Cutaneous leishmaniasis in Morocco: psychosocial burden and simplified diagnosis

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Cover design by Anita Muys (UA media service)

Description of cover photo

The cover photo shows a 6-month-old facial lesion of cutaneous leishmaniasis in a 7-year-old child living in the northern part of Morocco.
Cutaneous leishmaniasis in Morocco: psychosocial burden and simplified diagnosis

Cutane leishmaniase in Marokko: psychosociale belasting en vereenvoudigde diagnose

Dissertation submitted for the degree of Doctor of Biomedical Sciences at the University of Antwerp

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Abbreviations

ACL  Anthroponotic cutaneous leishmaniasis
AIDS  Acquired immunodeficiency syndrome
CBI  Cutaneous body image
CIMVC  Committee of integrated management of vector control
CL  Cutaneous leishmaniasis
DALY  Disability-Adjusted Life Year
DAT  Direct agglutination test
DCL  Diffuse cutaneous leishmaniasis
DELM  Direction de l’Épidémiologie et de la Lutte contre les Maladies (Directorate of Epidemiology and Disease Control)
DLQI  Dermatology life quality index
DNA  Deoxyribonucleic acid
ELISA  Enzyme-linked immunosorbent assay
EMR  Eastern mediterranean region
FGD  Focus group discussion
Fig  Figure
FR  Female respondent from Rissani area
FT  Female respondent from Tinejdad area
GBD  Global burden of disease
HCP  High Commission for Planning
HIV  Human immunodeficiency virus
HSP70  Heat shock protein 70
IFAT  Immunofluorescence antibody test
IFN  Interferon
IPM  Institut Pasteur du Maroc
IRS  Indoor residual spraying
ITN  Insecticide-treated net
ITS1  Internal transcribed spacer
KAP  Knowledge, Attitudes, and Practices
L.  Leishmania
LAMP  Loop-Mediated Isothermal Amplification
LCL  Localised cutaneous leishmaniasis
LLIN  Long-lasting insecticide-treated net
LMIC  Low and middle-income countries
LST  Leishmanin Skin Test
MCL  Mucocutaneous leishmaniasis
Summary

Introduction

Cutaneous leishmaniasis (CL) is a neglected tropical disease (NTD) affecting more than one million persons worldwide. In Morocco, reporting over the past decade on average more than 4000 cases per year, CL is considered a public health problem. CL is mainly poverty-related in Morocco. The social representations of CL were never explored, though efficient control requires understanding of people’s perception and community involvement. To fill this gap, and to facilitate early diagnosis of CL, our research aimed to document the psychosocial burden of CL in these regions, and to assess a new diagnostic tool for CL that is appropriate for use in remote endemic areas.

Methods

We first described a major outbreak of CL that took place between 2010 to 2012 in Errachidia province. We documented the intersectoral response measures including environmental management and reservoir control. The reservoir of L. major, a rodent, Meriones shawi, was targeted using strychnine-poisoned wheat baits.

The second part of the thesis documents social representations of CL in Southeastern Morocco, in both L. major and L. tropica areas. In 2015, we conducted qualitative research in a group of high school students living in L. major areas to study their perception of CL. Furthermore, we conducted focus group discussions on CL perception and its psychosocial burden in the adult population in Errachidia and Tinghir provinces. We related our findings on psychosocial burden to those in the international literature by conducting a scoping literature review.

The third part of the thesis assessed the accuracy of a new rapid diagnostic test (RDT) of CL in the real-life conditions of primary health centers. A “phase III” diagnostic accuracy study was performed in a consecutive series of patients with ulcerative skin lesions not older than four months and suggestive of CL. We compared the RDT results with a composite reference standard based on PCR and microscopy.

Results

Between 2004 and 2013, 7099 cases of CL were recorded in Errachidia Province. This outbreak due to L. major left many adolescents with permanent scar tissue on the face or other exposed body parts. Almost 20% of 448 high school students reported a CL lesion and 87% said it could lead to psychological consequences. The indelible CL scars lead to self-stigma and social stigma,
and the emergence of adverse psychological effects. Adult participants in the focus groups considered the impact of CL lesions and scars as important. Young women with CL scars in the face are stigmatized and may be rejected for marriage in these communities. People usually try a long list of folk remedies on the active lesions, but none was felt adequate. They expressed a real demand for better treatment. The scoping review confirmed that localized CL is a source of pronounced psychological suffering, stigmatization, and reduction of quality of life in several endemic countries.

In the third part, the RDT showed a sensitivity of 68% [95% CI, 61-74], a specificity of 94% [95% CI, 91-97], a positive predictive value of 95% [95% CI, 92-98] and a negative predictive value of 64% [95% CI, 58-70]. Species typing on a subsample of 87 PCR positives using PCR-RFLP and PCR-sequencing identified *L. tropica* (*n=40*), *L. major* (*n=35*) and *L. infantum* (*n=12*). Our findings suggest that this novel RDT for CL is a useful addition to clinical management in Morocco, especially in isolated localities far from provincial laboratories.

**Conclusion**

The high number of CL cases and related scars in Morocco should mobilize more public health attention to sustain preventive measures against vectors and animal reservoirs. The diagnostic management should be reviewed based on current evidence. We recommend the introduction of the RDT in all endemic CL areas with difficult geographical access and the psychological support of people suffering from CL indelible scars.
Samenvatting

Introductie

Cutane leishmaniasis (CL) is een verwaarloosde tropische ziekte (NTD) die meer dan een miljoen mensen over de hele wereld treft. In Marokko, met meer dan 4000 gevallen per jaar, wordt CL beschouwd als een probleem voor de volksgezondheid. CL is een ziekte die verband houdt met armoede. De sociale representaties van CL zijn nooit onderzocht in Marokko. We wilden de psychosociale belasting van CL in deze regio's documenteren, en we hebben een nieuwe test voor CL-diagnose in afgelegen en endemische CL-gebieden geëvalueerd.

Methoden

We hebben eerst de epidemie van CL beschreven die plaatsvond tussen 2010 en 2012 in de provincie Errachidia. We bestudeerden de intersectorale bestrijdingsmaatregelen, waaronder milieubeheer en reservoirbeheer. Het reservoir van *L. major*, een knaagdier, *Meriones shawi*, werd bestreden met lokaas dat behandeld was met strychnine.

Het tweede deel van het proefschrift richtte zich op maatschappelijke dimensies van CL in Zuidoost-Marokko, zowel in de gebieden van *L. major* als *L. tropica*. We deden kwalitatief onderzoek bij middelbare scholieren die in endemische *L. major* gebieden woonden. Daarnaast werden in maart en april 2015 focusgroep discussies gehouden in de provincies Errachidia en Tinghir met de volwassen bevolking. We hebben ook een literatuuronderzoek uitgevoerd om onze bevindingen te relateren aan die in de internationale literatuur.

Het derde deel van het proefschrift beoordeelde de nauwkeurigheid van een nieuwe snelle diagnostische test (RDT) voor CL in de context van negen eerstelijnsgezondheidscentra in vijf endemische provincies. Een "fase III" studie werd uitgevoerd in een opeenvolgende reeks van patiënten met ulceratieve huidletsels van minder dan vier maanden. We vergeleken de RDT-resultaten met een referentiestandaard op basis van PCR en microscopie.

Resultaten

Tussen 2004 en 2013 werden 7099 gevallen van CL geregistreerd in de provincie Errachidia. Deze epidemie door *L. major* heeft veel adolescenten met permanent littekens op het gezicht of op andere blootgestelde lichaamsdelen opgezadeld. Bijna 20% van de 448 middelbare scholieren meldde een CL-letsel en 87% zei dat dit psychologische gevolgen zou kunnen hebben. De permanente CL-littekens leiden tot zelfstigma, sociaal stigma en de andere negatieve psychologische effecten. Onze focusgroepen met volwassenen beschouwden de impact van CL-

In het derde deel toonde de RDT een sensitiviteit van 68% [95% CI, 61-74]), een specificiteit van 94% [95% CI, 91-97]), een positief voorspellende waarde PPV = 95% [95 % CI, 92-98] en een negatief voorspellende waarde NPV = 64% [95% BI, 58-70]. Species identificatie op een subgroep van 87 PCR-positieven met PCR-RFLP en PCR-sequentieanalyse toonde L. tropica (n = 40), L. major (n = 35) en L. infantum (n = 12). Onze studie suggereert dat deze nieuwe RDT voor CL een nuttige aanvulling is op klinisch management in Marokko, vooral in afgelegen plaatsen ver van provinciale laboratoria.

Conclusie

Het hoge aantal gevallen van CL in Marokko moet leiden tot meer aandacht voor de volksgezondheid. Preventieve maatregelen tegen vectoren en dierlijke reservoirs zijn nodig.

Het klinisch beleid moet opnieuw bekeken worden op basis van deze recente resultaten. We bevelen de introductie van de RDT aan in alle endemische CL-gebieden met een moeilijke geografische toegang en de psychologische ondersteuning van mensen die lijden aan CL-onuitwisbare littekens.
CHAPTER 1. General introduction
1. Leishmaniasis, an overview

Leishmaniasis are vector-borne diseases caused by obligate protozoan parasites from the genus *Leishmania* (Trypanosomatida: Trypanosomatidae) [1]. They are transmitted to humans through the bite of a female sand fly. *Leishmania* parasites are what is called “digenetic,” because they need two hosts to complete their life cycle. Within the insect vector, they present under the promastigote form, an extra-cellular form with a flagellum, and in the mammalian host, as the intracellular amastigote, a form without flagellum. This parasite can lead to a broad spectrum of diseases, both in humans and animals [2]. Depending on the *Leishmania* species, the reservoir of this parasite can be exclusively human, categorizing the disease as an anthroponosis, or, involve animal reservoirs (rodents, dogs, etc...), categorizing the disease as zoonotic [3].

More than twenty *Leishmania* species¹ are pathogenic for humans [1, 4]. The species classification within the genus *Leishmania* has always been a controversial matter, and there is not yet a definitively accepted taxonomy. Two genera of sand flies are involved in transmission: *Phlebotomus* and *Lutzomyia*, depending on the geographical region. Of the over 800 known species of sand flies, only 78 transmit *Leishmania* parasites to humans [1, 5]. Figure 1 gives an overview of *Leishmania* species and the sandfly species involved in their transmission in the Old World (Africa, Asia, Europe) or New World (the Americas).

The history of leishmaniasis can be traced back to 2,500 B.C., as several descriptions of the disease have been found in ancient writings, and recently molecular techniques confirmed the presence of the species in the archaeological material [1]. DNA of *L. donovani* was found in Egyptian and Nubian mummies dating back 4,000 years [6].

The leishmaniases are now considered as neglected tropical diseases (NTD) by the World Health Organization (WHO). The word leishmaniasis is often written as a plural noun because several presentations can clinically be considered as different diseases. In humans, we distinguish three main clinical forms: visceral, cutaneous and mucocutaneous leishmaniasis. Visceral leishmaniasis (VL) or kala-azar is a fatal systemic disease that is caused by species of the *Leishmania donovani* complex.

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¹ http://www.who.int/leishmaniasis/resources/who_wer9238/en/
Figure 1. An updated classification of *Leishmania* (Panel A) and sand fly (Panel B). The source of this diagram is Akhoundi et al. 2016 published in PLoS NTD under Copyright: © 2016 Akhoundi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. https://doi.org/10.1371/journal.pntd.0004349 [1].

NB: (*) and (+) signs indicate the synonym species name to the original one. No final classification for the underlined species names. Name between quotation mark means no official name is decided. Old world species are highlighted in blue and New world species are highlighted in red.
A recent update by the World Health Organisation (WHO)\(^2\) indicates that 97 countries and territories are endemic for leishmaniasis. This list includes 65 countries that are endemic for both VL and CL, ten countries that are endemic for VL only and 22 countries that are endemic for CL only. In 2015, 214,201 cases of CL and 23,865 cases of VL were reported to WHO. However, underreporting is frequent and higher CL incidence may be found at population level \([7]\). Correcting for this underreporting, the global annual incidence of CL is estimated at 0.6 to 1.0 million cases of CL and 40,000 to 90,000 cases of VL\(^3\).

As a metric to estimate the Global Burden of Diseases (GBD), and to compare disease burden between diseases and countries, health economists use Disability Adjusted Life Years (DALYs). The total number of DALYs lost due to a disease is calculated as the sum of years of life lost due to this disease (YLLs) and of years lived with disability (YLDs) \(^4\) see the box.

<table>
<thead>
<tr>
<th>DALY = YLL + YLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>YLL = N x L(_1)</td>
</tr>
<tr>
<td>YLD = I x DW x L(_2) or YLD = P x DW</td>
</tr>
</tbody>
</table>

\(N\) = number of deaths  
\(L_1\) = standard life expectancy at age of death in years  
\(I\) = number of incident cases  
\(DW\) = disability weight  
\(L_2\) = average duration of the case until remission or death (years)  
\(P\) = number of prevalent cases

The total number of DALYs per NTD are shown in Table 1 and 2 below, reproduced from the most recent Global Burden of Disease Report \([8]\).

Table 2 - reproduced from the same publication as above - shows a substantial increase in prevalent cases of Dengue and CL from 1990 to 2013.

\(^3\) http://www.who.int/mediacentre/factsheets/fs375/en/ accessed in 25 October 2017  
Table 1. Leading causes of DALYs resulting from the NTDs according to the Global Burden of Diseases Study (GBD) 2013 [8]

<table>
<thead>
<tr>
<th>NTD</th>
<th>DALYs (in millions) in 2013</th>
<th>Percent change for DALYs 2005-2013</th>
<th>YLDs (in millions) in 2013</th>
<th>YLLs (in millions) in 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral leishmaniasis</td>
<td>4.24</td>
<td>8.7%</td>
<td>0.008</td>
<td>4.23</td>
</tr>
<tr>
<td>Foodborne trematodiases</td>
<td>3.63</td>
<td>14.6%</td>
<td>3.63</td>
<td>0</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>3.06</td>
<td>−13.9%</td>
<td>2.86</td>
<td>0.2</td>
</tr>
<tr>
<td>Hookworm</td>
<td>2.18</td>
<td>−0.5%</td>
<td>2.18</td>
<td>0</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>2.02</td>
<td>−14.3%</td>
<td>2.02</td>
<td>0</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>1.27</td>
<td>−29.0%</td>
<td>0.93</td>
<td>0.34</td>
</tr>
<tr>
<td>Rabies</td>
<td>1.24</td>
<td>−14.6%</td>
<td>0.0001</td>
<td>1.24</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>1.18</td>
<td>−19.4%</td>
<td>1.18</td>
<td>0*</td>
</tr>
<tr>
<td>Dengue</td>
<td>1.14</td>
<td>17.0%</td>
<td>0.56</td>
<td>0.58</td>
</tr>
<tr>
<td>Trichuriasis</td>
<td>0.58</td>
<td>−12.3%</td>
<td>0.58</td>
<td>0</td>
</tr>
<tr>
<td>African trypanosomiasis</td>
<td>0.39</td>
<td>−54.3%</td>
<td>0.005</td>
<td>0.38</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>0.34</td>
<td>4.6%</td>
<td>0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>Cysticercosis</td>
<td>0.34</td>
<td>−16.4%</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>Cystic echinococcosis</td>
<td>0.18</td>
<td>−14.1%</td>
<td>0.08</td>
<td>0.1</td>
</tr>
<tr>
<td>Trachoma</td>
<td>0.17</td>
<td>−18.1%</td>
<td>0.17</td>
<td>0</td>
</tr>
<tr>
<td>Cutaneous and mucocutaneous leishmaniasis</td>
<td>0.04</td>
<td>35.9%</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>Leprosy</td>
<td>0.04</td>
<td>8.6%</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>Other NTDs</td>
<td>3.13</td>
<td>−11.8%</td>
<td>2.26</td>
<td>0.87</td>
</tr>
<tr>
<td>Total NTDs</td>
<td>25.17</td>
<td>NA</td>
<td>16.95</td>
<td>8.21</td>
</tr>
</tbody>
</table>

**Additional neglected diseases**

<table>
<thead>
<tr>
<th>DALYs (in millions) in 2013</th>
<th>Percent change for DALYs 2005-2013</th>
<th>YLDs (in millions) in 2013</th>
<th>YLLs (in millions) in 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhoid fever</td>
<td>11.13</td>
<td>−13.7%</td>
<td>0.16</td>
</tr>
<tr>
<td>Cholera</td>
<td>5.17</td>
<td>−20.1%</td>
<td>0.04</td>
</tr>
<tr>
<td>Paratyphoid fever</td>
<td>3.82</td>
<td>−8.0%</td>
<td>0.04</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>3.46</td>
<td>−29.6%</td>
<td>0.19</td>
</tr>
<tr>
<td>Venous animal contact</td>
<td>3.00</td>
<td>−3.4%</td>
<td>0.15</td>
</tr>
<tr>
<td>Scabies</td>
<td>1.71</td>
<td>4.8%</td>
<td>1.71</td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>0.38</td>
<td>−23.8%</td>
<td>0.03</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>0.11</td>
<td>8.2%</td>
<td>0.11</td>
</tr>
<tr>
<td>Total deaths from additional neglected diseases</td>
<td>28.78</td>
<td>NA</td>
<td>2.44</td>
</tr>
</tbody>
</table>

NOTE: For information on percent change calculations, see the Global Burden of Disease Study (GBD) 2013 capstone paper on DALYs [2]. The estimates presented in this table are also available on the Institute for Health Metrics and Evaluation (IHME) website and were previously published in [2–4]. Information on DALYs and YLDs for Cholera, Cryptosporidiosis, and Amoebiasis is not available from IHME website or capstone papers. **Abbreviations:** NA, non-applicable

Table 2. Prevalent cases of NTDs in 2013 and percent change from 1990 to 2013 according to the Global Burden of Disease Study (GBD) 2013 [8]

<table>
<thead>
<tr>
<th>Disease</th>
<th>Prevalent cases (in millions) in 2013</th>
<th>Percent change since 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascariasis</td>
<td>804.4</td>
<td>-25.5%</td>
</tr>
<tr>
<td>Trichuriasis</td>
<td>477.4</td>
<td>-11.6%</td>
</tr>
<tr>
<td>Hookworm</td>
<td>471.8</td>
<td>-5.1%</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>290.6</td>
<td>30.9%</td>
</tr>
<tr>
<td>Foodborne trematodiases</td>
<td>80.2</td>
<td>51.1%</td>
</tr>
<tr>
<td>Dengue†</td>
<td>58.4</td>
<td>610.9%</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>43.9</td>
<td>-32.1%</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>17.0</td>
<td>-31.2%</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>9.4</td>
<td>22.4%</td>
</tr>
<tr>
<td>Cutaneous/mucocutaneous leishmaniasis</td>
<td>3.9</td>
<td>174.2%</td>
</tr>
<tr>
<td>Trachoma†</td>
<td>2.4</td>
<td>-39.2%</td>
</tr>
<tr>
<td>Cysticercosis†</td>
<td>1.0</td>
<td>-26.3%</td>
</tr>
<tr>
<td>Cystic echinococcosis†</td>
<td>0.8</td>
<td>-15.4%</td>
</tr>
<tr>
<td>Leprosy</td>
<td>0.7</td>
<td>61.3%</td>
</tr>
<tr>
<td>Visceral leishmanias</td>
<td>0.1</td>
<td>35.1%</td>
</tr>
<tr>
<td>Rabies†</td>
<td>0.02</td>
<td>-40.4%</td>
</tr>
<tr>
<td>African trypanosomias</td>
<td>0.02</td>
<td>-71.1%</td>
</tr>
<tr>
<td>Other NTDs</td>
<td>59.7</td>
<td>-5.0%</td>
</tr>
<tr>
<td><strong>Total cases</strong></td>
<td><strong>2,322</strong></td>
<td>NA</td>
</tr>
</tbody>
</table>

**Additional NTDs**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Prevalent cases (in millions) in 2013</th>
<th>Percent change since 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomoniasis</td>
<td>67.1</td>
<td>45.6%</td>
</tr>
<tr>
<td>Scabies</td>
<td>66.1</td>
<td>24.8%</td>
</tr>
<tr>
<td>Typhoid fever*</td>
<td>11.0</td>
<td>-19.9%</td>
</tr>
<tr>
<td>Paratyphoid fever*</td>
<td>6.4</td>
<td>-27.9%</td>
</tr>
<tr>
<td>Venous animal contact*</td>
<td>5.5</td>
<td>-2.7%</td>
</tr>
<tr>
<td>Cholera*</td>
<td>2.3</td>
<td>6.1%</td>
</tr>
<tr>
<td>Cryptosporidiosis*</td>
<td>1.4</td>
<td>-19.4%</td>
</tr>
<tr>
<td>Amoebiasis*</td>
<td>0.4</td>
<td>17.0%</td>
</tr>
<tr>
<td><strong>Total cases of additional neglected diseases</strong></td>
<td><strong>160.2</strong></td>
<td>NA</td>
</tr>
</tbody>
</table>

* Incident cases in 2013 rather than prevalent cases.
† Symptomatic cases only.

NOTE: For information on percent change calculations, see GBD 2013 capstone paper on incidence, prevalence, and years lived with disability (YLDs) [3]. All data presented in this table (except for rabies, cholera, cryptosporidiosis, and amoebiasis) are also available from the Institute for Health Metrics and Evaluation (IHME) website and were previously published in [3]. Abbreviations: NA, non-applicable


Expressed over a population denominator, the global mean age-standardized DALYs for CL was 0.58 per 100 000. The highest level was observed in Afghanistan, 87.03 per 100 000, followed by Sudan (20.24), Syria (9.15), Yemen (6.25), and Iraq (5.95). Other African countries had a lower
burden, Burkina Faso (4.84), Morocco (1.92), Mauritania (1.67), Tunisia (0.66), Libya (0.61), Mali 0.42, Ethiopia (0.28).

However, the use of DALYs to quantify the burden of disease in non-fatal diseases was criticized [9] and will be discussed more in-depth in chapter 5 of this thesis.

Furthermore, following the resolution adopted at the 60th World Health Assembly in 2007, the global burden of Leishmaniasis and its control strategies were prioritized for countries where leishmaniases are considered a public health problem. Two reports published by WHO in 20165 and 20176 updated the information. WHO Eastern Mediterranean Region (WHO EMR) is the highest affected region, despite the underreporting bias and the lack of epidemiological information from some countries (due in general to political instabilities or weak monitoring system) [7].

The table 3 below, based on the global health observatory7, lists the overall number and the yearly average of CL cases reported in WHO EMR between 2006 and 2015.

**Table 3. Yearly and total CL cases declared in high burden WHO EMR countries between 2006 and 2015**

<table>
<thead>
<tr>
<th>WHO EMR CL high burden Countries*</th>
<th>Total CL cases declared between 2006 and 2015 (Number of years declared)</th>
<th>Average annual number of CL cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syria</td>
<td>444,995 (10)</td>
<td>44,499</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>276,940 (10)</td>
<td>27,694</td>
</tr>
<tr>
<td>Iran</td>
<td>222,229 (12)</td>
<td>18,519</td>
</tr>
<tr>
<td>Pakistan</td>
<td>43,393 (09)</td>
<td>4821</td>
</tr>
<tr>
<td>Tunisia</td>
<td>44,652 (10)</td>
<td>4465</td>
</tr>
<tr>
<td>Morocco</td>
<td>41,651 (10)</td>
<td>4165</td>
</tr>
<tr>
<td>Iraq</td>
<td>37,329 (12)</td>
<td>3110</td>
</tr>
<tr>
<td>Libya</td>
<td>19,655 (07)</td>
<td>2807</td>
</tr>
<tr>
<td>Yemen</td>
<td>27,855 (10)</td>
<td>2785</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>24,970 (10)</td>
<td>2497</td>
</tr>
</tbody>
</table>

5 http://www.who.int/leishmaniasis/resources/who_wer9122/en/ accessed in 10 april 2018
6 http://www.who.int/leishmaniasis/resources/who_wer9238/en/ accessed in 10 april 2018
7 http://www.who.int/leishmaniasis/resources/REH_38_TABLEAU_S1_S2_Version_finale.pdf
2. Cutaneous leishmaniasis

2.1 Clinical aspects

Depending on the species involved and the duration of the lesion, the clinical dermatological manifestations vary from nodule, papule to ulcer associated or not with nodular lymphangitis. In the Old World, CL is usually caused by *L. major*, *L. tropica*, and *L. aethiopica*. *L. infantum*, the agent of VL in children and HIV/co-infected patients in the Mediterranean region, may occasionally lead to a self-healing cutaneous lesion in adults, but the evolution of the cutaneous lesion is slower than that observed with *L. major* and *L. tropica* infections [10]. *L.aethiopica* leads to a more mutilating diffuse form in Ethiopia. In the New World, CL is mainly caused by *L. braziliensis*, *L. guyanensis*, *L. peruviana*, *L. amazonensis*. The clinical evolution is most severe in the mucocutaneous CL that is mutilating and challenging to cure [11].

The incubation period for symptomatic CL ranges from weeks to months, while for mucosal forms it may occur years after the initial infection. Asymptomatic infection is common and can be detected by the Montenegro skin test [12].

2.2 Diagnosis of cutaneous leishmaniasis

In endemic CL areas, clinicians make a CL diagnosis based on the suggestive skin lesions and a history of exposure to sand fly bites. However, the diagnosis of CL can sometimes be challenging because the lesion mimics both infectious and malignant conditions. The differential diagnosis of CL contains a long list of diseases as reported in table 4 [13].
### Table 4. Differential diagnosis of LCL, DCL, and MCL [13]

<table>
<thead>
<tr>
<th>Differential diagnosis of CL</th>
<th>Malignancies that may mimic CL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infectious</strong></td>
<td>Basal cell carcinoma</td>
</tr>
<tr>
<td>Ecthyma</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Furuncle</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Carbuncle</td>
<td></td>
</tr>
<tr>
<td>Sporotrichosis</td>
<td></td>
</tr>
<tr>
<td>North American blastomycosis</td>
<td></td>
</tr>
<tr>
<td>Paracocciomycosis</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis cutis</td>
<td></td>
</tr>
<tr>
<td>Syphilitic gumma</td>
<td></td>
</tr>
<tr>
<td>Yaws</td>
<td></td>
</tr>
<tr>
<td>Prototheca infection</td>
<td></td>
</tr>
<tr>
<td>Condyloma acuminata</td>
<td></td>
</tr>
<tr>
<td>Lupus vulgaris</td>
<td></td>
</tr>
<tr>
<td>Tuberculoid leprosy</td>
<td></td>
</tr>
<tr>
<td>Cutaneous furuncular myiasis</td>
<td></td>
</tr>
<tr>
<td>Tungiasis</td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Xanthoma tuberosum</td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td></td>
</tr>
<tr>
<td>Pyoderma gangrenosum</td>
<td></td>
</tr>
<tr>
<td>Insect bite</td>
<td></td>
</tr>
<tr>
<td><strong>Differential diagnosis of diffuse CL</strong></td>
<td></td>
</tr>
<tr>
<td>Lepromatous leprosy</td>
<td></td>
</tr>
<tr>
<td>Lobomycosis</td>
<td></td>
</tr>
<tr>
<td>Lupus vulgaris</td>
<td></td>
</tr>
<tr>
<td><strong>Differential diagnosis of mucocutaneous leishmaniasis</strong></td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td></td>
</tr>
<tr>
<td>Yaws</td>
<td></td>
</tr>
<tr>
<td>Rhinoscleroma</td>
<td></td>
</tr>
<tr>
<td>Oral squamous cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td></td>
</tr>
</tbody>
</table>

2.2.1 Classical methods

The use of light microscopy to visualize the amastigote form of the parasite after Giemsa coloration of a tissue smear remains the method of choice for laboratory diagnosis of CL. The moderate sensitivity of these microscopy readings is a severe limitation in clinical practice and will be discussed in Chapter 6 of this thesis.

