Cutaneous Leishmaniosis Situation and Predicting the Distribution of Phlebotomus papatasi and P. sergenti as Vectors of Leishmaniasis in Ardabil Province, Iran

Ali Khamesipour¹, Soheila Molaei², Navid Babaei-Pouya³, Eslam Moradi-Asl⁴,⁵,⁶

¹Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Tehran, Iran; ²Deputy of Research & Technology, Ardabil University of Medical Sciences, Ardabil, Iran; ³Nir County Health Centre, Ardabil University of Medical Sciences, Ardabil, Iran; ⁴Department of Public Health, School of Public Health, Ardabil University of Medical Sciences, Ardabil, Iran; ⁵Arthropod Borne Diseases Research Center, Ardabil University of Medical Sciences, Ardabil, Iran

Abstract: Cutaneous leishmaniosis (CL) is the most common form of leishmaniasis. CL caused by L. major and L. tropica is endemic in 17 provinces of Iran. This study was carried out to elucidate situation of CL in Ardabil province and to predict distribution of Phlebotomus papatasi and Phlebotomus sergenti (Diptera: Psychodidae) as vectors of CL in the region. In this cross-sectional study, data on CL patients were collected from local health centers of Ardabil province, Iran during 2006-2018 to establish a geodatabase using ArcGIS10.3. A total of 20 CL cases were selected randomly and skin samples were collected and analyzed by PCR method. MaxEnt 3.3.3 model was used to determine ecologically suitable niches for the main vectors. A total, 309 CL human cases were reported and the highest incidence rate of disease was occurred in Bilasavar (37/100,000) and Germi (35/100,000). A total of 2,794 sand flies were collected during May to October 2018. The environmentally suitable habitats for P. papatasi and P. sergenti were predicted to be present in northern and central areas of Ardabil province. The most variable that contributed ratio in the modeling were Isothermality and slope factors. Ardabil province is possibly an endemic area for CL. The presence of P. papatasi and P. sergenti justifies local transmission while the vectors of CL are existing in the northern and central areas of the province.

Key words: Phlebotomus papatasi, Phlebotomus sergenti, cutaneous leishmaniasis, vector, Iran

INTRODUCTION

Cutaneous Leishmaniosis (CL) is one of the neglected tropical disease caused by different Leishmania species which is endemic to more than 100 countries, annually 700,000-1,200,000 new cases of CL and visceral leishmaniasis (VL) in the world [1]. Four common clinical types of leishmaniasis are usually presented; CL, mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL) and VL. CL is the most common form of leishmaniasis, 2 types of CL were reported [2]. In Iran, 2 types of CL is endemic; zoonotic cutaneous leishmaniasis (ZCL) and anthroponotic cutaneous leishmaniasis (ACL) [3]. Annually, more than 20,000 cases of CL were reported in Iran. ACL is caused by Leishmania tropica and transmitted by Phlebotomus (Para-phlebotomus) sergenti [4,5]. ACL was reported from Tehran, Mashhad, Shiraz, Kerman, Yazd, Bam and etc. [6,7]. In Iran, ZCL is caused by L. major and transmitted by Phlebotomus papatasi and small rodents are the main reservoir. ZCL is endemic in 17 out of 31 provinces, Iran [3,8,9]. Over 800 species of sand flies are known but only over 30 transmit Leishmania [8]. 44 spices of sand flies are reported in Iran and 10 species is doubtful [9-11]. Ardabil province is located in northwest of Iran and is the main endemic areas of VL and annually 15-20 new cases were reported [12,13]. To our knowledge through search on internet, no study is conducted on CL in the northwest of Iran including Ardabil Province. Previously, Leishmania parasite has been reported from 2 spices of Sergentomyia dentata and S. sintoni as well as Leishmania was isolated from P. papatasi using molecular methods. The parasite species has not been identified [14,15]. This study aims to explain about CL situation in Ardabil province and to explore distribution of P. papatasi and P. sergenti (Diptera: Psychodidae) as possible vectors of leishmaniasis in the province.
MATERIALS AND METHODS

Study area

Ardabil Province is located in the north-west of Iran (37.45-39.42°N, 47.30-48.55°E). The province has an area of 17,953 km² and a population of 1,270,000, based on 2016 census. This province consists of 10 counties, 21 districts, 26 cities, 71 rural districts and 1,477 permanent villages [16].

