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The Epidemiological Study of Cutaneous Leishmaniasis in Patients Referred to Skin Lesions in Dashtestan District, Bushehr Province, Iran in 2013-2014

Majid Nozari [1], Marziyeh Ansari Shiri [1], Mohammad Reza Samaei [1], Mohammad Reza Shirdarreh [1], Ali Gholamnejad [1], Shahab Rezaeian [2]*

[1] *Department of Environmental Health Engineering, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran.*
[2] *Department of Environmental Health, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

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Corresponding Author:
Mohammad Reza Samaei

Email:
mrsamaei@sums.ac.ir

Tel:
+989177320737

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ABSTRACT

Introduction: Cutaneous leishmaniasis can lead to the loss of time, labor force, significant treatment costs and psychological problems. Also, cutaneous leishmaniasis is considered as an important health problem in Iran. This study was aimed at investigating the status of cutaneous leishmaniasis in Dashtestan district.

Materials and Methods: This cross-sectional and descriptive-correlational survey was performed on all confirmed leishmaniasis cases (86 person) following up in Dashtestan district health care centers during 2013-2014. Patient's general information such as age, sex, geographical region, number and site of ulcer, as well as the month and season of incidence were registered and analyzed by SPSS ver.24. All ethical issues were also addressed.

Results: During 2013, the maximum percent frequency of the incidence of cutaneous leishmaniasis was related to the age-group of 0-4 years (41.9%) and during 2014, was related to the age group of 15-24 years (30.2%). During 2013 and 2014, the incidence of cutaneous leishmaniasis was higher in males than in females and the face was the most common site of the ulcer. During 2013 and 2014, autumn and winter had the most seasonal incidence and most of the cases were observed during September and January, respectively.

Conclusion: Based on the study results, cutaneous leishmaniasis is high endemically in Dashtestan district. Therefore, control measures such as education, optimizing the environment and the application of appropriate coating for exposed age groups, especially children and young people are essential.

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Introduction

Cutaneous leishmaniasis (CL) is one of the ten known parasitic diseases in the tropical regions of the world and World Health Organization (WHO) has proposed and supported research and studies on its various aspects. CL is also a native Iranian parasitic disease, which is a common disease in humans and animals caused by protozoan flagellates groups, trypanosomatides family and leishmania genus. Symptoms of CL appear as ulcers that may remain on the body for up to one year [1, 2].

CL is caused by the vector-borne protozoan parasite Leishmania and is transmitted via infected female sandflies [3, 4]. Although not fatal, CL can cause significant morbidity as well as social stigma. CL can arise at any age and often causes cosmetic disfiguration [5]. However, since the disease is non-fatal, the attention given to the prevention and control has been relatively poor [6].

At least 42 species of sand flies have been identified and reported from the two genera of sergentumia and phlebotomus in different regions in Iran [7]. CL has also changed in most parts of the world; including Iran in such a way that the prevalence is increasing in many of these areas in an emerging and re-emerging fashion [8]. Numerous factors such as environmental changes, population displacement, out-migration and urbanization have played a major role in increasing the incidence of the disease cases [8]. Urbanization, household transmission cycle and manipulation of agricultural products and location of houses inside the forest areas are among such environmental changes that can affect the incidence of leishmaniasis. Leishmaniasis is sensitive to climate changes and is greatly affected by rainfall, temperature and humidity. The epidemiology of leishmaniasis is affected by both global warming and land degradation. Flood, famine and drought resulting from climate changes can cause massive migration to areas with leishmaniasis transmission and undesirable nutrition jeopardizes their safety. Lack of a diet containing proteins, vitamin A and zinc (Zn) increases the progression of visceral leishmaniasis [9]. Poverty increases leishmaniasis-induced injury. Housing condition (lack of sewage management or open sewers) may increase the birth of sand flies and their resting habitats and their proximity to human habitation. Sand flies are attracted to busy homes (a good source of blood meal). Human behavior (sleeping outside the house or on the ground) may increase the injury rate. The use of insecticides and mosquito net reduces the injury rate [9].