In the literature, the sensitivity of microscopy compared to PCR is variable. For example, in Pakistan, the sensitivity of microscopy was 60% in areas mainly dominated by *L. tropica*, *L. major*, and *L. infantum* [14]. In Iran, the sensitivity of microscopy was between 64% and 76% respectively in areas mainly dominated by *L. major*, *L. tropica*, *L. infantum* [15, 16]. 57% was the sensitivity found in a recent study from Palestinian districts were both *L. major* and *L. tropica* were found [17]. In Morocco, 69% of microscopy sensitivity was found compared to culture for *L. major*, *L. tropica* and *L. infantum* strains collected from different areas of endemicity [18]. Another study from Morocco reported a microscopy sensitivity of only 43% [19]. However, the National Institute of Hygiene in Morocco reached more than 85% of sensitivity and emphasizes that they have only 8% false positive and false negative microscopy results after a second control compared to PCR ITS1 [20]. This variability cannot be explained just by the species differences, but is mainly due to the sampling procedures, the sampling sites targeted in the suspected lesions [21] and the quality of the microscopy reading and the learner performance (e.g. optical microscopy versus virtual microscopy) [22].

Parasite culture of tissue samples obtained in biopsy or aspiration can be done in a well-equipped laboratory. However, there is a risk of culture contamination and the turn-around time, i.e. a one-week delay before results are available can also be frustrating. Identification of *L. major* or *L. tropica* parasites in blood from CL patients was also attempted. One study from Lebanon reported the successful detection of *Leishmania* parasites from whole blood in a culture tube with Nicole–Novy–McNeal (NNN) in confirmed CL patients [23]. However, no other study replicated or sustained such finding.

Histological examination is possible, but it depends on the quality of the biopsy and the experience of the anatomo-pathologist.

2.2.2 Immunological diagnosis

A review published in 2015 [24] listed all known immunological assays for leishmaniasis: Enzyme-Linked Immunosorbent Assay (ELISA), indirect immunofluorescent-antibody test (IFAT), western blot, lateral flow assay, and direct agglutination test. However, antibody responses in CL patients tend to be low in localized forms, which does not encourage the routine use of such immunological techniques [25, 26]. The antibody detection tests ELISA and IFAT were explored
in Iran in a CL endemic area (due to *L. major*). ELISA showed a sensitivity of 83.6% and 84.7% and specificity of 62.7% and 54.6%, respectively for detection of total IgG and IgM. The sensitivity and specificity of IFAT were higher: 91.6% and 81%, respectively [27]. A Direct Agglutination Test (DAT) based on homologous antigen showed a sensitivity and specificity of 90.5% and 91.8% respectively in *L. aethiopica* [28]. However, in LCL patients, antibody levels are generally rather low, unlike in DCL and VL patients. Therefore, no serologic tool is available for routine LCL diagnosis [28].

The Leishmanin Skin Test (LST) or Montenegro test detects the delayed-type hypersensitivity reaction to *leishmania* antigen with a sensitivity of 86.4% up to 100% [29, 30]. Some researchers recommended the use of LST as an alternative diagnostic method when there is a clinical doubt about a cutaneous lesion with no parasites found in direct smear microscopy examination [4]. However, LST remains positive for years after infection and does not allow discriminating a recent from an old *Leishmania* infection.

The success of the rK39 rapid diagnostic test for the diagnosis of VL encouraged researchers to develop a point-of-care test for CL. We report in Chapter 6 of this thesis the clinical evaluation of such test; the CL Detect™ Rapid Test, which is a membrane-based immunoassay for the detection of all clinically important *Leishmania* species that cause CL [24].

2.2.3 Polymerase chain reaction methods

Over the past few decades, several PCR-based methods, including real-time PCR assays, have been developed for *Leishmania* detection, quantification, and species identification, improving the molecular diagnosis of CL [31]. PCR is considered the most sensitive laboratory tool for CL diagnosis. One study reported that kinetoplast DNA (kDNA) PCR showed the highest sensitivity (98.7%), followed by the rRNA gene internal transcribed spacer 1 (ITS1) PCR (91.0%) and the spliced leader mini-exon PCR (53.8%) [32]. Other researchers found that kDNA PCR had a sensitivity of 90.7%, whereas an ITS1-based PCR had a sensitivity of 70.1% only [33]. A PCR based on Heat Shock Protein (HSP70) was reported with a moderate to high sensitivity depending on the performance of laboratories and the *Leishmania* species studied [34, 35]. Importantly, no standard single PCR is used by all laboratories. Nowadays, in low and middle-income countries, the most accessible and less expensive PCR is the one targeting internal transcribed spacers (ITS1) of the ribosomal DNA locus [31]. Loop-mediated isothermal amplification (LAMP) - also based on kinetoplast DNA detection- is a simplified PCR method that does not require a thermocycler. The amplified product is visualized by eye without requiring gels or fluorescence detection [36].

Quantitative PCR (qPCR) gives information about parasite load, which could be useful in following up the outcome of treatment. For example, quantitative reverse-transcriptase real-
time PCR (qRT-PCR) was used to assess the dynamics of parasite clearance in CL patients treated with miltefosine [37]. Multiplex qPCR was recently shown highly sensitive compared to Leishmania kinetoplast DNA, and more so than Giemsa-stained smears and parasite culture [38].

2.2.4 Identification of Leishmania species

Multi-locus enzyme electrophoresis (MLEE) has been considered the gold standard for Leishmania species typing for many years [39, 40]. Different assays for species typing in CL are reviewed by an article published in 2015 [4]. Restriction Fragment Length Polymorphism analysis (RFLP) is a PCR-based technique whereby the DNA amplification product is digested with one or several restriction enzymes (endonucleases) and the restriction fragments are then analyzed by electrophoresis [4, 41]. Several kinetoplast DNA (kDNA) assays are used in combination with RFLP analysis. A PCR based on the Heat Shock Protein 70 (HSP70) primers was also used in combination with RFLP analysis as well as sequencing. Parasite typing based on HSP70 sequencing showed a perfect agreement with MLEE [42]. With the advent of much cheaper sequencing techniques, this may become in the future the reference standard for parasite species identification. In contrast to MLEE, PCR based methods can be applied directly to clinical samples and do not require isolation/culture.

In practice, in CL endemic regions where only one available CL treatment or one CL species prevails, formal species identification often remains just an academic purpose. However, species identification is vital in outbreak investigations and in both endemic and non-endemic settings when travelers present with CL to a clinic.

2.3 Treatment of cutaneous leishmaniasis

The lack of an effective vaccine to prevent CL and the limited therapeutic options available were highlighted in two Cochrane reviews describing the available interventions for the Old and New World types [43, 44]. The absence of safe, efficacious and affordable treatment for CL is a matter for concern in low and middle-income countries (LMIC). The first-line therapy is based on the use of meglumine antimoniate or sodium stibogluconate. Other treatments are used as a second-line or for specific categories of patients: liposomal amphotericin B (AmBisome®), paromomycin (Paromomycin®), and miltefosine (Impavido®)[45]. Physical therapy, like cryotherapy and thermotherapy, are also used, however without much standardization of dose or technique.
A recent update of available treatments for CL localized forms was published as a Cochrane review and summarized the current evidence as follows [46]. The categories were:

A- Systemic and intralesional antimonials (Meglumine antimoniate and sodium stibogluconate) The most used drugs and well known for their side effects and resistance for some CL species.

B- Non-antimonial systemic treatments:

B-1- Oral treatments: Oral antifungal (fluconazole, ketoconazole, itraconazole); Oral dapsone; Oral allopurinol; Oral antibiotics (metronidazole, cotrimoxazole, rifampicin, azithromycin); Oral pentoxifylline; Oral miltefosine; Oral zinc sulphate; Oral artesunate.

B-2- Parenteral liposomal amphotericin B.

C- Non-antimonial topical or intralesional therapies:

C-1- Topical antifungals ( clotrimazole, miconazole, and ketoconazole); Topical paromomycin (aminosidine); Topical imiquimod; Topical aminoglycoside ointment (WR279,396); Topical miltefosine; Topical dapsone; Topical Thio-Ben; Topical 0.045% pharmaceutical chlorite (DAC N-055).

C-2- Intralesional zinc sulphate; Intralesional hypertonic sodium chloride; Intralesional interferon-gamma (IFN-γ); Intralesional metronidazole.

D- Physical therapies:

Laser carbon dioxide (CO2); Trichloroacetic acid (TCA); Cryotherapy; Thermotherapy; Topical photodynamic therapy; Mesotherapy.

E- Methods for promoting healing:

including dressing and antiseptics, are often employed in ulcerative lesions of CL to accelerate cure, normalize epithelialization, and reduce scarring, especially at cosmetic sites.

F- Alternative therapies:

Plants and herbal treatments; honey.
This Cochrane review concluded there is “very low-certainty evidence to support the effectiveness of itraconazole and paromomycin ointment for localized CL”. The authors considered it was impossible to conclude on the other interventions because of weak study design or incomparability of studies [46]. The authors highlighted the absence of evidence on the prevention of scarring.

3. Cutaneous leishmaniasis in Morocco

In Morocco, chronic skin lesions due to CL are a significant public health problem in some regions of the country where CL is caused by three species of the Leishmania parasite: L. major, L. tropica, and, sporadically, L. infantum. Epidemiologically, one distinguishes the L. major transmission area in the east and southeastern part of Morocco - where the rodent species Meriones shawi is the primary reservoir - from the L. tropica area that is concentrated in the center of Morocco and in the northern mountainous regions - where the transmission is from man to man (anthroponotic) [47]. Sporadic CL cases due to L. infantum occur mainly in the North of the country, whereas visceral leishmaniasis is endemic in the north and central northern region of Morocco [48]. Figure 2 shows the distribution of the three Leishmania species as documented during the past ten years within the map of poverty in Morocco defined by the poverty severity index as reported by the HCP. This index is known by the World Bank as “the squared poverty gap index” [49]. This index increases as the gap between the poverty line and the consumption expenditure of the poor increases. A higher index indicates more pronounced poverty.
Unfortunately, most maps in the literature showing the distribution of CL in Morocco overestimate the total surface of the endemic areas. Many maps show almost all parts of Morocco as endemic, except the south and the capital [47]. These flaws are due to technical limitations of the mapping software used or because of lack of detailed geospatial information on where cases occur. Other maps give the erroneous impression that cases occur in urban areas. Before 2013 there were no data about the number of CL cases reported by locality. It was therefore impossible to map the exact localization of the CL cases, and the case information was hence linked to the major town in each reporting area like in one recent publication showing the geographical distribution of ZCL and ACL incidence in Morocco [50].

How many new cases of CL are occurring in Morocco on an annual basis is still an unanswered question. Two recent reviews documented the CL incidence over the 2004–2014 period for both anthroponotic CL (ACL) and zoonotic (ZCL) based on the MoH Data [50, 51]. The Unit of Parasitic Diseases at the MoH provided epidemiological data on the period 1998 to 2017 (Figure 3), showing an epidemic peak in 2010 with an expected new peak in 2018. During this 20–year
period more than 66,000 citizens experienced CL in a total general population in Morocco of between 27 and 34 million inhabitants.

![Figure 3. Anthroponotic CL (ACL) and Zoonotic CL (CL) human cases reported between 1998 and 2017 in Morocco (Source of data: Unit of Parasitic Diseases, MoH)](image)

The official number of CL cases reported generates questions about possible underreporting or overreporting. Are cases diagnosed by private practitioners adequately notified to the official MoH reporting system? Little is known about this underreporting issue, and there are no prospective population studies that document the real incidence rate at the population level. According to Alvar et al., the underreporting factor for Morocco between 2004 and 2008 (period of the study data analysis) was considered mild with 2.8 to 4.6 for the notification system. According to the Moroccan law, CL is considered since 1996 a notifiable disease of Public Health importance and should lead to a vector control intervention using insecticides. Every public health facility must declare any new CL case to the central epidemiological surveillance unit within a week after it was diagnosed.

### 3.1 State of the art of research on CL in Morocco

Several masters and doctoral dissertations in both nursing and medical schools in Morocco have focused on CL in recent years, some of which have been published in peer review journals. Also, a growing number of papers on the topic have emerged over the past five years. We tried to summarize these studies below.

We performed a literature search from the 1950s to the present day in the PubMed database, combining the keywords "Morocco" and "cutaneous leishmaniasis," excluding the papers on

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8 [http://www.hcp.ma/](http://www.hcp.ma/)
visceral leishmaniasis (VL) and the papers that are included further in this Ph.D. thesis. On 15 October 2017, this search led to 85 articles, including 72 Morocco-specific publications. An additional search on Google scholar based on the names of first authors identified another 22 articles. Out of the 94 Morocco-specific papers (all listed in the references), 60% were published in the last five years. A timeline showing the evolution of publications on CL in Morocco is presented in Figure 4. However, of these 94 papers retrieved, 41 were published in non-indexed journals.

![Figure 4. Papers related to cutaneous leishmaniasis in Morocco from 1956 to 31 August 2017](image)

3.1.1 Transmission of CL: on host, parasite, and vector

Out of the 94 papers, 50 (54%) belong to the category ‘transmission of CL; on host, parasite or vector’. The first cases of the “Oriental Sore” in Morocco were described by Foley and Vialatte\(^\text{10}\) in 1914 -cited in Rhajaoui et al. (2004) [52]. The first human case of visceral leishmaniasis in Morocco was suspected in 1921 in Tanger by Remlinger\(^\text{11}\) cited in Rhajaoui et al. [53]. The first article on CL in Morocco included in our review was published in 1956 [54] during the French protectorate. The same research team from the Faculty of Montpellier (France) later continued its work under the supervision of Professor Rioux. Their publications documented the reservoir and vector of CL in southeastern Morocco. In 1982, Rioux et al. identified \textit{L. major} in the rodent species \textit{Meriones shawi} in Tata province and isolated it thereafter from human cases in south-eastern Morocco [55]. In 1987, Marty et al. confirmed the presence of \textit{L. tropica} in a CL lesion of a Moroccan child admitted to a French hospital after a stay in Tanant (100 km from Marrakech) [56]. In 1991, a CL case due to \textit{L. tropica} was reported for the first time in central Morocco [57,\text{".article"]


L. *tropica* has a very large distribution in Morocco. Several foci and outbreaks were observed in an area, extending from Agadir-Guelmim in the South via Essaouira and Chichaoua in the West, Beni Mellal, Azilal, Marrakech in the Centre, Ouarzazate in the East to Taza in the North [59].

The vectors for *L. major* and *L. tropica* in Morocco were identified as *Phlebotomus papatasi* and *Phlebotomus sergenti* respectively [57, 60]. At that time, only multilocus enzyme electrophoresis was available for species identification. Many different zymodemes (a group of parasites with the same isoenzymes) were identified in Moroccan samples, MON-1 for *L. infantum*, MON-25 for *L. major* and MON-102, MON-107, MON-109, MON-112, MON-113 MON-122, MON-123 for *L. tropica* as reported in the map (Figure 5) [61].

Figure 5. *Leishmania* *tropica*. Geographical distribution of zymodemes throughout the Moroccan endemic areas. The source of this map is Pratlong et al. 1991 in Annales de parasitology humaine comparée under Copyright © Masson, Paris [61]
At the end of the 90s, the enzymatic profile MON-102 of *L. tropica* was identified for the first time in Taza province by gel electrophoresis [62, 63]. Later, the existence of a canine reservoir of CL sustaining *L. tropica* MON-102 and MON-113 was suggested based on the previous work of Dereure et al. [58]. However, the role of dogs in the transmission of *Leishmania* species other than *L. infantum* was later disputed [64].

In 2015, a comprehensive review of the different rodent species and their epidemiological role as potential reservoir hosts of zoonotic CL in Morocco was published [65]. In 2017, the same team reported the presence of *L. infantum* and *L. tropica* in rodents caught in Essaouira, Chichaoua, Al Haouz and Marrakech provinces [66]. Molecular analysis revealed the presence of *Leishmania* species in 18 out a total of 197 rodents caught: six *Rattus rattus* (out of 80 captured; 7.5%), 11 *Mus musculus* (out of 50 captured; 22%), and one *Rattus norvegicus* (out of 9 captured; 11%) [66]. However, to identify *Leishmania* parasites in a mammal host does not necessarily mean that it is involved in the transmission cycle [64].

In recent years attention has shifted to entomological studies [67–69]. The seasonal fluctuations of phlebotomine sand fly populations and the correlation between the density of sand flies and temperature, humidity and rainfall were documented [70–74]. Guilvuard et al. documented two peaks of infectivity of *Phlebotomus sergenti*, one in August and another in October (9.9%) [57]. Other studies documented the feeding behavior of this sand fly [75, 76]. The relative abundance of males and females of *Ph. sergenti* varied according to the trapping method used [77]. Several studies identified the zymodemes MON-102, MON-107, MON-122 and MON-123 in sand flies infected by *L. tropica*. Ajaoud et al. detected *L. tropica* in human skin samples from CL patients and female *Ph. sergenti* in central Morocco, by using nested PCR for amplifying the ITS1-5.8S rDNA gene [78]. The diversity of parasite zymodemes in those sand flies was not correlated with parasite diversity in humans in Essaouira, Azilal, Taza where a regional genetic differentiation of *Ph. sergenti* exists [77, 78].

Ajaoud et al. corroborated that in the central Moroccan region where *L. tropica* is dominant, the most frequent phlebotomine species are *Ph. sergenti, Ph. longicuspis, Ph. perniciosus*, and *Ph. Sergentomyia* [76, 78]. There was higher diversity in *Ph. sergenti* lineages in Spain compared to Morocco [79]. Nonetheless, a study in Al Haouz province showed that *Ph. sergenti* in Morocco does not have the genetic characteristics of a single species [80]. In the Chichaoua area where the peak biting rate occurs at twilight with some seasonal variability, there was diversity in the nocturnal activity [75]. *Ph. papatasi* and *Ph. sergenti* were also studied in relation to climatic factors and CL risk [74]. In Sefrou province continuous sand fly activity was observed during six months (May–October) for *Ph. sergenti, Ph. perniciosus*, and *Ph. papatasi* with two peaks of transmission, the first peak in July and the second peak in September [81]. Other studies showed
the coexistence of toscana virus\textsuperscript{12} with \textit{L. tropica} specifically in \textit{Ph. sergenti} [82, 83]. Less common sandfly species such as \textit{Phlebotomus pernicious} have been observed [84]. An entomological investigation in the emerging focus of CL in Chichaoua was published in 2005 [85], followed by mathematical modeling of epidemic thresholds [86]. Other entomological surveys described the geographical distribution, current and predicted the distribution of different sand fly vectors of CL in different endemic-epidemic foci in central and southeastern Morocco [70, 74, 87–92]. Furthermore, unique parasite strains and atypical presentations of CL were documented [93–99].

In the past five years, molecular studies of \textit{Leishmania} parasite have gained more interest. Most of the work was published by the leishmaniasis research teams of the “Institut National d’Hygiène”, “Institut Pasteur Maroc” (IPM) and the Faculty of Medicine and Pharmacy of Casablanca, sometimes in collaboration with research units of other Moroccan universities and European institutions [18, 19, 48, 100–104]. In 2007, the first article using ITS1 primers to confirm the CL species was reported by Dr. Rajaoui in collaboration with the Faculty of Medicine in al-Quds University [105]. In 2009, biopsies from 26 patients were analyzed by PCR using primers from the small subunit ribosomal gene. This PCR assay had a good specificity (100%) with a higher sensitivity (84.6%) compared to microscopy (69.2%) and culture (69.2%) [18]. In 2013 a study using Multilocus Microsatellite Typing (MLMT) has suggested the existence of two different subpopulations of \textit{L. infantum} MON1 in Morocco [48]. A study of archived slides collected in the Chichaoua and Marrakech areas showed that the intra-focal occurrence of genetic variants of \textit{L. tropica} documented by MLMT is not due to genetic mutation but is explained by the importation of pre-existing variants of \textit{L. tropica} into Morocco [106]. Molecular methods using ITS1 PCR-RFLP confirmed that \textit{L. tropica} was the causal agent of CL in Azilal province [100]. An evaluation of this ITS1 PCR technique showed that the primers 13A and 13B gave the best sensitivity [19]. In Errachidia, a study based on ITS1 PCR-RFLP confirmed the presence of \textit{L. major} in most of this province but also identified \textit{L. tropica} for the first time [107]. In Sefrou province, an area well known as a focus of \textit{L. tropica} transmitted exclusively by \textit{Ph. sergenti}, the presence of \textit{L. infantum} within \textit{Ph. longicuspis} was confirmed in 2014 by nested PCR (amplifying the ITS-5.8S rDNA gene) [101]. ITS1 PCR-RFLP confirmed the coexistence of \textit{L. tropica} and \textit{L. infantum} in this province in the same year [104]. With the same technique, the presence of \textit{L. tropica} was confirmed in archived microscopic slides collected in Beni Mellal and Fkih Bensaleh [102]. The same technique was used to confirm the presence of \textit{L. tropica} as the causative agent of CL in Chichaoua [108]. A molecular investigation of a nation-wide collection of samples will be available soon [103].

\textsuperscript{12}Toscana virus is an RNA arbovirus (arthropod-borne virus). The virus can be transmitted to humans by the bite of an infected sandfly of the genus \textit{Phlebotomus} [154].
Climate change and its likely effect on the distribution and onset of new epidemic outbreaks of CL have mainly been studied by research teams from the University of Marrakech in collaboration with the National Aeronautics and Space Administration (NASA) [72, 87] or INH [71, 109]. Some of these studies have tried to define the risk factors of anthroponotic CL (L. tropica and L. infantum) and the link with the microenvironment [110, 111] and human behaviors [109].

3.1.2 Epidemiological burden

On one hand, the Global Burden of Disease study, as published in the Lancet Infectious Diseases in 2016 [112] ranks Morocco at the 14th position for CL over the World. On the other hand, according to the WHO global health observatory, Morocco is ranked the 6th with regard the yearly CL number of cases in the WHO EMR region (Table 3) and in the 4th among the 12 CL high burden countries with regard to the incidence rate of 5.62 CL cases/10 000 inhabitants in endemic areas in 2014\(^\text{13}\).

As discussed above several reviews have been carried out to describe the epidemiological burden of CL in Morocco. These were often based on routine data collected by the Ministry of Health [47, 50, 51, 53, 113–115] or data from the HCP [116].

A large number of publications are retrospective surveys, some of them summarised in the previous sections and others documenting the presence of new epidemic foci of CL as shown in table 5 [117–128].

\(^{13}\) http://www.who.int/leishmaniasis/resources/who_wer9122/en/ accessed in 10 april 2018
Table 5. List of the studies reporting Cutaneous Leishmaniasis in Morocco

<table>
<thead>
<tr>
<th>Year of publication</th>
<th>First author</th>
<th>Province of the study</th>
<th>Population sample</th>
<th>Confirmation of CL by</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Mezouari [127]</td>
<td>Commune Tamezmoute</td>
<td>23 cases (Nov-Dec 2013)</td>
<td>Microscopy</td>
</tr>
<tr>
<td>2016</td>
<td>Aasri [121]</td>
<td>Sidi Kacem</td>
<td>415 cases (2006-2014)</td>
<td>Microscopy</td>
</tr>
</tbody>
</table>

Other epidemiological observational studies based on microscopy and ITS1 PCR-RFLP-based leishmanial species identification, documented the geographical distribution of the epidemic and the effect of control measures against reservoir hosts (rodents) [107]. The discovery that more than one species circulates within a province leads to new questions for control, such as the identification of *L. tropica* and *L. infantum* in Errachidia, Ouarzazate and Zagora provinces where it was assumed previously they were endemic for *L. major* species only [107, 129]. Furthermore, the coexistence of both visceral leishmaniasis and CL in the same areas, as showed in the study from Taza, raises the question about the real reservoir of *L. infantum, L. tropica*: is it zoonotic or anthroponotic? [59].
3.1.3 Treatment

The healing potential of plants has been investigated by several recent studies in Moroccan universities [130–135]. No clinical research to assess novel therapeutic interventions in CL has been performed in Morocco. A single retrospective case study was recently published showing the effectiveness of laser in reducing erythematous facial scarring caused by CL in three patients [136].

Meglumine antimoniate (Glucantime®) is the only free treatment available in the public sector for all forms of leishmaniasis in Morocco. The dosage and the way of administration (intravenous or intralesional) depend on the number, size, and localization of CL lesions. Typically, the treatment is composed of two injections per week for four weeks. The standard protocol of leishmaniasis treatment as mentioned in the official MoH manual of leishmaniasis control is reported in table 6 [137].

Unfortunately, there is no well-defined outcome measurement, that would allow objective assessment of the success or the failure of this treatment. Usually, after four weeks of treatment, the ulcer starts healing but almost always leaves a permanent scar. Some CL lesions never come to the attention of clinicians as patients wait for the spontaneous healing.

