Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article https://doi.org/10.1016/j.apjtb.2017.09.011

Spatiotemporal clustering of cutaneous leishmaniasis in Fars province, Iran

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ARTICLE INFO

Article history:
Received 24 Jul 2017
Received in revised form 31 Jul 2017
Accepted 8 Sep 2017
Available online 20 Sep 2017

Keywords:
Cutaneous leishmaniasis
Scan statistics
Spatiotemporal clustering
Clusters within a cluster

ABSTRACT

Objective: To assess the spatiotemporal trait of cutaneous leishmaniasis (CL) in Fars province, Iran.
Methods: Spatiotemporal cluster analysis was conducted retrospectively to find spatio-temporal clusters of CL cases. Time-series data were recorded from 29 201 cases in Fars province, Iran from 2010 to 2015, which were used to verify if the cases were distributed randomly over time and place. Then, subgroup analysis was applied to find significant sub-clusters within large clusters. Spatiotemporal permutation scans statistics in addition to subgroup analysis were implemented using SaTScan software.
Results: This study resulted in statistically significant spatiotemporal clusters of CL ($P < 0.05$). The most likely cluster contained 350 cases from 1 July 2010 to 30 November 2010. Besides, 5 secondary clusters were detected in different periods of time. Finally, statistically significant sub-clusters were found within the three large clusters ($P < 0.05$).
Conclusions: Transmission of CL followed spatiotemporal pattern in Fars province, Iran. This can have an important effect on future studies on prediction and prevention of CL.

1. Introduction

Leishmaniasis is the second most noticeable vector-borne protozoa disease after malaria regarding both number of affected people and fatality. It is a parasitic infectious disease caused by species of genus *Leishmania* transferred by infected phebotomine sand fly bites. There are four main types of leishmaniasis, namely anthroponotic and zoonotic visceral leishmaniasis (VL) and anthroponotic and zoonotic cutaneous leishmaniasis (CL). Humans are supposed to be the only source of infection for sand fly vectors in anthroponotic types. In zoonotic types, animals are reservoirs that maintain and spread *Leishmania* parasites. The cutaneous type of *Leishmania* usually causes skin ulcers on open parts of body. It may bring about a great number of ulcers ending up in harsh disabilities and generate lasting ulcers, which can be a social stigma. The severity of medical pathology depends on parasitic species, species of the involved vector, and geographical site [1–8]. Since CL is associated with open-air activities, such as cultivation, mining, deforestation, and road building, it can be considered to be an earth-based phenomenon.

Involvement of humans with the vector's life cycle can induce a great risk of infection. In addition, vegetation density, poor sanitation, construction derbies, and building ruins have created an appropriate situation for sand flies to live and reproduce, which can help the disease to be transmitted faster [8]. Generally, more than 350 million people are at risk of leishmaniasis in 88 countries around the world and nearly 12 million people are currently contaminated. Indeed, approximately 2 million new cases occur yearly among which 500 000 cases are visceral leishmaniasis and the other 1 500 000 ones are CL. Moreover, nearly 90 percent of CL cases are located in Afghanistan, Algeria, Brazil, Iran, Peru, and Saudi [9–11].

CL is heavily correlated to poverty, and a large portion of efforts have been devoted to controlling the sources and reservoirs of infection. CL is the representative of highly-prevalent diseases in Fars province, Iran that is known to be an endemic [7]. Sand flies live in a large zone of Fars province, including Sepidan, Arsenjan, Neireez, and Estahban cities. In the last

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Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

year, almost 6 000 cases were reported in Fars province among which 2 000 cases occurred in urban and 4 000 ones in rural areas. The incidence rate of CL has been reported to be 106.01–144.00 cases per 100 000 inhabitants. Increase in the number of CL cases in the recent years can be partially due to an increase in poverty and lack of proper sanitation. In the capital of Fars province, Shiraz, the number of cases increased from 765 in 2012 to 1 386 in 2012 [7]. The same increasing pattern has also been observed in almost all cities of the province.

Investigation of features of CL can be used to improve disease management, including prediction and prevention. The spatial and temporal pattern of CL transmission has not been considered in Fars province yet. Therefore, it should be regarded as a problem of time and place simultaneously. Spatiotemporal statistical analysis used to detect significant clusters helps hypothesize the relations between CL and place and time. To make the best decision on public health costs and supervision, CL patterns as well as environmental factors (like time and space) need to be taken into account. In this framework, spatiotemporal statistical analysis of CL process that aims to find clusters (non-homogeneities) offers very beneficial knowledge for CL prediction and prevention planning and ultimately public health promotion. There are several methods of detecting clusters considering just time or space features of CL [7,12,13]. However, scan statistic regards both temporal and spatial features of CL simultaneously, which is most profitable. In a previous study, the results of spatiotemporal statistical analysis indicated that transmission of American cutaneous leishmaniasis followed a spatiotemporal pattern in Venezuela, USA [8].

