Spatial Patterns and Temporal Trends of Human Leishmaniasis Incidence in Khemisset Province, Morocco

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ABSTRACT

Leishmaniasis are parasitic diseases caused by Leishmania species and transmitted by the phlebotomine sandflies. In Morocco, Leishmaniasis are a major public health problem with two clinical forms: cutaneous and visceral Leishmaniasis. Khemisset Province is one of the Moroccan regions where cases of both cutaneous and visceral Leishmaniasis were reported without official data about epidemiological status. Epidemiological and entomological investigations were conducted in Khemisset Province based on 20-year epidemiological data (1997-2016). The results showed the presence of both clinical forms of Leishmaniasis in the study area. All ages and both genders were affected by these different forms of the disease. Five sandfly species were identified morphologically in the study area with the dominance of Phlebotomus sergenti (61.11%). The Province of Khemisset should be regarded as a potential focus for Leishmaniasis in the region because of the presence of all the components of the disease transmission cycles. It is suggested that the competent authorities monitor the Khemisset area constantly to prevent the danger and spread of this epidemic.

Key Words: Leishmaniasis, Phlebotomy, Sandfly, Epidemiology, Morocco, Epidemic

INTRODUCTION

Leishmaniasis is a complex of diseases caused by Leishmania species and transmitted by a Phlebotomine sandfly (Diptera:Psychodidae). These parasitic diseases present a wide range of clinical symptoms and epidemiological entities. It is among the second most apparent re-emerging vector-borne disease, after Malaria; that currently threaten 350 million people in 88 countries. In Morocco, Leishmaniasis outbreaks are endemic and constitute a major public health threat. The cutaneous (CL) and visceral (VL) forms coexist with three species of parasites (Leishmania major, L. tropica and L. infantum) which share responsibility for cases of cutaneous leishmaniasis, while only L. infantum is responsible for the visceral.

Three major sandfly vectors were found associated with dynamics of VL across Morocco; Phlebotomus ariasi, P. perniciosus and P. longicuspis, P. papatasi and P. sergenti are the proven vectors of L. major and L. tropica, respectively. Several mammals’ reservoirs were associated with the disease transmission across the country; dogs for zoonotic VL, rodents for zoonotic CL and human for anthroponotic CL.

The problem of Leishmaniasis has been identified as a priority for the national Ministry of Health. Since 1995. Leishmaniasis has become a notifiable disease and in 1997 our country invested in the fight against these diseases by implementing an action plan with general objectives as early management of cases of VL and disease control in outbreaks of cutaneous leishmaniasis.

Khemisset is one of the Moroccan regions where epidemiological status of human Leishmaniasis is unknown. In spite of the presence of canine Leishmaniasis, and its position beside the active Leishmaniasis outbreaks in central Morocco: in Fez-Meknes region, and in Benimellal-Khenifra region, no data about entomological or epidemiological investigations, in Khemisset region, were recorded.

The objective of our investigation is to study, for the first time, the epidemiological status of leishmaniasis in Khemisset province where cases of both forms of human leishmaniasis have been recorded.

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MATERIALS AND METHODS

Study area:
Administratively, Khemisset Province is part of Rabat - Salé - Kenitra region (Figure 1), the total area of the province is 781,000 hectares, of which 47.7% is agricultural land, 40.6% forest and 11.7% rangeland and wasteland. The province is made up of 4 Circles, 32 rural municipalities and three urban municipalities. In 2014, the total population was 542,025 inhabitants with the majority in rural municipalities.

The dominant climate type in the region is semi-arid to arid with some years characterized by a sub-humid climate when annual rainfall exceeds 600mm/year. Geographically; Khemisset province is composed of two entities: plateaus and depressions.

Epidemiological data:
The present study is a retrospective analysis of the leishmaniasis status at Khemisset province. Epidemiological data were obtained from the register of the Laboratory of Epidemiology and Environmental Health, in the local Delegation of Ministry of Health. The authorization has been obtained from the Regional and local Health Department to examine these registers.

All microscopic preparations concerning leishmaniasis cases, carried out in the different health districts of Khemisset Province, are sent and archived in this laboratory. Thereby, we collected 20-year epidemiological data (1997-2016) for analysis.

