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Epidemiological situation of leishmaniasis during the period 2020-2024 in the province of Ouezzane Northern Morocco

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Abstract: Leishmaniasis is a parasitic disease caused by protozoa of the genus Leishmania, transmitted to humans through bites of infected sandflies. In Morocco, this disease remains a significant public health concern, with an uneven distribution across different regions. The province of Ouezzane, situated in northern Morocco and characterized by mountainous terrain, a sub-humid climate, and a predominantly rural population, provides an environment conducive to the maintenance and transmission of leishmaniasis. Therefore, studying the epidemiological situation of leishmaniasis in Ouezzane is crucial not only for understanding the dynamics of disease transmission but also for guiding effective prevention and control strategies. An epidemiological study conducted in Ouezzane between 2020 and 2024 indicates a predominant occurrence of cutaneous leishmaniasis, accounting for 97.7% of cases, over the visceral form. The disease primarily affects rural areas, with notable outbreaks in Teroual, Zghira, and Sidi Bousber. Lesions are mainly located on the face and typically present as a single lesion, suggesting the predominance of Leishmania major. In contrast, multiple lesions, which are less common, may indicate the presence of Leishmania tropica. The disease mainly impacts children aged 0–14 years and, to a lesser extent, women. However, it is expected that by 2024, the gender distribution will become more balanced. Over the five-year period, the number of cases has doubled, reflecting both increased awareness and improved screening efforts, largely due to campaigns organized by the Ministry of Health.

Keywords: *Health, Leishmaniasis, Morocco, Ouezzane.*

1. Introduction

Leishmaniasis is a parasitic disease caused by protozoa of the genus Leishmania, transmitted by sand flies (Diptera: Psychodidae) [1]. It is one of the five priority diseases identified by the WHO [2]. Considered an emerging disease, it is closely linked to environmental conditions [3].

The leishmaniasis pathogen complex (parasite, vector and reservoir) develops in a geographical area determined by various bioclimatic parameters. Environmental changes influence the dynamics and spread of transmission foci. These disturbances can be anthropogenic or climatic in origin, often intertwined. The emergence of leishmaniasis is thus directly correlated with an increase in risk factors, mainly of human origin [4]. Climate change also plays a decisive role in the spread of vector-borne diseases, including leishmaniasis [5].

In Morocco, leishmaniasis was first described by Remlinger [6]. It became a cause for concern in the 1970s, with the launch of eco-epidemiological research programmes that provided a better understanding of the parasite cycles and an in-depth study of sand flies, mainly from a qualitative perspective [7-10].

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Our study will be based in the province of Ouezzane, in north-western Morocco, to highlight the epidemiological situation of the risk of leishmaniasis by identifying the forms and species of Leishmania and determining its clinical and epidemiological aspects in this region during the period 2020 to 2024. This study of leishmaniasis in its natural environment is therefore necessary in order to establish a plan to combat leishmaniasis in Ouezzane based on the results and discussions obtained.

This work is being carried out in collaboration between the Natural Resources and Renewable Energy Laboratory at the Faculty of Sciences in Kenitra and the Leishmaniasis Laboratory at the Provincial Hospital in Ouezzane.

2. Study Area

2.1. Geographical and Climatic Conditions

The study area covers the province of Ouezzane, located in north-eastern Morocco (Figure 1), which is protected from Atlantic influences by the surrounding medium-altitude mountains and enjoys a sub-humid Mediterranean climate with a dry summer season with temperatures ranging from 19°C to 32°C, and a cold to mild winter with temperatures ranging from 6°C to 14°C. The average annual rainfall is 800 mm. The distribution of this rainfall is irregular [11].

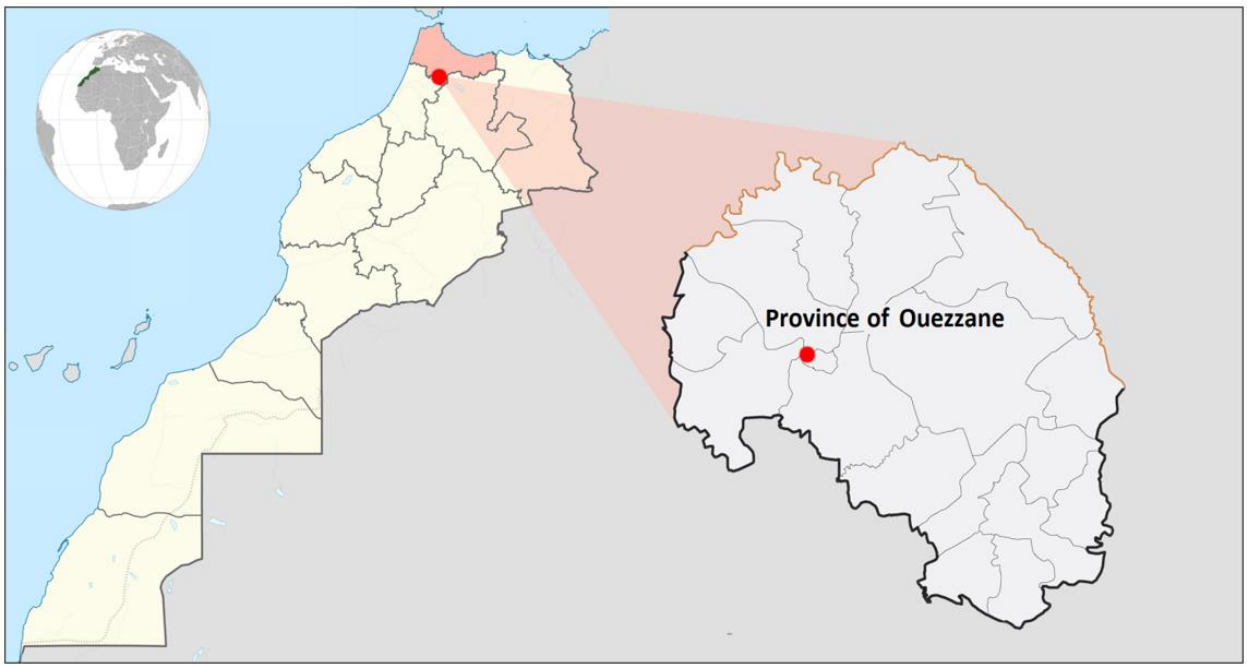

Figure 1.
The location of the province of Ouezzane on the national scale of Morocco.
Source: El Ghrifi [12].

2.2. Administrative and Health Situation

According to Moroccan administrative subdivisions, the province of Ouezzane is bordered by the province of Larache to the north-east, the province of Chefchaouen to the north-west, the province of Taounate to the east and the province of Sidi Kacem to the south. Its total area is 2,038.87 km2, comprising 17 municipalities, which are shown in (Figure 2) [11].