Health status changes in different geographical locations as well as different time periods. Thus, it is crucial to specify these differences and detect areas with an accumulation of health problems, which is of particular importance regarding epidemiological and public health viewpoints. Modern technologies, such as Geographic Information System and SaTScan, with help of scan statistics have enabled us to do disease mapping and spatiotemporal clustering in epidemiological researches.

The current study aims to evaluate spatiotemporal characteristics of CL in Fars province as an endemic area and to determine spatiotemporal clusters in this province from 2010 to 2015.

2. Materials and methods

2.1. Study design

Time-series design, including 29 201 incidence cases of CL recorded in Fars province from 2010 to 2015, was conducted retrospectively to verify if the cases were distributed randomly over time and space. To investigate the spatiotemporal features of CL, spatiotemporal permutation scan statistics was used.

2.2. Study area

Fars province, with Shiraz as its capital city, locates in the south of Iran, covering an area of 122 661 km^2 (7% of the total area of the country). Geographically, Fars province is located on 27°3′ and 31°40′ northern latitude and 50°36′ and 55°35′ western longitude. This province is composed of 26 cities, and geographical coordinates of each city were found through Google-Earth (US Department of State Geographer 2016). The province has three disparate atmospheric regions the first of which being the mountainous sites of north and northwest with mild chill winters and moderate summers. The second part includes central regions with rather rainy winters and hot dry summers. Finally, the third region is located in south and southeast with cold winters and hot summers. The average temperature of Shiraz is 16.8 °C ranging from 4.7 °C to 29.2 °C. Besides, its average altitude is 5 000 feet above the sea level. Based on 2011 census, the population of Fars province is 4.6 million people (6% of the total population of Iran), including 67.6% urban dwellers (urban areas and suburbs), 31% villagers (small towns and rural areas), and 0.3% nomad tribes. The current population growth rate was reported to be 1.3 percent in 2015. It should also be noted that there are migratory movements between the capital of the province and other cities [1].

2.3. Subjects

This study was performed on 29 201 confirmed CL cases. The cases were patients from 26 different cities of Fars province registered in the Contagious Disease Control center located in the main stance of School of Medicine, Shiraz, Iran. These cases showed positive CL through smear, culture, or polymerase chain reaction. Patients whose symptoms of CL began from 1 January 2010 to 31 December 2015 were included.

2.4. Population data

Clinical diagnosis of CL was obtained from the patient's medical records. Cases were recorded monthly from 1 January 2010 to 31 December 2015 for each city. Additionally, all cases recorded in each city were summed up to count the number of cases in that city. The geographical coordinates for each city were obtained through Google-Earth based on latitude–longitude coordinate system. Finally, the data were stored in Microsoft Excel 2007 and were exported to text format for further analysis.

2.5. Information processing

SPSS, version 22 was used for statistical analysis and ITSM2002 software was employed for detecting the general trend of CL over time. In addition, maps of CL cases were generated in ArcGIS, version 10. Finally, SaTScan, version 9.4.4 was used for spatiotemporal cluster analysis.

2.6. Statistical analysis

Median ± quartile deviation was used for continuous variables and minimum, maximum, relative frequency, bar charts, and frequency distribution tables were applied for qualitative variables. To testify the normality assumption, Kolmogorov–Smirnov test was used. In addition, *Chi*-square test was utilized to assess the equal frequency of malaria occurrence over time and place. Kruskal–Wallis test was also used to evaluate the equality of median of cases through different time periods and places. Significance level was considered to be 0.05 for all statistical tests. It should be noted that moving average method was applied to explore the general trend of CL occurrence over time.

2.7. Spatial pattern

Visual presentations of aggregated CL cases were generated using latitude–longitude coordinates of their related cities in different periods of time.

2.8. Spatiotemporal permutation scans statistics and clusters within a cluster analysis

To analyze a space-time featured variable, spatiotemporal permutation model introduced by Kulldorff was applied [14]. This model tests the null hypothesis whether cases are distributed randomly over time and space or whether they have constant risk over time and space. Scan statistics is explained by a cylindrical window with a circular geographical basis and the height akin to time. The core of the base is one of the located centroids over the study area, with the radius changing steadily in size. In this way, it varies from 0% to 50% of the number of expected cases. The height of the cylinder shows any possible time span of 50% or less of the total study period. The window moves in space and time, so that it covers each potential time span for each geographical location resulting in defining an infinitive number of overlapping cylinders of different forms and sizes that finally cover the whole study area. Scan statistics is used in a retrospective way to detect past clusters using retrospective data, and in a prospective way to detect clusters at present time. Scan statistics is used to identify clusters and their significance, which is evaluated by Monte Carlo hypothesis testing. This is done by comparing the likelihood ratio derived from observed data to the one obtained from Monte Carlo simulation. The method is valid and also able to manage potential confounders. It is not sensitive to spatial non-stationarity. Similar to Bernoulli, cases are supposed to have a random process defined for random locations in time. However, when the number of cases is very low compared to the total population, Poisson process offers a better approximation. These two distributions need the assumption of at-risk population per cylinder. Instead of working with at-risk population data, spatiotemporal permutation scan statistics calculates the expected number of cases using hyper geometric distribution with the assumption of defined total population. The windows where the observed number of cases exceeds the expected number of cases by the largest ratio are reported as clusters. Spatiotemporal permutation scan statistics is described as below:

