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ARAŞTIRMA / RESEARCH

Sand fly fauna and environmental parameters in a cutaneous leishmaniasis endemic region in Karaisali, Adana, Turkey

Bir kutanöz leishmaniasis endemik bölgesi olan Adana'nın Karaisalı ilçesinde kum sineği faunası ve çevresel parametreleri

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Abstract

Purpose: This paper presents the results of an entomological survey in an endemic focus of cutaneous leishmaniasis in Karaisali province of Adana in Turkey.

Materials and Methods: There were two field works in two consecutive years (2013 and 2014), which 1088 sand fly specimens were captured using 136 light traps which were conducted in 11 villages of Karaisali located in southwest part of Adana.

Results: Totally, six Phlebotomus species were described: Phlebotomus tobbi (41.54%), Phlebotomus neglectus/syriacus (9.74%), Phlebotomus papatasi (25.18%), Phlebotomus perfiliewi (3.49%), Sergentomyia dentata (18.38%) and Sergentomyia theodori (1.65%). The female/male rate was found to be 1.98. Phlebotomus tobbi, Phlebotomus papatasi, Phlebotomus neglectus/syriacus, Sergentomyia dentata and Sergentomyia theodori were dominant at 201-400m, 0-200m, 801-1000m, 401-600m and 601-800m respectively.

Conclusion: Environmental and climatic factors were compared for the presence of sand flies species especially vectors of cutaneous leishmaniasis by univariate binary regression analysis in PASW. Sand fly density maps were generated based on the elevation they were caught by using ARCMAP 10.2. The data also revealed a relationship between presence of the probable vector sand flies and several environmental factors such as altitude, temperature, trap location and humidity in Karaisali.

Key words: Sand fly, fauna, spatial distribution, cutaneous leishmaniasis, geographical information systems.

Öz

Amaç: Bu çalışmanın amacı, bir endemik bölge olan Adana'nın Karaisalı ilçesindeki kutanöz leishmaniasisin endemik odaklı entomolojik bir araştırmasının sonuçlarını sunmaktır.

Gereç ve Yöntem: Adana'nın güneybatısında bulunan Karaisalı'nın 11 köyünde İki yıl üst üste iki alanda (2013 ve 2014), 136 ışık tuzağı kullanılarak 1088 kum sineği örneği toplanmış, saha ile ilgili çevresel veriler kaydedilmiştir. Ayrıca Örnek toplanan alanlardaki çevresel ve iklimsel faktörler, vektör kum sineklerinin varlığı ile PASW'da tek değişkenli ikili regresyon yöntemi kullanılarak karşılaştırma yapıldı. Kum sineklerinin dağılım haritaları, ARCMAP 10.2 kullanılarak yakalandıkları yükseklere bağlı olarak oluşturuldu.

Bulgular: Toplanan örneklerin türleri; Phlebotomus tobbi % 41,54), Phlebotomus neglectus/syriacus (% 9,74), Phlebotomus papatasI % 25,18), Phlebotomus perfiliewi (% 3.49), Sergentomyia theodori (% 10,56) ve Sergentomyi adentata(% 9,46) olarak belirlenmiştir. Erkek/dişi oranı ise 1.89 olarak bulunmuştur. Phlebotomus.tobbi, Phlebotomus papatasi, Phlebotomus neglectus/syriacus, Sergentomyia ia dentata ve Sergentomyia theodori sırasıyla 201-400m, 0-200m, 801-1000m, 401-600m ve 601-800m yüksekliklerde baskın t ürler olarak belirlenmiştir.

Sonuç: Veriler, muhtemel vektör kum sineklerinin varlığı ile Karaisalı'da yükseklik, sıcaklık, tuzak konumu ve nem gibi birkaç çevresel faktör arasında bir ilişki olduğunu ortaya koymuştur.

Anahtar kelimeler: Kumsineği, fauna, mekansal dağılım, kutaöz leishmaniasis, geographical information systems.

