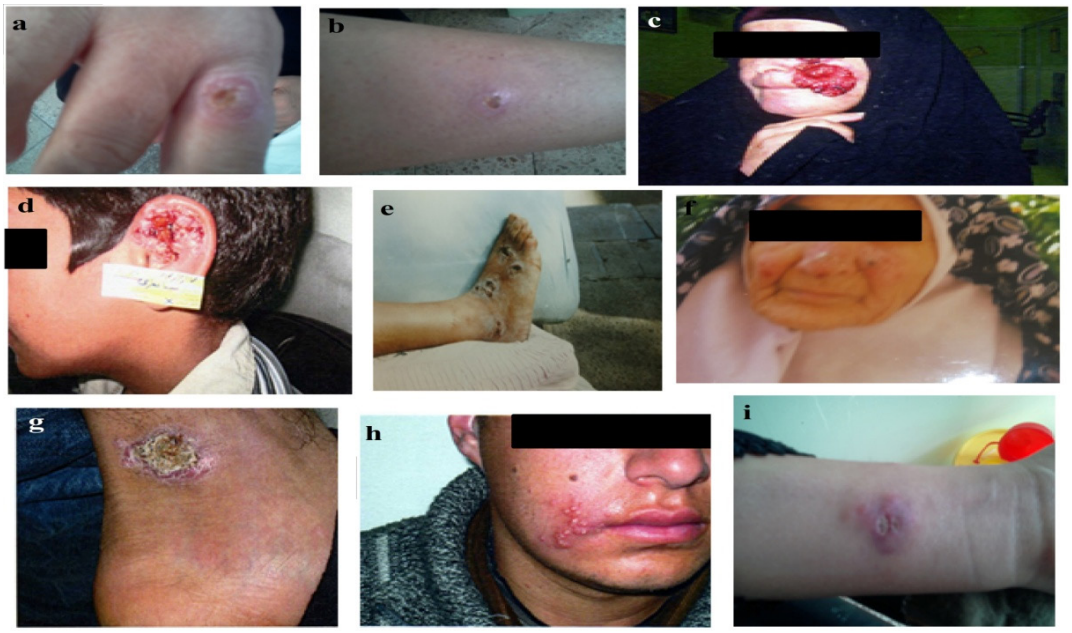


**Figure 3.** Images of the Clinical Manifestations of a Number of CL Patients Under Study: (a) Plaque such as form, (b) Nodular form, (c) Tumoral ahape, (d) Impetiginous form, (e) Ulcerative Form, (f) Erysipeloid form, (g) Sporotrichoid form, (h) Zosteriform, and (i) Nodular after injection. Note. CL: Cutaneous Leishmaniasis.

Overall, 729 adult mosquitoes (600 outside and 129 inside buildings) were collected and identified from May to November 2013. In the indoor bedroom and storage of houses and corridors, three species were collected, including *Phlebotomus papatasi* 94.2%, *Phlebotomus sergenti* 3.8%, and *Phlebotomus mascittii* 2%. In rodent nests, cracks in doors and walls, and bird nests, four species of sandflies were collected, which were *P. papatasi* 89.1%, *P. sergenti* 0.7%, *Phlebotomus mascittii* 3.3%, and *PPhlebotomus brevis* 0.6%. Most sandflies, indoor and outdoor and stagnant, were *Phlebotomus papatasi*. Sandflies appeared in late May and disappeared in late November. There were two active peaks in the *P. papatasi* curve, one from late May to late July and the other in late September.