Up to now, there are no plans for changing the current therapeutic guidelines for CL in Morocco. Moreover, current lack of evidence on Glucantime® resistance does not necessarily mean that it does not exist.
Table 6. Treatment standard protocol for Leishmaniasis in Morocco

<table>
<thead>
<tr>
<th></th>
<th>Systematic treatment</th>
<th>Local treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily intra-muscular injection of Glucantime* (20 mg of Sb5+/ kg without exceeding 2 vials)</td>
<td>Peri-lesional injections of Glucantime* (1 to 3 ml per lesion 2 times a week)</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>Indication of the treatment</td>
<td>Duration of treatment</td>
</tr>
<tr>
<td>Indication of the treatment</td>
<td>Duration of treatment</td>
<td>Indication of the treatment</td>
</tr>
<tr>
<td>Number of lesions ≥ 5 and/or</td>
<td>Size of lesion ≥ 4 cm and/or</td>
<td>Number of lesions &lt; 5 and/or Size of lesion &lt; 4 cm</td>
</tr>
<tr>
<td>Size of lesion ≥ 4 cm and/or</td>
<td>Periorificial lesion or peri-articular and/or lesions localized in finger and toe</td>
<td></td>
</tr>
<tr>
<td>Number of lesions &lt; 5 or Size of lesion &lt; 4 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL with L. tropica</td>
<td>3 weeks</td>
<td>4 weeks or more until complete cure</td>
</tr>
<tr>
<td>CL. with L. major</td>
<td>2 weeks</td>
<td></td>
</tr>
<tr>
<td>Visceral Leishmaniasis</td>
<td>3 weeks</td>
<td></td>
</tr>
</tbody>
</table>

Nb: Local treatment includes also the use of antiseptics (Eosine®, Betadine®) and antibiotic ointment (Aureomycine 3%).

The peri-lesional injection of Glucantime® is done by a syringe with a fine needle (Type insulin syringe). 1 to 3 ml of Glucantime® per session, 2 times a week until a whole cure reached generally between 3 to 4 weeks. The quantity injected of Glucantime® depends on the size of the lesion. For lesions due to L. tropica, the lesion should be covered during the treatment with an adhesive plaster.
3.1.4 Control strategies

Apart from the case study that we will report in this Ph.D. thesis, only three publications addressing control strategies were found, all published by the same team of Moroccan investigators. First, they assessed the insecticide susceptibility status of *Ph. sargentii* and *Ph. papatasi* to lambda-cyhalothrin, DDT, and malathion. All *Phlebotomus* species exposed were susceptible to all insecticides tested [138]. A second study was done in Oued El Biaza in Figuig province in collaboration with the U.S. Army [139]. It showed that rubidium incorporated into rodent baits could be used to demonstrate the level of blood feeding by sand flies. The ivermectin-treated rodent bait acts as a systemic insecticide that kills the adult female sand flies that feed on rodents before the sand flies become infective to vectors and take subsequent bloodmeals from other hosts [139]. The third study was a field evaluation of alpha-cypermethrin in indoor residual spraying for leishmaniasis control in the endemic areas in Boulmane and Taounate provinces. Significant reductions in leishmaniasis incidence and severity rate were observed when comparing sprayed and unsprayed localities. The residual activity of alphacypermethrin lasted for ten weeks after spraying [140]. Another field trial published in 2016 focused on the cost-effectiveness of the use of Indoor Residual Spraying (IRS) with a-cypermethrin, Long-Lasting Insecticide-treated Nets (LLIN) with and without a standard of care environmental management [141]. IRS with a-cypermethrin and LLIN distribution both reduced the incidence of CL in Morocco, though IRS was more effective. Indeed, CL incidence and sandfly abundance were significantly lower in the IRS arm (CL incidence rate ratio was 0.32, 95% CI [0.15–0.69], p=0.005 and sandfly abundance ratio was 0.39, 95% CI [0.18–0.85], p=0.022) [141]. However, a serious limitation of this study was noticed about the community who refused to use the bed nets at a significant level. Then, until the end of 2017, none of those interventions were scaled up. Furthermore, only authorization for research use and not for commercialization of the insecticides used in those studies was obtained.

### 3.2 The CL control policy in Morocco

In the 1980s, the Moroccan Ministry of Health (MoH) set up a unit for the management and control of parasitic diseases to address, amongst others, the CL problem. The main objective of the CL component of the National Programme for Control of Leishmaniasis is to prevent the disfiguring scars that are common sequelae of CL lesions. The MoH provides access to CL diagnosis and treatment free of charge in the public health facilities and does so by standardized protocols based on available evidence and the World Health Organisation expert advice. The national guidelines on the control of leishmaniasis, (1997\(^\text{14}\) and 2010) published by the Directorate of Epidemiology and Disease Control (MoH), consider CL predominantly a self-healing ailment in immunocompetent persons. Health professionals mostly refer to the most

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recent edition (2010) of the Leishmaniasis Control Guide which sets out four objectives for the control of cutaneous leishmaniasis [137]:

- Limit the spread of the parasite in *L. tropica* foci where humans are the reservoir host;
- Ensure rapid healing of CL lesions
- Avoid disfiguring scars
- Avoid visceralisation in CL due to *L. infantum*

4. Psycho-social burden caused by cutaneous leishmaniasis

In Latin America, several studies were conducted to assess the psychosocial burden of CL, and mucocutaneous leishmaniasis (MCL). Most of these researches are based on surveys of Knowledge, Attitudes, and Practices (KAP). In 1994 Weigel et al. published an article from Ecuador, where 208 adult persons participated in individual interviews to answer questions about CL perception. In this study, a gender difference was found. Males were three times more exposed to this disease, and CL reduced their work opportunities. Furthermore, women considered CL decreasing their self-esteem [142]. In Brazil, lack of information and misconceptions about MCL were found in a study of traditional remedies [143]. In North Eastern Peru, native citizens had a limited knowledge about MCL and CL. Some of them used various medicinal plants as remedies against leishmaniasis; while others practiced diet restrictions as a CL treatment [144]. Insufficient knowledge about CL was also the main finding in a study from Venezuela targeting residents in endemic CL areas [145]. In Paraguay, a KAP survey showed the need for a better prevention of new cases, better case recognition, better treatment-seeking behaviors in endemic MCL and CL areas [146]. A study aiming for an in-depth understanding of the perception of CL was done in Brazil by Reis et al. [147]. They assessed the impact of health education on the people’s adherence to biomedical explanations about MCL. For example, “wound” and “mosquito” were the core terms frequently cited by all residents to describe leishmaniasis [147].

To our knowledge, no study measured the psychosocial burden of CL in Morocco or North Africa, except one study published about Tunisia in 2016 [148]. Our scoping review (chapter 5) provides an overview of what has been published so far on the community perception of the localized form of this disease and compares this with similar work published for mucocutaneous and diffuse CL forms. In this thesis, we have studied CL stigma with qualitative methods. We provide a brief definition of the concept of stigma below.

The word “stigma” has its origins in ancient Greece where certain individuals or groups, such as criminals, burglars, or slaves were tattooed, to deny their membership to the civilized or well-
respected communities. Such physical marks defining the difference to others were a source of devaluation and elicited a range of aggressive behaviors towards the holders of those marks and sometimes their families.

Goffman was the first to attract attention to the mutual influence between the stigmatized victim and the stigmatizing persons. He defined stigma as: ‘The phenomenon whereby an individual with an attribute which is deeply discredited by his/her society is rejected as a result of the attribute. Stigma is a process by which the reaction of others spoils normal identity’ [149, 150].

Scambler in 1998 defined the concept of stigma in two major categories: 1) felt stigma where the stigmatized victims develop a fear of perceived discrimination, and 2) enacted stigma as a clear act of discrimination [151]. Then, Weiss further developed this concept to describe the social burden of neglected tropical diseases (Figure 6) [152].

![Figure 6. Hidden distress model of stigma as reported by Weiss [152]](image)

In our thesis project, we used the conceptual framework of the theory of social representations, as developed in the 80s’ by Miscovisci [153].

Miscovisci explained that social representations concern the “contents of everyday thinking and the stock of ideas that gives coherence to our religious beliefs, political ideas and the connections we create as spontaneously as we breath” [153].

As the stigma concept suffers from a certain lack of clarity, we have opted to document the psychosocial burden generated by CL in the larger sense. We documented not just the social burden of any stigmatizing condition, but also the associated concepts of psychological burden in the “daily-life” contextualized environment.
5 Thesis outline

5.1 General objective

The overall aim of this thesis was to contribute to the control of CL in Morocco by documenting the meaning of CL for the exposed population, including the psychosocial burden of CL and by assessing a new tool for CL diagnosis in remote and endemic CL areas.

5.2 Specific objectives

1. To describe the current epidemiological CL trend and describe the control intervention against CL reservoir in southeast Morocco.
2. To explore the perception of this disease in the young resident population in southeast Morocco
3. To evaluate stigmatization associated with CL in the general population of southeast Morocco
4. To review the concept of stigma, quality of life and psychosocial burden of CL in the literature
5. To assess the accuracy of a CL rapid diagnostic test in CL patient living in Moroccan endemic areas.
5.3 Overview of the thesis, by chapter

Each specific objective (SO) has resulted in a publication as presented below.

**SO1: Epidemiological weight of cutaneous leishmaniasis in Morocco.**

See chapter 2


The incidence of cutaneous leishmaniasis (CL) caused by *Leishmania major* has increased in Morocco over the last decade, prompting the Ministry of Health to take intersectoral response measures including vector and reservoir control. This article aimed to describe the CL outbreak response measures taken in the province of Errachidia, where the reservoir of *L. major*, a sand rat (*Meriones shawi*), was targeted using strychnine-poisoned wheat baits from 2010 to 2012. We analyzed routine surveillance data and other information using the data of the CL control programme. We present data on the evolution and the extension of CL in this province as well as the epidemiological profile of the disease. Between 2004 and 2013, 7099 cases of CL were recorded in Errachidia Province, gradually affecting all districts. Our results demonstrate that more women were affected than men and that all age groups were represented. Errachidia Province was the epicenter of the recent CL outbreak in Morocco. A notable decline in incidence rates was observed after 2011. The outbreak control measures may have contributed to this decline, as well as climatic trends or progressing herd immunity.
SO2: Psychosocial weight of cutaneous leishmaniasis scars in adolescents.

See chapter 3


In Morocco, cutaneous leishmaniasis (CL) is usually known to be a slowly healing localized skin disease, but in some cases, it can lead to mutilating scars. The outbreak of CL due to *Leishmania* major in the Errachidia province in southeastern Morocco between 2008 and 2010 left many adolescents with permanent scar tissue on the face or other exposed body parts. We studied the psychosocial impact of CL on these young people. In 2015 we conducted a cross-sectional survey of high-school students living in boarding schools in two CL-endemic areas of Errachidia: Rissani and Tinejdad. A self-administered questionnaire elicited a response about general knowledge of CL and related scars. An open-ended question focused on the possible psychosocial effects associated with these scars. The quantitative data were analyzed with Epi Info™ and the text data with NVivo software. Almost 20% of 448 respondents reported they had experienced a CL lesion and 87% said it could or lead to psychological consequences. The text analysis showed that girls more often than boys expanded on the adverse psychological effects of CL. The students considered CL as “dangerous,” “serious,” and “deathly,” and said it sometimes led to extreme suicidal ideations. The burden of CL in this age group is not negligible. The indelible CL scars lead to self-stigma and social stigma, and the emergence of negative psychological effects in this age group. While some students accepted their CL scars and related suffering as their “destiny,” others were eagerly demanding protective measures against CL and treatment for the scars.
SO3: Psychosocial weight of cutaneous leishmaniasis in general population.

See chapter 4


To document the social burden of Cutaneous Leishmaniasis in rural communities in Southeastern Morocco, between March and April 2015, we conducted qualitative research in communities exposed to *Leishmania major* or *L. tropica* in Errachidia and Tinghir provinces. Twenty-eight focus groups discussions (FGDs) were realized, with a stratification by gender and tradition of medicine (users of folk versus professional medicine). Data were analyzed using content analysis.

This rural population most exposed to CL in Morocco lacks access to health care in general and points out there are other significant public health issues that need to be resolved. Nonetheless, respondents consider the impact of CL lesions and scars as important and similar to that of burn scar tissue. Young women with CL scars in the face are stigmatized and will often be rejected for marriage in these communities. People usually try a long list of folk remedies on the active lesions, but none was felt adequate. There was a definite demand for better treatment as well as for treatment of the scars. The social impact of *L. major* and *L. tropica* is non-negligible, and the demand for better treatment should be addressed.
SO4: Concept of stigma, quality of life and psychosocial burden of CL in the literature.

See chapter 5


Cutaneous Leishmaniasis is a parasitic skin disease, linked to poverty, belonging to the group of Neglected Tropical Diseases. Depending on the severity, the type of lesions or scars, and the context, CL can lead to self- and social stigma influencing the quality of life and psychological well-being of the patient. This dimension is, however, little documented for the most common, localized type of CL. The population of interest for this review are patients with localized CL (LCL) or related scars and their entourage. We aimed to describe the current knowledge on the psychological burden and the stigma related to LCL. We searched the electronic databases PubMed, Web of Knowledge, PsycINFO, POPLINE, Cochrane Library, Science Direct, Global Health, and LILACS, for articles written in Arabic, English, French, Dutch, Portuguese, or Spanish, and published until the end of August 2017. Fifteen papers met our inclusion criteria. Psychological assessment based on standard scales and qualitative research asserts that LCL is a source of psychological suffering, stigmatization, and reduction of quality of life. The fact that the psychosocial burden generated by LCL is time-dependent makes it difficult to measure adequately. More adequate estimates are needed to document the full burden of this NTD specific form.
SO5: A New rapid diagnostic tool for cutaneous leishmaniasis in Morocco

See chapter 6


In Morocco, the diagnosis of cutaneous leishmaniasis (CL) is challenging in remote rural areas. We evaluated the accuracy of a rapid diagnostic test based on antigen detection (RDT), the CL Detect Rapid Test™ (Inbios International, Seattle, USA), in this setting. [Registered in ClinicalTrials.gov as NCT02979002]. We consecutively recruited patients with new skin ulcers in nine primary health centers. We took a dental broach sample for the RDT and two other tissue samples by scraping the border and the center of the lesion with a scalpel and smearing it on a slide. We duplicated each smear by pressing a clean slide against it and processed the slides by microscopy, ITS1 PCR, and kDNA PCR. In a subsample with positive PCR, Leishmania species was identified using PCR-RFLP and PCR-sequencing of hsp70 genes. A confirmed CL case was microscopy and/or PCR positive. We computed sensitivity and specificity of the RDT in comparison to this reference standard. Between December 2016 until July 2017 we included 219 patients, 50% of them were over 17 years old. The RDT showed a sensitivity of 68% [95% CI, 61-74], a specificity of 94% [95% CI, 91-97], a positive predictive value of 95% [95% CI, 92-98] and a negative predictive value of 64% [95% CI, 58-70]. Despite its low sensitivity, this novel RDT is a useful addition to clinical management of CL in Morocco, especially in isolated localities. Positive lesions can be treated as CL, but if negative, microscopy should be done in a second step. The sensitivity of the RDT can probably be optimized by improving the sampling procedure.
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Chapter 2. Control of Cutaneous Leishmaniasis Caused by Leishmania Major in Southeastern Morocco

Control of cutaneous leishmaniasis caused by *Leishmania major* in Southeastern Morocco

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Author Contribution

IB, VDB & MB designed the study. AI, SC & IB designed the intervention. IB & SC implemented the intervention. SC & IB collected all the data. IB, SH & MB analyzed the data. IB wrote the first draft of the paper with input from each listed author. VDB, MB, BA significantly contributed to the writing of different versions of the manuscript. AI & SH commented on drafts of the manuscript. All authors approved the final manuscript.
Abstract:

Objective: The incidence of cutaneous leishmaniasis (CL) caused by *Leishmania major* has increased in Morocco over the last decade, prompting the Ministry of Health to take inter-sectoral response measures including vector and reservoir control. The aim of this article is to describe the CL outbreak response measures taken in the province of Errachidia, where the reservoir of *L. major*, a sand rat (*Meriones shawi*), was targeted using strychnine-poisoned wheat baits from 2010 to 2012.

Method: We analyzed routine surveillance data and other information using the CL data control program.

Results: We present data on the evolution and the extension of CL in this province as well as the epidemiological profile of the disease. Between 2004 and 2013, 7,099 cases of CL were recorded in Errachidia Province, gradually affecting all districts. Our results demonstrate that more women were affected than men and that all age groups were represented.

Conclusion: Errachidia Province was the epicenter of the recent CL outbreak in Morocco. A notable decrease in incidence rates was observed after 2011. The outbreak control measures may have contributed to this decline, next to climatic trends or progressing herd immunity.

Keywords: Cutaneous leishmaniasis, Zoonoses, *Leishmania major*, Rodenticide, Morocco.
Introduction:

Leishmaniasis is a disease caused by protozoa of the genus Leishmania, a parasite that infects many mammals including humans [1] and is transmitted by an insect vector, the phlebotomine sand fly. The clinical spectrum of leishmaniasis includes cutaneous, mucocutaneous and visceral disease. While the latter is the most serious and fatal if not treated, cutaneous leishmaniasis (CL) causes considerable suffering because it can lead to disfiguring scars and social stigma. In North Africa and the Middle East, CL usually occurs in semi-arid and desert conditions, especially in Afghanistan, Algeria, Iran, Pakistan, Saudi Arabia, and Syria [2].

In North African countries, CL transmission has been increasing since the 1980s with the emergence of new foci [3]. Both anthroponotic CL, caused by L. tropica, and zoonotic CL (ZCL), caused by L. major are widespread [2]. While the former occurs mostly in urban areas, L. major affects rural populations and is the topic of this paper. In North-African countries, L. major belongs almost exclusively to the zymodeme Mon 25 [4]. The sand flies Phlebotomus papatasi or a closely related species is the insect vector [3]. The transmission cycle of L. major is complex and requires the presence of a rodent reservoir, such as the jird (Meriones spp), the fat sand rat (Psammomys obesus) or the great gerbil (Rhombomys opimus) [5]. P. obesus transmits L. major in most foci between Syria and Saudi Arabia in the east and Morocco in the west [6]. However, in the south of Morocco, M. shawi is the main reservoir [7, 8]. Meriones are granivorous and can cause important agricultural damage during their periodic population explosions [9]. Moreover, in Morocco it seems that Shaw’s gerbil has adapted to the peridomestic environment where it feeds on excreta and detritus, thriving close to garbage pits [8]. The burrows of these rodents provide optimal sand fly breeding conditions, as the terrestrial larvae of Ph. papatasi thrive in moist organic matter. The adult sand fly uses the well-insulated rodent burrows as diurnal resting sites [10, 11]. During spring, emergent sand flies become infected by feeding on infected reservoir rodents and transmit L. major to other rodents during subsequent feedings. Humans are an accidental host in this cycle and are usually infected during the sand fly season in summer/autumn. There is a close temporal association between the abundance of Ph. papatasi and the incidence of ZCL [12–14]. In Tunisia, the seasonal distribution of human ZCL cases showed one major peak in December, three months after the second peak of Ph. papatasi abundance [15].

The first symptoms of ZCL occur 1 to 6 months after the infective sand fly bite and persist until self-healing. ZCL lesions usually present as multiple ulcerated and often superinfected lesions. A presumptive diagnosis of CL is based on these clinical symptoms while a parasitological diagnosis remains the reference standard. It includes microscopic examination of Giemsa-stained biopsy smears based on the direct identification of amastigote forms with microscopy because more sophisticated techniques are expensive and rarely available in endemic areas [2, 16]. Spontaneous self-healing often occurs in CL after 2 to 6 months but leaves a scar. Depending on
the species, this tendency to self-cure usually occurs within approximately 2 to 6 months (e.g., *L. major*), or 6 to 15 months (e.g., *L. tropica*) of disease onset [2, 17]. Apart from species, the rate of spontaneous healing of the lesion depends on factors such as the parasite load, its virulence, the immune response of the host, the location of the lesion and the presence or absence of a bacterial infection [14, 18, 19].

ZCL cases usually fluctuate in an epidemic cycle with five- to ten-years intervals [6]. Fluctuating numbers of cases were attributed either to the cyclic development and loss of herd immunity or to natural disasters such as floods and famines [20]. There is evidence that the lack of IFN-gamma allows parasite multiplication and progression from infection to disease. Treatment of non-healing CL with IFN-gamma resulted in rapid and complete resolution of lesions [21]. However, the immunopathological and immune-protective mechanisms occurring during CL are difficult to establish without longitudinal studies accounting for the genetic heterogeneity of human and parasite populations [22].

The lack of an effective prophylactic vaccine suggests that we still do not fully understand the factors that regulate the induction and maintenance of anti-Leishmania immunity. Understanding these factors is critical for the design of effective vaccine and/or vaccination strategy against leishmaniasis [23].

According to the literature, the most effective way to control *L. major* is to combine reservoir and vector control, but the evidence of the effectiveness of this approach is limited to a small number of case studies [2]. Success stories include the destruction of rodent burrows as resting and breeding sites for *Ph. papatasi* between the 1940s and 1980s in the former Soviet Union as reported in the literature [24, 25]. Measures for controlling *Meriones* species were developed initially for agricultural purposes and are mostly based on anticoagulants and zinc phosphide as rodenticides [26]. Guidelines for Leishmaniasis control published by WHO in 1988 suggested introducing poisoned bait in burrow entrances to control the foci where *Meriones* species is the reservoir host [27].

Since 2008, an unusual increase in CL cases was observed in the province of Errachidia in Morocco that raised growing concern and frustration in the population and prompted the local health authorities to take extra control measures. An entomological survey conducted in Errachidia Province in July 2009 by the National Institute of Hygiene (INH Morocco) had demonstrated the presence of the species *Ph. papatasi* [28] as well as the reservoir *Meriones shawi*, but rodent control measures in the province had so far only been taken by the Ministry of Agriculture (MoA) in the framework of crop protection. From 2010 onwards, the local health authorities in Errachidia added the rodent control component to their ZCL outbreak response strategy. This paper describes the epidemiological pattern of the recent CL outbreak due to *L. major* in Errachidia Province and the results of the control strategy.
Materials and Methods

Study Area

Errachidia Province is located in the Ziz Ghriss Valley in the southeast of Morocco, including the Saharan areas, plains and highlands at an altitude above 1900 m, and covering a surface of 46 000 km². Errachidia has an arid climate with temperatures between -4 °C and 48 °C, with large daily and seasonal temperature variations. The annual mean temperature is 21 °C. Rainfall is scarce and usually occurs between February and March. The annual total precipitation is 134 ± 64 mm [29].

The province consists today of seven urban agglomerations (Errachidia, Erfoud, Goulmima Tinejdad, Moulay Ali Cherif, Boudnib, and Jorf) and 22 rural districts, with 496 localities (Suppl 1). An administrative reform in 2010 redesigned provincial boundaries and the total population of the province became 400 422 instead of 564 000 inhabitants. In this analysis, we have excluded the CL cases recorded in various localities that are no longer part of the province of Errachidia today.

Intervention

The CL control policy of the Ministry of Health (MoH) in Morocco recommends rodent control as one of the interventions against ZCL, next to screening and treatment of patients, vector control and enhancement of environmental sanitation [30]. All these actions should be supported by health education and intersectoral collaboration. However, in most provinces, local health authorities lack the resources for rodent control and limit themselves to promoting environmental hygiene and some limited insecticide spraying around urban and touristic centers.

Contrastingly, the Ministry of Agriculture (MoA) in Morocco actively implements rodent control in the framework of crop protection. The MoA produces the poisoned bait as the use of strychnine is strictly regulated and can be handled only by this Ministry. No strychnine is available in the commercial market. The technical staff of the MoA distributes the poisoned bait to farmers along with instructions for its use. Surveillance of the rodent population is based on sentinel site surveillance of the number of active borrows throughout the year.

In Errachidia Province, because of the alarming CL outbreak, the local health authorities of MoH decided end 2009 to join forces with the MoA workers in an attempt to control the rodent reservoir. The first joint control campaign with poisoned baits was conducted between early December 2009 and end of March 2010, and was from the outset inter-sectoral, with a strong commitment from both MoA and MoH. Technicians from both ministries working in close
collaboration supervised the community workers who applied the baits in the peridomestic environment. This was repeated every year thereafter for three consecutive years.

In brief, a squad of 2 to 4 control workers with a supervisor was dispatched to the affected village. The workers surveyed the full perimeter around the human settlement for rodent burrows up to the boundary where palm trees or cultivated fields started. Each active rodent hole was treated as follows. A worker wearing protective gloves and gear deposited six to twelve grains of strychnine-poisoned wheat about 10 cm inside the burrow using a spoon and the tip of a plastic bottle, under the supervision of the team leader. Three days later all dead rodents were disposed of (Figure 1). After 15 to 30 days, the remaining active burrows were counted, and the poisoned-bait application was repeated up to two times depending on the number of persistent active burrows. This control operation was repeated for three consecutive years with the same timing.

![Image of rodents and baits](image.png)

**Figure 1: Poisoned wheat introduced into one burrow (On the left). A colony of Meriones Shawi rodents killed by this technique in Errachidia Province (On the right)**

This intensive campaign was managed as a collaboration between local authorities, municipalities, MoA and MoH working together in the Committee of Integrated Management of Vector Control (CIMVC). Additional measures implemented from February 2010 onwards comprised health education messages to encourage people to use rodenticides at home against domestic rats and increase individual and community hygiene, including individual protection.
measures to avoid insect bites. In addition, complementary measures were introduced by the CIMVC in the seven urban districts and touristic areas of the province. These were vector control of mosquitoes using insecticide spraying between May and August and environmental sanitation around cities and villages involving local communities.

Data collection & analysis

This is a retrospective descriptive study of the ZCL epidemiology in Errachidia Province, dating back to the initiation of epidemiological surveillance in 1998 and including the intensive control measures started in 2010. We analyzed routine reports on the control program’s standard preventive measures, as well as anonymized data from the epidemiological surveillance system and reports at the provincial and national level. We consulted the central database to crosscheck the reporting of cases among Errachidia residents diagnosed or reported elsewhere. Information about laboratory diagnosis was only available for the period after 2008. The database was analyzed with the approval of the national health authorities in Morocco (MoH, Directorate of Epidemiology and Diseases Control).

We included only native Moroccan residents in rural and urban areas of Errachidia Province. The case definition of CL used in this study was the standard MoH definition: any person in an endemic area with at least one clinically active skin lesion reported as CL by a health professional [30]. Laboratory confirmation was only sought in the first five cases reported for all newly affected localities. Confirmation was achieved by direct microscopic examination of tissue scraping for the detection of amastigote forms. Most of the other cases were clinically diagnosed by medical staff in health centers or by dermatologists in hospitals, because only one clinical microbiology laboratory was located in the chief town of the province, far from most of the rural areas.