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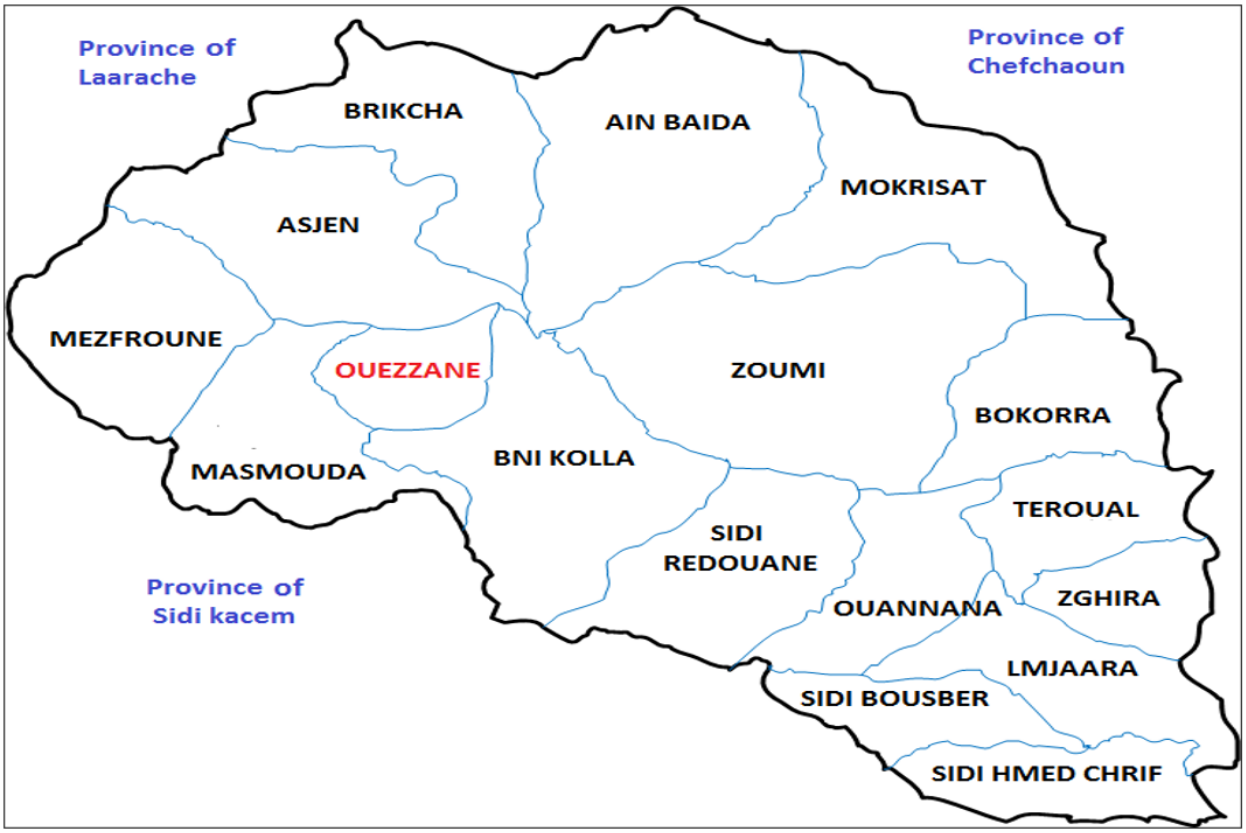

Figure 2.
Administrative and health subdivisions of the province of Ouezzane.
Source: El Ghrifi [12].

3. Study Methods

3.1. Identification of Leishmaniasis

3.1.1. Sampling

3.1.1.1. Cutaneous Leishmaniasis

Scraping is performed using a sterile vaccine needle in the infiltrated area, away from superinfected areas (Figure 3.a). The scraping and collection of serosities will be used to prepare smears and for cultures (Figure 3 b and c).

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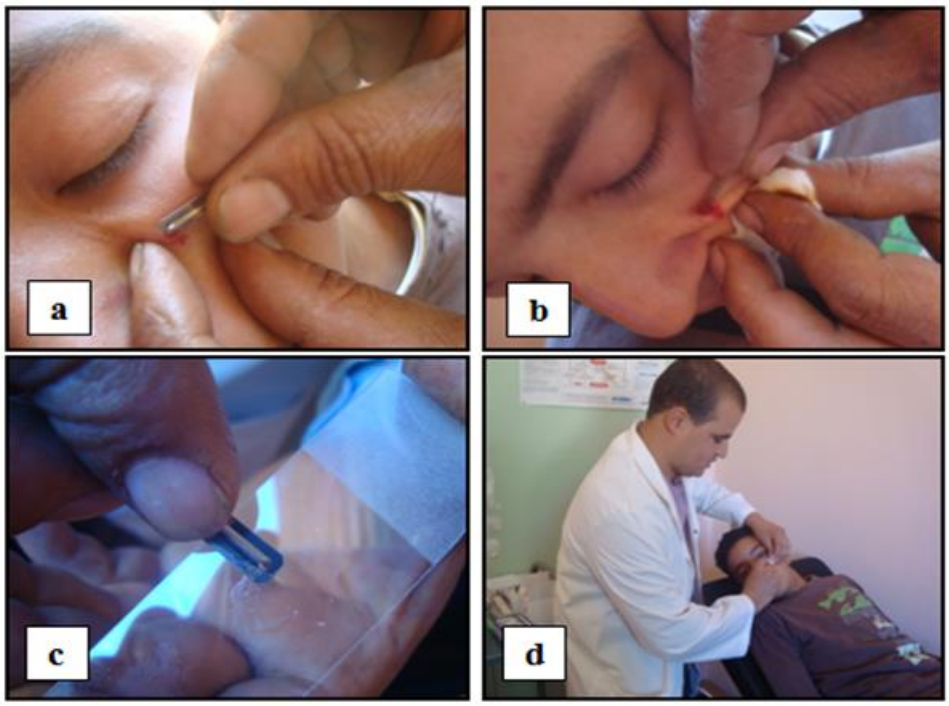

Figure 3.
Steps involved in scraping the lesion.
Source: El Ghrifi [12].

3.1.1.2. Visceral Leishmaniasis

Bone marrow sampling is the first-line treatment. It is performed either by sternal puncture or iliac crest puncture.

The procedure is painful and must be performed by experienced medical staff in a hospital setting, with strict adherence to aseptic techniques.

The bone marrow sample will be used to prepare thin smears or for culture.