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INTRODUCTION

Phlebotomine sand flies can transmit Bartonella bacilliformis bacterium and arthropod-borne viruses (Phlebovirus and Vesiculovirus) recognized as human pathogens (Toscana virus, Naples virus and Sicilian virus) and the Leishmania promastigotes causing leishmaniasis from one vertebrate to another vertebrate in the Old World. To date, genera of Phlebotomus, Sergentomyia and Chiniushave been identified in the Old World. For Neotropical sand flies, many researchers follow the classification of Lewis revised by Young and Duncan, which recognize three genera, 15 subgenera and 11 species groups. Cutaneous and visceral leishmaniasis are the two of the well-known leishmaniasis types caused by Leishmania species are commonly seen in Turkey[1-4].

P. tobbi is the proven vector of CL caused by Leishmania infantum in the East Mediterranean parts of Turkey. P. sergenti and P. similis are the probable vectors of Leishmania tropica in the south-eastern and western parts of Turkey, respectively. The current fauna research carried out in 40 cities of Turkey revealed 24 Phlebotomus, (18 of which were probable vector species) and five Sergentomyia species. According to the various entomological survey results;six Phlebotomus species (P. tobbi, P. syriacus, P. perfiliewi, P. papatasi, P. sergenti and P. simici) exist in Adana. In addition P. tobbi was found to be dominant species in Adana and Cukurova Region[5-9].

Cutaneous leishmaniasis is a notifiable disease in Turkey, and Ministry of Health (MoH) official records (2016) between 1990 and 2010reported 46.003 new cases[10]. Among these, 96% were from the Şanlıurfa, Adana, Osmaniye, Hatay, Diyarbakır, İçel and Kahramanmaraş provinces[11].One thousand nine hundred ninety-two (12-13%) patients were diagnosed with Cutaneous leishmaniasis between 2008 and 2016 in Adana. One hundred seventy two (8.63%) of these patients were reported in the same years from the Karaisali province. Thus, we studied in the area located in Adana having important characteristics such as proper ecological environment for the surviving of a sand fly vector population and the presence of the susceptible human population of Leishmania transmission.

Entomological surveys including the dynamics and changes in sand flies populations highlight the importance of climate and the environmental factors such as temperature and humidity respectively. Environmental variables, especially temperature and humidity, are the important factors for the distribution of pathogens and their vectors. The distribution of the vector sand fly species affected by climatic factors must coincide in space and time with the distribution of infections in humans[12,13].

GIS are techniques to input, store, retrieve, manipulate, analyze and output data that have spatial attributes associated with them. As such, they form an underlying tool for examining landscape epidemiology. They can be used to locate cases of disease, and establish the spatiotemporal relationships among the cases and selected environmental features[14].

GIS tools contribute to the generation of useful maps about vector sand flies distribution in CL endemic areas based on various geographical factors. Determination of the most proper environmental conditions for vector-arthropods is of great importance for better understanding the epidemiology of vector-borne diseases[15,16].

The aim of this entomological study was two fold; (1), to determine the new data of sand fly fauna in Karaisali province; (2) to identify the spatial distribution of vector sand fly species hosting these parasites and to better understand the effect of geographical factors.

MATERIALS AND METHODS

Our study areas were 11 villages (Emelcik, Yazibasi, Kirikli, Hacili, Corlu, Boztahta, Barakdagi, Hacimusali, Catalan, Sarimemetli and Altinova) in Karaisali province.The study area was 1.526 km2 in size. We chose this area due to its suitable environmental conditions for sand fly and the dominancy of proven leishmaniasis vector P. tobbi in Karaisali and Adana considered to be an endemic area for cutaneous leishmaniasis caused by L. infantum (Table 1). Karaisali is a district of Adana the fourth largest city in Turkey. The mountain range of Taurus is located in the northern part of the study area and constitutes the border between Karaisali and the inner Anatolian region (Figure 1).

Table 1. Geographical data of the different villages studied or of the different sampling areas.

No	Village	Latitude	Longitude	Altitude (m)	Temperature (℃)	Humidity (%)
1	Emelcik	37°13'16.78"N	35°10'1.34"E	178	36,2	53,65
2	Yazibasi	37°10'57.10"N	35°11'26.91"E	154	36,55	52,10
3	Kirikli	37°10'12.01"N	35°13'51.22"E	98	36,62	56,58
4	Hacili	37°17'44.31"N	35° 9'18.26"E	203	35,12	54,12
5	Corlu	37°19'5.34"N	35°10'13.11"E	448	37,16	49,58
6	Boztahta	37°22'7.07"N	35°27'36.62"E	367	37,08	50,08
7	Barakdagi	37°24'1.23"N	35°11'36.07"E	677	32,08	48,08
8	Hacimusali	37°18'39.82"N	35°16'38.41"E	267	37,33	50,66
9	Catalan	37°14'57.91"N	35°17'43.68"E	129	36,83	51,08
10	Sarimemetli	37°14'15.94"N	35°16'37.96"E	152	34,62	52,12
11	Altinova	37°10'51.73"N	35° 1'50.50"E	164	36,66	52,83