If c_{zt} is the number of cases in zone z and time t in a cylinder, the total number of observed cases in the whole geographical area over the study period is as follows:

$$C=\sum_z\sum_t c_{zt}$$

The expected number of cases for each zone and time is calculated as:

$$\mu_{zt}=\frac{\left(\sum_z c_{zt}\right)\left(\sum_t c_{zt}\right)}{C}$$

For any cylinder A, the expected number of cases is obtained by adding these expectations over the zones and time contained in A:

$$\mu_A=\sum_{z,t\in A}\mu_{zt}$$

Let c_A be the observed cases in A. c_A follows hyper geometric distribution; therefore:

If N is a finite population containing D successes, taking n random samples without replacement from N, the probability of observing k successes is:

$$p(c_A)=\frac{\binom{D}{k}\binom{N-D}{n-k}}{\binom{N}{n}}=\frac{\binom{\sum_{z\in A}c_{zt}}{c_A}\binom{C-\sum_{z\in A}c_{zt}}{\sum_{t\in A}c_{zt}-c_A}}{\binom{C}{\sum_{t\in A}c_{zt}}}$$

When C is large compared to $\sum_{z\in A}c_{zt}$ and $\sum_{t\in A}c_{zt}$, c_A can have Poisson distribution with mean μ_A. Thus, the Poisson Generalized Likelihood Ratio (GLR) is:

$$GLR=\left(\frac{c_A}{\mu_A}\right)^{c_A}\left(\frac{C-c_A}{C-\mu_A}\right)^{(C-c_A)}$$

This is used to estimate the likelihood of a cluster in a given spatiotemporal cylinder. This procedure is repeated for all cylinders. The main advantage of this method is that the population in each cylinder does not need to be known; it is estimated.

The significance test to determine whether a cluster is formed by chance; *i.e.*, its *P*-value, is done using Monte Carlo hypothesis testing. In doing so, timestamps of data points are shuffled and the statistics is calculated again. The process is repeated for 999 times. *P*-value is calculated by the following formula:

$$P-\text{value}=\frac{R}{S+1}$$

R is the rank of the maximum GLR from the real dataset and S is the number of replications.

When a detected cluster is large, it may be interesting to look for clusters within that cluster in the same way [8,15,16].

In this study, spatiotemporal permutation scan statistics was implemented in SaTScan software, version 9.4.4. Time precision of the study data was set to month. Additionally, spatiotemporal retrospective analysis was used and the circular spatial window shape was set to standard. The number of replications was 9 999 and the maximum spatial cluster size was 25 percent of the at-risk population. Indeed, the minimum and maximum temporal cluster size was 1 month and 50 percent of the study period, respectively. Clusters with high incidence rates were detected. Finally, only clusters with no geographical overlap were reported.

To find clusters within a cluster, retrospective spatiotemporal analysis was used to scan for clusters with high incidence rates. The spatiotemporal permutation model was applied from 1 January 2010 to 31 December 2015. Time aggregation units were set to month, time aggregation length was 1, and the coordinate system was latitude/longitude. Temporal data check was applied to ensure that all cases were within the specified time period. Geographical data check was also used to ignore observations that were outside the specified geographical area. Only one set of coordinates per location was allowed for multiple coordinate types. For spatial window, maximum spatial cluster size was 25 percent of at-risk population, and window shape was circular. For temporal window, maximum temporal cluster size was 50 percent of the study period, and the minimum temporal cluster size was 1 month.

3. Results

3.1. Summary information

This study was conducted on 29 201 CL cases which occurred from 2010 to 2015. The largest number of cases was observed in Marvdasht [30% (8 655/29 201)] followed by Shiraz [26% (7 738/29 201)] and Kharame [6% (1 700/29 201)]. The number of CL cases in 26 cities of Fars province has been shown in Figure 1.

3.2. Time trends

The number of CL cases varied over the study period. During 2010, the largest number of cases was 1 534. The maximum number of CL is 1 873 in 2011. The number of CL cases reached a peak of 1 673 in 2012. The maximum of 4 138 cases were observed in 2013. The number of cases reached the maximum of 1 541 in 2014. Finally, there was a maximum of 2 123 cases in 2015. In general, an increasing trend was detected in CL incidence rate during the study period. The annual incidence rate of cases accompanied by the smoothed curve of 2-month moving average has been presented in Figure 2.

During the study period, 20% (5 813/29 201) of the total cases occurred in 2015, 19% (5 388/29 201) in 2014, 18% (5 346/29 201) in 2011, 15% (4 457/29 201) in 2010, 14% (4 138/29 201) in 2013, and 14% (4 059/29 201) in 2012. Distribution of cases by year of onset has been depicted in Figure 3. Accordingly, a periodic trend was observed in the cases during 6 years from January 2010 to February 2015.