3.1.2. Detection of Parasites on the Smear

This operation takes place in the leishmaniasis laboratory at the provincial hospital in Ouezzane. It requires:

3.1.2.1. Giemsa Staining

The skin serous smears are fixed and stained with GIEMSA (Figure 4 a). This type of staining is carried out in the following steps:

- Fixing the smear with methyl alcohol for 2 to 5 minutes,
- Drying the slides,
- Staining with 10% Giemsa solution in distilled water or buffered water at pH= 7 for 15 to 20 minutes (Figure 4 b).
- Wash the slides with distilled water (Figure 4 c).
- Dry and then examine under a microscope with a 100x immersion oil objective (Figure 4 d) [12].

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Figure 4.
Steps in Giemsa staining.
Source: El Ghrifi [12] Leishmaniasis Laboratory, Ouezzane Provincial Hospital.

3.1.2.2. Slide Preservation

- Use Eukit diluted with xylene (or Canada balsam).
- Place 2 drops of diluted Eukit on the smear,
- Cover with a coverslip, avoiding the formation of air bubbles,
- Leave the slides to dry and then store them in a clean box [12].

3.1.2.3. Microscopic Examination of Parasites

Leishmania parasites are examined under an optical microscope (100 x magnification) using immersion oil, scanning the slides in a zigzag pattern vertically and horizontally (Figure 5a). If the patient's slides are positive, the leishmaniasis prescription form is completed with the required information (Figure 5 b and Figure 6)

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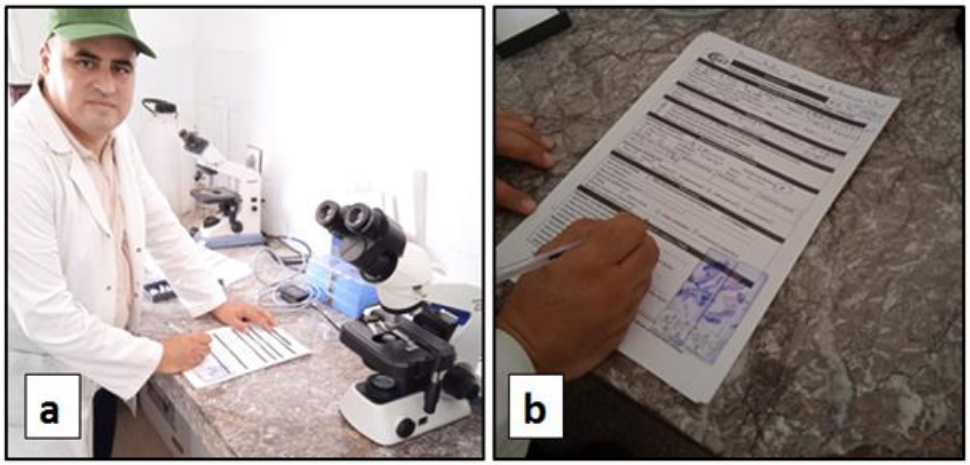

Figure 5.
Identification of parasites and collection of data on individuals affected by leishmaniasis.
Source: El Ghrifi [12] leishmaniasis laboratory, Ouezzane Provincial Hospital.

3.2. Screening Activity

The aim of screening activity in the Ouezzane area is to detect the epidemiological situation and monitor the evolution of leishmaniasis in this region.

This work has been carried out continuously for a long time at the leishmaniasis laboratory at the Ouezzane Provincial Hospital, but our study will be limited to the last five years (from 2020 to 2024), taking into account all information concerning leishmaniasis. This has enabled us to show specific results for the municipalities of Ouezzane.

This information is collected using the leishmaniasis prescription form, which is a form developed by the National Institute of Hygiene and contains precise data on cases of leishmaniasis (Figure 6).

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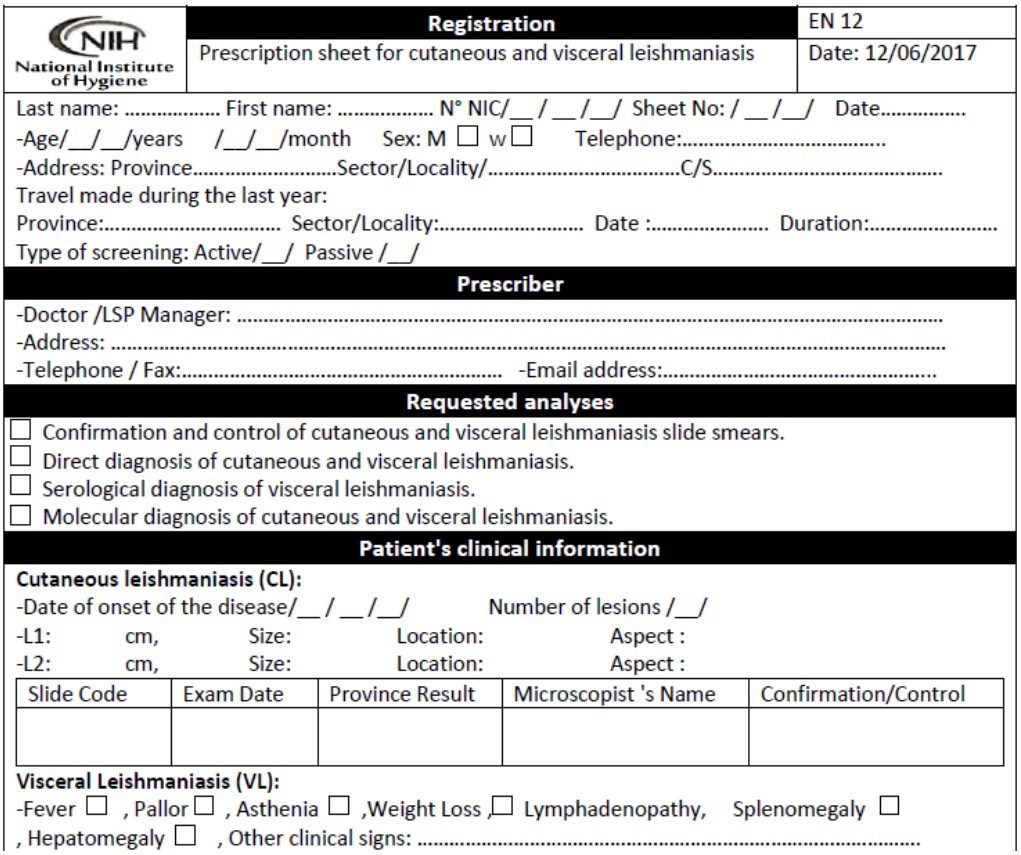

National Institute of Hygiene	Registration	EN 12
	Prescription sheet for cutaneous and visceral leishmaniasis	Date: 12/06/2017

Last name: ..... First name: ..... N° NIC/__ / __ /__/ Sheet No: / __ /__/ Date.....
-Age/__/__/years /__/__/month Sex: M ☐ w ☐ Telephone:.....
-Address: Province.....Sector/Locality/.....C/S.....
Travel made during the last year:
Province:..... Sector/Locality:..... Date :..... Duration:.....
Type of screening: Active/__/ Passive /__/

Prescriber

-Doctor /LSP Manager: .....
-Address: .....
-Telephone / Fax:..... -Email address:.....