Figure 1. Borders of study area

The average temperature of Adana province between 1950 and 2014 was 17.5°C (max 23.5; min 12.1°C). The annual average rainfall between 1950 and 2014 was 688.2 mm[17].

Sand fly collection procedure

Two field works were conducted in different periods of the summer season using CDC light traps (CDC light traps; John W. Hock Company, Florida, USA) in 11 villages of Karaisali. During the field work, at least three light traps were setup under or close to animal barn in each village. The first field work was carried out in 91 stations between 22 July 2015 and 29 July 2015. The second was carried out in 45 stations between September 5th and 8th September 2016. In addition, sticky papers were used to catch sand flies in seven villages.

The collected specimens were stored in 96% alcohol for identification based on the systematic characters of male and female using the several keys[18-20].

Data collection and statistical analysis

Through field observation we obtained two groups of data. The first group was related to the physical condition and the habitat of each station. During the field work, we noted the characteristics of light traps locations such as sampling site locations, number of ovine, cattle and poultry, wall construction type temperature and humidity.

The second group of data was related to the environmental variables of each location based on LPDAAC (Land Processes Distributed Active Archive Center) in NASA. Data on elevation, slope, aspect and Digital Elevation Models (DEMs) of the study area were derived from three arc second resolution Shuttle Radar Topography Mission (SRTM) NDVI, EVI, and LST were obtained from Landsat ETM + (path 175, row 34) and MODIS data set. For MODIS data, temperature layers (Day Land Surface Temperature, Night Land Surface Temperature, Band 31 emissivity, Band 32 emissivity) were from MOD11A2[21] and reflectance layers were from MOD13Q1 files[22]. The MOD13Q1 version six provides a Vegetation Index (VI) value at a per pixel basis. There were 2 primary vegetation layers. The first was the Normalized Difference Vegetation Index (NDVI) referred to as the continuity index to the existing National Oceanic and Atmospheric Administration-Advanced Very High-Resolution Radiometer (NOAA-AVHRR) derived NDVI. The second vegetation layer was the Enhanced Vegetation Index (EVI), which improved sensitivity over high biomass regions. Along with the Vegetation layers there were also MODIS Reflectance bands one (Red), two (NIR), three (Blue), and seven (MIR), as well as four observation layers. In addition, reflectance bands were combined to create, NDVI vegetation indices by using Landsat ETM + (path 175, row 34) data set for the years 2015 and 2016. All of the data was entered into the database of geographical information systems by using ARCMAP 10.0 software (ARCMAP; Esri, New York, USA). WGS 84 coordinate reference system was used for this purpose.

The statistical analysis (An univariate binary regression analysis) based on relationship between our environmental data and sand fly fauna results was administered using PASW software(PASW; SPSS Hong Kong Headquarters, Quarry Bay, Hong Kong).

RESULTS

We identified the sand fly fauna in the 11 villages in the north-west of Adana Province. In total, 136 CDC light traps were placed in each of the 11 study areas, and 1088 sand fly belonging to two Sergentomyia and four Phlebotomus species were collected. Specimens were identified as follows: P. tobbi (41.54%), P. neglectus/syriacus (9.74%), P. papatasi (25.18%), P. perfiliewi (3.49%), Sergentomyia(S.) dentata (18.38%) and S. theodori (1.65%). In addition, P. tobbi appeared to be the most abundant species in 4 of the 11 villages: Yazibasi (35.71%), Boztahta (78.62%), Catalan (61.36%) and Altinova (47.05%). P. papatasi was found to be the dominant one in 3 of the 11 villages in Karaisali: Emelcik (100%), Kirikli (80.15%) and Sarimemetli (36.36%). In 4 of the 11 villages Sergentomyia was detected to be dominant species in terms of population density: Hacili (44.44%), Corlu (52.45%), Barakdagi (40.00%) and Hacimusali (46.15%). P. neglectus/syriacus and P. perfiliewiwere not detected as dominant species in the study area. On the other hand, the female/male rate was 0.50 for all collected species, 0.48 for P. tobbi, 0.82 for P. neglectus/syriacus, 0.77 for P. papatasi, 0.35 for P. perfiliewi and 0.22 for Sergentomyia sp. (Table 2).

