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Epidemiological and parasitological approaches to human leishmaniasis in the Meknes region, Morocco

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Abstract: Leishmaniasis is a parasitic disease common to humans and animals, caused by the Leishmania parasite. It represents a major public health problem in Morocco and in many tropical and subtropical areas of the world. These parasitoses are transmitted by infected sandflies and are influenced by environmental, social, and biological factors. The aim of our research is to assess the epidemiological situation of leishmaniasis and to analyze the spatio-temporal distribution of new cases of leishmaniasis in the Meknes region. In light of this work, we carried out a retrospective study after Covid-19, collating all cases of cutaneous and visceral leishmaniasis recorded in the Meknes region. These epidemiological data were collected from special registers at the health establishment network service. In total, we collected thirty-one (31) registered infected cases. These epidemiological data show that there is a coexistence of the two forms of leishmaniasis: cutaneous (83.87%) and visceral (16.13%), with a predominance of rural areas. The sex ratio was 0.82, and the average annual incidence was 9 cases per year. These parasitoses affect all age groups and are not influenced by sex. Moreover, visceral leishmaniasis only infects cases under nine years of age (< 9). To combat these leishmaniases, especially in rural areas, it is crucial to adopt different epidemiological, parasitological, and sociological approaches.

Keywords: Cutaneous leishmaniasis, Epidemiological approach, Meknes, Parasitological approach, Visceral leishmaniasis.

1. Introduction

Leishmaniasis is an infectious disease caused by various parasites of the genus *Leishmania*. There are three main forms of the disease: cutaneous, mucocutaneous and visceral, also known as kala-azar. According to the World Health Organisation, these diseases affect almost 12 million people, with 700,000 to one million new cases reported each year. The incidence and prevalence of Leishmania vary according to geographical location and socio-economic conditions, with a predominance in tropical and subtropical areas. Leishmaniasis is transmitted by a complex cycle involving insects known as sandfly vectors and mammalian hosts, including humans. Mammalian hosts infected with the *leishmania* parasite play an essential role in the persistence and transmission of the infection in infected ecosystems. Phlebotomine vectors belong mainly to the genera *Phlebotomus*, which is characteristic of the Old World, and *Lutzomyia*, which is characteristic of the New. The best- known example is the vector of *Leishmania* in tropical regions, *Phlebotomus papatasi*, which is involved in the transmission of cutaneous leishmaniasis, while *Lutzomyia longipalpis* is the main vector of visceral leishmaniasis in Latin America. Geographical distribution, as well as the biology and behaviour of the vectors, are determining factors in the transmission of *Leishmania*.

2. Methodology

2.1. Study Environment

This study is being carried out in the Meknes region, which covers an area 1,786 km² according to the 2015 administrative boundaries. The region is characterised by two main geographical areas: the Saïs plateau and the Zerhoun pre-rifine hills, and by a semi- continental Mediterranean climate, with cool, rainy winters and hot, dry summers. According to the 2024 General Census of Population and Housing, the legal population of the province of Meknes is around 562,000, with an average annual growth rate of 0.79% between 2014 and 2024, which is lower than in other regions of Morocco. This rate is lower than the national average (0.85%) and that of the Fez-Meknes region (1.14%). This demographic slowdown, combined with urban growth, has led to the construction of new urban housing, some of which is still vulnerable, particularly on the outskirts of the city of Meknes and within the urban fabric. The province of Meknes is made up of 6 urban communes and 15 rural communes, representing 18.2% and 9.3% respectively of the total for the same type of commune in the Fez-Meknes region [1].



Figure 1. Administrative division of the Fez-Meknes region.

2.2. Materials and Methods

This research study recorded all new cases of cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) registered in the Meknes region during the period 2020-2023. The data for this retrospective study were collected from epidemiological bulletins published on the official website of the Moroccan Ministry of Health, covering the years 2020 to 2023 (Ministry of Health. However, these documents only provide the total number of cases of leishmaniasis reported in the Meknes region, without distinguishing between types of leishmaniasis or the socio-demographic characteristics of

infected individuals, such as age, sex, living environment (rural or urban), classification of cases (indigenous, imported, paradoxical) and type of screening (positive or negative). In order to complete the study data, additional information was collected from the case registers available at the Meknes SRES health facilities network department.

The epidemiological data was then analysed and interpreted using various statistical representations, such as tables, graphs and curves. The incidence rate of leishmaniasis calculated, corresponding to the number of new cases detected per 100,000 inhabitants in a given exposed population over the course of a year.

The statistical analysis of the data collected was carried out using Excel software (Microsoft), version 2010, to diagnose and interpret the epidemiological results relating to leishmaniasis in the Meknes region.