We collected data on age, sex, period of diagnosis for each case, which were subsequently anonymized. Data entry was performed using Microsoft Office Excel 2013 and analyzed by SPSS Software Version 19.1. Epidemiological curves are presented according to the Gregorian calendar. We used chi-square tests to compare proportions. Quantum GIS Geographic Information System (Open Source Geospatial Foundation Project) was used to design and develop CL distribution maps.
Chapter 2

Results

Since the start of the recording of ZCL cases in the Province of Errachidia in 1998, and up to 2003, 907 cases were reported. Between 2004 and 2013 the total number of cases in the Province reached 7099, with a peak number of 3483 cases (49%) in 2010 (Figure 2). Almost all cases were clinically diagnosed by health centers or hospitals, but 237 cases were parasitologically confirmed (out of a total of 303 smear examinations performed) (Table 1).

Table 1 Cases of cutaneous leishmaniasis in the province of Errachidia between 2004 and 2013 by age group, gender, area of residence, trimester of diagnosis and diagnosis confirmation

<table>
<thead>
<tr>
<th>Age groups* [0–10]</th>
<th>Total Cases</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>[0–10]</td>
<td>1854</td>
<td>26.1</td>
</tr>
<tr>
<td>[31–40]</td>
<td>782</td>
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<tr>
<td>[41–50]</td>
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<td>471</td>
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</tr>
<tr>
<td>&gt;60</td>
<td>348</td>
<td>4.9</td>
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<th>%</th>
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</thead>
<tbody>
<tr>
<td>Female</td>
<td>4184</td>
<td>58.9</td>
</tr>
<tr>
<td>Male</td>
<td>2915</td>
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</table>

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
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<td>Urban</td>
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</table>

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<td>Fall</td>
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</tr>
<tr>
<td>Winter</td>
<td>2453</td>
<td>34.6</td>
</tr>
<tr>
<td>Spring</td>
<td>304</td>
<td>4.3</td>
</tr>
<tr>
<td>Summer</td>
<td>520</td>
<td>7.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>Total Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>5250</td>
<td>94.6</td>
</tr>
<tr>
<td>Laboratory</td>
<td>303</td>
<td>5.4</td>
</tr>
</tbody>
</table>

*One missing data.
†Data available from 2009.
Figure 2: Number cases of Zoonotic Cutaneous Leishmaniasis per year in Errachidia Province and other affected Moroccan Provinces between 2004 and 2013.

The outbreak was first recognized as such in 2007 in the area of Merzouga in the southeast of the province, in Taous district bordering Algeria (Figure 3), where a total of 94 cases were recorded with an incidence rate (IR) of 2500 cases per 100,000 inhabitants. The following year, the outbreak spread to the north and west of the province. By 2010 all areas were affected, and the overall incidence rate was 869 per 100,000 inhabitants in that peak year. Incidence rates ranged between 11 and 9382 per 100,000 inhabitants in the rural districts Chorfa M’Daghra and Sidi Ali respectively.
Between 2010 and 2012, all affected urban and rural district areas in the province were treated with poisoned bait, covering approximately 5000 hectares. In these three years, 1287 kg of strychnine-poisoned wheat was used. The total number of active burrows observed before and after these operations are provided as additional information (Suppl 2). Initially, the campaign targeted 322 571 active burrows. Although only 80% to 89% of them could be treated, after the first round of poisoning in 2010, the number of active burrows decreased by 95%. After the launch of the campaign, we recorded a steep decrease in the number of CL-affected areas, from 28 areas in 2011 to 17 in 2012 and 5 areas in 2013 (Figure 3).

The overall incidence rate was reduced to 9 per 100 000 inhabitants in 2013 in the affected areas in the province. However, the city of Errachidia and its neighboring rural areas (Aoufous, Chorfa M’Daghra, Lkheng), which were not part of the rodent control campaign in 2010, recorded an increase in the number of CL cases from 54 in 2010 to 84 cases in 2011 (Suppl 3). After the rodent control intervention was equally applied throughout this area in 2012, the number of annual cases decreased to 12.
Figure 4: Seasonality pattern of zoonotic CL cases in Errachidia Province showing cumulative number per month for the period 2004 to 2013, with breakdown by rural and urban areas.

The maximum caseload was recorded in the fall and early winter season (Figure 4). The rural areas declared 83% of all registered cases. All age groups were affected, and the average age of cases was 24 ± 18 years. The most affected age group were the [11-20]-year-olds with 28% of cases, followed by the age group [0-10]-year-olds (26% of cases) (Table 1). Women were more often affected with 59% of the total number of cases. The female predominance of CL cases was present in all age groups, except in those older than 60 years (Figure 5).

Figure 5: Sex and age distribution of all cases of Zoonotic Cutaneous Leishmaniasis in Errachidia Province from 2004 to 2013.
Discussion

Our results describe a succession of yearly CL outbreaks in Errachidia Province, resulting in a total of 7099 cases between 2004 and 2013, with a peak incidence in 2010. After the launch of an intensive inter-sectorial campaign in 2010 including for the first-time rodent control to target the CL reservoir host, we observed a substantial reduction in the incidence rate of CL (from 869 to 9/100 000 inhabitants per year). However, the observed decline in the CL incidence rate in Errachidia Province could also be due to the natural cycle of the epidemic, with a decline in incidence when the number of susceptible persons in the human population has become too low. Climate factors may also play a role in the incidence trend of ZCL [29]. Whereas higher temperature may positively influence the abundance of the sand fly [31, 32], progressing desertification and lack of humidity could in turn force the rodent population to move to wetter areas and lead to decreases in incidence [10, 32]. Our data do not allow to disentangle those effects. Nevertheless, the systematic application of poisoned bait in the CL affected areas in Errachidia province has probably contributed to the decrease in CL incidence, as it led to a 95% reduction in the number of active rodent burrows in treated areas, which was sustained for each of the three years of the intervention. The effect observed with this strychnine-poisoned bait intervention was more important than that observed by others with anticoagulants or zinc phosphide, a 48% and 58% reduction respectively [26]. Another intervention in the valley of Turkmenia (Turkmenistan, Ex-Soviet Union) in 1941 succeeded in reducing human morbidity from 70% to 0.4% when chloropicrin bait was used in the burrows of large gerbils [25]. In Iran, poisoned bait applications led to a less impressive reduction in cases [26, 33]. A recent overview article about ZCL trends in Morocco confirmed that the peak incidence in the country was reached in 2010 with 6444 cases, and declined sharply to 2219 cases in 2011 and 740 cases in 2012 [34]. Errachidia Province was the major contributor to this caseload, accounting for 3483 or 54% of the total number of cases in the country in 2010 (Figure 2).

In this Province, the unique feature was the active collaboration of the local MoH leishmaniasis control program with the MoA in implementing the integrated management of vector control (IMVC) strategy into the affected areas. The MoH monitored the outcome of the strategy very closely, apparently with good results. The rodenticide strategy we describe has been used in Morocco for crop protection for decades and has been used elsewhere for ZCL control. Detailed knowledge of the animal and vector reservoirs of ZCL in a specific area is essential to use this chemical method with success. The rationale for rodent control in an integrated ZCL control strategy is based on the principle of the momentary reduction of the rodent population to reduce the infected reservoir [35]. If fewer sandflies have an infected blood meal it is expected that less will accidentally contaminate humans. The timing of the poisoned-bait intervention is essential, because the transmission from rodent to humans by the sand flies is most intensive in the summer months (May to September), and food habits of rodents are seasonal.
To be effective, the application of baits should take place from December to March, before the reproduction season of rodents and before the beginning of the sand fly season. Wheat bait is chosen because *Meriones shawi* stores grain reserves in its burrows and survives on it during winter [36].

The reported incidence of CL was higher among women than men, although men have a higher exposure level due to their occupations. In contrast, a higher CL incidence in men was found in a study in Iran [37] and in the Middle East [38]. Other studies from Saudi Arabia supported the hypothesis that the CL sex ratio is dependent on geographic location and cultural patterns [39, 40].

As men in Errachidia generally perceive CL as non-severe, this may have led to some under-reporting of cases and the true incidence rate may, therefore, have been higher than what we report here. Another limitation of our study was that cases were mainly ascertained clinically, though in each new area affected the first suspect cases were systematically confirmed by parasitological exam. Further surveillance thereafter was clinical. In addition, the poisoned bait strategy we describe will not work with *P. obesus*, as they do not feed on grain in the same way as *Meriones*. Finally, we fully acknowledge that the observed reduction in incidence cannot be directly attributed to the rodent control component, because of the observational nature of our study, and the fact that the intervention was integrated in a larger package of control measures. The absence of any control group does not allow establishing a causal effect.

The application of poisoned grains in the peridomestic environment for ZCL control may be hazardous as there is a lack of clear guidance on the methodology and precautions to take. Even if no cases of adverse events or fatalities of animals or humans have been reported to date with the strategy and dose applied, human fatalities have been reported at doses in excess of 5 to 10 mg per weight in the literature [41]. Moreover, questions can be raised about the environmental impact and sustainability of the strategy. We have no information about the effect of strychnine on protected species (e.g. *Rhombomys opimus*, hyraxes), about its efficacy in the longer term, or about its adverse effects on the ecosystem. There is no clear answer to these questions, and instead of promoting strychnine-based rodent killing on a large scale in similar contexts, we conclude that more eco-friendly alternatives for reservoir control are urgently needed. Reservoir control should include environmental surveillance to monitor the expansion of rodent populations after the rainy seasons as well as entomological surveillance to predict ZCL epidemics and target the control measures effectively [10].
Conclusion

It appears that the intersectoral ZCL control strategy adopted by the Province of Errachidia in 2010 contributed to a quick and major reduction in incidence in the context of the 2004-2013 outbreak. Given there is no vaccine for this disease, more appropriate and less toxic control measures are desperately needed. Meanwhile, health authorities should be aware of the rodenticide approach, and use it with caution as a response measure in similar contexts of ZCL outbreaks.

Acknowledgment

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Competing interests

The authors have no conflict of interest to declare. This manuscript is not submitted elsewhere for publication.

References


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27. WHO (1988) Guidelines for leishmaniasis control at regional and subregional levels.


Supporting information

Additional supporting information may be found in the online version of this article:

**Fig. S1.** Maps of the administrative division of districts in Errachidia Province - Kingdom of Morocco

**Table S1.** The number of burrows before and after operations to control the density of rodent Meriones Shawi by subdivision in the Errachidia Province.

**Table S2.** Distribution of cases and incidence of cutaneous leishmaniasis due to Leishmania major in Errachidia Province by districts.

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CHAPTER 3. Psychosocial impact of scars due to cutaneous leishmaniasis on high school students in Errachidia province, Morocco

Psychosocial impact of scars due to cutaneous leishmaniasis on high school students in Errachidia province, Morocco

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Abstract

Background: In Morocco, cutaneous leishmaniasis (CL) is usually known to be a slowly healing localized skin disease, but in some cases, it can lead to mutilating scars. The outbreak of CL due to *Leishmania major* in the Errachidia province in southeastern Morocco between 2008 and 2010 left many adolescents with permanent scar tissue on the face or other exposed body parts. We studied the psychosocial impact of CL on these young people.

Methods: In 2015 we conducted a cross-sectional survey among high-school students living in boarding schools in two CL-endemic areas of Errachidia: Rissani and Tinejdad. A self-administered questionnaire elicited responses about general knowledge of CL and related scars. An open-ended question focused on the possible psychosocial effects associated with these scars. The quantitative data were analyzed with Epi Info™ and the text data with NVivo software.

Results: Almost 20% of 448 respondents reported they had experienced a CL lesion and 87% said it could possibly or definitely lead to psychological consequences. The text analysis showed that girls more often than boys expanded on the negative psychological effects of CL. The students considered CL as “dangerous”, “serious”, and “deathly”, and said it sometimes led to extreme suicidal ideations.

Conclusion: The burden of CL in this age group is not negligible. The indelible CL scars lead to self-stigma and social stigma, and the emergence of negative psychological effects in this age group. While some students accepted their CL scars and related suffering as their “destiny”, others were eagerly demanding treatment to heal the scars and were claiming protective measures against CL.

Keywords: belief, body image, cutaneous leishmaniasis, *Leishmania major*, scars, self-concept, self-stigma, social stigma, students, adolescents, Errachidia, Morocco
Introduction

Leishmaniasis, a parasitic disease of the genus *Leishmania* transmitted by a sand-fly vector, is one of the most neglected diseases in the world, affecting the poorest of the poor [1]. In low- and middle-income countries, the affected population lives in precarious dwellings within vulnerable environmental conditions [2].

Cutaneous leishmaniasis (CL), the most frequent clinical presentation, usually presents as a localized lesion at the site of the sand-fly bite after a lapse of several days to months [2]. The lesion starts as an erythema that gradually transforms to a papule and later to a nodule. The nodule then increases in volume and progressively ulcerates [3]. The whole process generally takes between two and six months [4]. In immunocompetent persons, CL lesions are slowly self-healing but they often lead to scar tissue [5]. As the sand flies bite most often in the face or other exposed parts of the body, these disfiguring scars can lead to substantial psychological and social suffering, and economic losses [6]. Lesions affecting the central area of the face have a higher impact compared with others [7].

Studies conducted in Afghanistan, Pakistan, Syria, and Iran have demonstrated the serious social consequences of CL for young women. They may not be able to get married in the future [8–11] or not be allowed to stay with their partner if they contract CL after marriage [12]. Often, persons affected by CL during childhood only become aware of their affected body image during adolescence when the indelible scars become more visible in their own eyes and/or the eyes of their loved ones [13]. Indeed, the perception of the body, more precisely the self-perceived beauty of the face, is a major factor influencing self-awareness, especially, but not only, in women. In contrast, a similar facial scar in a male subject could be considered attractive in some communities [14].

Interestingly, a recent study quantified the CL burden by estimating the related disability-adjusted life years lost (DALY) based on physical disfigurement only. The authors of that study stated that the CL burden calculated in this way did not differ significantly in terms of age in the same geographic region, probably because this approach does not take into account the social stigma or the emotional or financial impact of CL [15]. However, as also demonstrated in the case of lymphatic filariasis, the disabling or disfiguring sequelae of some neglected tropical diseases can have a substantial impact on mental health [16].

In Morocco, CL is caused by two species, *L. major*, and *L. tropica*. The first is a zoonotic disease with rodents as a reservoir host, the second is anthroponotic [17]. CL due to *L. major* has recently led to important epidemics. The Errachidia province in southeastern Morocco has seen an epidemic peak in most of its districts between 2008 and 2010 [18, 19]. The most affected age group was those aged between 11 and 20 years, which is rarely studied in the literature.
Therefore, the aim of this article is to describe the psychosocial impact of CL on adolescents in Morocco’s L. major-endemic areas.

Method

Conceptual framework

Our concept of stigma is rooted in the framework proposed by Bos et al. [20], which was adapted from the one developed by Pryor et al. [21] (see Figure 1). Stigma is categorized by these authors as belonging to one of four types. The public (or social) stigma is at the core of the model and refers to the social and psychological reactions of society to the person who has the stigmatized condition [22]. Self-stigma reflects the impact of stigma on the stigmatized person and is partly internalized through a reduction in self-worth and psychological distress. Stigma by association reflects the negative reactions to family and friends of stigmatized persons and their attitudes to this [23]. Then, once the stigma becomes institutionalized within society, the authors define the fourth category as “structural stigma”, when the ideological systems of society perpetuate the stigmatized status [21].

![Figure 1: Conceptualization of ‘stigma’, based on the framework developed by Bos et al. (2013) and Pryor et al. (2004)](image)

As stated above, those who internalize stigma often experience a significant loss of self-esteem [22]. The relationship between self-concept and self-stigma is well documented in psychiatry. Stigmatized people have negative attitudes towards themselves as a reaction to their condition [24]. Self-concept should be distinguished from self-awareness and self-esteem. McConnell et al. [25] state that the awareness about the self is always influenced by the context.
Over time, the person develops a self-concept, which interacts with self-esteem, self-knowledge, and self-awareness within the social context. In this article, we use the term self-concept as the cognitive description of one’s self (self-awareness) added to the opinion about one’s self (self-esteem).

**Study population**

In April 2015, we carried out a cross-sectional questionnaire survey among boarding school students in two areas of the Errachidia province that reported high CL attack rates. The choice of these two areas was based on the reported incidence of CL cases, as published earlier [18]. One school was located in Tinejdad city with boarding facilities serving the surrounding districts of the Tinejdad Ferkla area. The second school was located in Rissani city serving the districts of the Rissani Sfalate area (see Table 1).

The two schools enrolled a total of 3,246 students including 561 boarding students with separate facilities for the girls and the boys. Henceforward, we will use the codes “FT” or “MT” to identify respectively the group of girls (F) and boys (M) from the Tinejdad (T) area, and the codes “FR” and “MR” to identify the respective groups of girls and boys from the Rissani (R) area.

**Table 1: Origin of interviewed boarding school students, stratified by gender (n=448)**

<table>
<thead>
<tr>
<th>District</th>
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</tr>
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<tr>
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<tr>
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<td>Sidi Ali</td>
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<tr>
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<td>70</td>
<td>101</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>190</td>
<td>258</td>
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Survey questionnaire

The survey was based on a self-administered questionnaire developed to assess the students’ general knowledge about CL. We pre-tested the questionnaire on 10 students from Errachidia city and found that the time it took to fill out the form was about 10 minutes. In each high school, the questionnaires were distributed on the same day to all present boarding school students. The questionnaire was in Arabic (English translation in Additional File 1, original Arabic form in Additional File 2).

The introductory part consisted of an information sheet and consent section for voluntary participation in the study, with some administrative information. The questionnaire itself consisted of 18 closed-ended questions and concluded with one open-ended question about the perceived psychological effects of CL scars (see Additional file 3).

As a start, the principal investigator (the first author) explained the purpose of the study and the confidentiality conditions to the students during an extracurricular study session. It was also stressed that the students were free to refuse to partly or completely answer the questionnaire. Then, he invited the respondents to select their answers and write a short paragraph for the last question. Students did not communicate with each other while answering the questions and a boarding master supervised each group. In the end, students returned the questionnaires to the investigator one by one (see Additional file 4 for all student responses).

Data analysis

Regarding the 18 closed-ended questions of the survey, the data entry, initial data cleaning, and descriptive analysis were done using Epi Info™ 7 (CDC Atlanta, USA) software. Three socio-epidemiological attributes were chosen for the comparative analysis: 1) participant’s gender: female vs. male; 2) a history of being affected by CL (personal experience with CL): yes or no; and 3) area of residence: Tinejdad or Rissani.

Students’ answers to the open-ended questions required the verbatim transcription in the Arabic language and then translation into French. The text analysis was performed with NVivo software version 10 (QSR International Melbourne Australia). The coding followed a deductive approach based on the conceptual framework (see Figure 1). An inductive analysis was done to extend the generated codes (see Figure 2) in order to define the factors which influenced the impact of CL scars on the patient’s psychological state. Five major themes were elucidated: perception of body image, self-stigma, social stigma, self-concept, and health-seeking behavior. Attributes of gender, area of residence, and personal CL experience were also used to classify answers in order to examine relationships within these qualitative data.
Results

From the 454 students present on the day of the survey, 258 boys and 190 girls (448 in total) participated in the study, while six boys refused. The mean age of the respondents was 18.1 years ± 2.3 for boys and 17.2 years ± 1.6 for girls. Table 1 shows the origin of the students stratified by gender. All participants were from rural districts that declared CL outbreaks between 2008 and 2010 [18].

Affliction with and general knowledge about CL

In total, 362 out of 448 respondents (80.8%) said they knew about CL and 60% correctly indicated that an insect transmits CL to humans. Eighty-eight participants (19.6%) reported having been personally infected with CL (38 girls, 50 boys) and among the others, 159 knew at least one person who has been affected by CL. In the group of 88 students that had been personally infected with CL, 71 remembered the specific year that they were affected. Most cases appeared between 2008 and 2010.
When asked if they knew CL by any local name, 181 participants out of 448 replied “Leishmania”. Twenty wrote a name connected with a “mosquito” and 15 described CL as a boil or a sore such as the “boil of Ata”. (The Ata is an ethnic group living outside the Errachidia province). Finally, two respondents noted “azmoulen”, an Amazigh word for “scar”. A minority of 26 students wrote the name of a non-related skin disease, such as measles, eczema, syphilis, jaundice, or urticaria.

The majority of respondents (n=356) stated that CL leaves marks and scars on the skin, usually on the face (n=315). Girls more often than boys pointed out that CL leaves scars and that these scars are on the face.

Half of the students evoked the existence of medical treatment against CL scars, while one third quoted traditional remedies such as henna, eucalyptus, saffron, aloe vera, tea, Artemisia, Sahara grass, among others. Some mentioned food items such as olive oil, lemon, eggs, honey, onion, and garlic. Others evoked traditional practices such as burning the diseased skin, the use of tar, acids (bleach, alcohol, bleach water), or urine of animals. In addition, four students referred to the use of expired drugs (pills blended together or the application in powder form of an outdated injectable antibiotic) in order to treat CL lesions. However, no one reported any specific traditional remedy against CL scars.

Almost all respondents (86%, n=384) wrote that CL possibly or definitely has psychological effects. Table 2 shows that there was no significant difference in response to this question related to gender or personal experience with CL, although almost twice as many students from the Rissani area (12.8%) considered there were no psychological consequences than students from the Tinejdad area (6.6%).

The text analysis of the open-ended question about the possible CL impact is presented below in three thematic sections: 1) CL severity and body image; 2) CL scars and stigma; and 3) dealing with scars. A total of 52 boys (20.2%) and 24 girls (12.6%) did not answer the open-ended question.
Table 2: Potential psychological effects of CL scars on students, stratified by gender, area of origin, and personal CL experience (n=422)

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Psychological effects of CL scars*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Girls (n=178)</td>
<td>82</td>
</tr>
<tr>
<td>Boys (n=244)</td>
<td>99</td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td></td>
</tr>
<tr>
<td>Rissani area (n = 164)</td>
<td>74</td>
</tr>
<tr>
<td>Tinejdad area (n=258)</td>
<td>107</td>
</tr>
<tr>
<td><strong>Personal CL experience</strong></td>
<td></td>
</tr>
<tr>
<td>Personal CL experience (n=86)</td>
<td>42</td>
</tr>
<tr>
<td>No Personal CL experience (n=333)</td>
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</tr>
</tbody>
</table>

*26 missing data
*Two non-respondents to this question
*One non-respondent to this question

CL severity and body image

The students’ perception of the severity of CL can be summarized in three interrelated words: “dangerous”, “serious”, and “deathly”.

**Dangerous:** CL is perceived by many as dangerous because of the fear of contagion and the risk of transmitting CL to relatives: “It will lead to social isolation and the person will live away from others due to fear of contaminating them.” (MT305). The fear is also given as an explanation of the rejection by their own relatives: “The person affected is afraid by this disease as there is a risk it could worsen and not heal. He will be disturbed by its appearance if seen [by others] and be afraid that his friends and family will become distant from him and reject him for fear of being affected by the same disease.” (MR081). Another boy wrote: “He will not be able to share meals with his family.” (MT360).

**Serious:** One girl wrote: “The affected person will be disgusted to see himself in the mirror.” (FT186). Another wrote: “Sometimes the affected people end up hating themselves.” (FR044). Differences according to gender were observed in terms of use of the word “scar”. Many girls but very few boys thought that CL scars are considerably worse for a woman than for a man. According to our respondents, girls are more concerned about the effects of CL scars, the appearance of their face, and the beauty of their body. Three boys reported that men are more heavily affected by CL, as they lose their masculinity as a result of the disease. One boy evoked the need for psychological support: “… Also it causes the patient psychological and dermatological effects over a long period of time, which requires a visit to a psychiatrist.” (MR121). One girl noted that both sexes suffered equally due to the disease (FR031).
Deathly: A few respondents thought (erroneously) that CL can have a fatal outcome and wrote that death is the natural outcome of the disease. Interestingly, others evoked a fatal outcome in the context of suicidal thoughts, as stated by one boy: “This disease often influences some people because it leaves scars from the beginning that lead a person to commit suicide.” (MR123). In addition, four girls cited the terms “depression” and “suicidal ideations” in their answers. One girl noted: “Cutaneous leishmaniasis influences the psychological state and leads to death.” (FT289).

CL scars and stigma

The fear of facing others was frequently mentioned by both sexes. “…The affected person cannot talk about his disease due to fear of being rejected by people,” noted one boy (MR132). “The person affected cannot show this to his friends because they will not want to sit with him,” said another boy (MR160). The attitude of friends and family influences the psychological state of the affected person, as reported by one girl: “She will have depression, a durable fear, and shame. She does not have the absolute courage to sit with her friends for fear of their mockery.” (FT237).

Additionally, girls noted components of self-stigma such as shame, embarrassment, depression, and self-contempt. One girl noted: “The affected person is very worried and feels ashamed in the presence of people. She prohibits herself to go to rallies because of this disease.” (FT 225). Another wrote: “The sequelae of leishmaniasis negatively influence the condition of the affected person. A psychological complex will develop, and she will be ashamed to appear in front of friends because it is a mark of shame and contempt.” (FT265).

The answers show how self-stigma and social stigma are linked. When the scar is formed, people tend to stare at it and the gaze of others induces social rejection of the affected person. “It affects me every time I meet my friends. They look at my scar, it diminishes my value in front of people,” reported a girl who experienced CL (FR009). Consequently, communication barriers emerge: “The scar is a mark of shame and contempt. The affected person will be unable to cope with society due to fear of social discrimination and contempt.” (MT413). One girl noted: “She will become closed off (introverted) and she will not talk to people and she is not going to love looking at herself because of these scars.” (FT243). The affected person no longer has the same appearance as before, and she/he is now different from others. The negative behavior of others leads to a feeling of isolation and difficulty in everyday life: “There is a feeling of isolation and loneliness, and a lack of stability in daily life because of the negative attitude of society towards the patient,” wrote one boy (MR120). Another girl wrote: “For a girl when the disease leaves a mark on the face, for example, the girl will think that is dangerous for her beauty, which will influence her psychological state. Especially in our traditional society that is absolutely not lenient towards those who have spots on the face because they think it is hereditary.” (FT194). CL can lead to ‘social death’ as explained by one girl: “Moroccan society is not merciful and judges
people’s appearance.” (FT241). Indeed, some participants linked CL scars with diminished chances of getting married: “Often the scars in affected men and especially in affected women are a barrier to getting married because the scars are visible, something that is not tolerated by fiancés.” (FR049). Another girl noted: “Women have more fear on their faces, afraid that the young man who comes forward to ask for her hand will disappear after having seen these stains.” (FR001). A girl who previously experienced CL noted that the possibility of not getting married is a problem only for women: “She will be afraid about her future especially for the wedding. Meanwhile, in our society, an affected boy remains a man. There is no harm if he has scars.” (FT202).

In addition, the fear to meet others leads to a lower self-concept: “The girl is ashamed to show her face; scars can prevent her to leave the house, thereby increasing her psychological suffering” (FR018). “Why me?” is a question that a person affected by CL often asks: “Why am I the person who has this disease and carries this mark on the face?” reported a boy previously affected by CL (MR153).