P. tobbi was found to be dominant at 201-400m, P. papatasiat 0-200m, P. neglectus/syriacusat 801-1000 m, S. dentata and S. theodori at401-600m and 601-800m (Figure2). The geographical data for each study area are displayed in Table 3. The minimum and maximum temperature were between 22 ^{o}C and 53 ^{o}C, and average temperature was found to be 34.23 ^{o}C. The humidity was between %20 and %90, and average humidity was measured to be %52.91. Trap location, Cattle number, max. and min. temperature, EVI, NDVI, DEM, MIR, LANDSAT NDVI, EMIS31 and LSTNIGHT values were associated with P. tobbi distribution ($p<0.05$). We also observed statistical relationship between the presence of P. neglectus/syriacus and trap habitat, trap location type, poultry number, EVI, NDVI, DEM, MIR, LANDSAT NDVI, EMIS31, EMIS32, LSTNIGHT values ($p<0.05$). Trap habitat, ovine number, max. temperature, min. moisture, EVI, EMIS31, EMIS32 and LSTNIGHT values were to be statistically significant for P. perfiliewi distribution (Table 3).

Table 2. The sand fly species collected in the area.

Species / Villages	Emelcik	Yazibasi	Kirikli	Hacili	Corlu	Boztahta	B.dagi	H.musali	Catalan	Sarimetli	Altinova	Total	%	♂	♀	♂/♀
P. tobbi	0	10	28	16	42	228	42	10	54	6	16	452	41.54	148	304	2.05
P. neglectus/syriacus	0	0	0	2	10	34	52	2	0	4	2	106	9.74	48	58	1.20
P. papatasi	2	6	210	8	2	4	2	2	20	8	10	274	25.18	130	144	1.10
P. perfiliewi	0	8	8	4	4	10	0	0	0	0	4	38	3.49	10	28	2.80
Sergentomyiadentata	0	1	8	11	29	7	30	4	10	2	1	103	9.46	23	80	3.47
Sergentomyiathe odori	0	3	8	13	35	7	34	8	4	2	1	115	10.56	17	98	5.76
Total	2	28	262	54	122	160	160	26	88	22	34	1088	100	376	712	1.89

Table 3. Characteristics of localization and environmental factors related to the presence of three sand fly vector species.

	P. tobbi		*P. neglectus/syriacus*		*P. perfiliewi*	
	p	**Cl (95%)**	**p**	**Cl (95%)**	**p**	**Cl (95%)**
Trap habitat	**0.121**	**0.931-1.848**	**0.021***	**1.071-2.353**	**0.018***	**0.233-0.871**
Centre	0.009*	0.129-0.745	0.007*	0.0136-0.733	0.998	0.00-
Edge	0.003*	0.105-0.637	0.029*	0.160-0.906	0.998	0.00-
Other	-	ref	-	ref	-	ref
Trap Location	**0.030***	**1.030-1.759**	**0.183**	**0.901-1.723**	**0.087**	**0.490-1.050**
Barn	0.003*	0.80-0.594	0.050*	0.367-0.135	0.998	0.00-
Garden	0.033*	0.121-0.917	0.026*	0.296-0.102	0.998	0.00-
House	0.007*	0.1260.715	0.012	0.347-0.152	0.998	0.00-
Other	-	ref	-	ref	-	-
Trap Location Type	**0.497**	**0.936-1.146**	**0.042***	**1.133-1.005**	**0.593**	**0.893-1.219**
Briquette	0.158	0.87-1.488	0.701	0.120	0.999	0.00-
Concrete+Briquette	0.999	0.00-	0.513	0.250	0.999	0.00-
Concrete+Stone	0.442	0.141-2.349	0.972	0.172	0.999	0.00-
Concrete	0.997	0.85-1.225	0.467	0.102	0.999	0.00-
Stone+Briquet	0.507	0.369-7.526	0.211	0.537	0.999	0.00-
Stone	0.999	0.00-	0.128	0.618	0.999	0.00-
Wooden+Briquet	0.337	0.134-1.992	0.433	0.377	0.999	0.00-
Wooden+Stone	0.999	0.00-	0.999	0.00	0.999	0.00-
Wooden	0.999	0.00-	0.999	0.00	0.999	0.00-