The epidemiology of leishmaniasis is dependent on the characteristics of the parasite species, the ecological characteristics of the transmission sites and the exposure of the present and past human populations to the parasite. There are 70 species of animals, as well as humans as natural reservoirs of leishmania parasites [9]. In a study on the epidemiology of CL in Dashti and Dashtestan cities, Hamzavai et al. showed that a rural focus of CL was identified in these two cities and the methods recommended for inhibition of rural CL must be used in order to control the disease [10]. Behbahani et al. also demonstrated in their research that the focus of CL was of rural type in Khuzestan, Bushehr, and some parts of Ilam [11]. In recent years, many studies have been conducted in neighboring provinces of Bushehr, such as studies on epidemiology of leishmaniasis in Andimeshk [12], Hoveizeh [13], Omidieh [11], Hajiabad [14], Yasuj [15], Boyer Ahmed [16], Luristan [17], and Shiraz [18], indicating the endemic foci of leishmaniasis in the southwestern provinces of Iran.

Considering the attitude to the history and statistics of human case studies in the Bushehr province (North of the Persian Gulf), it is observed that the endemic form of the disease existed many years ago. The emergence of relatively important epidemics over the past years in the province shows the need for a serious fight and control of this disease, especially in the rural areas in which the statistics of the patients is high [10]. Furthermore, considering the view that there is little information published on the epidemiological status of CL in Bushehr Province, there is an obvious need for the present research. The aim of this study was to investigate some epidemiological aspects of CL and to show its importance in Dashtestan province.

Materials and Methods

In this cross-sectional and descriptive-correlational study which began from the beginning of March 2013 to the end of March 2014, 210 people were referred to healthcare centers for their wound-induced injury, 43 of whom were diagnosed after being examined by a physician in the health center, and simple direct smear was taken from the lesions. Then smears were placed on a microscopic slide. Smears after staining by Giemsa were examined

by a light microscope in order to perform amastigote detection. Patients' information were collected by experienced staff. In the event of a malfunctioning of the information, the information was completed by contact or presence in the patients' living space. Afterward, their information was recorded in epidemiological abstract datasheet of CL. The statistical population included 86 patients with CL (43 cases per year). To use the data from these sheets in this study, a permission was obtained from Dashtestan healthcare network and the required data for each patient such as age, gender, geographical area (city or village), date of onset of the disease (month and season), and the number and site of the ulcers were extracted from the sheets and analyzed using SPSS ver. 24.

In this research, Chi-square test and Fisher's exact test were used for statistical analysis and alpha less than 0.05 was considered as significant. Chi-square test was performed in age groups of 0-4, 5-14, 15-24 and more than 25 years. The ulcer site was divided into four groups of face, legs, hands and others for the test report. The number of ulcers was also divided into four groups: one, two, three, four and more than four.

Considering that the population of the city of Dashtestan was registered according to the official statistics of 247319 people and the cases of the patients have been discovered to be 43 per year, the incidence rate per 10,000 people was calculated as follows:

$$\text{Incidence rate} = \frac{\text{Number of new cases of disease per year}}{\text{Total population}} \times 10000 \quad (1)$$

Ethical issues

The protocol of the study was conducted in accordance with international rules on human testing and approved by the Shiraz University of Medical Sciences Ethics Committee (ethics code: Ir.sums.REC.1393.310).

Results

According to the given number of new cases of the disease and the total population, the incidence rate of the disease was calculated in 10,000 people, which was equal to 1.7.

The maximum incidence of CL in years 2013 and 2014 was 18 (41.9%) and 13 (30.2%), respectively in the age groups of 0-4 and 15-24 years. The minimum incidence rate of CL in years 2013 and 2014 was (0%) in the age groups of 55-64, 45-54 and 35-44 years (Table 1). Also, the statistical test showed that there was no significant difference between the incidence of disease in different age groups ($p < 0.05$).

The incidence of CL in men in years 2013 and 2014 was 25 (58.1%) and 24 (55.8%), respectively. The incidence of CL in women in 2013 and 2014 years was 18 (41.9%), and 19 (44.2%), respectively (Table 1). Also, the statistical test revealed that there was no significant difference between the incidence of the disease and gender ($p < 0.05$).

The maximum frequency of ulcer site in CL in years 2013 and 2014 was seen in the face with frequencies of 15 (34.9%) and 20 (46.5%), respectively. The least frequent ulcer site in cutaneous leishmaniasis was observed in the trunk with frequency of (13.2%) in year 2013. The least frequent ulcer site in cutaneous leishmaniasis was observed in the hands and face simultaneously (0%) in year 2014 (Table 1). Also, the statistical test showed that there was no significant difference between the incidence of the disease and the ulcer site ($p < 0.05$).