3. Results

This section of our research presents two complementary aspects, one epidemiological and the other parasitological, based on diagnosis and evolution of scarring in infected individuals.

The first part focuses on an epidemiological study of new cases recorded in the Meknes region.

3.1. Annual Distribution of Leishmaniasis According to Environment (2020-2023)

Figure 2 shows the results of our retrospective analysis of the annual incidence of leishmaniasis.



Urban environment Rural environment

Figure 2.

Annual distribution of leishmaniasis according to environment (2020-2023)

Analysis of this figure shows that leishmaniasis is more prevalent in rural areas than in urban ones. This increase is due to the appearance and spread of environments favourable to the development of vectors of the *leishmania* parasite, in particular sandflies. During this research period, we noted that the number of new cases of leishmaniasis in the urban environment did not exceed 1 case/year, except for the year 2021. However, the number of new cases of leishmaniasis reached a maximum value (7 cases/year). This difference can be explained by awareness campaigns for the local population and the application of the ministerial programme to combat leishmaniasis [2].

In some cases, the population of rural origin settles in urban areas and brings domestic animals with it, and may even breed livestock on the spot, providing suitable environments for the multiplication of

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sandflies that carry the *leishmania* parasite [3].

These results are somewhat similar to those of the study we carried out during the period 2014_2019 in the Meknes region, which showed that certain peri-urban and urban stations are at the origin of the appearance of this form of parasitic disease.

3.2. Distribution of Cutaneous Leishmaniasis Cases by Environment (2020_2023)

Figure 3 shows the distribution of cases of cutaneous leishmaniasis by area, with 83.33% in rural areas and 16.66% in urban areas.



Distribution of cutaneous leishmaniasis by environment (2020-2023).

Analysis of this graphic presentation largely confirms the previous results: rural areas contain environmental conditions that favour the multiplication of insect vectors of leishmania parasites and leading to the appearance of a high number of new cases of cutaneous leishmaniasis [3].

3.3. Breakdown of New Cases of Visceral Leishmaniasis by Environment (2020-2023)



Distribution of visceral leishmaniasis environment (2020-2023).

The figure above shows that visceral leishmaniasis occurred only in rural areas, with a percentage of 100%.

These results show that the rural environment contains both forms of cutaneous and visceral leishmaniasis, with remarkable epidemiological rates.

3.4. Indigenous Spatio-temporal Distribution of Cases of Cutaneous Leishmaniasis by Municipality (2020-2023)



CU Meknes: urban district of Meknes; S.A. Khayat: Sidi Ablellah Khaya Figure 5. Distribution of cases of cutaneous leishmaniasis by commune (2020-2023).

Analysis of this graph shows that most of the communes are affected by cutaneous leishmaniasis with the exception of Ouisslane, Sidi Abdellah Khayat and Boufekrane. The communes of Oualili, Mhaya, Oued jdida and Moulay Driss zerhoun recorded only one new case of cutaneous leishmaniasis during the study period (2020-2023). The number of cases of cutaneous leishmaniasis (CL) varies from one commune to another and does exceed 2 cases/year in any of the communes in the study area.

3.5. Spatial and Temporal Distribution of Indigenous Cases of Visceral Leishmaniasis by Commune (2020-2023)



Figure 6.

Distribution of visceral leishmaniasis cases by commune (2020-2023).

The graph illustrates the spatial distribution of visceral leishmaniasis by commune. Visceral leishmaniasis is more prevalent in rural areas than in urban ones.

Figure 6 shows that the highest number of new cases of visceral leishmaniasis is recorded in the communes of Mhaya, Nezala bni Ammar and Dkhissa (1 case/year). However, the incidence rate of visceral leishmaniasis is zero in the other communes of Oualili, Oued jdida, Mejjat, Sidi Bouzekri, Al Anouar and Toulal (0 cases/year).

3.6. Monthly Trends in Cases of Cutaneous and Visceral Leishmaniasis (2020-2023)



Figure 7. Monthly change in cases of cutaneous and visceral leishmaniasis (2020-2023)

According to the graph analysis above, there is a very remarkable peak in visceral and cutaneous leishmaniasis in February (11 cases), followed by simultaneous variations in cases of leishmaniasis (LC) and (LV) during the months April to December. The variation observed did not exceed 3 cases/month for each form of leishmaniasis [4].