**Dealing with scars**

Half of the respondents wrote that treatment for CL scars was available in hospitals from general practitioners or dermatologists. However, the affected person slowly understands after trying various treatments that these scars will never disappear: “The psychological state of the affected person could become worse after receiving treatment because the problem is that scars never disappear [even after treatment],” noted a boy previously affected by CL (MR116). “The fear and worry regarding the lack of treatment for this disease is the real problem for a person affected by it,” added a girl also previously affected by CL (FR011). The ineffectiveness of delayed treatment was also something stressed upon: “We need prompt treatment for this disease to avoid any effect on the psychological state of the person affected.” (MR174). In addition, one girl mentioned the problem of unaffordable treatment (FT224). Nevertheless, a number of responses included questions about what was indeed, an effective treatment against CL scars. “Is there a way to heal the scars?” wrote one girl (FR 068). Another girl wrote: “Is there a treatment for scars? I wish there was a yes answer, there was a cure!” (FT195).

Various coping strategies were described as a solution to deal with the scars, especially among girls, focusing on ways to hide the scars temporarily: “I am personally affected by this disease. I suffer from its consequences. The scar on my face has created a big problem in my life. I am obliged to put cream to try to hide it before going anywhere.” (FR035).
Alternatively, spiritual factors such as ‘God’s will’ and ‘destiny’ were the third care-seeking explanations. Two boys and one girl mentioned that God decided who is to be affected by the disease and who will be healed. One boy also suggested these scars need to be accepted “…After a while, he will get used to those scars and it will become normal.” (MR096). Contrastingly, other students asked for direct government intervention: “This disease could leave psychological trouble in the affected person. That is why the government and the concerned commission must find a solution for this disease, it’s not the duty of the population,” wrote one boy (MR093). Another boy noted that the population along with the policymakers should be involved in the prevention of this dangerous disease (MT355).

Discussion

This study showed that 20% of the boarding school students who were surveyed in this CL-endemic region of Morocco had been affected by CL in the past and almost all believed there were potentially psychological implications. CL was found to be relatively well known. Our findings show that CL and its ensuing scars led to a considerable psychosocial burden in this adolescent age group. Girls seemed to be more affected than boys, but both genders equally expressed their concerns and demands for treatment. In this context, adequate dermatological care was beyond the reach of most of the participants due to the poverty of their families. It should be noted that in this Moroccan sociocultural context, scarification marks are common in rural and remote areas in the elderly population and people also contract scars frequently in agricultural work. Traditional tattoos are considered a sign of beauty, a mark of tribal identification, or a protection against sin and malicious witchcrafts [26]. However, nowadays this cultural practice is less commonly accepted by the younger generation living in the same area, and none of the adolescents referred to it. A large majority of them highlighted the negative psychological consequences and the stigmatizing effect of CL and its scars.

Students pointed to a large spectrum of negative psychosocial effects, ranging from slight shame to suicidal thoughts. Social exclusion of CL patients has also been reported elsewhere in the literature [8]. In its most extreme form, students equated CL with ‘social death’ due to the visibility of CL scars, which has also been reported by other authors [27–29]. If a CL scar is located on the face, it is perceived as a barrier to marriage and in this traditional society, this can imply ‘social death’ for women. The participants in this study made comments to this effect spontaneously, without any trigger related to marriage built into the questionnaire. This therefore reflected a deep concern in the adolescent age group. The finding is consistent with previous research reporting that CL scars had a deeper impact for women who remained unmarried, especially in a society where early marriages for girls are common [12].
In addition, while several respondents viewed CL scars as a curse, others accepted them within a spiritual context, which requires praying, asking for forgiveness, and having patience and faith that God has a treatment for every disease or a plan for it whether it will be treated or not.

The findings in this study illustrate the conceptual model of stigma developed by Pryor and Bos and colleagues (see Figure 1). In this traditional Moroccan society, the presence of skin lesions on the face and other exposed body parts leads to public (social) stigma, at least in the adolescent age group, as the cognitive, affective, and behavioral reactions of people to a skin lesion affecting the face are negative and lead to social avoidance [30]. Notions of contagion, of congenitally transmitted infections, and of fatal illness are associated with lesions. In this context, the presence of a CL scar leads to a depreciation of body image in the affected person, which is, of course, dependent on age and gender, but female adolescents are highly vulnerable, as shown in the literature [31, 32]. The ensuing self-devaluation [28, 33] and deterioration of self-concept [23] leads to self-stigma that is perpetuated due to the protracted nature of the scars. Hence, the explicit demand for this age group is to treat CL more quickly and effectively.

Our study had some limitations. Firstly, the self-administered questionnaire method inevitably provides less detailed information than an individual in-depth interview would generate. For instance, we do not have in-depth explanations for the underlying reasons of shame and contempt induced by CL, and we see a need to conduct focus group discussions in the future to better document the social representations of this disease. However, we chose the method of using a self-administered questionnaire format for ethical reasons, as it would have been difficult to arrange individual interviews in an anonymous way in this environment of a boarding school. We wanted to avoid enhancing harassment and mockery among students in such a closed environment. Secondly, there was a relatively large group of non-respondents to the open-ended question (n=76). This could be explained for 59 participants by the fact that they did not know about the disease or any person affected by it. Thirdly, the previous personal CL scar experience was based only on the self-declaration of the participants and we had no formal verification of this information.

**Conclusion**

This is the first study done in Morocco documenting the psychological effects of CL scars in the adolescent population living in CL-endemic areas. The indelible CL scars lead to self-stigma and social stigma, and the emergence of negative psychological effects in this age group. Preventing the avoidable burden of CL and mitigating its dermatological and psychosocial consequences should be a priority for health authorities. We suggest that the control of CL in the North African region should be envisaged in an interdisciplinary, multi-sectoral approach, preferably in a regional framework to prevent as much as possible the avoidable suffering.
Additional files

Additional files 1: Multilingual abstracts in the five official working languages of the United Nations. (PDF 763 kb)

Additional files 2: English translation of the self-administered questionnaire. (PDF 327kb)

Additional files 3: Original version of the questionnaire in Arabic (PDF 1639 kb)

Additional files 4: French translation of participants’ responses to the last question in the questionnaire: Could you write a small paragraph about the probable psychological state of the person (woman or man) affected by those scars? (PDF 404kb)

Additional files 5: Access database file including full questionnaire and student responses (ACCDB 1416 kb)

Abbreviations

CL: cutaneous leishmaniasis; FR: female from Rissani area; FT: female from Tinejdad area; MR: male from Rissani area; MT: male from Tinejdad area

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Availability of data

The data sets supporting the conclusions of this article are included in the article and its additional files.

Authors’ contributions

IB, ST, HF, VDB, HS, and MB drafted the study protocol. IB conducted the data collection. IB, HF, ST, VDB, and MB analyzed the qualitative part of the data. IB, ST, and MB analyzed the quantitative data. IB wrote the first draft of the paper and all authors participated in its critical review. All authors endorse the final version and approve the submission.

Competing interests

All authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Approval for the study was obtained from the representatives of the Ministry of Education and Ministry of Health in Errachidia province and from the two participating high schools’ education officials. The study, as part of a social representations project, was approved by the CERB Biomedical Research and Ethics Committee in Rabat, Morocco, and by the Institutional Review
Board of the Institute of Tropical Medicine in Antwerp, Belgium. Written informed consent was obtained from each participant. Students were free to refuse to partly or completely answer the questionnaire and no names were asked for nor recorded.

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References


Psychosocial impact of CL scars on students | 103


“The mosquitoes that destroy your face”. Social impact of Cutaneous Leishmaniasis in South-eastern Morocco, a qualitative study.

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Keywords: Cutaneous leishmaniasis; Scars; Stigma; Qualitative research; psychosocial burden; Morocco

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Abstract

Objective: To document the psychosocial burden of Cutaneous Leishmaniasis (CL) in rural communities in Southeastern Morocco.

Method: Between March and April 2015, we conducted qualitative research in communities exposed to *Leishmania major* or *L. tropica* in Errachidia and Tinghir provinces. Twenty-eight focus groups discussions (FGDs) were realized, with a stratification by gender and tradition of medicine (users of folk versus professional medicine). Data were analyzed using content analysis.

Results: This rural population most exposed to CL in Morocco lacks access to health care in general and clearly points out there are other major public health issues that need to be resolved. Nonetheless, respondents consider the impact of CL lesions and scars as important and similar to that of burn scar tissue. Young women with CL scars in the face are stigmatized and will often be rejected for marriage in these communities. People usually try a long list of folk remedies on the active lesions, but none was felt adequate. There was a clear demand for better treatment as well as for treatment of the scars.

Conclusions: The psycho-social impact of CL due to *L. major* and *L. tropica* is substantial, especially for young single women with facial scars. These generate social and self-stigma and diminish their marriage prospects. CL is well known, but not considered as a major health priority by these poor rural communities in Southeastern Morocco where gender discrimination is still an issue and access to basic health care is as neglected as CL. Early CL diagnosis and new treatment options with better skin outcomes are urgently needed.
Introduction

Leishmaniasis is a vector-borne disease caused by protozoan species of the genus *Leishmania* and transmitted by different sand fly species of the *Phlebotomus* and *Lutzomyia* genus. The disease manifests as one of three main clinical presentations: visceral, cutaneous or mucocutaneous [1, 2]. Cutaneous Leishmaniasis (CL) is quite common in Morocco and is caused by the anthroponotically transmitted *L. tropica* or the zoonotic *L. major* species [3, 4]. In some areas, both causal species coexist [5, 6]. CL has led to epidemic outbreaks in Morocco in the past, the most recent one occurring between 2004 and 2013 in the Southeast of the country with more than 10,000 recorded cases [7, 8].

Most CL lesions develop on the uncovered parts of the body as those are most exposed to the sand fly bites [9]. In Moroccan patients, CL lesions are of the “localized” type and start as one or multiple slowly progressing nodules that subsequently ulcerate. The healing of this skin ulcer usually leads to substantial scar tissue, which can be quite disfiguring (Figs 1 and 2). CL tends to be considered as a minor illness when compared to the visceral and mucocutaneous forms of leishmaniasis that have fatal or severely mutilating outcomes. However, little research has actually been done about how the affected communities perceive CL, what their explanatory model is for it, and how they deal with this illness. A number of Knowledge, Attitude, and Practices (KAP) surveys in different countries and age groups showed a general lack of knowledge regarding CL symptoms, transmission mode, and reservoir [10–18]. We observed in our previous work in adolescent youth living in the region affected by the CL epidemic that in this age group the psychological impact is real and the demand for prevention and care, especially for the repair of scar tissue was clearly formulated [19]. We wanted to expand this work to the general population, as a contribution to the formulation of sound control CL policy.
The aim of this study is to describe the social burden generated by CL in Morocco, by exploring the community perspective on this illness and social consequences related to CL in the adult population living in rural endemic areas in Southeastern Morocco.
Method

We followed the Consolidated Criteria for Reporting Qualitative research (COREQ) to report our findings. A checklist is provided as supporting information (S1.file).

Research team

Our research team was multidisciplinary, composed of three public health experts with a medical background, two psycho-social scientists and one veterinary scientist. The principal investigator (IB), who is a male Arabic-speaking researcher with a medical background and special training in qualitative research methods, conducted the fieldwork. He was not involved in clinical care in the area and was not known to the study participants. He was accompanied by a male health worker or a female community member known to the respondents.

Study design

Participant selection and setting

Our primary aim was to describe the community perspective on CL following a qualitative explanatory case study approach [20]. We conducted Focus Group Discussions (FGDs) between March and April 2015 in two provinces in Southeastern Morocco: Errachidia and Tinghir (Fig 3), the first endemic for L. major and the second predominantly for L. tropica [7, 21]. Table 1 shows the recently reported caseload of CL by species in these provinces. According to the High Commission for Planning in Morocco, poverty rates in both provinces are high (18-24%) and unemployment rates (9-16%) are higher than in other regions of Morocco.
Table 1. Number of cases of CL due to *L. major* and *L. tropica* in southeastern Morocco as notified to the Ministry of Health (2013-2016)

<table>
<thead>
<tr>
<th>Province</th>
<th><em>L. major</em> cases</th>
<th><em>L. tropica</em> cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Errachidia</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Tinghir Province</td>
<td>155</td>
<td>90</td>
</tr>
<tr>
<td>Ouarzazate</td>
<td>89</td>
<td>170</td>
</tr>
<tr>
<td>Total Morocco</td>
<td>537</td>
<td>460</td>
</tr>
</tbody>
</table>

In each province, we purposefully chose the two districts with the highest number of CL cases notified between 2009 and 2013. Within each district, we used snowballing as purposive sampling strategy to select study participants from the CL endemic areas aiming for maximum diversity in age, gender, and socioeconomic background.

We pre-planned a stratification of the FGDs on two dimensions: gender and a cultural preference for the tradition of medicine (folk or allopathic medicine). The purpose of this stratification was to allow a free and uninhibited discourse within groups. The users of the allopathic sector were invited by approaching people who accompanied their relatives to the health centers in villages. To reach the people who prefer folk healers, we used a snowballing approach starting from key informants in the villages who put us in touch with traditional healers and their networks.

In total, we conducted sixteen FGDs: in each of the four districts, there were four FGDs: one with male and one with female clients of the formal health sector (henceforward indicated as ‘allopathic’), and one with male and one with female users of folk medicine (‘folk’). Based on our findings in Errachidia province, however, we decided to add twelve more FGDs to include the perspectives of specific communities that were living in very remote and difficult to reach areas. A total of 251 participants attended the 28 FGDs.
FGDs included between seven and fourteen participants in each session. Four men and ten women in Tinghir and seven men and nineteen women in Errachidia, all users of the allopathic sector, refused our initial invitation. Reasons invoked were a lack of time, impossibility to leave their children in the care of someone else, or failure to obtain approval from their spouses. No participant received any incentive.

We organized the FGD sessions based on what our respondents proposed as suitable time and location. The discussions took place in quiet rooms that provided sufficient privacy, such as coffee houses, mosques, schools, private houses or local NGO offices.

**Data collection**

During the FGD we used a topic guide inspired by Brown et al., [22] who analyzed the determinants of quality of life in patients with scar tissue (see supporting information S2.file). Topics included knowledge of CL, physical and psychological consequences, behavior, social impact, treatment outcome and knowledge of preventive measures. After completion of the first two FGD, we adapted the topic guide slightly, as we wanted to better understand the perception of CL scars and the attitude/behavior towards people with CL scars better. Thus, we added two questions: ‘How can you make the scars go away?’ and ‘How do people, in general, behave with those affected by this disease?’

The two main languages used for these discussions were the Amazigh (local language) and Moroccan Arabic. Each FGD was recorded with audio and video recording except for three FGDs for which we only used audio recording at the request of the participants. At the end of each FGD, the researcher took notes after discussion with the observer. An average of 39 minutes (range 28-64) duration was observed for the initial 16 FGDs and an average of 31 minutes for all FGDs (Table 2).
Table 2. FGD’s duration and participants’ characteristics

<table>
<thead>
<tr>
<th>Health facility (Allopathic) clients</th>
<th>Tinghir Province</th>
<th>Errachidia Province</th>
<th>Additional FGD *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of FGD</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>FGD mean duration in minutes (min-max)</td>
<td>41 (31-60)</td>
<td>32 (28-35)</td>
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<tr>
<td>[age range in years]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of male participants</td>
<td>16 [24-57]</td>
<td>22 [21-60]</td>
<td>22 [20-67]</td>
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<tr>
<td>[age range in years]</td>
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</table>

<table>
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<tr>
<th>Traditional medicine (Folk) clients</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of FGD</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>FGD mean duration in minutes (min-max)</td>
<td>42 (37-46)</td>
<td>40 (29-64)</td>
<td>23 (14-32)</td>
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<tr>
<td>[age range in years]</td>
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<td></td>
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</tr>
<tr>
<td>Total number of male participants</td>
<td>16 [24-57]</td>
<td>22 [21-60]</td>
<td>22 [20-67]</td>
</tr>
<tr>
<td>[age range in years]</td>
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</tbody>
</table>

FGD: Focus group discussion

(*) done in Errachidia province to reach saturation

Data Analysis

We followed the process of directed content analysis to analyze the text data [23]. FGDs were transcribed and translated into French. Two persons (IB, HF) independently coded data, one using NVivo 10 software and the other one coded manually. We adopted a single coding framework, including themes and subthemes, see Table 3. The codes were informed by our previous work targeting the adolescents [19] and the conceptual model by Brown et al., 2008 [22]. We used the main themes as a structure for reporting our findings.
### Table 3. Themes and subthemes in the coding framework

<table>
<thead>
<tr>
<th>CL Knowledge</th>
<th>Gender discrimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL name</td>
<td>Marriage opportunities</td>
</tr>
<tr>
<td>CL causes</td>
<td>Stigma</td>
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<tr>
<td>CL symptoms</td>
<td>Coping strategies</td>
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<td>CL transmission ways</td>
<td>Prevention strategies</td>
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<tr>
<td>CL Perception</td>
<td>Health seeking patens for CL</td>
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<tr>
<td>CL severity</td>
<td>Treatment accessibility</td>
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<td>Skin outcome</td>
<td>CL traditional remedies</td>
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<tr>
<td>Psychological effects</td>
<td>CL conventional therapies</td>
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<td></td>
<td>CL scar treatment</td>
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</tbody>
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#### Ethical considerations

The Institutional Review Board of the Institute of Tropical Medicine in Antwerp Belgium (956/July.2014) and the Ethical Committee of Biomedical Research of the Faculty of Medicine and Pharmacy in Rabat Morocco (1139/04_Nov.2014) have approved the study. We obtained the authorization to conduct the study from the national and local Ministry of Health authorities.

Participation in the FGD was entirely voluntary. Invited participants were given an information sheet (Supporting information S3.file) to read, and asked to present themselves at the appointment for the FGD at a specific day and time if they agreed to participate. At the specific setting, their oral consents were recorded on audiotape. The participant’s identities were anonymized in the data. Feedback on our findings will be given to the communities after the results of this work will be published.
Results

Without having put ‘young age’ as an exclusion criterion, all respondents in our FGD were more than 19 years old, and this was the case in both male and female groups (see Table 2). The FGD participants did not allow the young and adolescents to sit with them and to participate in this discussion. We present the results within four themes: CL knowledge; CL perception; gender discrimination; health-seeking patterns for CL.

CL Knowledge

The people in this region know CL as the “disease of the scars” and describe the natural evolution rather accurately, as a spot or several bumps on the skin you develop after a mosquito bite, sometimes blistering or ulcerating but not healing for four to six months and eventually leading to a permanent bluish/black mark or scar. In this rural society, almost everybody has some scar tissue, but the local names for CL as the “mosquito sore” or the “mosquito scar” clearly distinguish them and link the skin lesion to an insect vector. All FGDs said that the sore was caused by the bite of a mosquito (chniwla), and the distinction between mosquito and sand fly was not made. However, the causal chain between an insect bite leading to CL and later scar tissue was very clear for everybody. One person summarized this as “the mosquitoes that destroy your face”. Other names were used as well, usually referring to the locality where the problem was first observed, such as “sore of Touroug.”

Respondents also spontaneously used the terms ‘leishmania’ or ‘leishmaniasis’. In Errachidia province, the term seemed familiar to many in all the allopathic FGD groups and in some of the folk medicine ones. Contrastingly, in Tinghir, the term ‘leishmaniasis’ was only used in two allopathic FGD and not in the others. People in Tinghir seemed less familiar with CL in general and did not think it was common in their area, whereas in Errachidia many respondents had experienced CL themselves or had relatives and friends affected by it.

FGDs respondents stated that CL is not contagious by direct contact, nor transmitted by water. Stagnant water, manure, dead rats, and pollution, in general, were repeatedly evoked as risk factors, as they increase mosquito density. People acknowledge that the risk is seasonal, and higher in summer. In summer, people tend to sleep outside which exposes them to more mosquito bites. Insect nuisance was said to be substantial in the villages, and a quiet night of sleep hard to get by because of this. Women were considered most exposed to mosquito bites as they engage in farming activities taking care of cattle and dealing with manure and waste. Little children and tourists were thought to be more vulnerable as well, and a few respondents attributed this to their lack of immunity, sweeter blood or softer skin.
CL perception

Several women in the Errachidia area compared the severity of CL to that of acne and stated that CL is more severe. “Leishmania is more unpleasant than acne. Acne is not pitting, but leishmania digs the skin” (FGD15 Women-Allopathic). In some cases, the lesions can be truly incapacitating depending on the location, such as eyes, foot soles or hands. Active lesions on the hands impair the daily work of women. “If the disease is not on the hands you use to prepare the bread and meals, it’s not a problem.” (FGD10 Women-Folk).

All FGDs agreed that the scar tissue of CL can be a real problem, mostly for women. “The most serious is the scar in the middle of the face. If it is on the side of the face it can be hidden by the veil” (FGD26 Women-Allopathic). A CL scar was considered similar to a burn scar: “The scar does not disappear. It leaves a mark as if you were burned by fire and even worse. Look at my own hand for example. I have a Leishmania scar and that is another scar from a burn. There is no difference! Because of the burn, the muscles located under the skin also disappeared.” (FGD22 Men-Allopathic).

Several participants expressed anxiety about CL, especially that it would affect their children. “We are afraid that this disease would come back in the future because the mosquito is still there.” (FGD21 Women-Folk). In addition, three participants worried about the possible transformation of the scars into skin cancers in the near future. “…maybe this scar will change in the future into a bad disease [cancer]” (FGD04 Women-Folk).

Contrastingly, some individuals in the male groups considered CL as a benign problem as it is not contagious and not fatal. One of them said “An epidemic of leishmania occurred in the past, there were many complaints. Now people forgot this disease. Nowadays if you ask someone something about leishmaniasis, he or she will say (s)he does not know anything about it…” (FGD14 Men-Folk). Moreover, several participants, mainly in Tinghir, raised other health issues which were of more direct concern to them than CL. Access to obstetric care was definitely considered a bigger problem in these remote rural villages than CL.

Gender discrimination

In these rural societies, marriage is considered as a goal in life and an award for every woman. All FGD respondents highlighted that a young woman risks never to get married if her face is affected by CL. “At work, it is not a problem but for the marriage, a man will see the scar on the girl’s face and he will go away. It’s possible that the girl will never get married” (FGD01 Men-Allopathic). This was even more clearly expressed by the women in the folk medicine tradition-FGDs who said they would stay polite with a young woman affected by CL and would share household chores such as cleaning and cooking but that this did not imply they agreed to get...
their sons married to her. “It’s ok for day-to-day exchanges, some polite words, good morning, good night. We pay attention not to hurt her feelings. But marriage? No way.” (FGD04 Women-Folk). Others pointed to CL being a reason for revoking marriage vows. “There was the case of the girls who were engaged and later got this disease. They were told, we stop [the engagement], go treat yourselves and get cured before we can go on with the marriage. These girls were affected either on their faces or on their foreheads.” (FGD20 Women-Folk). As stated above, some male respondents held contrasting views and considered that: “this was not a huge problem if the girl will never get married” (FGD01 Men-Allopathic). It seems also that within the context of customary intra-family marriage, the man cannot reject the woman for this reason. Interestingly as well, CL scars that would appear after marriage are no reason for divorce. “The husband cannot divorce his wife if she is affected by this disease. Because she became ill in his house.” (FGD19 Men-Folk).

The extent to which CL leads to social stigma beyond limiting the marriage prospects for women is not uniform. In both provinces, people incriminated the nearest locality to have had the disease earlier before contaminating them via mosquito or rodents. Participants from the area where the disease first appeared in Errachidia mentioned strong social rejection by other groups in the neighborhood. Some said to be ashamed to live in a village with CL “Men and women of the neighboring locality start insulting us and laughing at us.” (FGD28 Men-Folk). Some feel also rejected by their relatives living abroad who contracted CL during their holidays in Morocco.

Some women affected by CL tend to hide their lesions for fear of stigmatization. Others felt uncomfortable to share a meal with somebody with an open sore, mostly for fear of contamination. Nonetheless, many participants described a change in attitudes over time, towards less discrimination “At the beginning, many persons who saw me told me I must remove this. My face became less beautiful than before. I was also upset. Every time you touch it, you feel something on your face. You feel it and it is disturbing. After a while, you forget its presence” (FGD04 Women-Folk).

Several times the male respondents expressed they did not see any reason for social rejection, as it is quite common to see scars from various causes in everyday life in this rural context. A scar becomes a part of the person and the society is accustomed to it. Moreover, CL does not change their personal relationships and interactions. “What is the matter to have an additional scar? It’s normal!... Even for women, it is the signature stamp of our area.” (FGD01 Men-Allopathic).
Health seeking patterns for CL

Everybody had an opinion about CL treatment, best summarized as ‘we try everything, but nothing works’. More than thirty ways of self-treating the skin bumps were cited including food items (honey, oil, eggs, saffron, salt, tea, vinegar, chillies), herbs (henna, Aloe-Vera, fenugreek, basil, rose water), but also harmful products (white spirit, gasoline, tar, bleach water, crushed outdated pills) or alcohol and soap. People try to get rid of the bump by scratching, cutting or burning it. Folk healers often apply burning as well, e.g. using sulfur powder. Besides, they apply water from natural sources, sand, and soil to cure the lesion. The ulcers are treated differently, with different mixtures, such as dust, hair and crushed glass, or salt and tobacco. Scars are treated with a long list of different products (lemon to lighten the color, several oils such as oil of cade, but also used motor oil, white spirit, rose water, salt, ...).

Conventional treatment in health facilities was a matter for debate. Participants noted that protocols varied, from ointments to a variable number of injections. Some worried about side effects “They said that the injections have bad effects on the kidneys” (FGD09 Men-Folk).

Though some acknowledged you have to be treated early to see some effect, several pointed out that the results were often disappointing “She said to me that she followed the treatment for four weeks every two days per week. But now after the end of those treatments, she has a blue color.” (FGD02 Women-Allopathic). “Do you see the spot on the hand of this man sitting near to me, even if it appears like it is healed the margins are full of the disease, if you touch it, it is not yet cured” (FGD09 Men-Folk). The demand for scar treatment was more pronounced in Errachidia than in Tinghir province.

Mostly, respondents highlighted the lack of effective remedy for the disfiguring scars. “I tried everything to make the scar disappear. I became like an herbalist. Until I get tired and I accept the presence of this scar on my face. For me cosmetic surgery is dangerous, it may even lead to the development of cancer. So better leave the scar where it is.” (FGD24 Women-Allopathic). Very few participants, mainly from the allopathic groups, said progressive healing is possible, especially in children who get early medical treatment.