Entomological data:
Specimens were collected from ten stations in the study area using 30 sticky traps by station during the entomological season (May-July 2018). Thus, traps were placed in the evening, in different biotopes, and recovered the next morning. Traps were prepared with A4 sheets coated with castor oil. The collected specimens were stored in 70% alcohol for lightening and rapid assembly in the chloral balm for species identification.

The identification was made morphologically by examining the male genitalia, female spermathecae and pharynges based on the key for the determination of Moroccan sandfly Mahjour et al. (1997), and referring to Boussaa Samia, (2008), for the identification of species of the subgenus Larroussius.

RESULTS

Epidemiological status of leishmaniasis in Khemisset:
According to epidemiological data, both forms of leishmaniasis (CL and VL) were recorded in Khemisset Province (Table 1). According to epidemiological data (Table 1), both forms of leishmaniasis (CL and VL) were recorded in Khemisset Province. The rural area is more affected by leishmaniasis cases (76%) compared to (15%) in urban areas. The epidemiological profile of leishmaniasis in our study area assumes that the disease affects both sexes and different age groups (Figure 2). The epidemiological profile of leishmaniasis in our study area assumes that the disease affects both sexes and different age groups (Figure 2). Figure 3 shows the distribution of leishmaniasis forms according to age.

Spatiotemporal trends of leishmaniasis in Khemisset:
Figure 4 presents maps illustrating the spatial evolution of visceral leishmaniasis (a) and cutaneous leishmaniasis (b) in Khemisset Province based on epidemiological data. Analysis of secular trend of leishmaniasis (both VL and CL) cases (Figure 5) shows that the highest number of 6 cases was noted in 2010. The seasonal fluctuation of leishmaniasis cases recorded in the study area (Figure 6) shows difference in the number of visceral leishmaniasis cases recorded by season.

Sandfly species composition in Khemisset:
Thus, our entomological investigations were conducted in urban and rural municipalities of Khemisset Province where leishmaniasis cases were detected in the study area as shown in Table 2. The study area covered an altitude range of 225-1221m.

Table 3 shows the percentages of different species collected in our study area where five species were morphologically identified. Five species were identified morphologically. Phlebotomus Sergenti was the most dominant species (61.11%), followed by P. longicuspis (20.37%), Sergentomyia yamiuta (11.11%), P. ariasi (5.56%) and P. perniciosus (1.85%).

DISCUSSION

As noted recently in Morocco, both forms of leishmaniasis coexist in several northern and central regions, such as Taza, Moulay yaacoub, Sidi Kacem, Chefchaoune, El Haouz and Lhocaïma. In the study area, the recorded VL cases are due to Leishmania infantum while L. tropical and L. major are responsible for the cases of CL. The visceral leishmaniasis by L. infantum is the dominant form (52%) of leishmaniasis in the province. Results suggesting the local transmission cycle since Natami et al. (2000), reported canine leishmaniasis by L. infantum this area with a serape valence of 16.71%. Concerning the rural area is more infected with leishmaniasis than the urban area, this results is in line with the WHO reports confirming the rural nature of this disease. Thus far, leishmaniasis affects the rural sector more than the urban sector in Khemisset Province.
Morocco, as confirmed by different investigations in leishmaniasis foci in northern and central Morocco.17

The results show that there is no relationship between sex and leishmaniasis. It is statistically confirmed, whether visceral (P=0.19) or cutaneous (P=0.23) form. But results confirmed a relationship between leishmaniasis and patient age. Our result is in agreement with those of other works. In Morocco as 81% of VL cases are less than 9 years old (Mahjour et al. 1997).3 In Tunisia, Aounet al.(2009), concluded that children under five years old are the most affected group by VL. Globally, VL is known as infantile disease but it can affect adults, particularly in HIV-Leishmania co-infection condition.3, 18, 19 On the other hand, the age-specific risk depends on the parasitic species and the population’s exposure history. Thus, in endemic area of L. infantum, the median age of clinical cases of VL tends to be lower (generally < 5 years), while it occurs in adults mainly in cases of severe immune deficiency.20, 21

Concerning CL distribution according to age groups (Figure 3), 52% of cases range between 15 and 49. These results are consistent with the results found in other regions in Morocco by Arroub et al. (2012), in Tunisia by Abda et al. (2010) and in Iran by Fazaeli et al. (2009) showing that the young population is the most affected by CL.22-24