The number of ulcers in patients with cutaneous leishmaniasis in years 2013 and 2014 was respectively equal to 1 (most frequent), 2 and 3. The least frequent ulcers were seen in people with cutaneous leishmaniasis in years 2013 and 2014 (more than 3) (Table1). Furthermore, the statistical test revealed that there was a significant difference between the incidence of the disease and the number of ulcers ($p < 0.05$).

The maximum monthly-based incidence of cutaneous leishmaniasis in years 2013 and 2014 was respectively 7 (16.3%) in September and 9 (20.9%) in December. The minimum monthly based incidence of cutaneous leishmaniasis in years 2013 and 2014 occurred respectively in March and June (0%) (Table 2). Also, the

statistical test showed that there was no significant difference between the incidence of the disease and different months of the year ($p < 0.05$).

The maximum season-based incidence of cutaneous leishmaniasis in years 2013 and 2014 was 15 (32.8%) and 20 (46.5%) in the autumn and winter seasons, respectively. The minimum season-based incidence of CL in years 2013 and 2014 occurred in spring with frequency of 3 (7%) and in summer with frequency of 5 (11.6%), respectively (Table 2). In addition, the statistical test indicated a significant difference between the incidence of the disease and different seasons of the year ($p < 0.05$).

The incidence rate of CL in year 2013 in urban and rural areas was 22 (51.2%) and 21 (48.8%), respectively. Also, the incidence of CL in year 2014 in urban and rural areas was 17 (39.5%) and 26 (60.5%), respectively (Table 2). Moreover, the statistical test showed that there was no significant difference between the incidence of the disease and geographical areas ($p < 0.05$).

Table 1: Demographic and epidemiological data of patients with CL in Dashtestan Province, Bushehr Province in 2013-2014

	(%) Frequency of disease		
Demographic data	**2013**	**2014**	**P_{Value}**
Age group (years)			0.071
0-4	18 (41.9)	12 (27.9)	
5-14	1 (2.3)	7 (16.3)	
15-24	9 (20.9)	13 (30.2)	
25-34	6 (14)	9 (20.9)	
35-44	6 (14)	0 (0)	
45-54	1(2.3)	0 (0)	
55-64	0 (0)	1 (2.3)	
≥ 65	2 (4.7)	1 (2.3)	
Sex			0.828
Male	25 (58.1)	24 (55.8)	
Female	18 (41.9)	19 (44.2)	
Site of ulcer			0.696
Face	15 (34.9)	20 (46.5)	
Leg	9 (20.9)	8 (18.6)	
Hand	14 (32.6)	10 (23.3)	
Hand and leg	2 (4.7)	2 (4.7)	
Hand and face	2 (4.7)	0 (0)	
Trunk	1(2.3)	3 (7)	
Number of ulcer			0.042
1	29 (67.4)	18 (41.9)	
2	4 (9.3)	10 (23.3)	
3	3 (7)	9 (20.9)	
4	1(2.3)	3 (7)	
5	0 (0)	2 (4.7)	
6	2 (4.7)	0 (0)	
7	1(2.3)	0 (0)	
8	1(2.3)	0 (0)	
9	0 (0)	1(2.3)	
10	1(2.3)	0 (0)	
15	1(2.3)	0 (0)	

Table 2: Demographic and epidemiological data of patients with CL in Dashtestan province, Bushehr province in 2013-2014

	Frequency of disease (%)		
Demographic data	**2013**	**2014**	**P_{Value}**
Month			0.146
April	0 (0)	3 (7)	
May	3 (7)	5 (11.6)	
June	5 (11.6)	4 (9.3)	
March	3 (7)	0 (0)	
August	2 (4.7)	1 (2.3)	
September	7 (16.3)	1 (2.3)	
October	5 (11.6)	3 (7)	
November	5 (11.6)	6 (14)	
December	5 (11.6)	4 (9.3)	
January	5 (11.6)	9 (20.9)	
February	1 (2.3)	5 (11.6)	
March	2 (4.7)	2 (4.7)	
Season			0.010
Spring	3 (7)	11 (25.5)	
Summer	12 (27.7)	5 (11.6)	
Autumn	14 (32.4)	20 (46.5)	
Winter	15 (32.8)	7 (16.4)	
Geographical region			0.279
Rural	22 (51.2)	17 (39.5)	
Urban	21 (48.8)	26 (60.5)	