Generally, CL has a 4–6 month-incubation period in Fars province. Based on Figure 2, peaks of occurrence were detected

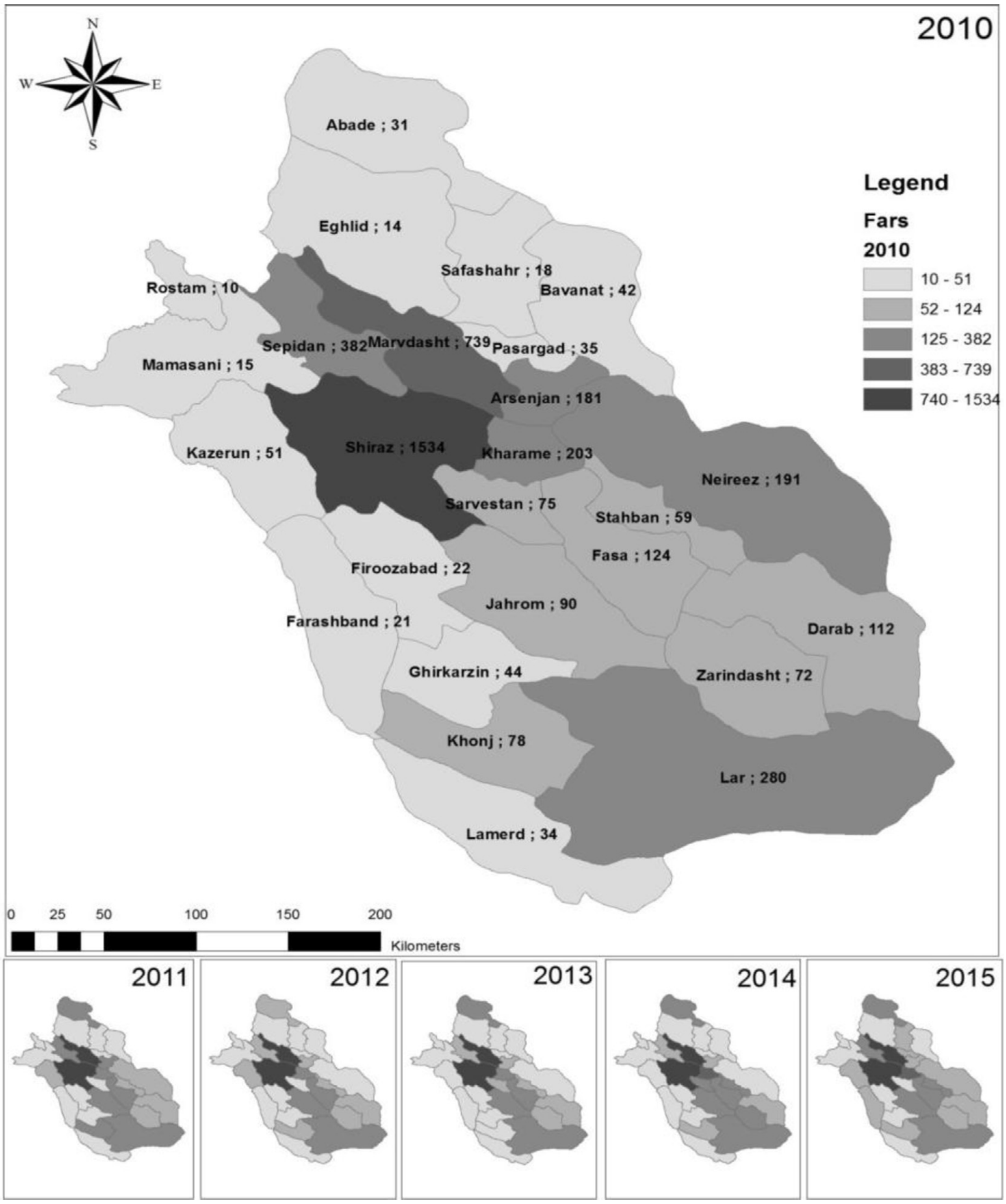

Figure 1. Geographical distribution of CL cases for each year.
Note: Scale bar and legend scales are the same for all 6 maps.