Requested analyses

☐ Confirmation and control of cutaneous and visceral leishmaniasis slide smears.
☐ Direct diagnosis of cutaneous and visceral leishmaniasis.
☐ Serological diagnosis of visceral leishmaniasis.
☐ Molecular diagnosis of cutaneous and visceral leishmaniasis.

Patient's clinical information

Cutaneous leishmaniasis (CL):
-Date of onset of the disease/__ / __ /__/ Number of lesions /__/
-L1: cm, Size: Location: Aspect :
-L2: cm, Size: Location: Aspect :

Slide Code	Exam Date	Province Result	Microscopist 's Name	Confirmation/Control

Visceral Leishmaniasis (VL):
-Fever ☐ , Pallor ☐ , Asthenia ☐ ,Weight Loss ☐ Lymphadenopathy, Splenomegaly ☐ , Hepatomegaly ☐ , Other clinical signs: .....

Figure 6.
Leishmaniasis prescription form.
Source: El Ghrifi [12] Leishmaniasis Laboratory, Ouezzane Provincial Hospital.

4. Results and Discussion

4.1. Identification of Leishmania

During microscopic examination of smears prepared at the leishmaniasis laboratory at Ouezzane Provincial Hospital, several amastigote forms of Leishmania were observed inside Giemsa-stained macrophages. These amastigotes, which are small (2–5 µm), appear ovoid in shape, with a central nucleus and a characteristic kinetoplast, confirming the presence of the parasite in the infected skin tissue. The image (Figure 7) clearly shows a parasitised macrophage containing multiple amastigotes, indicating an active infection [13]. This result constitutes direct parasitological evidence of cutaneous leishmaniasis in the province of Ouezzane and corroborates the epidemiological data collected between 2020 and 2024.

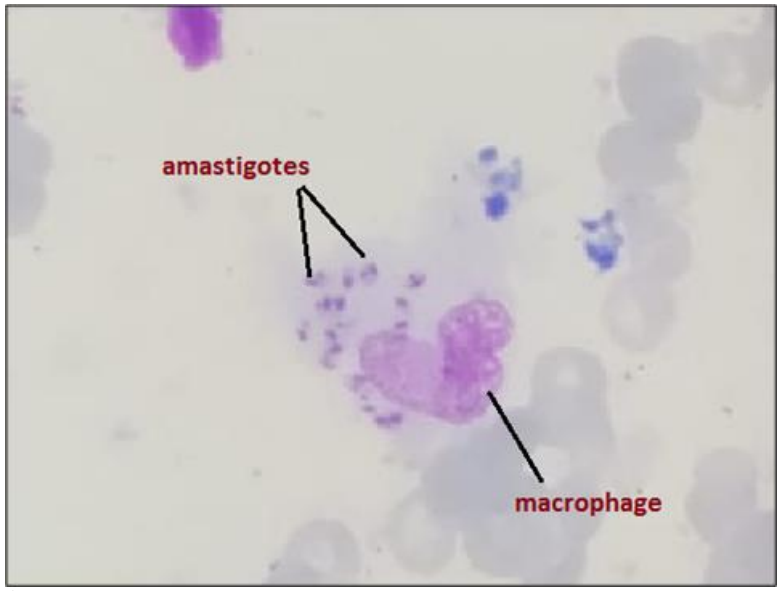

Figure 7.
Microscopic photo of amastigote leishmania (x100).
Source: El Ghrifi [12] leishmaniasis laboratory, Ouezzane Provincial Hospital.

4.2. Screening Activity

During these five years, 90 positive cases of leishmaniasis were recorded, of which only two cases of visceral leishmaniasis (VL) were recorded and the rest (88 cases) or 97.7% were cases of cutaneous leishmaniasis (CL) in our study area (Figure 8).

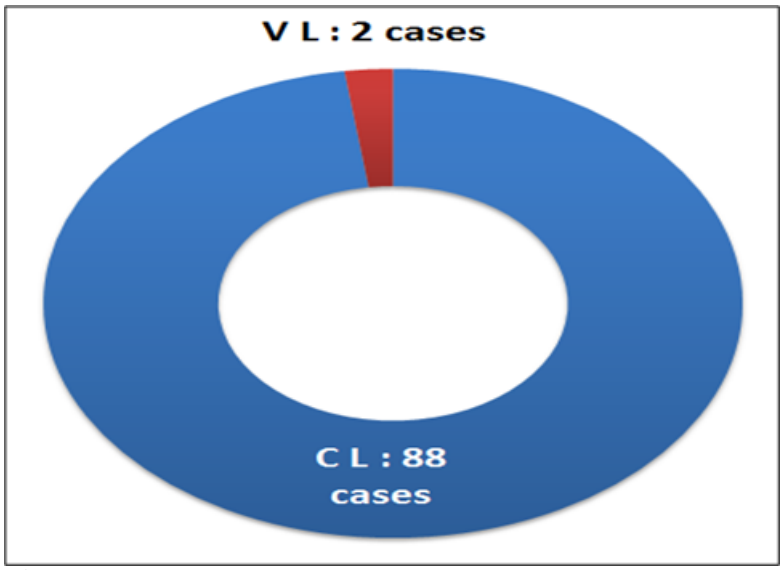

Figure 8.
Proportion of leishmaniasis cases in the province of Ouezzane.
Source: El Ghrifi [12] Ouezzane Regional Hospital.

The low proportion (2 cases) of visceral leishmaniasis recorded during this period indicates that living conditions are probably unfavorable for the leishmaniasis complex (parasite, vector and reservoir) causing visceral leishmaniasis.

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The remaining positive cases recorded (88 cases) concern cutaneous leishmaniasis and have been classified according to:

- Clinical aspects (location, number and size of lesions).
- Epidemiological aspects (area, gender, age and time).

4.2.1. Clinical Aspects

According to the location of the lesions, 78 cases affected the face, which represents the majority compared to other parts of the body (Figure 9), with only one case affecting the back and another affecting the neck. This is probably due to people not covering their faces while sleeping, when sand flies become active as they are nocturnal creatures. Therefore, the face is most often uncovered and consequently easily accessible to sand fly bites.