Some respondents emphasize that cosmetic surgery is not accessible and not affordable “…the specialists in dermatology are far away. You should go to another town. The doctors there bite you more than the mosquito-bite (Laugh). People are different, there are some who have money and some without. Then, many people accept the reality and ignore the presence of this disease. They use natural treatments which is more economical and better than looking for aesthetic surgical interventions” (FGD08 Women-Folk). Moreover, cosmetic surgery was considered of little benefit compared to the huge cost. “Leishmania made muscles under the skin disappear.”
That is why there are doctors who say that even cosmetic surgery is not going to give good results for the scar of Leishmanio” (FGD22 Men-Allopathic).

Discussion

Our extensive discussions with members of these rural Moroccan communities learned us that CL is definitely not the main health problem in these remote areas, but nonetheless generates substantial suffering, mainly in women, due to the disfiguring skin outcomes and the social stigma surrounding it. Our respondents described the psychosocial impact of CL as more severe than that of acne and as similar to that of scar tissue from burns. We observed substantial gender differences related to this perceived burden. Women bear the highest burden of CL, as, beyond the physical discomfort of active lesions on hands etc., they seem more stigmatized. The emotional well-being of young single women with facial lesions was said to be strongly affected by CL scars, and this corroborates our earlier findings in adolescent youth [19]. Moreover, our respondents considered women at higher risk for CL due to their agricultural and household chores.

The explanation used by these rural community locates the origin of CL clearly in the natural world: the causal link between the insect bite and the skin lesion is made very clearly and specifically, even if the insect is mostly (and erroneously) identified as a mosquito, whereas it is, in fact, a sand fly. Risk factors for CL are also very correctly identified as all factors that increase vector abundance. One could ask if the link with rodents is made sufficiently strong in the *L. major* areas. Unfortunately, there is so far, no effective and sustained community involvement in CL control. Any ongoing reservoir or vector control interventions target perceived immediate risks, like the threat of wild rodents to the agricultural production or the insect nuisance preventing a quiet night sleep in summer days.

At the closer examination of the notion of the stigma surrounding CL, it seems that this is mostly linked to scars on the face, and then mostly in young women. This type of lesions is only a small subgroup of all the CL lesions. In our study, CL seemed a lesser problem in the group of users of “folk medicine”, as they seem to care less about the scars. Due to their very harsh everyday life conditions, another scar may then make little difference. The main contrast between users of the folk and allopathic tradition was that the latter considered the CL scars as an unsatisfactory sequela requiring an effective medical solution. It may be interesting to conduct further research on differences in perception according to causative species. The larger size of CL and CL scars may generate more concern in the *L. major* than in *L. tropica* areas, though the latter lesions are more protracted.
Social impact of CL

Health seeking behavior patterns for CL and CL scars were multiple and overlapping encompassing the health care traditions of self-help, folk, and allopathic medicine. The effect of the toxic and abrasive products and burns used in self-remedies and by traditional healers on the extent of scarring should not be underestimated, and there is a scope for better information and education of the community in this field.

Our findings are consistent with the few available qualitative studies on CL from elsewhere in the world. In Kabul, Afghanistan, women were at higher risk than men to contract CL and affected people were excluded from social life leading to emotional and physical isolation. Similarly to our findings, women faced difficulties to get married if they had a CL lesion or CL scars [24]. Likewise, in Yemen, a review emphasized the stigmatizing potential of disfiguring CL in women [25]. In contrast, in Suriname, where CL mainly affected men, the only factor that generated stigma was the presence of a big (or multiple) CL lesion(s), but no barriers to the marriage were evoked [26]. Contrastingly, mucocutaneous CL in Latin America was linked to constant stigma and permanent psychological effects [27–31].

Our qualitative approach derives its strength from a conceptual framework, with an iterative process used in the development of the data collection tools, and the pursuit of saturation in the information reached with the use of additional FGD. The findings from this set of FGD combined with informal interviews and observations on the ground is considered by our team more valid to capture the socio-cultural diversity in interpretations of CL-illness than the use of a restricted number of in-depth interviews with former or actual CL patients. Our study had some limitations though. Some statements by respondents may have been subject to social desirability bias, e.g. when people were informed that the moderator works as a researcher in a national public health institute, this triggered their interest about national health policies and specific health needs in the area. However, we do not think it had a major effect on the way the groups voiced their perception of CL. Secondly, our categorization of FGD into preferred health care tradition (folk medicine versus allopathic) was not mutually exclusive and may not have captured the full spectrum of care traditions, but it certainly helped us to explore the community perspective across a range of socio-cultural groups in this context.

In conclusion, this qualitative study is the first in Morocco to address the perspective of the population on CL and CL management. As all qualitative research, its main limitations lie in the generalizability or what is called its external validity. Said otherwise, to what extent are our findings representative for other provinces in the country and region? As our findings are consistent though with a larger body of evidence from quantitative KAP studies and psychological assessments by questionnaire surveys and with qualitative research based on FGD conducted earlier in Afghanistan and Surinam, we believe the findings are robust and should inform policy. CL is a true problem in this region, very much intertwined with gender discrimination and lack of access to basic health care in remote rural areas. In dialogue with the
community, sound prevention and control policies should be designed. One of the main implications of our findings was that innovation in CL early diagnosis and treatment is desperately needed. The management of existing disfiguring CL scars remains also a subject of further research. In summary, CL is well known but not considered as a major health priority by these poor rural communities in Southeastern Morocco with lots of competing health problems. Its psychosocial impact is substantial though and can be in some cases very important, especially for young single women with facial scars, generating social and self-stigma and diminishing their marriage prospects. A new treatment for CL with better skin outcomes is urgently needed.

Supporting information

S1_File.pdf: COREQ Checklist (DOCX)
S2_File.pdf: Translated FGD topic guide (DOCX)
S3_File.pdf: Original participant Information sheet (PDF)

Acknowledgments

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Author Contributions

IB, the principal investigator was involved in the concept, design, development of the study and the instruments, as well as in the implementation, collect and analysis and reporting aspects of the study. He wrote the first draft of the manuscript.

LB, HF, HS provided expert advice for the study design and writing of the manuscript.

HF, VDB, and MB revised the protocol, assisted in data analysis and provided suggestions to improve the study design and manuscript.

All authors have read and approved the final version of the manuscript.

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**Competing Interests**

The authors have declared that no competing interests exist.

**Data Availability Statement**

Public deposition of our data would breach compliance with the protocol approved by our research ethics boards, as we promised to ensure confidentiality of our participants. Our data are audio and video recordings of our group discussions and written transcripts of those. The former allows for easy identification of participants and cannot be shared. The written transcripts allow also for identification in combination with the information on time and location of the session and knowledge of the village.

We are willing to make the transcripts available to qualified researchers on condition of signing a data sharing agreement. Qualified researchers can contact the chair of the Antwerp IRB, Prof. Dr. A. Buve, (abuve@itg.be) in case they would like access to the transcripts.

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication

**References**


CHAPTER 5. Psychosocial burden of localised cutaneous leishmaniasis: a scoping review.

Psychosocial burden of Localised Cutaneous Leishmaniasis: a scoping review
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Abstract

Background
Cutaneous Leishmaniasis (CL) is a parasitic skin disease, linked to poverty, and belonging to the group of Neglected Tropical Diseases. Depending on the severity, the type of lesions or scars, and the context, CL can lead to self- and social stigma influencing the quality of life and psychological well-being of the patient. This dimension is, however, little documented for the most common, localized form of cutaneous leishmaniasis (LCL).

We aimed to describe the current knowledge on the psychological burden and the stigma related to LCL.

Methods
The population of interest for this scoping review are patients or their relatives with localized LCL or related scars.

We searched the electronic databases PubMed, Web of Knowledge, PsycINFO, PLoLINE, Cochrane Library, Science Direct, Global Health, and LILACS, for articles written in Arabic, English, French, Dutch, Portuguese, or Spanish, and published until the end of August 2017.

Results
From 2485 initial records, fifteen papers met our inclusion criteria. Dermatology life quality index was the most frequent used scale to assess LCL psychological impact in quantitative studies. Six qualitative studies used individual interviews and/or focus groups discussions to explore the psychological and/or the social burden of this disease. Quantitative assessments using standard scales as well as qualitative research asserts that LCL is a source of psychological suffering, stigmatization, and decreased the quality of life (QoL).

Conclusion
Most studies showed that LCL has a significant negative effect on the QoL and mental health. However, the fact that the psychosocial burden generated by LCL is time-dependent makes it hard to measure. We recommend to develop a more specific and validated assessment scale to appreciate the full burden of this disease and enhance comparability of findings.

Keywords:
Cutaneous leishmaniasis, Cicatrix, Psychology, Social Stigma, Quality of life
Background

Cutaneous leishmaniasis (CL), a neglected tropical disease (NTD), is a parasitic infection of the skin characterized by slowly healing ulcers that can leave indelible scars. There are various clinical presentations of CL: localized, diffuse, disseminated and mucocutaneous CL.

The global mean age-standardized disability-adjusted life years (DALYs) lost by CL were estimated at 0.58 per 100,000 people [1]. However, several authors have criticized the way the global burden of disease tools, such as DALYs, have quantified the burden of CL. A recent viewpoint drew attention to the fact that only active forms of CL are included in DALY calculations. It suggests to consider also the psychological impact linked to the disfiguring scars of CL [2]. Moreover, the same authors proposed to multiply by ten the actual burden of CL, to take into account the effect of CL scars [2]. Under this scenario, the global number of people living with LCL scars is estimated at 40 million. Such claims call for a higher prioritization and resource allocation to CL [2].

Localized CL (LCL) is by far the most common presentation. Localized CL (LCL) is by far the most common presentation. The localized form of CL is typically a single papular or nodular skin lesion that progressively ulcerates and usually self-heals within a few months [3].

In 2013, LCL was primarily reported from fifteen countries. Namely, Afghanistan, Algeria, Brazil, Colombia, Honduras, Iran, Morocco, Nicaragua, Pakistan, Peru, Saudi Arabia, Syrian Arab Republic, Tunisia, Turkey and Yemen [4].

LCL is often presented in the literature as a dermatological disorder that is “associated with stigma” and psychological suffering. That is quite obvious for the severely mutilating or disseminated types of CL. Severe scar tissue from any cause (acne, burn, injury, Buruli ulcer, lymphatic filariasis, podoconiosis, a.o.) reduces the quality of life in a direct way but also through a process of social rejection and self-deprecation [5–13]. In some skin disorders, this psychosocial effect is predominant, as in vitiligo patients, who experience a reduced quality of life because of their social rejection [14–18]. The psychological distress generated by skin ailments is complex and may depend on the severity and visibility of the lesions. Skin disorders are no exception to the general rule that the degree of fear inspired by a disease is mainly associated with the perception of severity and also lack of adequate treatment [19]. In the past, the typical examples were smallpox and leprosy that led to a total isolation of those affected, and more recently, Ebola, HIV/AIDS, psychiatric and mental illnesses continue to generate this stigmatizing behavior in many communities. The resulting social isolation affects a human being psychologically and this was shown in the case of the recent Ebola epidemic to have a negative impact on survival [20, 21]. However, the experience in psoriasis shows that the relationship between symptom severity and psychosocial distress reported by psoriasis patients is not straightforward [22]. Nevertheless, a systematic review showed that clinical depression was 1.5 times more frequent in psoriasis patients than in non-affected peers [23].
In the literature on the psychosocial burden of disease, stigma is a central concept. Mostly stigma manifests itself in discriminatory attitudes against those who are different and is related both to the conception of the ‘other’ as well as the ‘me’[24]. This conceptual complexity makes stigma difficult to determine. Goffman defines stigma as: ‘The phenomenon whereby an individual with an attribute which is deeply discredited by his/her society is rejected as a result of the attribute. Stigma is a process by which the reaction of others spoils normal identity’ [19, 25]. Other authors define stigma as a social process that is characterized by exclusion, rejection, and blame resulting from the anticipated perception or expression of an adverse social judgment about a person or a group [26, 27]. This judgment can be related to the mental disorder, ethnicity or physical handicap of the stigmatized persons [25]. Stigmatisation also affects people who are not perceived as a threat to others but who present a disfigured body [28, 29]. The judgments made are often the result of comparisons to a perfect, idealized body image [30]. Often shaped on the pictures of ‘celebrities’ as the ideal social appearance, this idealized body image increases the pressure on those who appear different, pushing some of them to seek cosmetic surgery [31]. Stigma is also typically attached to those conditions in which people are blamed for their appearance [32]. The social rejection generates suffering, as the stigmatized person interiorizes what others think of her/him, leading to loss of self-esteem and what is called “self-stigma“ [33]. Once the individual perceives the stigma, it can affect his/her quality of life [15]. The more general concept of “stigma” has, therefore, two dimensions: how others relate to the stigmatized person and how that person relates to himself/herself. Henceforward in this text, we distinguish these two dimensions based on the model presented previously by Deacon [34] and adapted by Weiss in 2008 [27]:

1. Social stigma (public stigma) occurs when society actively adopts negative attitudes of rejection or exclusion against the stigmatized who undergo inequitable or discriminatory treatment. Enacted stigma, which is about passive discrimination, is one expression of social stigma. Even if some people disagree with the stigmatizing behaviors of others, they often remain passive about it, without trying to stop it.

2. Self-stigma is an internalized mechanism in the stigmatized person, who is anticipating the social rejection or is adapting to a stigmatizing environment. In this category, we can include: felt stigma, experienced stigma, internalized stigma, internal stigma, anticipated stigma, imagined stigma, perceived stigma.

Then, whether the painless nodules, ulcers, and scars of LCL contribute to the global burden of CL beyond the local discomfort they generate is an open question. So far, little research has been done to underpin this general statement about LCL, nor to differentiate the burden according to CL type.
Our review aimed to describe the current evidence related to the psychosocial burden of LCL. More specifically, our objectives were to (i) explore which quantitative scales were used to assess this burden; (ii) summarize qualitatively the extent of psychosocial suffering (specifically self-stigma, social stigma, quality of life) attributable to LCL. Our research question was “Do persons experiencing a LCL skin lesion suffer from any psychosocial burden?”

Methods

A scoping review is mainly based on a preliminary assessment of the amount/ the extent of available research in the literature [35]. Following Arksey and O’Malley’s framework, we worked through five stages, listed hereafter. Stage 1: identifying the research question; Stage 2: identifying relevant studies; Stage 3: study selection; Stage 4: charting the data; Stage 5: collating, summarizing and reporting the results [36, 37].

Inclusion and exclusion criteria

Inclusion criteria

Population: Persons (patients or their relatives) experiencing a skin condition linked to LCL
Range of Concepts: LCL-related stigma and its psychological consequences in different LCL skin conditions, including LCL scars.
Context: All countries.

All articles discussing entirely or partially the stigma types, psychological and social consequences and quality of life, psychological or psychosocial effects related to Leishmaniasis skin condition. All types of publications were eligible: original articles, literature review, editorial, comments, correspondence, abstracts of workshop or conferences, grey literature, as available online.

Exclusion criteria

As listed in additional file 1, we excluded articles for which no full text was available; books; articles targeting veterinary studies, vectors, laboratory or in-vitro research; studies describing CL epidemiology, outbreaks or trends without or with a little indirect information about psychosocial outcomes; articles exclusively targeting HIV or mental illness or other stigmatising diseases without any link with CL; articles treating only the visceral form of leishmaniasis or post dermal kala azar form or exclusively the CL mucosal or tegumentary form; articles on diagnostic methods for CL; treatment assessment or methods not targeting psychosocial interventions in CL (e.g. drug effectiveness).
Search strategy

Eight databases were selected: PubMed, Web of Knowledge, Science Direct, PsycINFO, POPLINE, LILACS, Global Health CABI and Cochrane Library. We searched these databases from their inception until the end of August 2017 with language restriction to Arabic, English, French, Dutch, Portuguese, and Spanish.

The search in the PubMed and Web of Knowledge databases followed the three steps recommended in Peters et al. [38]. (1) Firstly, we did a limited exploratory search in PubMed combining three keywords: Cutaneous leishmaniasis AND (Stigma OR Quality of life). Then, we built a logical grid after analyzing text words contained in the title and abstract with adding the subject heading and index terms. (2) Secondly, we organized them into blocks by using the advanced search composed of MeSH terms and free synonymous words categorized into three blocks (1#Population, 2#Concept, 3#Context) which helped us to build the query question and perform the primary search strategy (as listed below). For the other databases, shorter search equations were set to find relevant references to our topic (See Additional file 1). (3) Thirdly we performed a hand search on the reference list of the selected articles and searched for any publication by the first, second, third and last authors in those selected articles and meeting the same inclusion criteria but initially not detected by our initial search strategy. Also, we looked for overall articles that cited the ones we selected. We repeated this process iteratively until no other relevant articles were found.

Query search (PubMed) and (Web of Knowledge):
This search strategy was done end of September 2016 and updated on 1st September 2017.

(((Leishmaniasis OR Dermal Leishmaniasis OR Cutaneous Leishmaniasis OR Mucocutaneous Leishmaniasis OR Diffuse Cutaneous Leishmaniasis NOT Visceral Leishmaniasis)) AND (Social problems OR Public opinion OR Social discrimination OR Social isolation OR Social exclusion OR Social distance OR Stereotyping OR Social perception OR Social conditions OR Social adjustment OR Social behavior OR Social behaviour OR Social behavior disorders OR Social environment OR Social support OR Social marketing OR Cost of illness OR Stigma* OR Public stigma OR Social Stigma OR community stigma OR Enacted stigma OR Self-stigma OR Self stigma OR Experienced stigma OR Perceived stigma OR imagined stigma OR Anticipated stigma OR Felt stigma OR Self-report OR internalized stigma OR Health related stigma OR Gender Identity OR Quality of Life OR Perception OR Rejection OR Discrimination OR Parasitic skin diseases/psychology OR Skin diseases, bacterial/psychology OR Neglected Diseases/psychology OR Psychosocial OR Psychosocial)) AND (("1920/01/01"[PDat] : "2017/08/31"[PDat]) AND (Arabic[lang] OR English[lang] OR French[lang] OR Dutch[lang] OR German[lang] OR Spanish[lang]))
Data collection, extraction, and analysis

Two investigators (IB, ZB) independently reviewed the titles and abstracts of all retrieved records and selected the articles for full-text review based on eligibility criteria as defined below. EndnoteX7 (Thomson Reuters 2014) was used as a reference manager software. This software served to avoid duplication, achieve the first screening of titles or titles and abstracts, download and manage full texts of selected references for a complete reading step and provide a digital backup for archiving and enhancing traceability of the search process for this review. In case of disagreement, the last author (MB) served as the tiebreaker. The other authors, all experts in the field of CL, stigma and conducting systematic reviews, rechecked the inclusion and exclusion criteria of selected articles.

The results of the literature search are presented in a flow diagram (Figure 1) describing the scoping review process [38]. The additional file 1 includes more details on the search: activities performed with date, the query equation employed, and the reference list found, including justification for excluding references after full-text reading and the final references of the included articles.

We extracted the data and mapped them in an Excel sheet (Windows, Microsoft Corporation). We used a narrative synthesis approach to compile the data and examples from individual studies. We focused on how studies addressing a different aspect of the same phenomenon can be narratively summarized to provide a bigger picture of that phenomenon. Using Nvivo11 QRS International, we classified them under main conceptual categories (Additional file 1).

We followed the fifth steps of reporting scoping reviews with the use of PRISMA flowchart for reporting the whole search strategy [36, 37, 39].
Results

Included papers
We included 15 articles matching our criteria for a full review (Table 1) [40–54]. The studies were done in nine countries: Afghanistan, Iran, Morocco, Sri Lanka, Surinam, Syria, Tunisia, and Yemen. Nine papers used quantitative research methods, including eight cross-sectional descriptive surveys and one randomized controlled trial of a psychotherapy intervention in LCL. One was based on mixed methods while five were based on qualitative research: one of them was based on focus group discussions, one was a review, and the others were based on interviews.
Psychosocial burden of LCL measured quantitatively

**Anxiety and depression:** Seven quantitative studies measured the psychosocial outcome on a standard scale (Table 2). Yanik et al. [41] assessed the psychological impact of LCL in three groups of patients in Turkey (LCL-active lesions, LCL-scars, and a control group). They used three different scales to quantify 1. Anxiety and depression (HAD), 2. Body Image Satisfaction (BIS), and 3. Dermatology Life Quality Index (DLQI). They concluded that anxiety and depression were frequent symptoms in patients with LCL at both the active lesion and scar stage and affected their quality of life. Patients with LCL scars had better quality of life scores than those with active lesions. Simsek et al. [43] in Turkey used the DSM classification scale of mental illness (DSM-IV) to study predictors of mental disorders in a sample of women from a rural South-eastern region in Turkey. In multivariate analysis, LCL was found to be an independent predictor of mental illness (Odds Ratio (OR) 2.15, 95% C.I. 1.25 to 7.31) at the same level as domestic violence (OR 2.03, 95% C.I. 1.17 to 4.28). In 2015, Turan et al. [50] in the same endemic area in Sanliurfa, used four scales in children and adolescents from 7 to 18 years and their parents. In the patients with LCL, depression scores were significantly higher than in the control group, and this was also the case in the group of mothers of children with LCL. No difference was found in anxiety scores.

**Low quality of life:** Two studies were conducted in Iran [45, 48] using the DLQI scale to assess the QoL in LCL patients under treatment. Vares et al. [48] found that the type of LCL lesion had a significant effect on patient’s QoL. The DLQI score in patients with papular LCL lesions was better than in those with nodular and plaque lesions. Those with ulcerated lesions had the lowest QoL score. Nilforoushzadeh et al. [45] randomly allocated 40 women with LCL active lesions to either the standard LCL treatment or standard treatment plus psychotherapy. The intervention arm showed a more pronounced improvement in DLQI score at the end of the study after eight weeks (p=0.001). Hanjdani et al. [49] used the Family DLQI scale to assess the impact of chronic skin disorders in a family member and on the other –non-affected- household members. A variety of chronic skin disorders of at least one-month duration (vitiligo, pemphigus, psoriasis, and LCL) were included. There was a reduction of QoL, without a significant difference between disorders, but the sample size was small. The main problems mentioned were emotional distress, spending much time with the patient, and reactions of the others to the patient’s condition. The main complaint in families with a LCL case was the time spent looking after the patient.

**Stigma:** In Tunisia, Chahed et al. [52] assessed the level of stigma (using the Psoriasis Life Stress Inventory and QoL (Revised Illness Perception Questionnaire and the World Health Organization QoL26) in a sample of 41 women with LCL scars and reported a wide range of psychological effects. 73% of the women had suffered social exclusion and stigmatization. They reported broken relationships, interpersonal conflicts, reduced employment opportunities, and reduced marriage prospects for men and women. LCL was a constant source of stress for the study participants. They thought their scars were degrading, and a source of shame. The “experience of social rejection” and the “anticipation and avoidance of stress” were significantly and negatively correlated with age. The younger the person, the more impact CL had in terms of...
stigma. QoL was correlated with the “anticipation of stress” score but negatively correlated with knowledge about CL. The more a person knew about LCL, the less likely they were to view their quality of social life negatively.

**Fear of scars:** The two other quantitative studies included in this review [46, 47] were both based on a general questionnaire targeting knowledge, attitudes, and practices of LCL patients in endemic areas from Syria and Sri Lanka. In both studies, no direct information about QoL was provided except the adverse effect of fear about LCL scars, especially on the face.

**Psychosocial burden of LCL reported qualitatively**

Very recently five qualitative studies provided more information on the CL psychosocial burden from the perspective of the general population and affected patients living in LCL endemic areas (Table 3).  

**Expectation of stigmatization:** The most important effects were linked to LCL patients holding scars on visible parts of their body, specifically on women faces’ more than men. Chahed et al. [52] reported feelings of inferiority and the idea that the disease is equal to an apparent social disadvantage. Fifty percent of all women with scars said scars alter their beauty.  

**Perception of changed body image:** The beauty loss concept has been a frequent feature of all these studies, especially for young bachelors. The impact of unwanted changes in body image is considered as a curse or bad luck that will diminish the marriage opportunities of the person suffering from LCL scar.  

**Self-stigma:** A broad spectrum of psychosocial suffering was reported such as being shy, ashamed, stressed, anxious, depressed. Feeling embarrassment, sadness, suicidal thoughts and a decrease of self-concept (self-confidence, self-esteem, self-contempt, self-awareness) lead patients to perceive or interpret negatively some direct or indirect reactions from partners, relatives, and the general population. Some women affected by LCL try to hide their lesions from their partner and look for more permanent solutions by surgical repair or aesthetic surgery. As Chahed et al. [52] have pointed out, the extent of the stigma that patients experience is associated with their anticipation of rejection.  

**Social stigma:** From emotional isolation and emotional distress to firm social rejection and discrimination within the same household, with friends or/and workmates, or abroad family visitors. Some affected people felt their chances to find employment, studies or marriage jeopardized. However, simultaneously, the perceived stigma which was expressed or enacted by the neighborhood led to psychological consequences as well. In Afghanistan [40], women with LCL frequently felt rejected. As LCL was relatively new in Kabul, there was a widespread misconception about its origin, with fear of contagion and an association of LCL with dirt and low personal hygiene. People attributed LCL to “the fault and sin carried by strangers living in neighboring provinces or cities.” Such misconceptions raise the level of rejection and social exclusion.
Mitigating factors of stigma (Resistance to stigma): The tendency to isolate LCL patients from others contributes to more self-stigma. The patients try to hide or mask the lesion, by using homemade or commercial makeup, or by wearing the traditional veil. The qualitative studies also reported that LCL being for some people only a minor source of suffering, for the following reasons. Firstly, people living in rural areas consider it quite normal to have scars because of the forestry and agriculture activities which expose them to several abrasions and wounds. Thus, an ulcer that self-heals over time is not a major problem for them. Such scars are sometimes even considered as a mark of belonging and membership to a community, even as a mark of glory and pride. Secondly, religious people tend to accept this kind of ailments, as a submission to the will of the Almighty. Finally, old people have less fear about aesthetic outcomes than younger people.

Discussion
The main finding of our review is that LCL indeed generates psychological suffering and mental ill-health, as shown in several countries. LCL leads to the social stigma that eventually causes self-stigmatization, which amplifies the feelings of fear, anxiety, and depression in those affected. Extreme self-isolation and self-contempt can sometimes even lead to suicidal ideation. Moreover, young single women with facial scars face the most substantial psychosocial impact of LCL [40, 52, 54, 55]. Of course, there is a contrast with the ill-health generated by the more severe diffuse, disseminated and mutilating mucocutaneous CL forms, which can even be life-threatening. Getnet’s work in Ethiopia [56] and in Latin America [57–61] showed the psychosocial burden of these non-localized CL types for which the prognosis is much more severe than that of LCL.