For the visceral form we can see that in 1997, five municipalities were affected by the disease while between 1998 and 2016, the number of affected municipalities increased from 5 to 14 (Figure 4-a) For cutaneous form we can see that in 1997, one municipality was affected by the disease while between 1998 and 2016, the number of affected municipalities increased from 1 to 10 (Figure 4-a). As a secular trend of leishmaniasis cases (both LV and CL) the highest number of 6 cases was noted in 2010 as well. According to the same distribution (Figure 5), only VL was recorded between 1997 and 1999, while CL was introduced since 2000. In 1997-2016 period, VL cases peaked in 2003 whereas CL cases peaked ten years later in 2013 (Figure 5). We noted also the dominance of the visceral form until 2010, then, CL cases became more frequent.

Most cases of visceral leishmaniasis were detected in December and July. This seasonality could be related to the seasonal dynamics of vector species and the incubation period of the disease. For cutaneous leishmaniasis, the recording of cases is spread over a precise period, with an absence of cases in April, August and September. Studying the sandfly species composition and its bionomics is an interesting tool for a better understanding of the disease transmission dynamic and it contributes to the planning for the prevention and control against leishmaniasis particularly in the areas where the risk of leishmaniasis extension is thought to be significant.

The vector species for two clinical forms of leishmaniasis in Morocco coexist in our study area; namely Phlebotomus sergenti, the proven vector of CL caused by L. tropica and the three species (P. longicuspis, P. ariasi and P. perniciosus) of VL caused by L. infantum.4 Results explain the presence of cases of both clinical forms of leishmaniasis in the Khemisset Province and confirm the hypothesis of local transmission cycles. The Zorisk of leishmaniasis can be characterized by vector abundance (The abundance of vector species is an important risk factor for characterization of a leishmaniasis endemic area). According to biotopes, entomological data (Table 1) shows the presence of sandfly in both urban and rural areas, with altitudes between 225 and 1221m. The five species were collected in rural area; while only P. sergenti collected in urban area. The sandfly species diversity in rural areas is favored by the presence of reservoir animals coupled with poor hygiene and sanitation facilities, and the abundance of organic matter.2

In Morocco, sandfly seasonality is widely studied. In semi-arid area of Marrakech (at 466m), P. sergenti was active especially during the period of April–May–June and absent in the rest of the year.13 In semi-arid area of Chichaoua Province (at 1148 m), P. sergenti population reached peak density during July and August and was lowest from September through November.25

For Larroussius species activity, in semi-arid area of Ourika (at 850 m), P. perniciosus, P. longicuspis and P. ariasi activities show a bimodal seasonal trend with two abundance peaks, in summer (May–June–July) and in autumn (September–October), while in Yabora (at 1155 m), their activities show a mono-modal annual pattern with a long activity period extending from March to November with the highest density recorded in June.25 P. sergenti and Larroussius species activities determine necessarily the seasonal fluctuation of leishmaniasis cases recorded in the study area (Figure 6).

CONCLUSION

The number of cases recorded in our study area is not alarming but obviously informative on the local presence of all the components of the disease transmission cycle. Consequently, the Province of Khemisset should be regarded as a potential focus for leishmaniasis because of the following tangible reasons:

- Leishmania species, confirmed by recorded human cases;
- Animal reservoir, confirmed by canine leishmaniasis cases;
- Proven vectors of CL and VL in Morocco
- Active human leishmaniasis foci in the region with human population movements;

Taking into consideration the under-reporting of human cases, particularly in rural areas, the results of the present study are enough for a continuous epidemiological surveillance to prevent the risk.
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Authors’ Contribution:

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| Hanna Marwa         | Writing the article                               |
| Boussaa Samia       | Critical revision of the article                  |
| Raghay Kawtar       | Data analysis and interpretation                  |
| Marbouch Insaf      | Interpretation of data                            |
| Mohamed Fadli       | Final approval of the article                     |