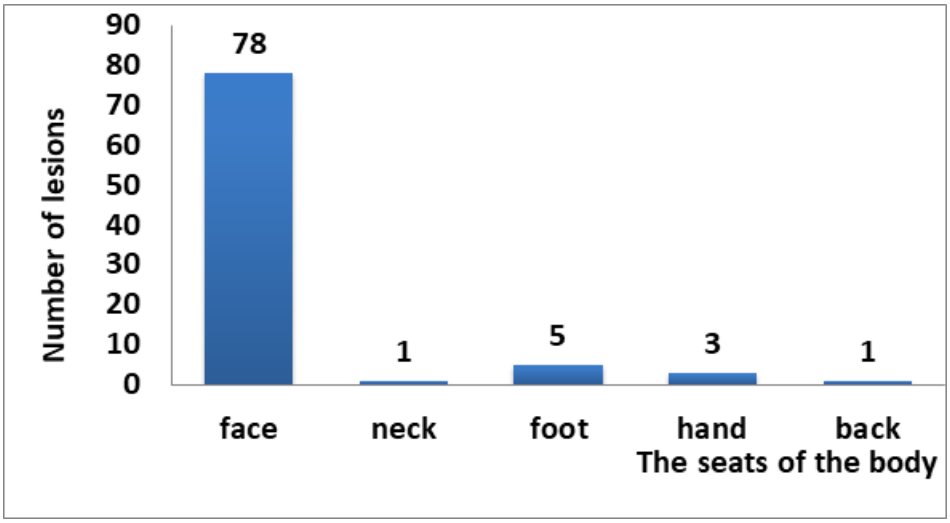

Figure 9.
Number of cutaneous leishmaniasis lesions according to location on the human body.
Source: El Ghrifi [12] Ouezzane Regional Hospital.

According to the diagnosis of the lesion, its size varies from 0.2 to 2.5 cm, and in terms of their number, all sites had a single lesion except for the face, which showed a multiplication of lesions, ranging from 1 to 5 lesions, with 70 cases having a single lesion, 6 cases having 2 lesions, one case had 3 lesions, and the last case had 5 lesions with an absence of 4 lesions (Figure 10).

Therefore, the majority of cases (almost 90%), which have a single lesion on their faces, present the frequent characteristic of cutaneous leishmaniasis due to Leishmania major in the local dynamics, and the rare cases of multiple lesions ranging from 2 to 5 lesions can be explained by repeated exposure to multiple bites from sand flies carrying Leishmania parasites or to low immunity in the human victim of this parasite, and the case of 5 lesions may indicate infections with Leishmania tropica, which is often responsible for polymorphic and sometimes multifocal forms in certain foci, and with regard to the visceral form of leishmaniasis , the latter suggests the presence of *Leishmania infantum*, which is also responsible for atypical cutaneous forms of leishmaniasis [13].

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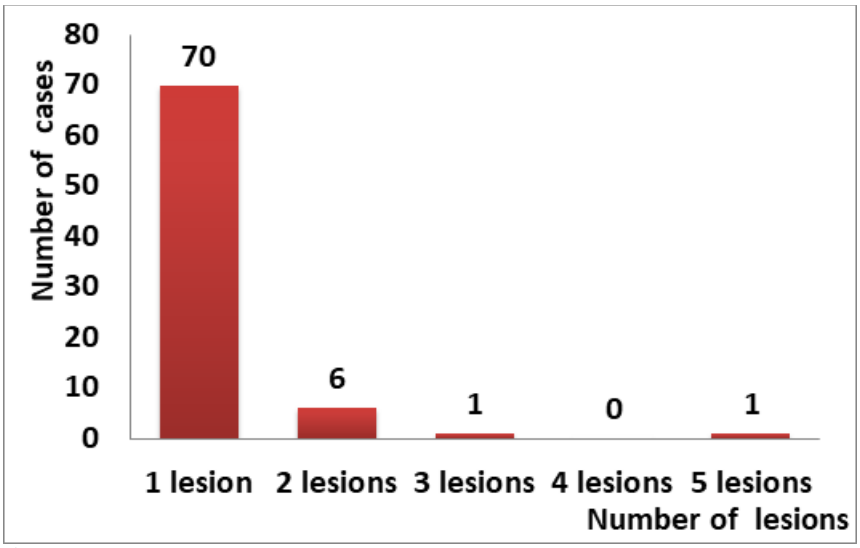

Figure 10.
Variation in the number of cutaneous leishmaniasis lesions on the human face.
Source: El Ghrifi [12] Ouezzane Regional Hospital.

Depending on the healing of the lesion, there were cases where the lesions developed into scars (Figure 11 a) and other cases where the lesions healed (Figure 11 b).

Lesions that did not heal show that the treatment period was exceeded. Obviously, if the lesion is not treated at an advanced stage, it will leave a permanent mark.

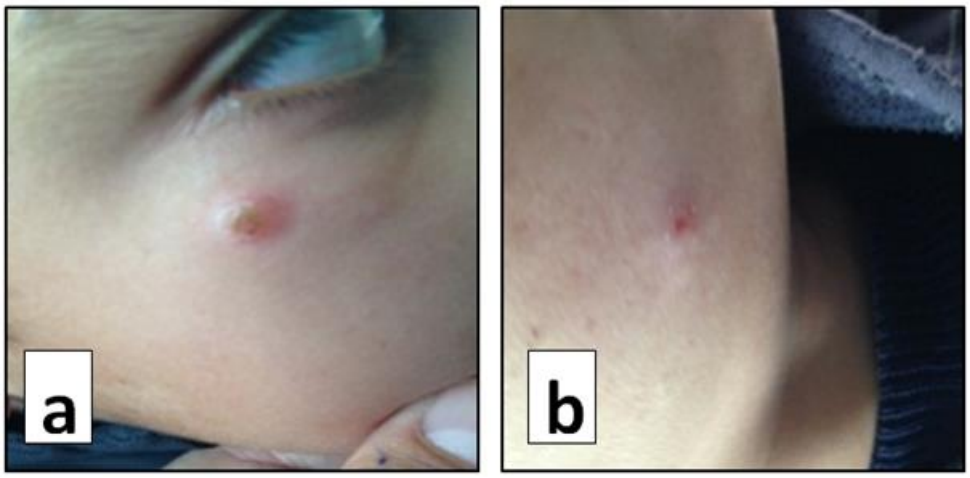

Figure 11.
Lesions caused by cutaneous leishmaniasis due to *Leishmania tropica*.
Source: El Ghrifi [12].