Data collection

CL patients’ data are collected from the source document of each patient from Health Center of Ardabil province from 2006 to 2018, the information in regard to demographic, travel history, site and number of lesion was recorded and analyzed. Sand flies were collected from 30 areas during May to November 2018. The study areas were selected based on the frequency of human CL in the past 10 years. Five urban areas and 25 villages were selected, and their geographic coordinates were recorded using Global Positioning System. Sand flies were collected using caster oiled papers (60 papers for indoors and outdoors of each area). Then, the collected sand flies were put in acetone and then stored in the 70% alcohol. The specimens were mounted by addition of a single drop of Puris’ medium and identified using relevant identification keys [17,18].

PCR method

A sample was prepared from the slide which was used for direct smear, the skin materials were isolated from the slide by scratching, DNA was extracted and used for PCR amplification according to the procedure previously described [19,20]. PCR will be carried out using the primers for leishmania-specific pair fragment of kinetoplast DNA, F: (5’TCGCCAGACGCCCCATACC, 3’ ) and R: (5’AGGGGTGGGTAAATAGG 3’). Amplification; 35 cycle, denaturation at 94°C for 1 min, annealing at 60°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 7 min.

Modeling

The MaxEnt version 3.3.3 was used to predict the presence of *P. papatasi* and *P. sergenti* sand flies in order to prepare the distribution map at 72 locations (30 locations from *P. sergenti* and 42 locations for *P. papatasi*) in Ardabil Province northwest of Iran (Table 1). Jackknife test was used to analyze the relationship between weather variables and distribution of sand flies.
flies and the relevant variables were identified with percentages and non-relevant variables were assigned zero. The output of the MaxEnt model was included in the ArcMap 10.4.1 software and was analyzed by Jackknife test and descriptive statistical.

RESULTS

Aspects of cutaneous leishmaniasis

Totally, 309 cases of CL were reported during 2005-2018 in Ardabil Province. The highest number (31 cases) of CL was reported in 2009 and the lowest number (13 cases) in 2011 year (Fig. 1). CL were reported in 9 of 10 counties in Ardabil Province and the highest number was reported in Bilasavar region for 37/100,000 and Germi 35/100,000, about 50% of CL patients were reported from the north part, 39% from the central parts and 11% from south parts of Ardabil Province (Fig. 2), 71% of CL were males and 29% were females. The age range of the patients was 86% over 10 years and 14% under 10 years, 53% of the patients mentioned a history of travel outside the province. Annually, 20-30 CL cases were reported from Ardabil Province which 74.5% of CL reported from 13 urban areas and 24.5% from 46 rural areas. The clinical picture of the lesions showed that 65% of the lesions were dry with no secretion and 35% of the lesions were wet with secretion, most of the lesions (35.5%) was seen in hands and 30% in the face (Fig. 3), 47% of the patients present only a single lesion and 53% more than one lesion. The PCR results showed that the causative agents were *Leishmania tropica* in line 7 and *L. major* in lanes 1, 4, and 6 (Fig. 4).

Sand flies collected

A total of 2,794 sand flies were collected from 30 locations of Ardabil province, 40% of the total sand flies were belong to

![Graph](image1.png)

**Fig. 1.** Trend of CL cases reported in urban and rural areas in Ardabil Province, northwest of Iran (2005-2018).

![Graph](image2.png)

**Fig. 2.** Frequency of *Phlebotomus papatasi* (A) and *Phlebotomus sergentii* (B) in northwest provinces, Iran, 2018.

![Image](image3.png)

**Fig. 3.** The frequency and location of lesion of CL in different areas, Ardabil Province, northwest Iran (2005-2018).

![Image](image4.png)

**Fig. 4.** 1.5% agarose gel electrophoresis of PCR amplification for identification of *Leishmania* species on Giemsa-Stained slides. Lanes 1,4,7: Standard *Leishmania major*; Lanes 2,3,6: negative control; Lane 5: DNA Ladder (50 bp); Lane 8: *Leishmania tropica*. 

*Khamesipor et al.: New focus of cutaneous leishmaniasis in Iran*
and the highest number of the spices were collected from Germy and Bilasavar counties (Fig. 2). Monthly activity of *P. sergenti* in Ardabil Province was started in 15 May and ended in 30 September (when density of sand flies reaches to zero) with one peak in June (Fig. 5).