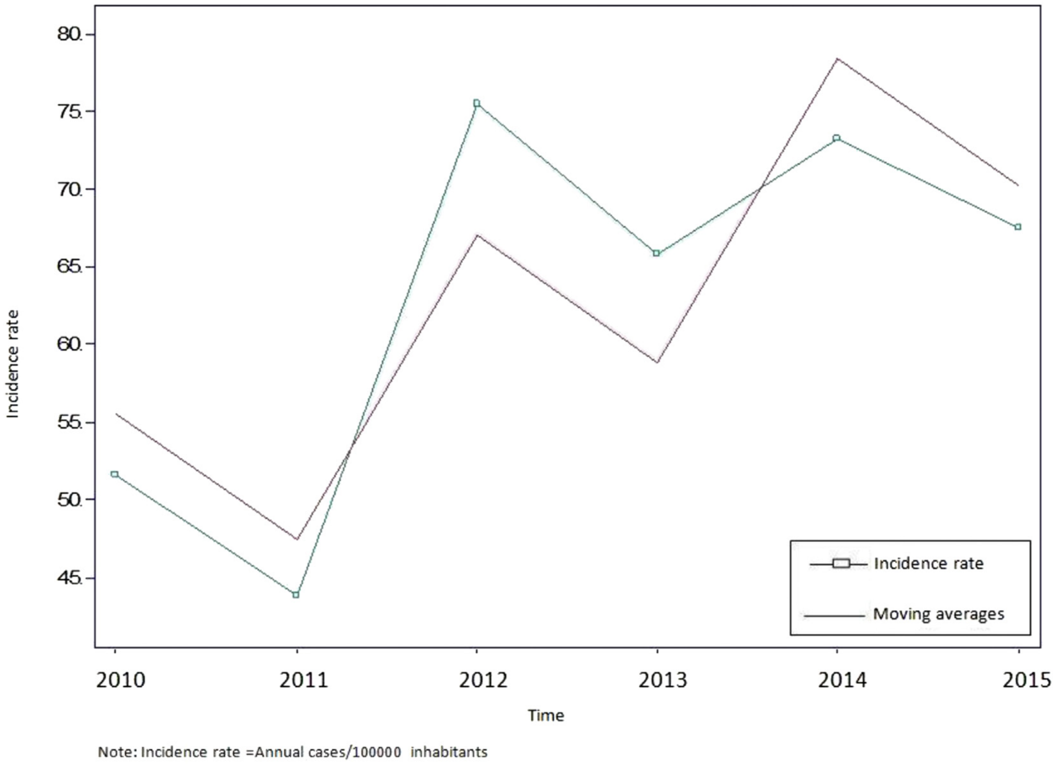

Figure 2. Incidence rate of cutaneous leishmaniasis in Fars province, Iran from 2010 to 2015.

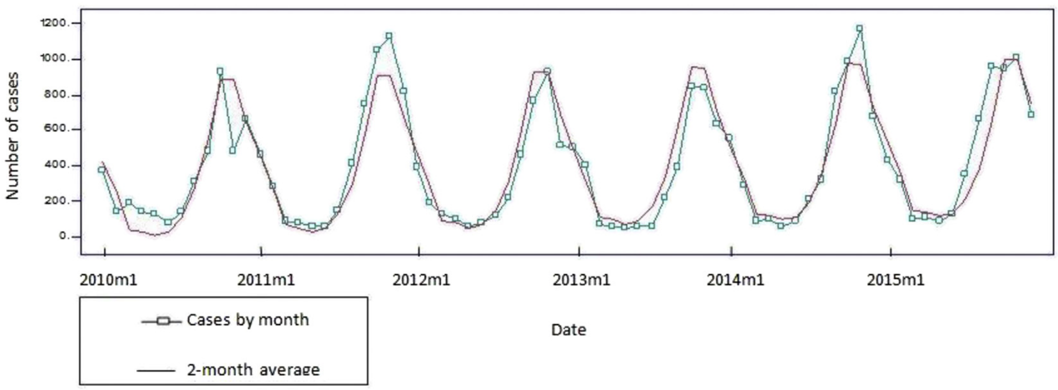

Figure 3. Number of CL cases by month and year of onset in Fars province, Iran from 2010 to 2015.

from August to December (in fall and partially in winter) following insects' bites from March to August (incubation period of CL).

3.3. Geographical distribution

The geographical distribution of CL cases for each year has been shown in Figure 1.

3.4. Spatiotemporal analysis

The results of spatiotemporal permutation analysis revealed six spatiotemporal clusters ($P < 0.001$), including the most likely cluster and five Secondary Clusters (SCs). SCs contained almost one-tenths [10% (2 918/29 201)] of the total cases over the study period. The related geographical extent varied from 0.00 to 76.04 km in radius. The results of permutation scan statistics analysis have been presented in Table 1. Besides, the related cluster locations have been depicted in Figure 4.

3.4.1. Most likely cluster

The most likely cluster occurred from 1/7/2010 to 30/11/2010 and contained almost 13% (350/2 721) of all cases in that period. The cluster is composed of two cities; *i.e.*, Rostam and Sepidan. Sepidan contained almost 97% (340/350) of the total cases which occurred mostly in September, October and November. The remaining 10 cases that made approximately 3% (10/350) of the total cases emerged in Rostam. The cases were partially close geographically within a radius of 12.45 km.

3.4.2. Secondary clusters

The five SCs comprised 87% (2 568/2 918) of the total clustered cases. The SC2 contained 25% of the total cases that occurred from 1/11/2013 to 31/12/2014, with the largest number of cases being related to 2014 (5 388/6 859). During this period, three-fourths of the total cases occurred in September, October, November and December. SC2 contained 5 cities, including Estahban (36%), Jahrom (22%), Khareme (18%), Fasa (13%) and Sarvestan (11%) (Percentages represent fractions of total

Table 1

Results of spatiotemporal permutation analysis on cutaneous leishmaniasis in Fars province from 1 January 2010 to 31 December 2015.