### *Results of Determining the Sex and Species of the Reservoir of CL in the Ardestan Region*

Fifty mice were caught from Zavareh, Mahabad, Ardestan, Amiran, Moghar, Talakabad, and Gachorstaq during summer and autumn. The most caught rodents were *Rhombomys opimus* (90%) and *Meriones libycus* (10%). These two rodents live socially. *R. opimus* is active during the day, while *M. libycus* is active at night. These two rodents were active in the same area and have been caught many times from adjacent nests. *R. opimus* had two activity peaks. The maximum activity of this rodent was in summer, and it had a smaller peak in late autumn. The average length of rhomboids was 301 mm, and the average length of *Rhombomys opimus* and *Meriones libycus* was between 278-301 mm, and the average head and body lengths of these rodents were between 145-156 mm. In addition, the average tail length was 151 mm (Figure 5), while the average length of the *M. libycus* was 290 mm.

## Discussion

In this descriptive-analytical cross-sectional study, using the available statistics and demographic information, the status of CL disease was investigated in terms of some epidemiological aspects among 121 CL patients in Ardestan, Iran during 2015-2016. In terms of gender, the prevalence of the disease was higher in men than women, which can be due to different factors, including men's jobs; as known, men are usually outside the home, especially in the areas of agriculture and animal husbandry. Farm workers and livestock grazers in the desert are more susceptible to diseases transmitted by mosquito bites. Women are less exposed to mosquito bites than men due

to more protective clothing. In their study, Hamzavi et al found that the prevalence of the disease was significantly higher in males than in females.[26] In a study conducted in Kerman province, the incidence of the disease was reported to be relatively high in men compared to women (93.8% vs. 6.2%).[27] In the present study, the highest incidence of the disease was observed in the age group of 21-30 years, while the lowest rate was related to the age group of 31-40 years. Young people in the statistical population were exposed to the disease for reasons such as work and activity in the infected centers.

According to the results of this study, the highest seasonal incidence of CL was related to autumn, and to a lesser degree, it was observed in summer. Basically, in the regions of the world where the development of adult sandflies is seasonal, the pattern of CL infection in humans also follows a seasonal trend. In areas where adult mosquitoes develop in the spring and early summer months, new cases of CL usually appear in late summer and autumn. In another study, Khazaei et al found that most CL cases were observed in eastern, central, and southern provinces. In general, 2031 cases (55.13%) were males, and 2,306 (62.6%) cases were living in large towns. The age of the patients was 27 ± 18 years old. More than 31% of them were under 14 years of age. Approximately 62.75% of the wounds formed on the hands, 24.8% of them were detected in the head and neck, and only 2.7% were found in the body,[28] which is consistent with the results of the present study. Karami et al reported the highest incidence of the disease in the fall. According to the findings of the current study, the highest rate of infection was related to Kaveh town and was observed in students. This area is a part of hyperendemic areas due to the existing habitat and the reproduction environment for the disease reservoir, as well as the entry of non-native reservoirs into it. In addition, the building of scattered new residential and military areas has led to the migration and settlement of non-native people, who are probably more susceptible to getting the disease compared to resident people, possibly increasing the prevalence of the disease in Kaveh town. Most houses in this area are renovated, and most people with CL are living in these renovated buildings. There are also a number of schools and student dormitories nearby. Karami et al reported the majority of wounds (36.5%) in Isfahan in the form of nodules and the greater prevalence of wounds in the upper extremities (48.3%), especially in men (32.4%), which corroborates the result of the present study. They further indicated that 54.5% of patients had more than one wound on their body, of which 81.2%, 16.7%, and 2.1% were Iranian patients, Afghans, and other nationalities, respectively.[29]