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Table 1: Leishmaniasis cases dispatched by form and gender

| Year | Visceral leishmaniasis cases | cutaneous leishmaniasis cases |
|------|-------------------------------|-------------------------------|
|      | Female | male | Female | male |
| 1997 | 1      | 0    | 0      | 0    |
| 1998 | 0      | 1    | 0      | 0    |
| 1999 | 0      | 2    | 0      | 0    |
| 2000 | 0      | 2    | 0      | 1    |
| 2001 | 0      | 0    | 0      | 0    |
| 2002 | 1      | 0    | 0      | 0    |
| 2003 | 1      | 3    | 0      | 1    |
| 2004 | 0      | 0    | 0      | 1    |
| 2005 | 0      | 0    | 0      | 0    |
| 2006 | 0      | 2    | 0      | 0    |
| 2007 | 2      | 1    | 0      | 0    |
| 2008 | 0      | 2    | 0      | 0    |
| 2009 | 0      | 0    | 0      | 0    |
| 2010 | 0      | 0    | 0      | 0    |
| 2011 | 0      | 0    | 2      | 1    |
| 2012 | 1      | 1    | 1      | 1    |
| 2013 | 0      | 0    | 3      | 2    |
| 2014 | 1      | 0    | 1      | 0    |
| 2015 | 0      | 1    | 3      | 0    |
| 2016 | 0      | 0    | 0      | 2    |
| Total| 7      | 15   | 10     | 9    |

Table 2: Entomological data in the province of Khemisset

| Area    | Station  | Altitude (m) | The percentages of phlebotomes collected from each area | number of specimens collected | density per m² | Sex-ratio (M/F) | Sandfly species | Dominant species |
|---------|----------|--------------|--------------------------------------------------------|-----------------------------|----------------|----------------|----------------|------------------|
| Urban   | Khemisset| 451          | 7.14                                                   | 4                           | 6.45           | -              | Phlebotomus sergenti | P. sergenti     |
| Rural   | Maaziz   | 411          | 3.57                                                   | 2                           | 3.22           | -              | Phlebotomus longicuspis | P. longicuspis |
|         | Ait ouribel | 471         | 10.71                                                  | 6                           | 9.6            | 5              | P. sergenti     | P. sergenti |
|         | Sfassif  | 391          | 26.7                                                   | 15                          | 24.1           | 0.85           | Sergentomyia minuta | P. sergenti |
|         |          |              |                                                        |                             |                |                | P. longicuspis | P. longicuspis |
|         | Oulmess  | 1221         | 1.78                                                   | 1                           | 1.61           | -              | P. sergenti     | P. sergenti |
|         | Ain johnra | 225         | 3.57                                                   | 2                           | 3.22           | -              | P. sergenti     | P. sergenti |
|         | Brachoua | 247          | 21.42                                                  | 12                          | 19.35          | 1.4            | P. sergenti     | P. longicuspis |
|         | Ain sbit | 586          | 19.64                                                  | 11                          | 17.7           | 4.5            | S. minuta       | P. longicuspis |
|         |          |              |                                                        |                             |                |                | P. sergenti     | P. longicuspis |
|         | Ait mimoun | 664         | 5.35                                                   | 3                           | 4.83           | 2              | S. minuta       | S. minuta |
|         |          |              |                                                        |                             |                |                | P. sergenti     | P. longicuspis |
Table 3: percentages of different species collected in study area

| Species                              | Percentage |
|--------------------------------------|------------|
| Phlebotomus sergenti                 | 61.11 %    |
| Phlebotomus longicuspis              | 20.37 %    |
| Phlebotomus Sergentomiya minuta      | 11.11 %    |
| Phlebotomus ariasi                   | 5.56%      |
| Phlebotomus perniciosus              | 1.85%      |

Figure 1: Map of Rabat Salé Kenitra region, specifying the study area.

Figure 2: Distribution of visceral (a) and cutaneous (b) leishmaniasis cases according to gender in Khemisset period 1997-2016.

Figure 3: Distribution of cases of cutaneous (a) and visceral (b) leishmaniasis according to age groups in Khemisset Province period 1997-2016.
**Figure 4:** Spatiotemporal distribution of cutaneous (a) and visceral (b) leishmaniasis cases in Khemisset Province between 1997 and 2016.

**Figure 5:** Evolution of number of visceral (a) and cutaneous (b) leishmaniasis cases recorded in Khemisset Province between 1997 and 2016.

**Figure 6:** Distribution of leishmaniasis cases by month in Khemisset Province from 1997 to 2016.