4.2.2. Epidemiological Aspects

4.2.2.1. Distribution of Indigenous Cases by Sector

During the period from 2020 to 2024, the provincial hospital in Ouezzane recorded 88 positive cases of cutaneous leishmaniasis, which are divided into two parts: the rural part, which accounts for a large percentage (89.6%) compared to the urban part. This reflects that rural areas are conducive to the proliferation of Leishmania parasites due to stables, domestic and agricultural wastewater, and uncontrolled household waste, which provide breeding grounds and vectors.

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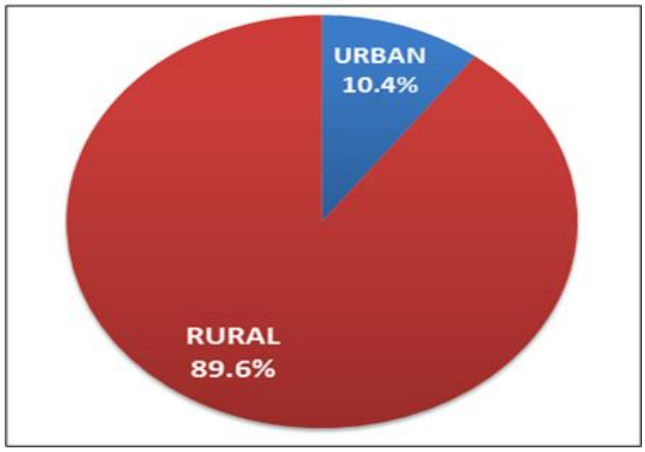

Figure 12.
Distribution of indigenous cases according to urban and rural areas in Ouezzane during the period from 2020 to 2024.
Source: El Ghrifi [12] Ouezzane Provincial Hospital.

During the five years of our study, there was a sharp increase in indigenous cases, from 1 to 11 cases in the municipality of Teroual, alongside a slight increase in Zoumi from 1 to 3 cases and in the municipality of Ain Dorrij from 1 to 2 cases. However, the other municipalities showed a decrease in indigenous cases, particularly Ouezzane and Sidi Bousber, which experienced a sharp decline (Ouezzane from 5 to 1 case and Sidi Bousber from 6 to 1 case). However, there was a single case in Sidi Redouane in 2023, which was cancelled in 2024, and two cases in Ouannana in the same year.

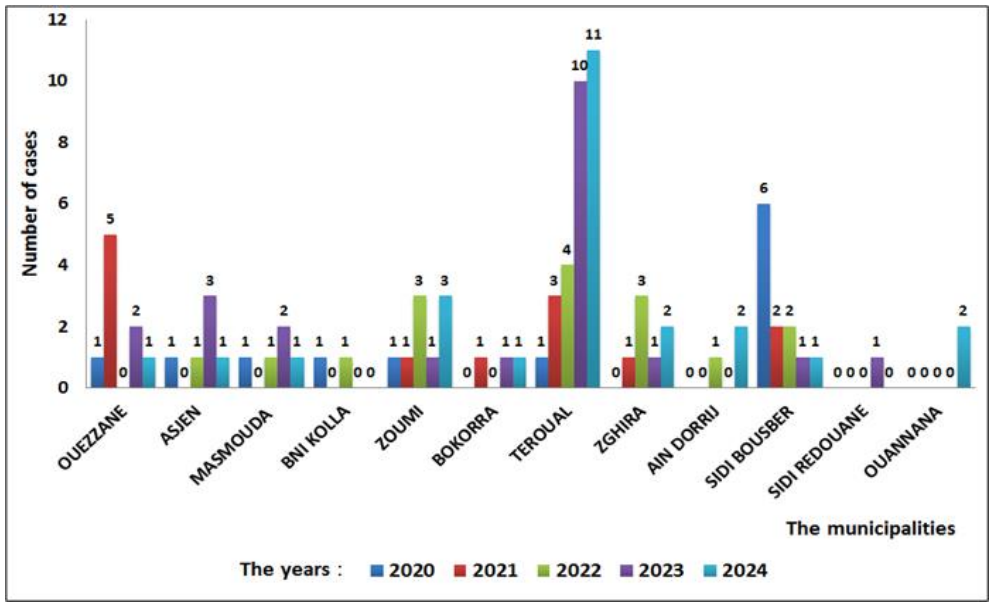

Figure 13.
Evolution of indigenous cases by municipality in the province of Ouezzane during the period from 2020 to 2024.
Source: El Ghrifi [12] Ouezzane Provincial Hospital.

According to the screening results, the municipality of Teroual ranks first with 33% of cases, or one third of the total number of indigenous cases. It is therefore the main endemic focus in the province. It is followed by the municipality of Sidi Bousber (13%) and that of Zoumi (11%), which also have significant

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foci, together accounting for nearly a quarter of cases. It should be noted that these areas are neighboring and are located in the south-east of the province of Ouezzane, adjacent to the province of Taounate, according to the map of subdivisions of our province under study.

On the other hand, the municipality of Ouezzane Centre accounts for 10% of the total, which is a significant proportion, probably reflecting urban concentration and/or population movements. Other municipalities such as Zghira (8%), Asjen (7%), Masmouda (6%) and Bokorra (5%) are intermediate centres, suggesting moderate dissemination.

Finally, marginal municipalities such as Ain Dorrij (3%), Ouannana (2%), Bni Kolla (2%) and Sidi Redouane (1%) showed low transmission or better local control (Figure 14).

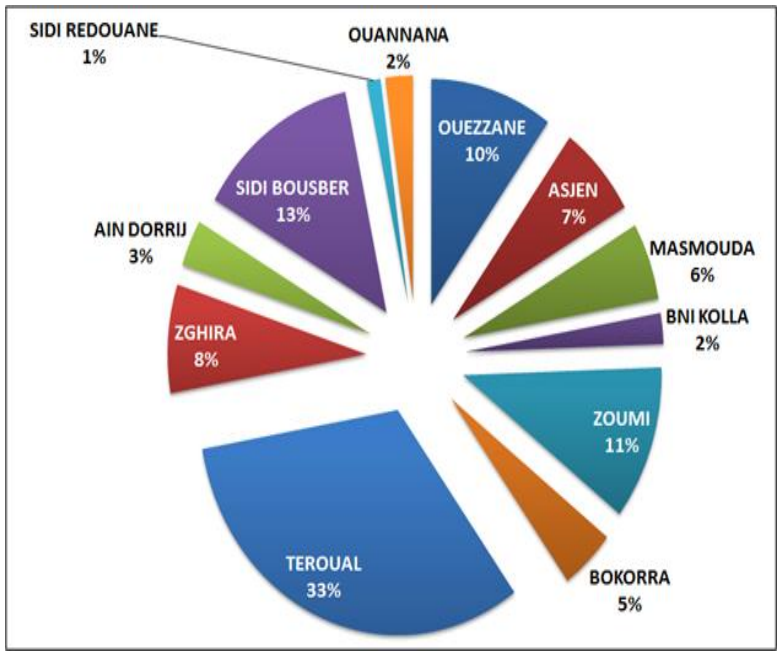

Figure 14.
Percentages of indigenous cases by municipality in the province of Ouezzane during the period 2020 to 2024.
Source: El Ghrifi [12] Ouezzane Provincial Hospital.