**Ecological niche modeling**

**P. sergenti**

The environmental suitability for *P. sergenti* was predicted to be present in most parts of northern Ardabil Province (Fig. 6A), although there are some areas of high environmental suitability in northeastern, northwestern regions as well. Warmer colors show areas with higher environmental suitability for *P. sergenti*. The areas under receiver operating characteristic (ROC) curve (AUC) for training and testing data was calculated as 0.904 and 0.933, respectively. The most variable seems to contribute the ratio in the modeling of *P. sergenti* was Bio3 (Isothermality) factor and Jackknife test results showed that the environmental variable with the highest gain in the predicting power of the model if used in isolation was Slope variable (Fig. 7A).

**P. papatasi**

The results of this study showed that the environmental suitability habitat for *P. papatasi* was in northern and central parts of Ardabil province (Fig. 6B). There are some areas of high environmental suitability in northern regions in borderline of Iran and Azerbaijan Republic country. The area under receiver operating characteristic (ROC) curve (AUC) for training and testing data was calculated as 0.904 and 0.933, respectively. The important variable which contributed ratio in the modeling of *P. papatasi* was slope and NDVI factors. The results of jackknife test showed that the Bio8 (Mean Temperature of Wettest Quarter) factors with highest gain in the predicting power of the model if used in isolation (Fig. 7B).
DISCUSSION

Annually, 20-30 cases of CL were reported from Ardabil province and in 47% of the cases showed no history of travel outside the province. Out of human cases, 17% were under 10 years old and 74.5% of CL reported from 13 urban areas as well as 65% of lesions were dry and without secretion that these symptoms are probably related to ACL. The important clinical symptoms for ACL including dry ulcer that most occur in face in urban areas [17].

In The results of this study show that dominant species of Ardabil province is a *P. papatasi*. In a study which was completed by Absavaran et al. [19], Dehkordi et al. [22], Ghorbani et al. [23], and; Moradi-Asl et al. [24] confirmed the current results. *P. papatasi* and *P. sergenti* are the main vectors of ZCL and ACL in Iran respectively [3]. In this study *P. papatasi* was collected from all regions of Ardabil Province with a high frequency in north and central parts and *P. sergenti* from north part of Ar-
in the Ardebil province, the same areas of CL reports in Bilasavar and Germi counties. This result showed that the vectors of CL is present in the Ardebil province with activity in June to July. Since 45% of \textit{P. papatasi} and 24.5% of \textit{P. sergenti} were collected from indoors, it seems that the sand flies have a feeding and resting indoors places but invers, \textit{P. sergenti} is more likely to outdoors places. In study of central Iran, 52% of collected sand flies were \textit{P. papatasi} that 33% of them were captured in indoor places and the seasonal activity was reported May to September [25]. The average temperature and altitude level in the central and northwest of Iran has a significant difference and have a greatest impacts on seasonal activity and distribution of sand flies [17-26]. The results of this study showed that

![Fig. 7. Variable importance (upper) and the most important variable by jackknife test (down) for two vectors of cutaneous leishmaniasis in Ardabil Province. (A) \textit{Phlebotomus sergenti}. (B) \textit{Phlebotomus papatasi}.](image)

the suitable habitat for \textit{P. sergenti} in Ardabil Province were located in north part that this area have a lower than 1,500 m altitude. The results of MaxEnt model and jacknife test showed that isothermality and slope factors had the greatest effect on the distribution of \textit{P. sergenti} in north part of Ardabil Province, respectively. With the slope increasing from 10 to 88 degrees, also frequency of \textit{P. papatasi} has increased and after the 89 degrees the frequency of this spices has decreased. In one study, \textit{Lutzomyia whitmani} the vector for CL in Southern Brazil was affected the east slope of the Andes [27] and the results of ecological niche model of \textit{P. alexandri} and \textit{P. papatasi} in the Middle East showed that elevation, minimum temperature in the coldest month, mean temperature in the wettest quarter, and
precipitation in the driest month were the most important role in modeling of this spices [28]. For *P. papatasi* in Ardabil Province the suitable habitat was identified in north and central parts. The important factors that impacted on ecological niche model of *P. papatasi* were slope and NDVI. In central of Iran, lowland areas provide good ecological niches for *P. papatasi* and max-min temperature were impacted on ecological niche model [16]. According to the findings, cutaneous leishmaniasis might be endemic in Ardabil province with low incidence. Moreover, *P. papatasi* and *P. sergenti* as vectors of CL are present in northern and central parts of the province, and there are the potential of transmission of CL in the areas. It is recommended to more studies be done in the area in respect of CL reservoir and vectors.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest related to this study.

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