Discussion

In the present research, the incidence rate of CL was 1.7 cases per 10,000 people in both 2013 and 2014 years. One of the causes of the injury is traveling to areas where the disease is native. If people and especially non-indigenous people are exposed to carriers for any reason, the incidence of the disease will increase. Migration and demographic changes cause a change in the disease trend and in fact, population displacement is a very prominent factor in causing CL epidemic [19].

According to the results of the current research, the scattered age distribution of patients showed that the incidence of CL in the 0-4 age group was higher in years 2013 and 2014, which could be due to various reasons, including higher outdoor exposure seen in this age group because of the child's play and safety and the lower safety of this age group [20]. Also, the high incidence of CL in the age group of 15-24 years can be due to a variety of reasons such as illiteracy, and working in fields and gardens at night (during which sand flies are active) without protective cover. Since people in this age group are mostly farmers and workers in the studied area and have low income levels, they are more likely to be exposed to sand flies [17, 19]. The least incidence of CL was seen in the age group of over 45 years in the years 2013 and 2014. The reduction in the incidence of the disease in the age group of over 45 years in years 2013 and 2014 could be due to many reasons, including less exposure with outdoors, lower workload and finding permanent safety because of the development of the disease at an earlier age [13]. In addition, the disease incidence in the age group of 35-44 years in years 2013 and 2014 was 6 (14%) and zero, respectively. Increased expertise and awareness level about preventive measures may be the reason for the minimum incidence of the disease in this age group. There is a reduction in the number of patients at older age in most researches, which poses more seriously the need to design preventive measures against CL. According to the results of studies conducted by Talary in

Kashan [21], Hanafi-Bojd in Hormozgan [22], Yaghoubi in Yazd [23], Soleimani in Hormozgan [24], Gorel in Sanliurfa [25], and Babaei in southern part of Lorestan [26], the highest incidence of CL has been observed in the age group under the age of 10 years and is consistent with the results of the present study. Also, in a study on CL epidemiology in Damghan, Mohammadi Azni et al. showed that the mean age of the patients was 33.5 years and their age ranged from 1 to 94 years. The highest and lowest prevalence rates were observed in the age groups of 20-29 and more than 70 years, respectively. There was also a significant relationship between the incidence of the disease in different age groups ($p < 0.0001$) [27]. In a study on the prevalence of CL infection in the city of Mohammad Abad, Jiroft city in Kerman province, Porsmaeilian et al. reported the highest and lowest infection rate in the age groups of 11-20 (10.5%) and 21-30 years (3%). The most common age group was 15-24 years and 7.85% of patients were in the age group of 15-45 years [8]. In a study on the epidemiology of CL in 210 patients in Hamedan province during the years 2002-2007, Zahirnia et al. showed that 44.3% of patients were in the age group of 15-24 years [19]; however the results of some other studies such as those conducted by Sdqyany in Tehran [28] and Sadeghi Nejad in Khuzestan [29], are not consistent with the results of the present research. In general, by measuring the results of numerous studies, it can be concluded that CL incidence rate in different age groups is dependent on different foci, and the age distribution is related to the native nature of the disease.