This cluster of leishmaniasis cases, which makes the municipalities of Teroual, Sidi Bousber and Zoumi the major hotspots, may be due to the geographical location of their territory, which favors ecological conditions conducive to the proliferation of sandflies (climate, humidity, forest cover and watercourses). Teroual (the first infected region) is located near the Louahada dam and the province of Taounate, which are likely hotspots for leishmaniasis (see map, Figure 1).

Furthermore, sand fly populations find their optimum development and reproduction in sub-humid forests, allowing *L. tropica* to reach altitudes of up to 1600 m and be capable of triggering an active and stable outbreak of leishmaniasis, which confirms our results because the areas studied are located in the Rif mountain range, which has sub-humid forests. In terms of health, leishmaniasis appears to be an indicator of climate change [14].

Socio-economic conditions (precarious housing, high population density) and proximity to isolated rural areas may also contribute to the spread of cutaneous leishmaniasis in these regions.

For the municipality of Ouezzane, an urban environment, the presence of 10% of cases in urban areas reflects an spread of the disease to semi-urban areas, possibly linked to internal mobility (rural exodus, trade) or nearby landfills.

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For secondary foci (Zghira, Asjen, Masmouda, Bokorra) and other marginal areas (Ain Dorrij, Ouannana, Bni Kolla, Sidi Redouane) which have successively intermediate and low endemicity rates, this means that they have less favorable ecological conditions for vectors, or are subject to better health surveillance. However, under-reporting of cases cannot be ruled out.

4.2.2.2. Distribution of Indigenous Cases by Age

The childhood category, which includes children under the age of 14, accounts for a large proportion ranging from 13% to 20%, with the 10-14 age group being the most affected (20%). These proportions add up to 47%, which is the largest share of the diagram.

The adolescent category (15-24 years old) and the young adult category (25-39 years old) have low percentages of 8% and 7% respectively, compared to the other age categories.

The 40-59 age group ranks second after the childhood category with a percentage of 23%, while the over-60 age group has 13%, ranking second to last (Figure 15).

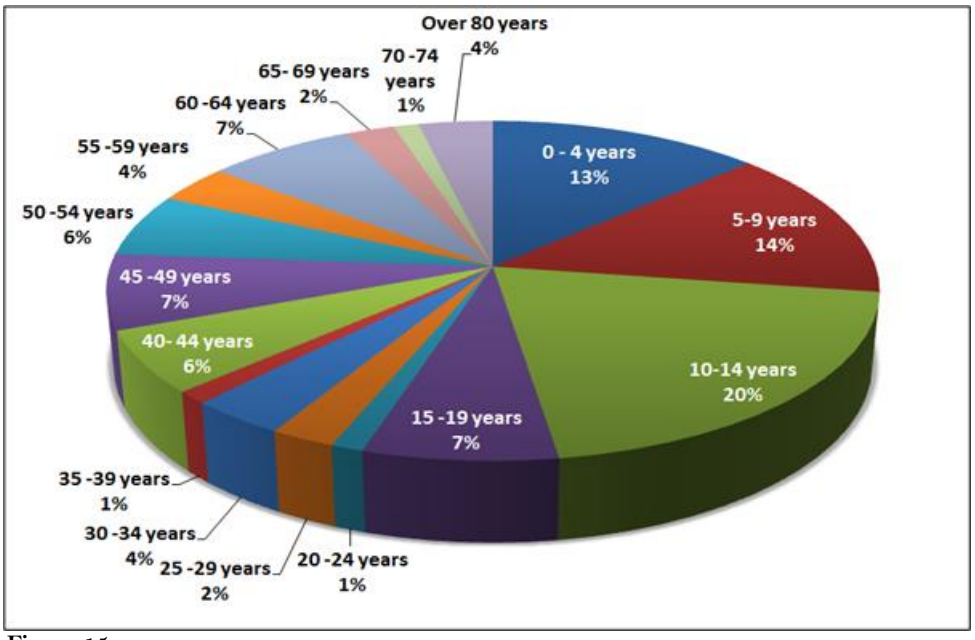

Figure 15.
Distribution of indigenous cases of cutaneous leishmaniasis by age group during the period 2020 to 2024.
Source: El Ghrifi [12] Ouezzane Provincial Hospital.

Various surveys indicate that people under the age of 14 spend more time in environments favorable to sand flies, such as stables, rubbish dumps and sewage, which increases their exposure to sand fly bites. The second reason is that their immunity is being strengthened, making them more susceptible to the Leishmania parasite, not to mention that these individuals receive a great deal of care from their parents, which allows them to consult doctors frequently.

For the 15-39 age group, which includes those least affected, this may reflect that these individuals had acquired immunity or were less exposed due to travelling for work or study.

The 40-59 age group, which ranks second in terms of the epidemic, indicates that these individuals were stabilizing in their areas to carry out agricultural, rural and forestry activities where leishmaniasis rates are increasing.

Other age groups over 60 suffer from a weakened immune system or are likely to be busy with their daily lives and rarely consult doctors about skin lesions.

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4.2.2.3. Distribution of Indigenous Cases by Gender

The histogram shows that for each year of our study period, the number of female patients is always higher than the number of male patients, except in 2022, when the number of male patients is equal to the number of female patients, resulting in a higher percentage of female cases (58%) than male cases (42%) (Figure 16).