Other	-	ref	-	ref	-	ref
CattleNumber	0.000***	1.053-1.155	0.597	0.941-1.035	0.637	0.923-1.050
OvineNumber	0.600	0.994-1.010	0.184	0.997-1.014	0.001**	1.006-1.025
PoultryNumber	0.555	0.981-1.010	0.035*	0.958-0.998	0.482	0.987-1.028
Max. Temp.	0.001**	0.891-0.971	0.468	0.935-1.032	0.000***	0.796-0.915
Min. Temp.	0.011*	0.769-0.966	0.162	0.795-1.039	0.334	0.948-1.171
Max. Moisture	0.919	0.967-1.031	0.187	0.943-1.011	0.713	0.958-1.065
Min. Moisture	0.167	0.991-1.053	0.063	0.922-1.002	0.000***	1.072-1.166
EVI	0.000***	15339.8-2.258E8	0.000***	152.714-4213030.2	0.024*	2.629-969351,703
NDVI	0.000***	822.9-543869.04	0.000***	509.2-716405.14	0.060	0.848-3529.14
DEM	0.005*	1.000-1.003	0.000***	1.006-1.009	0.390	0.997-1.001
MIR	0.000***	0.000-	0.026*	0.000-0.363	0.963	0.000-15631.00
LANDSAT NDVI	0.000***	111.4-4953969.04	0.000***	1.007-2.712E8	0.068	0.000-1.747
EMIS31	0.008*	0.000-0.005	0.000***	0.000-	0.008*	0.000-
EMIS32	0.292	0.000-264166.154	0.014*	22925.33-7.811E38	0.007*	7.864E12-1.371E8
LSTNIGHT	0.006*	0.611-0.921	0.000***	0.282-0.520	0.001**	1.257-2.547

EVI: Enhanced Vegetation Index, **NDVI:** Normalized Difference Vegetation Index, **EMIS:** Emissivity, **MIR:** Middle Infrared, **LSTNIGHT:** Land Surface Temperature of Night, **DEM:** Digital Elevation Models

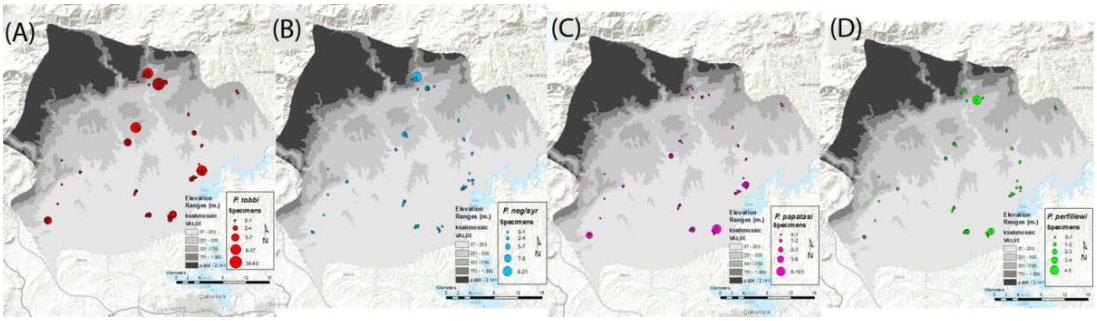

Figure 2. Sand fly distribution shown on an elevation model: (A) P. tobbi (B) P. papatasi (C) P. neglectus/syriacus (D) P. perfiliewi.

DISCUSSION

Environmental factors can be effective in ecological modification and in the behavior of sandfly vectors. In addition, the fauna in particular areas may undergo changes that can lead to the loss of biodiversity and/or an increase in the number of species[23,24]. Geographic information system and remote sensing, is used to compare the occurrence and epidemiological data of diseases to the environmental data. These technologies allow analysis and detection of spatiotemporal patterns by monitoring and mapping risk areas of diseases, especially vector-borne diseases[25].