Cluster	Location	Radius (km)	Observed/expected	Time frame	Test statistic	*P*-value*
Cluster 1	Sepidan, Rostam	13.25	2.96	1/7/2010–30/11/2010	149.337	< 0.001
Cluster 2	Fasa, Estahban, Jahrom, Sarvestan, Kharame	69.36	1.47	1/11/2013–31/12/2014	121.104	< 0.001
Cluster 3	Zarindasht, Darab, Lar	76.04	2.94	1/1/2010–30/6/2010	112.949	< 0.001
Cluster 4	Neireez	0.00	3.88	1/7/2010–31/12/2010	105.304	< 0.001
Cluster 5	Farashband	0.00	16.05	1/1/2015–31/1/2015	75.337	< 0.001
Cluster 6	Arsenjan	0.00	1.63	1/6/2010–30/11/2011	36.592	< 0.001

*Statistical significance was evaluated using Monte Carlo hypothesis testing.

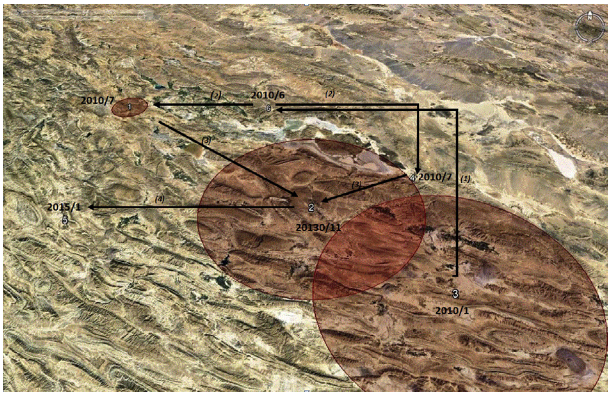

Figure 4. Detected spatiotemporal clusters from 2010 to 2015. Parenthesized numbers on black arrows show progression of clusters in time and space.

cases in each city in the related period). SC2 was the largest cluster containing 60% (1 175/2 927) of the total cases. The cases were not geographically close within a radius of 65.36 km.

SC3 contained 44% (268/603) of the cases that occurred from 1/1/2010 to 30/6/2010. It contained three cities, including Zarindasht containing 9%, Darab containing 28%, and Lar containing 62% of the total cases. Almost 38% (101/268) of the cases occurred in January. Nonetheless, the cases were not very close geographically within a radius of 76.04 km.

SC4 contained 100% (171/171) of the total cases during 1/7/2010–31/12/2010. It contained a city called Neireez, and almost 92% of the cases occurred in September, October, November and December. The cases were closely distributed within a radius of 0 km.

SC5 contained 100% (41/41) of the total cases between 1/1/2015 and 31/1/2015. It contained a city named Farashband, and 100% of the cases occurred in January. The cases were closely distributed within a radius of 0 km.

SC6 contained 100% (354/354) of the total cases from 1/6/2010 to 30/11/2011. It contained a city called Arsenjan, and almost 80% of the cases occurred in September, October, November and December. The cases were closely distributed within a radius of 0 km.

3.4.3. Clusters within a cluster analysis

The results of sub-cluster analysis have been presented in Table 2. Cluster locations have also been depicted in Figure 5.

Cluster 1: Out of the 1 266 cases occurred in Sepidan and Rostam during the study period, 9% (112/1 266) were observed in Sepidan and 2% (11/1 266) in Rostam from 1/11/2012 to 28/2/2014. Rostam had 0.2% (11/6 439) and Sepidan had 2% (112/6 439) of all cases in this period. The results of the analysis revealed that Rostam was a statistically significant cluster in 1/11/2012–28/2/2014. Rostam contained 11 cases with a test statistic equal to 6.82, which was greater than the critical value of 5.95 for 0.01 significance level.

Cluster 2: From the 5 001 cases observed in Kharame, Fasa, Estahban, Jahrom and Sarvestan during the study period, 6% (325/5 001) occurred in Kharame during 1/7/2015–31/10/2015, 8% (428/5 001) in Fasa during 1/9/2011–28/2/2013, and 5% (264/5 001) in Estahban during 1/5/2014–31/3/2015. Out of the 2 924 cases observed during 1/7/2015–31/10/2015, 9% (325/2 924) occurred in Kharame. Besides, from the 8 685 cases detected during 1/9/2011–28/2/2013, 5% (428/8 685) occurred in Fasa. Finally, from the 5 160 cases observed during 1/5/2014–31/3/2015, 5% (265/5 160) occurred in Estahban.