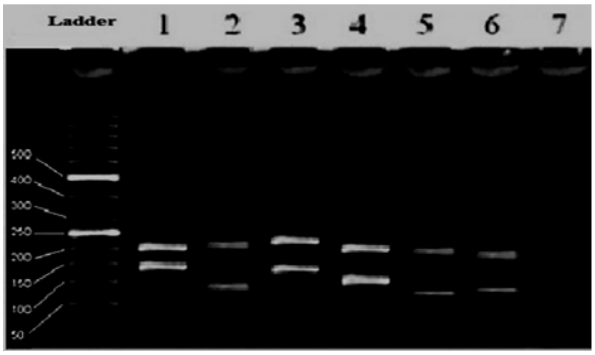


**Figure 4.** Band Profiles Created by *Hae III* Enzyme With Different Species of *Leishmania* by the PCR-RFLP Method. *Note*. PCR_RFLP: Polymerase chain reaction-restriction fragment length polymorphism; M: Molecular marker 50 bp; Line 1: *L. major* standard; Line 2*: L. tropica* standard; Lines 3 and 4: *L. major* two isolates according to sample standard; Lines 5 and 6*: L. tropica* two isolates according to sample standard; Line 7: Negative control.

On the other hand, considering that new buildings are often built in deserted areas and suburbs, near reservoir and carriers habitats, and due to the lack of proper mosquito nets and insecticides, bites of more sandflies occurred in them, and more incidence of this disease was expected in this studied population. In this area, brick kilns and sand dunes, construction debris, or barren lands inside the city are also the places of active colonies of mice (disease reservoirs). Zavareh is another highly contaminated area of Ardestan, as it is also a location of the disease reservoirs living, and the scattered colonies of mice are found in it. Jalali et al conducted a study in some cities and reported a high prevalence of the disease in new buildings,[30] which is in line with our findings. Momeni et al stated that 4 species of sandflies were caught in human habitats in 5 areas of Isfahan, including *P. ansarii*, *P. papatasi*, *P. caucasicus*, and *P. sergenti*. They concluded that CL in 5


**Figure 5.** *Rhombomys opimus* (a) and *Meriones libycus* (b) Caught in This Study.

areas of this city can be of rural (wet) and urban (dry) types. For example, in the Malekshahr neighborhood, the population of *P. papatasi*, *P. caucasicus*, and *P. ansarii* was 86%, 13%, and 1%, respectively, while in the Shahinshahr neighborhood, these population rates were 84%, 14%, and 2%, respectively. Moreover, they reported the most activity of the *R. opimus* rodent in Isfahan province from 1 August 1 to 1 December,[31] which conforms to the results of the present study.

Doudi et al conducted a study on patients with CL in the two regions of Isfahan and Bam using the PCR-RFLP technique. Based on the information obtained from the cleavage of the sequence amplified in the ITS1 region by using two primers LITSr and L5.8s, they reported 4 different genotypic patterns, two patterns (LmB, LmA) belonged to *L. major*, and two patterns (LtB, LtA) belonged to *L. tropica*.[32] Shiee et al detected three species, including *L. tropica* Eu727198, *L. major* EF653296, and *L. infantum* FJ497004 in Kashan by applying the PCR-RFLP method.[33] Azizi et al, in a molecular study on the parasite in Hormozgan province, found amplified fragments with the size of 560 and 720 bp, respectively, as PCR products for *L. major* and *L. tropica* ITS1 gene. All the mentioned molecular studies are in accordance with the PCR-RFLP technique used in the present study for the evaluation of the ITS1 gene to identify the species of this parasite.[34] Khezzani and Bouchemal isolated about 4800 cases of CL during 13 years. The 10-19 year cases were approximately 31.41%. In their study, most patients (65%), among other cases, were males.[35] Eventually, Holakouie-Naieni et al found about 589 confirmed CL cases; the annual outbreak was nearly 30.9 per 100 000, and most cases happened in central areas.[36]

## Conclusion

The high incidence of skin lesions caused by CL in Isfahan province led to the establishment of this study. The existence of epidemiological complications in the leishmaniasis transmission ring (various reservoirs and carriers) made the care and control of this disease highly important. Unfortunately, the results demonstrated that the prevalence of CL has been increasing annually in some provinces of Iran, especially in Isfahan Province. Nevertheless, further studies are required to determine the vectors, reservoirs, and species of this disease and to design appropriate strategies to control it.

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### Competing Interests

The authors declare that there is no conflict of interests.

### Ethical Approval

Not applicable.

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