Climatic, environmental and population changes can cause alterations in the ecology and the behavior of sandflies and in the epidemiology of leishmaniasis. Adana has become an immigration centre for Syrian refugees. Thus, it has been reported that cutaneous leishmaniasis incidence has increased in this area due to the Syrian refugees[26,27].

According to the previous entomological survey results18 probable sand fly species have been identified in Turkey[5,8,28,29]. Five of them (*P. papatasi, P. sergenti, P. neglectus/syriacus, P. perfiliewi*and *P. tobbi*) were detected in our study area[8,30]. Our results are associated with the results of previous studies in that there is a relationship between environmental factors and presence of vector species. On the other hand, we were not able to analyze *P. perfiliewi* statistical values accurately owing to the fewer than the expected number of the specimens.

We divided our data into two major classifications. The first group data came from the traps status, while the second group from environmental factors. (MODIS and non-MODIS). Trap habitat data were statistically significant for the presence of*P. tobbi* and *P. neglectus/syriacus* ($p<0.05$). Habitats especially, in the centre of the villages where more people lived are preferred to spread by anthropophilic sand fly species *P. tobbi* and *P. neglectus/syriacus*prefer the barns including animal dung is suspected to provide ideal conditions for sand fly breeding[31].However, in our study, we did not observe any relationship between presence of *P. perfiliewi* and trap habitat status data (Table 3).

In addition, we compared presence of sand flies with a lot of environmental data such as EVI and cattle number in the present study. *P. tobbi* distribution showed a positive correlation with five MODIS (EVI, NDVI, MIR, EMIS31 and LSTNIGHT) and five non-MODIS (DEM, LANDSAT NDVI, cattle number, maximum and minimum temperature) data groups ($p<0.05$).We also observed the relationship between the presence of *P. neglectus/syriacus*and six MODIS data (EVI, NDVI, MIR, EMIS31, EMIS32 and LSTNIGHT) and four non-MODIS data (poultry number, DEM and LANDSAT NDVI) ($p<0.05$). *P. tobbi* and *P. neglectus/syriacus* considered to be the probable vector species for cutaneous leishmaniasis are present in every altitude with different densities. Especially DEM data including the sand fly habitat altitude were reported, to be associated with the distribution of cutaneous leishmaniasis infections in humans[32].

The presence of low number of *P. perfiliewi* specimens in this study were resulted from three MODIS data (EVI, EMIS31 and EMIS32) and four non-MODIS data (ovine number, maximum temperature, minimum moisture and LSTNIGHT) (Table 3) (Figure 2).mong specific variables, trap location is not an important factor for the presence of the vector species. In other studies, *P. neglectus* species has been collected both from sylvatic and domestic or from peridomestic environments[33]. *P. neglectus* was found a higher presence at the edge of settlements in Aydin province, Turkey[12]. However, we did not determine any positive correlation between, the presence of all species and trap location type (Table 3).

Cattle, ovine and poultry number are of great importance in terms of visceral and cutaneous leishmaniasis reservoir. *P. sergenti*, *P. papatasi*, *P. major* and *P. syriacus* are considered to be the probable vectors, and dogs are the main reservoir of *L. infantum*, while *P. sergenti* is the main suspected vector of *L. tropica*[1,34,35].

We also observed that the effects of various geographical factors such as temperature, humidity, and emissivity on distribution of sand fly species in Karaisali (Table 3). Environmental factors are known to be common effect in sand fly distribution. It has been reported that especially, vegetation and altitude play an important role for determining the habitats and vector capacities of sand flies[12,14,36-39].

We could not determine all of Adana's sand fly fauna since it will take a very long time to work. However, our the relationship findings between sand fly distribution and geographical parameters provides us some predictions in terms of distribution of sand fly and leishmaniasis in Adana and its surroundings. In addition, we didn't dissect the sand fly midguts for monitoring the live promastigotes in the field.

Karaisali province has the richest sand fly fauna in Southern Anatolia. *P. tobbi* is a dominant sand fly species, and it could trigger CL transmission Yazibasi, Boztahta, Catalan and Altinova villages. We suggest that in addition to experimental laboratory studies the effects of sampling location and geographical factors on the distribution of sand fly species be determined to better understand the biology of vector species. Estimating the impact of emerging foci and dynamics of vectors and diseases may also be helpful.

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