Scattered gender distribution of patients shows that the incidence of CL in men and women in years 2013 and 2014 was 25 (58.1%) and 24 (55.8%), 18 (41.9%) and 19 (44.2%), respectively. There was no significant change between men or women in terms of the incidence rate of the disease in years 2013 and 2014; however, the disease incidence rate was generally higher in men than in women, which may as a result of higher exposure to the outdoor environment for occupational purposes, sleeping outdoor and in uncovered areas, less body-coverage in men than in women and more frequent commuting in abandoned and desert areas, which have increased the likelihood of contact with sand flies and bites in men as compared to women [19]. In a study on CL epidemiology in Damghan, Mohammadi Azni et al. showed that out of 465 patients with CL, male and female subjects accounted for 263 (56.5%) and 202 (43.5%) of participants, indicating that the disease was more prevalent in men than in women [27]. Likewise, in a study on CL epidemiology in Khorasan Razavi province, Khajeh Deloyi et al. demonstrated that out of 3,558 patients with CL, 52% were male [30]. In a research on the epidemiologic factors of skin leishmaniasis in 727 patients referred to health centers in Kermanshah province, Hamzavi et al. showed that the prevalence of the disease in men was 12.2 times that of women [31]; however, the results of some researchers are not consistent with the results of the present study; for example, Poursmaeilian et al. investigated the prevalence of CL infection in the city of Mohammad Abad, Jiroft city in Kerman province. They performed a physical examination on a total of 3516 people, including 1743 female (49.6%) and 1773 (50.4%) male subjects; the results showed that the prevalence of CL infection was 5.3% (6.2% and 4.5% for female and male genders, respectively), and a significant difference was observed between the two genders ($p < 0.05$) [8]. Also, Talary in Kashan [21], Ebadi in Isfahan [32], Karimi Zarch in Sarakhs [33] and Drodgar in Kashan [7]showed in their researches that the infection distribution was higher among females. The higher prevalence of the disease among female participants in these studies was attributed to the economic activities of women and carpet weaving in dimly lit rooms and basements; since sand flies are also dynamic during days in these places and continue to feed from human blood [23]. The inconsistency between the results of these studies and those of the present study may be due to the fact that most of the men are engaged in agricultural activities outside the house during the night hours and do not have adequate clothing due to the warm weather owing to the warm and dry

desert climate of Dashtestan, which lead to more prevalence of the disease among men than women; however, weather conditions are much better in provinces such as Isfahan, Kashan, Kerman and Khorasan Razavi as compared to Dushtestan, and men have better clothing. There is also a higher incidence of the disease in women than in men in some provinces, which can be attributed to cultural differences in different provinces.

According to the results, the maximum frequency of ulcer site in CL in 2013 and 2014 was seen in the face with frequencies of 15 (34.9%) and 20 (46.5%), respectively, which may be due to lack of insufficient skin coverage in this part of the body in men and women. The least frequent ulcer site in CL was observed in the trunk with frequency of 1 (2.3%) in year 2013, which can be due to sufficient skin coverage in this part of the body in men and women. The least frequent ulcer site in cutaneous leishmaniasis was observed in the hands and face simultaneously (0%) in year 2014. Also, in year 2014, the frequency of ulcers site in the hands and face was low at the same time, indicating that the ulcer site was mainly seen in one member during the onset of this disease. Reduced ulcer sites in the hands and face in 2014 may be due to increased awareness of people about the disease and the recognition of ways to prevent the disease, which has made people to cover open areas of their body such as the face and hands. The most affected organ was the face in researches conducted by Ebadi in Isfahan [32] and Karimi Zarch in Sarakhs [33] which is consistent with the results of the present study. Considering the short mouthparts or proboscis of mosquitoes that allow the blood to be absorbed from the covered parts of the host's body, it is thus likely that the upper and lower extremities will be bitten by sand flies [34, 35]. Furthermore, sand flies use odor and chemical attractions such as carbon dioxide to select and choose the proper host and the intended site, which directs the insect to find the proper host and the intended site and these absorbent materials are more abundant in the hands and legs than in the other parts of the body [36, 37]. In a study on the prevalence of CL in the city of Mohammad Abad, Jiroft city in Kerman province, Porsmailian et al., showed that most of the skin ulcers were seen in the face (47%), then in the hands (34%), legs (5%) and several other sites (14%) (4); however, the results of researches conducted by Talary in Kashan [21], Hamzavi in Kermanshah [31], Zahirnia in Hamedan [19] and Kasiri in Khorramshahr [34], are not consistent with the results of the present study and the most affected organs included the hands and face. In a study on the incidence of CL in Chabahar province, Moghateli et al. showed that most of the skin ulcers were seen in the hands and legs (94%), then face (35%) and other parts (14%) in 2008 and there was a decreasing trend in the percentage of ulcers by 2010 [38]. In a study on CL epidemiology in Damghan, Mohammadi Azni et al. showed that out of 465 infected individuals, hands, face and leg ulcers accounted for 49, 35 and 8.6% of cases, respectively and the hands and legs accounted for 7.4% of ulcers site [27]. The reason for the inconsistency between the results of these researches with the present study may be due to the fact that the bite location and subsequent the ulcer site was also different depending on the type of clothing used by individuals in various climatic zones. Therefore, depending on the rituals and dressing styles of the inhabitants of different areas and the bloodthirsty habits of sand flies, the organs with the highest CL ulcers are different in different parts of the world and even in one country. In addition, the results showed that the maximum frequency of CL-induced ulcers (N = 1) in 2013 and 2014 were observed with frequency of (67.4%) and 18 (41.9%), respectively. Also, the maximum frequency of ulcers (N = 2) was observed with frequency of 4 (9.3%) and 10 (23.3%) in the same years, respectively, which is consistent with the results of studies conducted by Ebadi [32], Babaei [26], and Hamzavi [31]; however, Zahirnia in Hamedan [19], Rafati in Damghan [36] and Hamzavi in Bushehr [10] indicated in their research that more than 60% of the patients had more than one ulcer in their body. The number of CL-induced ulcer is multiple, with the least frequency of (more than 3). The cause of multiple ulcers can vary, which includes the method of blood-sucking