Cluster 3: From the 2 532 cases observed in Zarindasht, Darab and Lar over the study period, 2% (52/2 532) occurred in Darab during 1/3/2010–31/5/2010 and 9% (222/2 532) occurred in Zarindasht during 1/4/2014–31/12/2015. Additionally, in 458 cases occurred during 1/3/2010–31/5/2010, 11% were related to Darab. Finally, from all the 10 250 cases occurred during 1/4/2014–31/12/2015, 2% were observed in Zarindasht.

Table 2

Results of sub-cluster analysis on detected clusters of cutaneous leishmaniasis in Fars province from 1 January 2010 to 31 December 2015.

Cluster	Location	Radius	Start date	End date	Test statistic	Observed/expected	*P*-value*
Cluster 1	Rostan	0	1/11/2012	28/2/2014	6.83	3.90	< 0.01
Cluster 2	Kharame	0	1/7/2015	31/10/2015	47.84	1.79	< 0.001
	Fasa	0	1/9/2011	28/2/2013	47.55	1.64	< 0.001
	Estahban	0	1/5/2014	31/3/2015	27.77	1.62	< 0.001
Cluster 3	Darab	0	1/3/2010	31/5/2010	16.19	2.47	< 0.001
	Zarindasht	0	1/4/2014	31/12/2015	13.06	1.42	< 0.001

*Statistical significance was evaluated using Monte Carlo hypothesis testing.

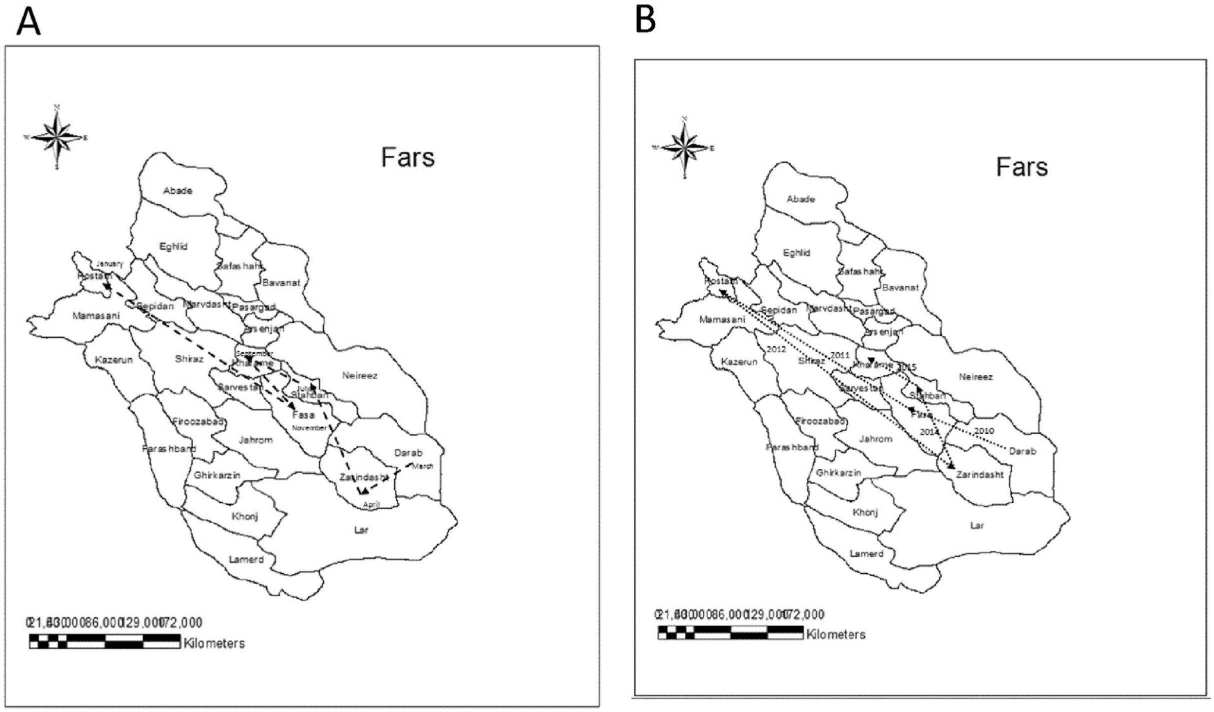

Figure 5. Detected sub-clusters from 2010 to 2015.
A: Black dashed arrows show progression of sub-clusters by month and space. B: Black dotted arrows show progression of sub-clusters by year and space.

4. Discussion

Based on the results of the current study, there were CL outbreaks in Fars province. Indeed, CL followed the spatio-temporal feature in the area. Moreover, 6 spatiotemporal clusters were statistically significant during 2010–2015. The most likely cluster contained 13% of all the cases that occurred from 1/7/2010 to 30/11/2010 and more than 1% of the total cases during the study period.

The aggregated cases moved from southeast toward north-west of the province. As we moved from south to north, aggregation of plains decreased and mountainous areas increased. This was in favor of larger clusters detected in southeast, because high hills and mountains could be barriers to spread of the disease. This movement was also in agreement with Nomads' migration. They change their residency from south to east and from spring to winter (and vice versa) to find more productive areas. They have livestock, dogs, cats, and some other rodents as potential reservoirs of CL, which can spread the disease more easily on their path [8].