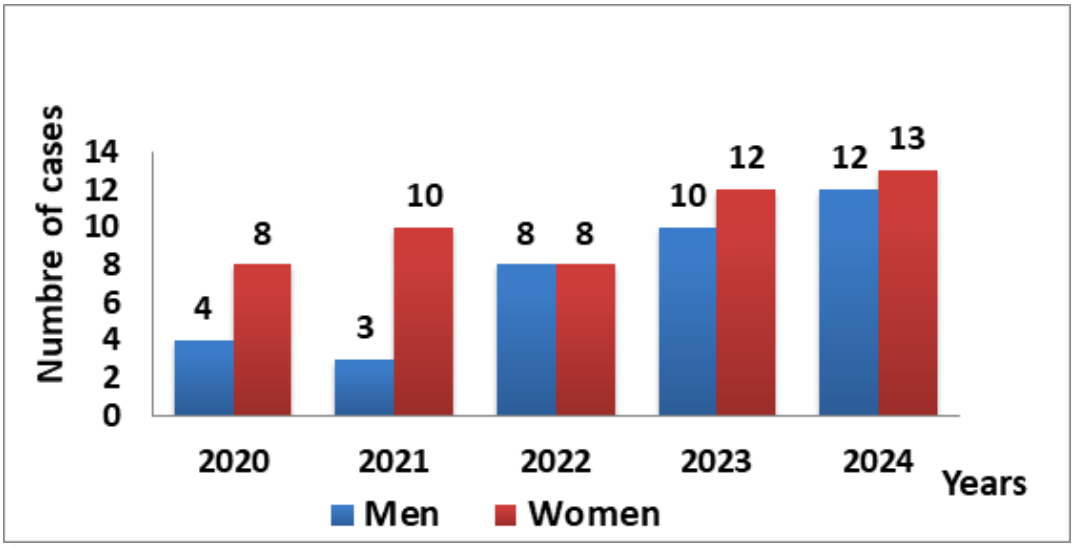

Figure 16.
Distribution of indigenous cases of cutaneous leishmaniasis by gender in the province of Ouezzane during the period 2020 to 2024.
Source: El Ghrifi [12] Ouezzane Provincial Hospital.

The increase in both sexes may be due to an expansion of the endemic area, ecological conditions favorable to the proliferation of vectors (sand flies), or improved screening.

The high percentage of females reveals that women consult doctors much more than men and also that women always remain in a single environment (the home) to carry out their domestic activities, which depend on stables, rubbish dumps, etc., exposing them to sand fly bites. Men, on the other hand, always change environments for their work, which may reduce their risk of being bitten by sand flies.

4.2.2.4. Evolution of Indigenous Cases Over Time

The number of cases has increased year on year, rising from 12 to 25 indigenous cases, more than double in five years, with a sharp increase to 22 cases in 2023 (Figure 17).

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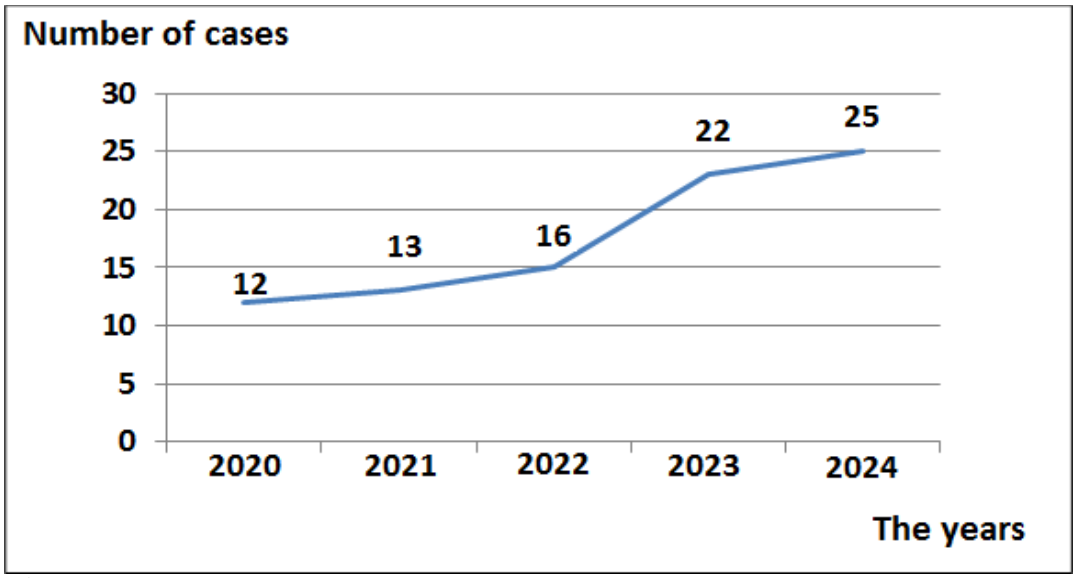

Figure 17.
Evolution of indigenous cases over time in the province of Ouezzane during the period from 2020 to 2024.
Source: El Ghrifi [12] Ouezzane Provincial Hospital, 2025.

The most marked growth occurred between 2022 and 2023 (+37.5%), which could be linked to environmental factors (climate favorable to sand flies), socio-economic factors (housing conditions, poverty, proximity to forest areas), or health factors (delayed diagnosis, inadequate vector control).

In 2024, the increase continues but at a slower pace (+13.6% compared to 2023), which may suggest either saturation of the local epidemic or the beginning of the effectiveness of the control measures put in place.

This increase over the last two years may also be due to the efforts made by the Ouezzane public health and social protection delegation to combat leishmaniasis by raising public awareness of the importance of consultations to check whether or not they have leishmaniasis and to cure them definitively, and by strengthening its epidemiological surveillance programmes

5. Conclusion

The study conducted on the epidemiological situation of leishmaniasis in the province of Ouezzane during the period 2020-2024 shows the predominance of cutaneous leishmaniasis (97.7%) over visceral leishmaniasis.

Cutaneous leishmaniasis has a high incidence in rural areas, particularly in the municipality of Teroual, reflecting the presence of favorable environmental conditions for one or all components of the leishmaniasis complex (parasite, vector and reservoir).

During the 2020-2024 period covered by our study, cutaneous leishmaniasis mainly affects the face compared to other sites, which remain in the minority. This shows that the face is the area most accessible to vector bites, as it is always exposed during the night.

This form of leishmaniasis mainly manifests itself as a single lesion, which presents a clinical appearance similar to *leishmania major*. However, cases of multiple lesions, which are rare, may reflect the presence of *leishmania tropica*.

On the other hand, the evolution of cutaneous leishmaniasis cases during this period highlights a heterogeneous distribution, dominated by the rural municipalities of Teroual, Zghira and Sidi Bousber, while the other municipalities show low or sporadic endemicity, with uneven geographical distribution

since the Teroual, Sidi Bousber and Zoumi areas account for more than half of the cases, making them priority hotspots.

In terms of age groups, cutaneous leishmaniasis mainly affects children (0–14 years old), making it an endemic pediatric disease; and in terms of gender, cutaneous leishmaniasis affects women more than men, especially in the early stages. Nevertheless, the difference tends to decrease over the years, resulting in an almost equal distribution in 2024, revealing that these children and women are most at risk of leishmaniasis.

The province of Ouezzane has seen a significant increase in cases of cutaneous leishmaniasis between 2020 and 2024, with a doubling in 5 years. This probably reflects improved detection thanks to screening campaigns and public awareness initiatives.

These results highlight the importance of implementing an integrated control programme combining prevention, epidemiological surveillance, vector control, awareness-raising among rural populations and scientific research in order to limit the spread of this disease.

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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