Our review adds some new insights to the stigma theory presented previously for HIV by Deacon & al [34] and adapted for neglected tropical diseases by Weiss in 2008 [27]. We elaborated further on this model by including the reflection about body image and its distortions presented by Thompson & al [62]. We also introduced “change over time” as an important determinant of the perception of the severity and the psychosocial impact of any acquired skin disorder. Time adjustments influence the degree of self-stigma on the one hand, and on the other hand, also influence the attitudes (negative or positive) society has towards the patient (Figure 2).

Like for burn injuries, the evidence shows an adjustment over time independently of the injury size [63]. Contrastingly, older people will tend to heavily personalize negative social and self-images within age identity and global wellbeing. Then, change of self-perceptions that could happen within age is a matter of debate. For some people self-concept could be stable but also could change in positive and negative ways [64], [65]; depending on the unforgettable memories [66] and how emotions and memory interact and how these emotions change across lifespan [67, 68].
Fig. 2 Time adjustment of stigma types and psychosocial impact of unwanted skin conditions adapted from Deacon & al [34]; Weiss [27]; and Thompson & al [62] by taking the example of localized cutaneous leishmaniasis (LCL)
This scoping review can only be as strong as the studies it included, and in our case, there were some limitations. A small sample size characterized most of the included studies, and not all had appropriate control groups. E.g., the Kassi et al. [44] record is a case report presented at a symposium, telling the case of one woman with CL. The reports are not all of the same quality and are sometimes lacking detail. Reithinger et al. [42] is a short communication, describing summary results of a study that has not yet been fully published in an indexed journal. The majority of our records had descriptive cross-sectional quantitative survey designs with one single participant contact. Not all studies distinguished the LCL lesion from the LCL scar, though there is evidence that the effect is not the same, and that even within LCL it depends on lesion type.

Future studies should pay more attention to potential confounding factors such as the physical aspect of the lesion or scar, the socio-economic position of the person with LCL and context related factors determining the CL-related knowledge, attitudes, and practices of society [69]. Indeed, lack of knowledge about this disease and its transmission patterns can lead to collective fears, which could fuel stigmatization, rejection, and isolation such as observed in Kabul, Afghanistan. A mixed methods approach is strongly advised, as so far in many endemic areas only quantitative patient surveys were done, leaving a lot of unanswered questions about the community perception. To enhance our understanding, we recommend the development of a standard validated stigma scale for CL. Furthermore, prospective follow-up studies will more accurately describe the time-dependent changes in perception. Future qualitative research should examine stigma and quality of life components from the perspective of the patient and the healthcare provider and give orientations for interventions.

Our review contains some limitations. Firstly, we acknowledge that we may have missed some relevant studies because of language restrictions that excluded large parts of the world where CL is common as well (especially articles published in Chinese and Russian). Secondly, as a common limitation of scoping reviews, we did not grade the quality of evidence, to avoid loss of information, but we may have included weak evidence in this way.

**Conclusion**

The literature on CL in the past 20 years claims that CL lesions like it is the case in any other neglected infectious diseases related to poverty, influences the psychological state of those affected and can lead to a reduction in their quality of life and stigma [27, 70–75]. However, previous literature reviews assessing the burden of disease caused by leishmaniasis usually did not distinguish between the localized CL self-healing form and the other non-localised forms of CL (mucocutaneous, diffuse). Our review showed that most qualitative studies concur that LCL has indeed a negative effect on quality of life and mental health, through a process of social and self-stigmatization.
List of abbreviations

BIS: Body Image Satisfaction scale; CL: Cutaneous Leishmaniasis; DALYs: Disability-Adjusted Life Years; DLQI: Dermatology Life Quality Index; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th. Edition; HAD: Hospital Anxiety and Depression scale; LCL: a localized form of cutaneous leishmaniasis; MeSH: Medical Subject Headings; NTD: Neglected Tropical Disease; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; QoL: Quality of Life.

Declarations

Ethics approval and consent to participate
We followed the relevant standards while conducting this review.

Consent to publish
Not applicable.

Availability of data and materials
The data sets supporting the conclusions of this article are included in the article and its additional file.

Competing interests
The authors declare that they have no competing interests.

Funding
No specific funding was received for this work.

Authors' contributions
IB, VB, and MB developed the idea for the paper. IB and HS conducted searches. IB and ZB assessed citations. IB, ZB, and MB assessed the included records. All authors rechecked the inclusion and exclusion criteria of the selected records. IB, VB, and MB wrote the first draft of the manuscript. All authors commented on the manuscript. All authors read and approved the final manuscript.

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References


54. Al-Kamel MA (2017) Stigmata in cutaneous leishmaniasis Historical and new evidence based concepts. Our Dermatology Online


150 | Chapter 5
Additional file 1 Logbook, search history, excluded articles, included articles and Data extraction labels of the main conceptual categories
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year of publication</th>
<th>Country of the study</th>
<th>Methods</th>
<th>Study design</th>
<th>Timing</th>
<th>Study Population</th>
<th>Sample size</th>
<th>Sex ratio (M/F)</th>
<th>Age range (years)</th>
<th>Patients with CL scars included</th>
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<tbody>
<tr>
<td>Al-Kamel et al.</td>
<td>2017</td>
<td>Yemen</td>
<td>Qualitative</td>
<td>Interview</td>
<td>May 2016</td>
<td>CL patients</td>
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<td>Bennis et al.</td>
<td>2017</td>
<td>Morocco</td>
<td>Qualitative</td>
<td>Questionnaire</td>
<td>April 2015</td>
<td>Boarding school students</td>
<td>448</td>
<td>1.36</td>
<td>16-20</td>
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<td>Tunisia</td>
<td>Quantitative</td>
<td>Questionnaire survey (scale)</td>
<td>Not defined</td>
<td>Women with CL scar</td>
<td>41</td>
<td>All female</td>
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<td>Ramdas et al.</td>
<td>2016</td>
<td>Surinam</td>
<td>Qualitative</td>
<td>Ethnography</td>
<td>Sep 09 to Dec 10</td>
<td>CL patients. General population 205 CL patient &amp; 321 people</td>
<td>205</td>
<td>8.3 &amp; 1.4</td>
<td>20-49</td>
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<td>Turan et al.</td>
<td>2015</td>
<td>Turkey</td>
<td>Quantitative</td>
<td>Questionnaire survey (scale)</td>
<td>May 11 to Apr 13</td>
<td>Paediatric CL patients and healthy controls 54 CL patients</td>
<td>54</td>
<td>1.16</td>
<td>7-18</td>
<td>No</td>
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<td>Handjani et al.</td>
<td>2013</td>
<td>Iran</td>
<td>Quantitative</td>
<td>Questionnaire survey (scale)</td>
<td>2013</td>
<td>Relatives of dermatological cases 50 relatives</td>
<td>50</td>
<td>0.85</td>
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<td>Vares et al.</td>
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<td>Iran</td>
<td>Quantitative</td>
<td>Questionnaire survey (scale)</td>
<td>Not defined</td>
<td>CL patients</td>
<td>124</td>
<td>0.59</td>
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<td>Abazid et al.</td>
<td>2010</td>
<td>Syria</td>
<td>Quantitative</td>
<td>KAP-survey</td>
<td>Nov 06 to Oct 07</td>
<td>CL patients or the caregiver seeking treat 70</td>
<td>70</td>
<td>0.46</td>
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<td>Fernando et al.</td>
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<td>Sri Lanka</td>
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<td>KAP-survey</td>
<td>Sep 06 to Feb 08</td>
<td>CL patients</td>
<td>120</td>
<td>2.75</td>
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<td>Niiforouzadeh et al.</td>
<td>2010</td>
<td>Iran</td>
<td>Quantitative</td>
<td>RCT</td>
<td>2007</td>
<td>Female CL patients 2 groups of 20</td>
<td>2 groups of 20</td>
<td>All female</td>
<td>&gt; 10</td>
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<td>Kassi et al.</td>
<td>2008</td>
<td>Afghanistan</td>
<td>Qualitative</td>
<td>Case report</td>
<td>Not defined</td>
<td>Woman with CL scar</td>
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<td>All female</td>
<td>28</td>
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<td>Authors</td>
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<td>Study Population</td>
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<td>Sex ratio (M/F)</td>
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<td>Simsek et al.</td>
<td>2008</td>
<td>Turkey</td>
<td>Quantitative</td>
<td>Questionnaire survey (scale)</td>
<td>2006</td>
<td>Women in general population</td>
<td>247</td>
<td>All female</td>
<td>15-47</td>
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<td>Reithinger et al.</td>
<td>2005</td>
<td>Afghanistan</td>
<td>Mixed</td>
<td>KAP &amp; FGD</td>
<td>Oct 2002</td>
<td>KAP: household head; FGD: women</td>
<td>KAP: 252;</td>
<td>FGD All female</td>
<td>Not defined</td>
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<td>Yanik et al.</td>
<td>2004</td>
<td>Turkey</td>
<td>Quantitative</td>
<td>Questionnaire survey (scale)</td>
<td>Sep 02 to Aug 03</td>
<td>CL patients, persons with CL scar, healthy controls</td>
<td>3 groups of 33</td>
<td>1:1</td>
<td>12-35</td>
<td>Yes</td>
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<td>Reyburn et al.</td>
<td>2000</td>
<td>Afghanistan</td>
<td>Qualitative</td>
<td>FGD</td>
<td>Feb to Jul 1998</td>
<td>CL patients and unaffected spouses</td>
<td>8 groups of 6 to 10</td>
<td>1:1</td>
<td>≥28</td>
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<td>Reference</td>
<td>Measurement scales of psychological, social and QoL burdens</td>
<td>Main findings</td>
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<tr>
<td>Chahed-2016</td>
<td>Revised Illness Perception Questionnaire (IPQ-R), World Health Organization Quality of Life-26 (WHOQOL-26)</td>
<td>Large range of negative psychological consequences of LCL it is a source of constant stress and shame. Leading to social rejection and anticipation/avoidance behavior.</td>
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<td>Turan-2015</td>
<td>The Child Depression Inventory (CDI), The State-Trait Anxiety Inventories for Children (STAIC), The Paediatric Quality of Life Inventory Parent and Child Versions (PedQL-P and C)</td>
<td>Depression scores were significantly higher in children and adolescents with CL compared to age-matched controls. No significant difference was found in anxiety scores. Depression scores were also higher in mother of children with LCL.</td>
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<td>Handjani-2013</td>
<td>Family Dermatology Life Quality Index (FDLQI)</td>
<td>Family members of LCL cases face a reduction in QoL, mainly due to the time they have to spend looking after the patient.</td>
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<td>Vares-2013</td>
<td>Dermatology Life Quality Index (DLQI)</td>
<td>The type of LCL lesion determines the degree of QoL reduction. Most reduced in ulcers, least in papular LCL.</td>
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<tr>
<td>Nilforoushzadeh-2010</td>
<td>Dermatology Life Quality Index (DLQI)</td>
<td>The effect of adjunct psychotherapy to drug treatment on QoL was tested in a randomized controlled trial on women with LCL. QoL was improved in the intervention group.</td>
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<td>Simsek-2008</td>
<td>The Turkish version of the Structured Clinical Interview for Axes I of the DSM-IV (SCID-I)</td>
<td>This study on mental health of women in rural Turkey identified CL as one of the main independent predictors of mental disorders.</td>
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<td>Yanik-2004</td>
<td>Hospital Anxiety Depression Scale (HAD), Body Image Satisfaction Scale (BIS), Dermatology Quality of Life Scale (DQL)</td>
<td>Depression and anxiety are higher in LCL compared to control. Impaired body satisfaction in LCL. LCL have reduced QoL compared to LCL scars and controls.</td>
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<td>1st Author name, Year of publication and country of the study.</td>
<td>Types of stigma noticed</td>
<td>Types of psychological consequences related to CL</td>
<td>Reasons for stigma</td>
<td>Reasons for the Psychosocial burden</td>
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<td>Al-Kamel-2017 (Yemen)</td>
<td>Social stigma Aesthetic stigma Psychological stigma</td>
<td>Stress Depression Anxiousness Shame</td>
<td>Socially denied</td>
<td>Feels shame. Fears of infecting their children and infecting others through food and drink. Fears of deformity, disability, and death. Fear of malignancy.</td>
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<td>Bennis-2017 (Morocco)</td>
<td>Self-stigma Social stigma</td>
<td>A large spectrum of negative psychosocial effects: Anxiety, Embarrassment, Sadness, Shame, Suicidal ideas</td>
<td>Social stigma (Family relationships, social contempt, fear, marriage difficulties, avoidance by others). Self-stigma (embarrassment, shame, sadness, anxiety, depression, suicidal ideas)</td>
<td>The emergence of psychological consequences due to perceived stigma.</td>
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<tr>
<td>Chahed-2016 (Tunisia)</td>
<td>Social exclusion and stigmatization</td>
<td>Loss of self-esteem, feelings of inferiority and the idea that the disease is equal to an obvious social disadvantage. There was also a strong sense of shame.</td>
<td>The nature of stigma that patients experience is associated with certain general fears and anticipation of rejection, rather than the possible rejection that patient truly experiences from society. It reduced marriage prospects for men (75%) and women (59%). ZCL has had no positive effects on their lives. The consequences have rather been negative. About 73% suffered social exclusion and stigmatization. Their relationships have been broken and they face more interpersonal conflicts in society, regardless of the context (family, social or professional). The consequences were seen also in their chances of getting employment.</td>
<td>Respondents are dissatisfied with their self-image and appearance. Scars alter women’s beauty (58%). Drugs were seen to have little or no effect, and the risk of getting the disease again is likely. The more a person looks at the world anticipating segregation and rejection, the more they perceive ZCL as a disease with harmful effects on them-The more they see it as a mysterious and incomprehensible illness, and the more they talk about suffering and emotional difficulties.</td>
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<tr>
<td>Author</td>
<td>Year</td>
<td>Country</td>
<td>Type of Stigma</td>
<td>Description</td>
<td></td>
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<tr>
<td>Ramdas 2016 (Surinam)</td>
<td></td>
<td></td>
<td>Aesthetic stigma</td>
<td>In the literature: 1) experienced or enacted stigma; 2) anticipated, felt, or perceived stigma; and 3) internalized or self-stigma. 38 CL patients who reported having experienced negative reactions related to CL expressed feeling bad about their looks. Some said they were &quot;disgusted&quot; by their sores, others reported feeling &quot;shy&quot; or &quot;ashamed&quot;. Health-related stigma is linked to patients’ own illness experiences’. These feelings were experienced when sores became bigger, or when they had multiple sores. The gruesome appearance of CL sores contributed to patients’ fear of the disease. Aside from a sore's appearance, the growth of a sore, the increase in a number of sores, and their visibility could also cause overt negative reactions from those in a patient’s environment.</td>
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<td></td>
</tr>
<tr>
<td>Reyburn 2000 (Afghanistan)</td>
<td></td>
<td></td>
<td>Enacted stigma</td>
<td>Feeling rejection and isolation Some women expressed anger at the situation. Feeling bring shame to the whole family. One girl said that she sometimes felt suicidal. Women tended to express feelings of rejection and isolation more frequently than men. Widespread and inappropriate fear of direct or indirect transmission of CL to others. Fear of contagion and an association with dirt, low personal hygiene, sinfulness, and disgust. If you commit a sin or you have done something wrong, you will get the punishment from God later on. An insulting word, “soldana” was commonly reported to be used against CL sufferers, leading to a general feeling of victimization. Some respondents were required to eat separately from the family with his/her own plate, cup, utensils and not to share a bed with healthy family members. Women with CL often reported that they were not allowed to cook for the family. Had lost authority or felt disempowered from interacting in public or managing their affairs. It seemed that the perceived need to isolate CL sufferers from others readily developed into a more personal rejection. One of the major concerns for young people, especially women, was their appearance and adherence to an ideal of beauty. CL was felt to seriously undermine their prospects for marriage. Both active lesions and scars were a concern, particularly on the face. Several men reported that leishmaniasis caused problems in taking their ablutions and staying ritually clean throughout the prayer. They felt that others saw them as unclean and they were deterred from praying at the mosque.</td>
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</table>
CHAPTER 6. Accuracy of a rapid diagnostic test based on antigen detection for the diagnosis of cutaneous leishmaniasis in patients with suggestive skin lesions in Morocco.

Accuracy of a rapid diagnostic test based on antigen detection for the diagnosis of cutaneous leishmaniasis in patients with suggestive skin lesions in Morocco

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Key words: Sensitivity and specificity; diagnostic accuracy; rapid diagnostic test for antigen detection; cutaneous leishmaniasis; Morocco
Abstract

In rural areas in Morocco, diagnosing cutaneous leishmaniasis (CL) can be challenging. We evaluated the accuracy of a rapid diagnostic test based on antigen detection (RDT), the CL Detect Rapid Test™ (Inbios International, Seattle, USA), in this setting.

We consecutively recruited patients with new skin ulcers in nine primary health centers. We took a dental broach sample for the RDT and two other tissue samples by scraping border and center of the lesion with a scalpel and smearing it on a slide. We duplicated each smear by pressing a clean slide against it and processed the slides by microscopy, PCR ITS1, and kDNA minicircle PCR. In a subgroup with positive PCR, the *Leishmania* species was identified using PCR-RFLP and PCR-sequencing of *hsp70* genes. A participant with positive microscopy and/or PCR was considered a confirmed CL case. We computed sensitivity and specificity of the RDT compared to this reference standard [ClinicalTrials.gov registration: NCT02979002].

Between December 2016 and July 2017, we included 219 patients, 50% of them were under 18 years old. RDT sensitivity was 68% [95% confidence interval (CI), 61-74], specificity 94% [95% CI, 91-97], positive predictive value 95% [95% CI, 92-98] and negative predictive value 64% [95% CI, 58-70].

Despite its low sensitivity, this novel RDT is a useful addition to clinical management of CL in Morocco, especially in isolated localities. RDT-positive lesions can be treated as CL; but when RDT-negative, microscopy should be done in a second step. The sensitivity of the RDT can probably be optimized by improving the sampling procedure.
Introduction

Cutaneous leishmaniasis (CL) is a vector-borne disease which usually leads to slowly self-healing skin lesions and residual scars on those parts of the body exposed to sandfly bites [1]. In 2016, Morocco reported 4951 CL cases. In the southeast of the country, CL is mainly due to *Leishmania major*, and in the central and northern regions to *L. tropica*. Some sporadic cases of CL due to *L. infantum* have also been described [2, 3]. CL scars on the face can cause considerable psychosocial suffering. In young women in North Africa and the Middle East, social rejection, diminished marriage and employment prospects, and suicidal thoughts have been documented.[4, 5] Women typically use traditional remedies and several cosmetics to mask the scar tissue or seek plastic surgery [4, 6].

Early diagnosis and prompt and adequate treatment may improve the healing process and prevent the development of stigmatizing lesions and scars [7]. However, current diagnostic methods for CL are far from optimal and there is no single gold standard diagnostic technique. In resource-limited settings, the most used diagnostic test for CL is direct microscopy, i.e. searching for amastigote forms of the *Leishmania* parasite in Giemsa-stained skin smears or biopsies [7]. However, the sensitivity of this test is moderate, between 53% and 76% [8–11] depending on the disease duration, sampling technique, and skills of the microscopist [12]. In patients with suggestive lesions and negative microscopy results, the polymerase chain reaction (PCR) can help to confirm or rule out the diagnosis of leishmaniasis [13]. In Morocco, a PCR based on the internal transcribed spacer (ITS1) primer showed a sensitivity between 84% and 100% [14, 15]. Furthermore, Restriction Fragment Length Polymorphism (PCR-RFLP) analysis of the amplification product allows species identification of the *Leishmania* parasites [16–18]. Conditions for these diagnostic methods are not always available/optimal in the remote areas where the disease is endemic [7]. In rural areas in Morocco, patients often have to travel up to two days to a center where the CL diagnosis is possible, and sometimes the laboratory request and the sample have to be sent to the national reference laboratory [19]. Therefore, the diagnosis of CL in the *L. major*-endemic areas in the southeast is, in practice, often based on the clinical features of the lesion, whereas in *L. tropica* and *L. infantum*-endemic areas in the northern, central, and mountainous regions, the diagnosis is more often based on a direct microscopic exam. An accurate and user-friendly rapid diagnostic test for antigen detection (RDT) would be welcome in these settings.

In general, RDTs are quick and easy to perform, with the ability to give results at the point of care, meaning that results are available during the first patient visit. RDTs can be used in settings with limited laboratory infrastructure and staff [20]. Several years after the successful development of an RDT for visceral leishmaniasis,[21–23] an RDT for CL has been developed. The CL Detect Rapid Test™ (Inbios International, Seattle, USA) is an antigen detection assay that detects a membrane-based amastigote antigen (peroxidoxin). The test is recommended for ten *Leishmania* species (*L. tropica, L. amazonensis, L. donovani, L. infantum, L. mexicana, L. guyanensis, L. major, L. braziliensis, L. panamensis, L. peruviana*), and is to be used on a sample
taken with a dental broach from a skin ulcer not older than four months. Based on a study in
Tunisia (unpublished), the manufacturer claims a 100% sensitivity for CL due to \textit{L. major}
compared to microscopy. The present study aimed to estimate the diagnostic accuracy of the CL
Detect\textsuperscript{TM} Rapid Test compared to a composite reference standard test (direct examination of skin
smears by microscopy and/or PCR), in patients with skin ulcers attending primary health centers
in CL-endemic areas in Morocco.

\section*{Materials and methods}
\subsection*{Study design}
This was an observational, prospective, phase III diagnostic accuracy study.

\subsection*{Participants}
Study participants were recruited in nine primary health centers located in five CL-endemic
provinces where previous studies had established the dominant species, namely, Ouarzazate
(\textit{L. major})\cite{24} Tinghir (\textit{L. tropica}), Errachidia (\textit{L. major})\cite{25} Sefrou (\textit{L. tropica} and \textit{L. infantum})
\cite{26} and Sidi Kacem (\textit{L. tropica} and \textit{L. infantum}) \cite{27}.

Between December 2016 and July 2017, nurses and physicians working in these primary health
centers consecutively enrolled patients with ulcerative skin lesions. To be included, patients had
to have at least one ulcerative skin lesion suggestive of CL. This lesion had to be of recent onset,
defined as a duration reported by the patient of fewer than four months. In case there were
multiple lesions, the most recent and none superinfected was selected for this study.

Patients were excluded if they had already received antimony treatment or any other physical
(e.g. cryotherapy, thermotherapy, laser) or traditional (e.g. burn, acid, traditional scraping)
treatment for their skin lesion during the last two months. Patients with apparent other diseases
or patients requiring hospitalization or any other anti-parasitic treatment were also excluded, as
were those with ulcerative lesions of suspected bacterial origin which healed completely after a
short course of antibiotic treatment.

\subsection*{Study procedures}
After verification of the eligibility criteria and after obtaining informed consent,
sociodemographic and clinical information was collected from each patient. Next, a picture was
taken of the selected lesion. A health professional then took a small sample of the ulcer using a
dental broach, after cleaning the skin with sterile 0.9\% saline solution and discarding the crust
or superficial skin with a small sterile scalpel. This first tissue sample was used for the RDT. Then,
two additional skin smear samples (Mic 1 and Mic 2) were taken from the same lesion, from the
center and the border of the ulcer as recommended by Suárez et al \cite{28}. Each smear was put on
a slide and duplicated by pressing a clean slide against it. The four resulting smears were used
for microscopy and PCR analyses. The slides were put in a clean and dust-free area for one to
four hours, far from direct sunlight exposure, to make sure that they dried well at room
temperature. Two slides (Mic 1 and Mic 2) were transferred to the nearest provincial laboratory
for microscopy reading. The two remaining slides, resulting from the duplication, were sent to
laboratories in Casablanca and Antwerp (Belgium) for PCR testing (as shown in supporting
material 1).

Index test
The CL Detect Rapid Test™, in package CL025 with lot identifier UA1259 and manufacturer
specifications available online (http://www.inbios.com/wp-content/uploads/2016/06/900159-
00-IVD-CL-Detect-Rapid-Test-Package-Insert.pdf) was done on the sample collected by the
dental broach. The health professional recorded the RDT results on a separate sheet, as well as
the time to perform the entire procedure from starting the RDT until reading the result, and the
prevailing room temperature. We followed manufacturer’s instructions regarding reading and
interpretation of results. The RDT was considered positive if both control and test bands were
visible and became red, and negative if only the control band was visible (Figure 1).

![Image of CL Detect Rapid Test™](image)

(1) CL Detect Rapid Test™ showing a positive result.
(2) CL Detect Rapid Test™ showing a negative result.

Figure 1. Interpretation of CL Detect Rapid Test™
Reference standard tests

Microscopy
In the provincial laboratory, two slides per participant were fixed by dipping them in methanol for 2 to 3 minutes and staining them with Giemsa 10%, before examination under a light microscope (x1000 magnification). Both slides were examined by the same microscopist, who reported the number of fields, and the total number of macrophages and *Leishmania* amastigote forms found. Then, the best slide of the two (Mic1 or Mic2, depending on the ease to visualize macrophages if negative and to identify the number of *Leishmania* amastigote forms if positive) was sent to the National Institute of Hygiene (NIH) to be verified by a laboratory engineer, who had no knowledge of the patients or any previous test results. At the NIH, all slides were assessed for their quality (and scored as bad, average, excellent) according to the standard operating procedures (SOP). If the provincial laboratory reported a positive and the NIH a negative result, the other slide was also sent to the NIH. In this case, we took the conclusion from the reader at the NIH based on the two slides as the final microscopy result.

ITS1-PCR
DNA extraction from the third slide smear was carried out manually according to the phenol-chloroform method [14, 18]. As a quality control measure, a NanoVue Plus spectrophotometer (Biochrom US, Massachusetts, USA) was used to measure the DNA concentration in 100 samples. As described previously, [14, 18] the internal transcribed spacer 1 of the ribosomal DNA repeat unit (ITS1) was amplified in a Biometra GmbH (Whatman Biometra, UK) thermocycler using primers L5.8S: 5′-TGATACCACCTTATCGCACTT-3′ and LITSR: 5′-CTGGATCATTTTCCGATG-3′ (Biolegio, Nijmegen Netherlands). Amplification was carried out in 25 μl. Cycling conditions were as follows: denaturation at 94°C for 4 minutes, next at 94°C for 40 seconds, 53°C for 30 seconds, 72°C for 1 minute, 72°C for 10 minutes (40 cycles). The obtained amplification products were revealed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under ultraviolet (UV) light. The ITS1-PCR was considered positive for the *Leishmania* genus if a band of the expected size (~300-350 base pairs) was obtained.