In a study done in Fars province [17], the results of Ordinary Least Square regression and Geographic Weighted Regression models indicated a spatial relationship between CL and rainy days, minimum temperature, maximum relative humidity, and population density as the most important eco-environmental predictors. In another study [17] done in Sudan, altitude was the most important factor of CL. One other study [3] performed on spatial relations between environmental and meteorological factors in Spain showed that shelters against wind lands and lower temperature in summer were important factors in sand flies frequency. These results were partially in agreement with the sequence of the main cluster and the two largest SCs in the current study since as we moved from south to north of the province, the climate got colder, the area got mountainous, and the number of rainy days increased. It could also explain why two of the largest cities (Shiraz and Marvdasht) with the maximum number of CL cases in each year were not included in the detected clusters. These two cities had a high population density (both geographical area extent and population number) that is ignored in permutation scan statistics analysis in which clusters are ranked by their likelihood ratio scores. Such a high frequency of CL could also be attributed to the fact that Shiraz and Marvdasht are important industrial and advanced cities in Fars province with advanced medical services, and many patients from nearby cities go there to get health services.

A previous study [6] demonstrated that seasonal transmission of disease was inclined toward summer and spring. This partially approved the occurrence of outbreaks in the current study. In addition, occupation could play a main role in CL infection. Most people in rural parts of Fars province are workers and farmers and, consequently, are more prone to be exposed to insect bites and infection.

A previous study [13] showed a descending trend in CL occurrence during drought periods, which is consistent with the trend seen in the incidence rate of CL in the present study. There is a drought situation in Fars province every other year. This might also be attributed to herd immunity caused by CL infection in the society. Yet, the definite reason for the observed trend is not clear [8,13], and further researches are required to be conducted in this regard.

This study had some limitations that could have affected the results. First, the surveillance system of CL in Iran is a passive system and many patients with small lesions or lesions in masked parts of their bodies do not refer to health centers or surveillance systems. Therefore, the incidence rate of CL might be underestimated. This could affect prediction of the disease load and the required health services as well as detection of potential clusters. Another study limitation was variety in climatic factors and disease pattern in Fars province that could make it difficult to predict CL outbreaks in future. Moreover, genetic factors in addition to sero-epidemiological features of agents and vectors should be regarded to evaluate the true spatiotemporal nature of CL.

Similar to other cluster detection methods, scan statistics used to detect spatiotemporal clusters can discover more than one cluster in a given area and time period. In this respect, prioritizing clusters is quite difficult, especially in case of distinction between statistical and clinical significance. Detected clusters are ranked by their likelihood-ratio scores and can neglect areas with a moderately high relative risk but a large population size and time period. On the other hand, the statistical test is not performed for relative risks. Therefore, significant clusters may or may not have a high relative risk. That is why some large frequent CL cases were not statistically significant in our study. Since relative risk is an important value in epidemiological viewpoint, there should be another statistic [*e.g.* population attributable risk] beside statistical significance value to decide between significant and non-significant clusters. population attributable risk is a number between 0 and 1; therefore, clinical comparison of clusters can be somehow logical.

One other limitation of scan statistics is that the precise limits of detected clusters remain uncertain and some sites within the detected cluster may not have a high risk. This is due to limited sample size in the sites. This happens when the cluster does not have a circular shape, but circular scan window or cylinder has been used. Even if scan windows are not used, the same situation may happen and increasing sample size to get a better estimation is not always possible. To cope with this problem, Oliveir-F measure derived from SaTSsan software is used. However, it is available in purely spatial clustering (Poisson distribution), but not for spatiotemporal permutation analysis (hyper geometric distribution). Oliveir-F measure is a value between 0 and 1 calculated for each location. In case this value is closer to 1, the related location can be more likely to be a part of the true cluster. In our study, risks and population sizes were not available for each location. Thus, population attributable risk could not be calculated to compare the clusters. On the other hand, Oliveir-F measure could not be calculated since spatiotemporal permutation analysis was used to detect the clusters. To deal with this problem, subgroup analysis was used to detect clusters within clusters. When a large cluster composed of several locations is significant, it may be interesting to find real significant locations within that cluster. In this way, it becomes clear which cluster caused the main cluster to be significant. Non-significant locations can be detected, as well. This method can partially solve the prioritizing problem among statistically significant clusters.

Overall, further researches are needed to investigate the spatiotemporal traits of CL, including environmental and genetic factors predicting CL cases clusters in future.

Conflict of interest statement

All authors declare that there is no conflict of interest.

Acknowledgements

This article was extracted from the PhD dissertation (proposal No. 12439) written by Marjan Zare and approved by Research Vice-chancellor of Shiraz University of Medical Sciences. Hereby, the authors would like to thank Ms. A. Keivanshekouh at the Research Improvement Center of Shiraz University of Medical Sciences for improving the use of English in the manuscript.

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