3.7. Breakdown of New Leishmaniasis Cases Age, Sex and Type of Leishmaniasis

Table 1.	
Distribution of leishmaniasis according to age, sex	and type of leishmaniasis

Age Total			otal	Children	Children	Adult	Adult [30-39]	Adult [40-49]	Adult [50-59]	Aged
Туре		N= 31		[0-9]	[10-19]	[20-29]	N=1 (3,22%)	N=4 (12,90%)	N=5 (16,13%)	[60 and over]
		(10	00%)	N=8 (25,80%)	N=7 (22,58%)	N=3 (9,67%)				N=3 (9,67%)
Cutaneous	Male	13	41.93	3	7	3	1	4	5	3
leishmaniasis (C.L.)	Female	13	41.93	9.67%	22.58%	9.67%	3.22%	12.90%	16.13%	9.67%
Visceral leishmaniasis	Male	1	3.22							
(V.L.)	Female	4	12.90	5						
				16.13%	0	0	0	0	0	0

The table above shows the distribution of cases of cutaneous and visceral leishmaniasis according to age, sex and type of leishmaniasis. Cutaneous leishmaniasis affects all visceral leishmaniasis infects mainly the 0-9 age group, with a percentage of 16.13% for both sexes. Cutaneous leishmaniasis and visceral leishmaniasis are frequent in females, with percentages of 41.93% and 12.90% respectively.

The second part focuses on a parasitological study based on diagnosis and evolution of the healing of skin lesions.

3.8. Parasitological Diagnosis

The method used to diagnose cutaneous leishmaniasis in the diagnostic laboratory for parasitic diseases in the province of Meknes is the parasitological examination, which consists of direct microscopic identification of the leishmania body (amastigote form) after staining with MAYGRÜNWALD GIEMSA (MGG).

All the skin smears and stains will be read by a specialist team to identify the leishmaniasis body at the provincial laboratory and determine the species of parasite at the National Institute of Hygiene in Rabat.

Dermal sampling is carried out either by scraping the lesions or by skin biopsy. During this sampling phase, we used a document under the name "Individual Examination Voucher" for each patient with cutaneous leishmaniasis [5].





Figure 8.

(a; b): Scraping of a cutaneous leishmaniasis lesion on the nose (health centre, 2023)

The images below show the different types of skin lesions in patients who came to the health centre before the Covid19 period.



A: ulcero-vegetative lesion



B: ulcerative plaque

C: papulo-nodular lesion



D: ulcer-crust nodule

E: ulcerative lesion



F: ulcerative lesion Figure 9. Size and appearance of lesions in patients (health centre, 2019)

3.9. Wound Healing in a Person Affected by Cutaneous Leishmaniasis

In this section, we followed a treatment protocol involving the use of peri-lesional injections of Glucantime® in the nose of an elderly woman. This treatment concerns all cutaneous leishmaniasis lesions of which there are fewer than 5 and each of which is less than 4 centimetres in diameter.





This treatment consists of a peri-lesional injection into healthy skin, 1cm from the edge of the lesion, using a syringe fitted with a fine needle (insulin syringe type), of 1 to 3 ml of the product per session. The quantity to be injected depends on the size of the lesion. The treatment is repeated 2 times a week until complete healing is achieved, which generally takes 2 to 4 weeks. Infiltration should involve entire inducated peripheral zone at the base of the lesion, where the density leishmania is very high (Figure 11).



a: Glucantime injection; Figure 11. Technique for peri-lesional injection of 1.5ml of Glucantime®.



b: Theoretical technique for peri-injury injection

4. Discussion

The data collected during this study highlights the importance of an integrated approach to controlling human leishmaniasis, whether cutaneous or visceral. These parasitoses generally affect both sexes and all age categories with the exception of visceral leishmaniasis, which only infects individuals under the age of 9. This finding confirms the results obtained during research carried out by BENCHAHID and colleagues during the 2014–2019 study period in the Meknes region.

With regard to the progress of healing, we noted that the use of peri-lesional injections of 1.5ml of Glucantime®, twice a week, in an elderly woman gave impeccable results after 4 weeks. This enhances and confirms the results obtained by Mr. Zakaria Al Alaoui and his colleagues (from 2 to 8 weeks of treatment) in 2017 [6].

5. Conclusion

Cutaneous leishmaniasis, which often progresses slowly and heals spontaneously, leaving unsightly scars, remains a real public health problem in our country. The incidence of new cases and the severity of the disease are influenced by eco-epidemiological conditions and the immune status of patients affected by this parasitosis, with the possibility of secondary bacterial superinfection. Faced with the emergence of severe and resistant forms of the disease, it is crucial to step up prophylactic measures by combating reservoirs and the spread of the disease, in particular parasite vectors. In addition, it is essential to optimise treatment by adopting standardised protocols and a consensus-based reference system.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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