of sand flies (because these insects carry out various bites for each stage of blood sucking), the manner in which people are covered and high abundance of the infected sand flies in a region [19]. Also, the number of ulcers may be due to getting infected bites at various times, or by spontaneous inoculation by scratching.

The best temperatures include 23-28 ºC and the best moisture content is 70-100% [19]. According to the results of the present study, in years 2013 and 2014, the maximum CL incidence was seen in September and January with frequency of 7 (16.3%) and 9 (20.9%), respectively. Considering air temperature and humidity in other studies, the peak of the disease incidence has been reported in other months. In a study on CL epidemiology in Damghan during the years 2006-2015, Mohammadi Azni et al. showed that the highest incidence was in the months of October, November and December, and attributed it to the fact that there are significant changes in the incidence of rural CL in different months of the year, and the highest incidence rate is observed in the foci of the country in October, November and December [27].

According to the results of this study, the maximum incidence of CL disease was observed in autumn of 2013 with the frequency of 15 (32.8%). This conclusion is consistent with the studies conducted by Hanafi-Bojd in Hormozgan [22], Nejati in Andimeshk [12] and Nilforoushzadeh in Isfahan [39]. Furthermore, the highest incidence of CL in the winter of 2014 was 20 (46.5%), which is consistent with the researches carried out by Talary in Kashan [21] and Hamzavi in Kermanshah [31]. Considering that the Latency period of CL is between two weeks to two month, it is predicted that the ulcers will be more frequent in the winter and autumn. According to the results of the current research, the incidence of CL in 2013 in urban areas with a frequency of 22 (51.2%) was higher in rural areas with a frequency of 21 (48.8%), which is consistent with the conclusion made by Nejati in Andimeshk [12]. Considering that the health status in urban areas is better than rural areas, the elevated CL incidence in urban areas in Dashtestan province in year 2013 may be attributed to the droughts of previous years and the migration of large numbers of villagers to Dashtestan city, construction of affordable buildings around the city and close to low-income mountainous areas, population growth, increased frequency of carriers and unhealthy waste disposal. The results obtained in year 2014 were contrary to that of 2013; the incidence of CL in urban and rural areas was 17 (39.5%) and 26 (60.5%), respectively. The reduced incidence of CL disease in urban areas in year 2014 could be due to increased public awareness by health centers and the prevention of this disease.

In this research, CL disease incidence rate was determined in Dashtestan district. However, further studies are required in order to identify the animal reservoirs of this disease in this district and determine the optimal methods to combat female sandflies.

Conclusion

Based on the results of the current research, the incidence of CL disease in Dashtestan was equal to 1.7 per 10,000 in both 2013 and 2014. Overall, the highest incidence rate in children, adolescents and young adults has been observed in years 2013 and 2014. The incidence of the disease was higher in urban areas than in rural areas and in men than in women. The highest incidence of the disease was also observed in the winter and autumn and in September and January. It can be concluded according to the results of this research that CL is highly endemic in Dashtestan province and is considered as a health challenge in this city; therefore, it is necessary to consider planning for disease prevention and perform further research to reduce the incidence of the disease.

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Conflict of interest

The authors declare that there is no conflict of interest.

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