For species identification, positive samples were subjected to restriction fragment length polymorphism (RFLP) analysis using the restriction endonuclease HaeIII (BioLabs, USA) [14, 18]. Reference strains of *L. infantum* (MHOM/ES/90/LEM2205), *L. tropica* (MHOM/MA/2010/L02) and *L. major* (MHOM/IL/81/Friedlin) were included.

kDNA-PCR
Two slides per lesion were used for molecular testing. One of the two slides was systematically analyzed by ITS1-PCR in Casablanca and the second slides were divided into two groups to be analyzed by ITS1-PCR in Casablanca or by kDNA-PCR in Antwerp as explained in supporting material 1. The kDNA-PCR was used as an additional method to detect *Leishmania* DNA in the samples. Because this PCR amplifies the parasite’s kinetoplast DNA minicircles, of which 10000
to 20,000 copies are found in each cell, it is more sensitive than ITS1-PCR. DNA was extracted using either the phenol-chloroform method described above or (for most samples) using the QiaAmp DNA Mini Kit (Qiagen, www.qiagen.com). Lysis was achieved by 3 hours incubation at 56°C in Tissue Lysis Buffer, and the DNA was eluted in 50 µl elution buffer AE. Primers JW11 and JW12 were used,[29] in a reaction of 25 µl containing 1x QuantiTect SYBR Green PCR Master Mix (Qiagen); 1 µM of each primer; 0.1 mg/ml acetylated BSA; and 2.5 µl DNA. Cycling was performed in a Light Cycler 480 instrument (Roche, molecular.roche.com) using an initial denaturation of 15 minutes at 95°C, followed by 50 cycles of 10 seconds at 95°C, 20 seconds at 57°C, and 30 seconds at 72°C. The Tm of the amplicons was determined in a melting curve analysis from 67°C to 95°C. A standard curve was included in each PCR, ranging from 10⁳ down to 10⁻⁴ pg *L. infantum* DNA, determined spectrophotometrically. The kDNA-PCR was considered positive for *Leishmania* if more than 0.2 parasite genome equivalents were detected on the slide, and a melting peak was observed between 79.5°C and 81°C.

**Analysis**

Data were entered in duplicate in Microsoft Excel 2016 and a duplicate check was done to detect typing errors. Data analysis was performed using Stata 13 (Stata Corporation, Texas, USA). Differences in proportions were tested using Fischer’s exact test. The Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the RDT were calculated comparing the results of the index test to the composite reference standard. The composite reference standard was defined as follows. A patient was considered as a confirmed case of CL if microscopy results and/or PCR results (be it kDNA or ITS1) were positive. Else, the patient was considered a non-case. Test accuracy was first calculated in the whole study population and then compared across subgroups defined on the basis of geographical origin, *Leishmania* species, and slide quality.

We estimated the required sample size (n = 240) based on an expected frequency of CL in the patient series of 50%, an anticipated sensitivity of the CL Detect Rapid Test™ of 97% and a specificity of 84%, an α error of 5%, and a power of 80%.

**Quality assurance**

The study was registered on clinicaltrials.gov with the identifier NCT2979002 in November 2016. Before the start of the study, Standard Operating Procedures (SOPs) were developed (available on request). These SOPs were carefully explained and validated during three training days for all health professionals involved in the study procedures. The diagnostic tests in this study were done blinded to the results of other tests, i.e. readers of the index and reference tests did not have access to any other test results. Each package of RDTs was subject to internal quality control, using a positive and negative control following the manufacturer’s recommendations. These controls of all RDT packages were done during a training workshop prior to the study. We report the results in compliance with the STARD (Standards for Reporting of Diagnostic Accuracy studies) checklist.[30]
**Ethical aspects**
The protocol of this study was approved by the Institutional Review Board of the Institute of Tropical Medicine in Antwerp (Reference1060/15), the Ethics committee of Antwerp University Hospital UZA (Reference15/51/557) and the Comité d’éthique en recherche biomedicale CERB (Reference41/16) in Rabat. The results of the index test under evaluation and the molecular tests (PCR) were not communicated to the patients or their physicians. Hence, no therapeutic decisions were based on the index test results, and CL case management followed current clinical guidelines in Morocco.

**Results**

**Participants**
In total, 219 patients were included (Figure 2), amongst whom there were 113 men (52%) and 106 women (48%). Half of the participants (n = 108; 50%) were under 18 years old. Seventy-four patients (34%) had two to ten lesions. The diameter of the selected lesions ranged between 0.1 and 5 cm. Most lesions were small: 142 participants (65%) had lesions with a diameter of one cm or less. Table 1 shows the demographic and clinical characteristics of the study population. None of the health professionals reported any adverse events from the sampling, even if they did not systematically use lidocaine ointment (Emla®) or injectable xylocaine. There were no withdrawals from the study.

**Test methods**
For the RDT, there were no missing data, nor any invalid results. The RDT gave 97 positives and 122 negative results. The average time to perform the RDT was 32 ± 7 minutes (n = 217) with a range between 15 minutes and 48 minutes.
For the reference standard tests, some data were (partially) missing, as, during transportation of microscopy slides between the primary health facilities and provincial laboratories, 17 slides broke, and one of them was irrecoverable. Moreover, 32 slides were not well fixed, which limited the second reading by the NIH supervisor. For PCR tests, three partially broken slides were recoverable and two completely irrecoverable. The mean time (±SD) to obtain the result of microscopy from the provincial laboratories was 2.7 ± 3 days (n = 198).
Figure 2. Flowchart of the study

Potentially eligible participants
N = 769

Excluded
N = 503
- Age less than 05 years old (307)
- Non-ulcerative CL form (50)
- Received traditional or physical treatment of the lesion (21)
- Received antimoniate or anti-parasite treatment (42)
- Lesion duration of more than four months (81)
- Other reasons, unspecified (02)

Eligible participants
N = 266

No RDT
N = 47
- Lesions near to the eye (13)
- Refusal to participate (07)
- Impossibility to sign informed consent (27)

RDT
N = 219

RDT Negative
N = 122

Reference standard done
N = 122

Final diagnosis
Absence of CL (N = 78)
Presence of CL (N = 44)

RDT Positive
N = 97

Reference standard done
N = 97

Final diagnosis
Absence of CL (N = 05)
Presence of CL (N = 92)
Table 1. Demographic and clinical characteristics of 219 patients enrolled

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Provinces historically endemic for <em>L. major</em></strong></td>
<td></td>
</tr>
<tr>
<td>Errachidia</td>
<td>42</td>
</tr>
<tr>
<td>Ouarzazate</td>
<td>35</td>
</tr>
<tr>
<td><strong>Provinces historically endemic for <em>L. tropica</em></strong></td>
<td></td>
</tr>
<tr>
<td>Tinghir</td>
<td>65</td>
</tr>
<tr>
<td>Sefrou</td>
<td>67</td>
</tr>
<tr>
<td>Sidi Kacem</td>
<td>10</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>219</td>
</tr>
<tr>
<td><strong>Patient age (years)</strong></td>
<td></td>
</tr>
<tr>
<td>[05 - 17]</td>
<td>108</td>
</tr>
<tr>
<td>[18 - 87]</td>
<td>110</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>106</td>
</tr>
<tr>
<td>Men</td>
<td>113</td>
</tr>
<tr>
<td><strong>Lesion on the face</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>112</td>
</tr>
<tr>
<td>No</td>
<td>107</td>
</tr>
<tr>
<td><strong>Lesion duration (days)</strong></td>
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<tr>
<td>≤ 50</td>
<td>105</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>107</td>
</tr>
<tr>
<td><strong>Lesion diameter (cm)</strong></td>
<td></td>
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<tr>
<td>≤ 01</td>
<td>142</td>
</tr>
<tr>
<td>&gt; 01</td>
<td>76</td>
</tr>
<tr>
<td><strong>Number of lesions per patient</strong></td>
<td></td>
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<tr>
<td>01</td>
<td>145</td>
</tr>
<tr>
<td>02-10</td>
<td>74</td>
</tr>
</tbody>
</table>

(⁎) Information missing for one participant (**) Information missing for seven participants

**Diagnostic accuracy**

The reference standard classified 136 (62%) participants as CL cases: 75 had both positive microscopy and positive PCR results, 26 had positive microscopy only, and 35 had positive PCR only (Figure 3). The frequency of confirmed CL in this patient series varied by region: in the areas known to be endemic for *L. major*, it was 70%, compared to 58% in *L. tropica* regions. The *Leishmania* species could be identified in samples of 87 participants: 35 were *L. major*, 40 *L. tropica*, and 10 *L. infantum*. The geographical distribution of the species is shown in Table 2.
Table 2. Place of inclusion and index test results according to *Leishmania* species, n = 87

<table>
<thead>
<tr>
<th>Provinces historically endemic for</th>
<th><em>L. major</em></th>
<th><em>L. infantum</em></th>
<th><em>L. tropica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Errachidia</strong></td>
<td>N</td>
<td>RDT +</td>
<td>N</td>
</tr>
<tr>
<td>19</td>
<td>12</td>
<td>00</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ouarzazate</strong></td>
<td>14</td>
<td>10</td>
<td>00</td>
</tr>
<tr>
<td><strong>Provinces historically endemic for</strong></td>
<td><strong>L. tropica</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tinghir</strong></td>
<td>02</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td><strong>Sefrou</strong></td>
<td>00</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td><strong>Sidi Kacem</strong></td>
<td>00</td>
<td>-</td>
<td>01</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>35</td>
<td>23</td>
<td>12</td>
</tr>
</tbody>
</table>

Species identification based on ITS1 RFLP (n = 80) or *HSP70* (n = 7).

When compared to the composite reference standard, the RDT showed a Se = 68% [95% confidence interval (CI), 61-74], Sp = 94% [95% CI, 91-97], PPV = 95% [95% CI, 92-98] and NPV = 64% [95% CI, 58-70] in the whole study population (n = 219). The diagnostic accuracy varied according to the geographical origin of the samples, though this difference was not statistically significant. The Se of the RDT was higher in regions historically endemic for *L. tropica*, i.e. 73% [95% CI, 66-80], versus 59% [95% CI, 48-70] in *L. major* regions (Table 3). This species effect was supported by comparing the results of the RDT after typing of the samples: 83% of the confirmed *L. tropica* and 66% of the *L. major* samples were RDT positive (Table 2); However, in this comparison, the observed differences in Se and Sp did not reach statistical significance (Fisher exact = 0.32 and 0.10 respectively). On the other hand, we did find a significant association between RDT accuracy and smear slide quality: RDT Se was significantly higher among patients in whom microscopy slides of better quality were obtained compared to the rest of the population (Table 3) (Fisher exact = 0.001).
<table>
<thead>
<tr>
<th>Provinces historically endemic for</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. tropica</em></td>
<td>142</td>
<td>73% [95% CI, 66-80]</td>
<td>92% [95% CI, 87-96]</td>
<td>92% [95% CI, 88-97]</td>
<td>71% [95% CI, 64-79]</td>
</tr>
<tr>
<td><em>L. major</em></td>
<td>77</td>
<td>59% [95% CI, 48-70]</td>
<td>100%</td>
<td>100%</td>
<td>51% [95% CI, 40-62]</td>
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<thead>
<tr>
<th>Slide quality <em>(φ)</em></th>
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<tr>
<td>Excellent quality</td>
<td>42</td>
<td>88% [95% CI, 78-98]</td>
<td>100%</td>
<td>100%</td>
<td>71% [95% CI, 58-85]</td>
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<tr>
<td>Average quality</td>
<td>92</td>
<td>64% [95% CI, 54-74]</td>
<td>96% [95% CI, 92-100]</td>
<td>98% [95% CI, 95-100]</td>
<td>50% [95% CI, 40-60]</td>
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<tr>
<td>Bad quality</td>
<td>69</td>
<td>53% [95% CI, 42-65]</td>
<td>90% [95% CI, 83-97]</td>
<td>80% [95% CI, 71-89]</td>
<td>72% [95% CI, 61-82]</td>
</tr>
</tbody>
</table>

*(φ)* 16 cases were impossible to assess by the laboratory engineer at the NIH due to the poor fixation of the smear slides.
The room temperature during the RDT implementation varied between 8°C and 38°C. Nine RDTs were performed at a room temperature above 30°C. In total, only 47 samples (21%) were examined at a room temperature between 20°-30°C, i.e. corresponding to the manufacturer’s instructions.

Figure 3. Distribution of index and reference test results in the study population (n = 219)

Discussion
We report the first evaluation of an RDT for CL in a series of Moroccan patients with skin lesions suggestive of CL. In remote and rural endemic areas in Morocco, we can recommend for patients presenting skin ulcers suggestive of CL to use at first the RDT, followed by microscopy for those who are RDT negative. Indeed, the sensitivity of this RDT was moderate, and the specificity was excellent, leading to a high PPV of 95%. This means that 19 out of 20 patients with an RDT-positive ulcer truly have CL, and that treatment is therefore warranted in this group. The RDT-negative patients will need a second test to rule out CL, as the NPV was low. The turn-around time (i.e. the rapidity of getting the results) of less than 40 minutes, the absence of invalid results and the short time required for training the health center staff are also supporting the use of this RDT in remote rural areas. The study followed a phase III design, recruiting suspect patients in real-life conditions in nine different health facilities, and this is representative of the context in which the RDT will be used in the future. This study design provides a solid basis for eventually reformulating the diagnostic policy in CL in Morocco.
Current limitations of this RDT are that its stability in ambient temperature is only assured for two years, as well as the cost. In Morocco, the current (2016) unit price of this RDT was US$ 8 per patient, which is not affordable in comparison with microscopy that costs less than US$ 1 per patient. However, a recent study in Sri Lanka using the same RDT for *L. donovani* mentioned a lower cost, US$ 4 per patient,[31] and future cost may evolve as a function of demand and offer.

We acknowledge that our study has some limitations. Firstly, our reference standard classification was probably not 100 percent perfect. The sampling procedures for microscopy and PCR were the main factor that may have limited the sensitivity of the reference standard. Secondly, the kDNA-PCR was not systematically done on all the second slides. A suboptimal sensitivity of the reference standard may have decreased somewhat the specificity estimate of the RDT, though this seems not so apparent from our results. Thirdly, it was not possible in this study to systematically determine the alternative diagnoses in patients who did not have CL. Fourthly, because the age of lesions was based on the subjective declaration of the patient, we may have unwittingly included a few patients with lesions older than four months [12].

Our results are consistent with those from a study in Afghanistan, in which the same CL RDT was used in 257 patients with mostly nodular lesions, living in Kabul, where *L. tropica* is known to be the predominant species [32]. The accuracy of the RDT compared to a reference standard based on qPCR and microscopy was: Se = 65% [95% CI, 59-71]; Sp = 100%; PPV = 100% [95% CI, 98-100]; NPV = 16% [95% CI, 09-24] [32].

An economic evaluation of the same RDT compared to microscopy in a population living in *L. tropica* areas, reported that the incremental cost-effectiveness ratio per DALY averted was less than US$ 10.15 for the RDT compared to microscopy, which makes it an attractive option.[33] Other studies suggest that loop-mediated isothermal amplification (LAMP) could be an alternative as a point of care diagnostic, though the required equipment, training and technology is less user-friendly than an RDT. Reverse transcriptase LAMP seems especially interesting for CL diagnosis in regions where a species-specific diagnosis is required because several species circulate simultaneously [34–37].

Some questions remain unanswered. The study was not powered to show any species-dependent differences in sensitivity, though our results suggest there is such an effect. If this is explored further and a species effect is confirmed, further work would need to verify if the antigen targeted by the assay is not slightly different in *L. major* areas in Morocco. In the Sri Lanka study, the low sensitivity of the RDT (36%) was linked to the low expression of the peroxidoxin antigen in Sri Lankan *L. donovani* [31]. Alternatively, the ‘species’ effect could simply be due to differences in parasite load, which could obviously influence the assay’s performance. The moderate sensitivity of the RDT in this study could also be explained by a low parasite yield.
within the sample taken by the small saws of a dental broach. There is scope for optimizing the sampling technique, for example with the use of a sterile disposable punch, as recently published, or by repeating the procedure.[38] Further work is required to explore these hypotheses.

In conclusion, this novel RDT for CL in its current format is a useful addition to clinical case management in Morocco, especially in isolated localities far from provincial laboratories. A positive RDT has a very good PPV (95%) and warrants treatment in endemic CL areas. This RDT is a powerful decision-making tool for nurses and other health professionals to improve the management of CL in remote areas. The RDT can be part of an algorithm that uses microscopy in a second step. Nevertheless, we recommend improving the sensitivity of this RDT, and this could probably be reached by optimizing the sampling procedure.

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Competing interests
None declared. The manufacturer of the RDT did not interfere with the decision to publish nor with the content of the publication.

Previous presentation of the results of this study
The preliminary results of this study were presented as part of a poster communication (n° = C-1606) at the 6th World Congress on Leishmaniasis in Toledo, Spain, in May 2017 and as a poster (n° = 3P17) at the 10th European Congress on Tropical Medicine and International Health in Antwerp, Belgium, in October 2017.

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References


Supplemental file 1: Repartition of the samples
CHAPTER 7. General discussion
1. Cutaneous leishmaniasis control

The first article in this thesis (Article1) [1], describes the epidemiological trend of CL in the province of Errachidia as reported by the Moroccan health system and analyses the effect of a rodent control intervention in this province. The intervention consisted in the use of poisoned wheat baits to reduce the number of rodents (*Meriones shawi*), the reservoir host for *L.major* [2]. This intervention led to an observed decrease in human CL cases, but, as it was a public health intervention, there were no randomly selected control groups, and only before/after comparisons could be made, or comparisons between areas where the intervention started earlier vs. later. To ensure the success of this control intervention, it was necessary to align the efforts of teams from several ministries i.e. the Directorate of Epidemiology and Disease Control (Ministry of Health), Directorate of Local Communities (Ministry of the Interior), the National Office of Food Safety (Ministry of Agriculture), as well as the local authorities. Civil society members organized themselves into support groups and deployed the poisoned wheat in the rodent burrows, in a radius of about 500 meters around the houses. This operation had to be repeated at least twice or three times a year until there were no further CL cases. The inter-sectoral collaboration was a success, as the different ministries worked efficiently together and the coverage of the at-risk area by the intervention was excellent. Whether the intervention was effective and cost-effective is not so easy to derive from our data. Our study was descriptive in nature, and no experimental study has ever been done with these poisoned baits. We concluded that there was an effect on CL incidence, without providing hard causal evidence. Nonetheless, the effectiveness of CL control strategies by rodent poisoned bait does not come only from Morocco. In 1973 zinc phosphide was used against rodents in Iran based on a similar intervention from Azerbaijan (Ex URSS), but it was not successful to reduce the incidence of CL [3]. However, other studies from Iran, published in 2000, 2005 and 2014, claimed that zinc phosphide-treated baits placed inside rodents’ burrows led to a decrease of both *Ph. papatasi* density and CL human incidence [4–6]. Zinc phosphide or photostoxin showed similar effectiveness [5]. Authors suggested that rodent control operations using zinc phosphide within a 500-meter circle around houses should be deployed once every two years before the start of the sand fly season [6].

In Morocco, the cyclicity of the outbreaks is documented for ZCL cases as shown in the updated figure 1 from our previous article published [1]. The fluctuation in an epidemic cycle with five to ten years intervals is attributed by some researchers to loss of herd immunity or to natural disasters such as floods or dryness. The 2017 outbreak is dominated by the ZCL cases noticed in three provinces from southeastern Morocco (Errachidia n=1436; Tinghir n=1091 and Zagora n=1702) representing 90% of all cases reported in the country (n=4737). As explained before, the Errachidia province had less tendency of cases compared to the neighboring provinces. The 2018 tendency will give a hypothetic answer about the success of the rodents control strategy if the number of ZCL cases in Errachidia province will remain less important than in other provinces. However, the relationship is difficult to establish. Longitudinal studies accounting for the genetic
heterogeneity of human and parasite populations with regard to the global environmental vulnerability could bring some answers [7].

![Figure 1: Number cases of Zoonotic Cutaneous Leishmaniasis per year in Errachidia Province and other affected Moroccan Provinces between 2004 and 2017.](image)

We did not document the environmental effects of the strychnine baits in Morocco. In other studies, strychnine use was found harmful to the environment [8, 9]. Moreover, the scope for replicating this intervention elsewhere depends on its acceptability by the local population as well as on the quality of inter-sectoral collaboration, which is not necessarily the same in each province. Furthermore, this control strategy requires sustainable funding and the political endorsement of the Ministry of Health (MoH). This type of intervention is often seen as an exclusive task for the Ministry of Agriculture with the primary aim to protect crops and palm trees by reducing the rodent population, and the link with CL is not always made spontaneously. High staff turnover furthermore compromises the sustainability of this intervention. Young public health staff is not motivated to get involved in vector control operations, because of the unattractive living conditions typical of these remote areas.

How to be less harmful to the environment when carrying out rodents’ operations should be addressed by adopting a new technique based on the use of toxic fumes (carbon monoxide, nitrous oxide, sodium oxide) by gas cartridges to fumigate rodent burrows\(^\text{15}\). A 2013 report from the USA agricultural department cited “There are no secondary poisoning risks involved with the use of gas cartridges as the gas produced dissipates into the atmosphere shortly after activation. Primary risks to non-targets would be minimized by treating only active burrows”\(^\text{16}\).

\(^{15}\)http://www.wildearthguardians.org/site/PageServer?pagename=priorities_wildlife_war_wildlife_kill_methods_denning accessed in 10 april 2018


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The manual for case management of cutaneous leishmaniasis in the WHO EMR recommends data collection and analysis with the aim to measure the performance of the health intervention at different levels of the health system [2]. However, the rodents’ operations quality control or monitoring system are absent in the Moroccan leishmaniasis guideline, except for an indicator: the number of active burrows x 5 / mammalogical surveillance station per hectare [10]. A protocol describing technical standard operating procedures should be developed before starting field trials to assess the effectiveness of such new preventive measure against rodents’ population in *L. major* endemic areas.

For the Moroccan MoH, developing a CL control policy is complex. The fight against ZCL, which occurs predominantly in scantily populated rural areas, proved relatively successful so far, while control measures were less effective in fighting the anthroponotic CL form (ACL), which affects peri-urban and urban agglomerations with a higher population density. The National Institute of Hygiene (INH) in Morocco and the Directorate of Epidemiology and Disease Control conducted two trials to evaluate the effect of prevention strategies. The primary outcome measure of the first trial of vector control methods was CL incidence. Three intervention arms were compared: 1) the use of long-lasting insecticide-treated nets (LLINs) (Permanet2®; Vestergaard Frandsen, Lausanne, Switzerland) or the insecticide-treated bed nets (ITNs) impregnated with deltamethrin at 55 mg/m² with distribution of ITNs or LLINs to cover all sleeping areas with environmental management; 2) indoor residual spraying (IRS) using α-cypermethrin with environmental management; and 3) environmental management that consisted of campaigns to promote personal protection against exposure to sand flies, cleaning of animal sheds, waste disposal, and maintenance of hygiene. The study concluded that IRS with α-cypermethrin and LLIN distribution both reduced the incidence of CL [11]. IRS was highly effective and more cost-effective for the prevention of CL in *L. tropica* and *L. infantum* areas [11].

The second field trial evaluated the use of systemic ivermectin-baited traps in rodent reservoirs to control the sand fly vector of *L. major*. This trial showed excellent results on the reduction of vector density with a preservation of the environment [12]. However, IRS and ivermectin-baited traps are not yet adopted for widespread use in endemic CL areas in Morocco as the health authorities require more evidence of their effectiveness under real-life conditions. In the current context, the rodent control intervention with strychnine baits is still the intervention of choice in several zoonotic CL endemic areas in Morocco in case of a new outbreak. Similarly, the only ACL control strategy currently recommended by the MoH in its national Leishmaniasis Control Guide is a rapid clinical diagnosis of CL patients and permanent covering of lesions with plaster strips to prevent sand flies getting infected through uncovered ones and subsequently transmitting the disease to humans. This type of recommendation is neither realistic nor based on evidence of its effectiveness. A large number of those infected by CL and seeking treatment in primary health centers are children younger than 10 years of age who are tempted to remove the plaster strip after only a few hours. Furthermore, the cost of the frequent plaster changes
over a long period (days or rather months) before the scars start healing is substantial and must be covered by the patient. Finally, the lesions are often located in areas that are difficult to cover with an adhesive plaster.

In 2015 a Cochrane review [13] was published about vector and reservoir control interventions for preventing both visceral and cutaneous leishmaniasis. For CL, the authors reviewed the effectiveness of 14 interventions. Two Iranian trials studied permethrin-impregnated soldier uniforms as a personal protection measure, but the sample size was insufficient to conclude on any effect. An intervention trial of LLINs (Olyset®) showed a decrease of indoor female *Ph. sergenti* density compared to a control group [14, 15]. The Cochrane review concluded that insecticides may be effective at reducing the incidence of CL. However, the level of evidence remains insufficient to discriminate between IRS, or the use of insecticide-treated bednets, bedsheets, or curtains [13].

In summary, few data exist on prevention of ACL, and even if the effect of insecticidal interventions was demonstrated on entomological outcomes in proof-of-concept studies or small-scale field trials, their effectiveness in real-life conditions is not guaranteed. Moreover, no studies about the acceptability of these preventive measures are available, while acceptability is an important determinant of the success of any disease control strategy. A recent study analyzing risk factors of CL in Southwestern Morocco showed a correlation between ACL and vulnerable socio-economic conditions [16]. The poverty rate of the affected communities and their behaviors should be addressed to break the anthropogenic transmission. Like in the case of one study from Iran demonstrating that the “chance” to get ACL increases when the poor population lives close to the cattle sheds [17]. Such example matches the social vulnerability in endemic Moroccan ACL areas where the use of bed nets by rural population does not seem adapted to their behaviors.

Unfortunately, a new preventive measure against ACL in Morocco cannot work without two conditions: (1) downsizing the damage of the presence of animal cattle around the shelters and people at risk of this disease; (2) introducing a long lasting easy rechargeable insecticide tool that could be portative on necklace and/or bracelet to decrease risk of human contact with the ACL vector. Such tools should be distributed in mass with the aim to stop the transmission cycle.
2. Cutaneous leishmaniasis social representations and psychological suffering

So far none of the studies carried out in Morocco did directly address the psycho-social burden of CL on Moroccan individuals and/or communities. In southeastern Morocco, the disfiguring scars resulting from this disease, which are particularly distressing for young girls, led to repeated demands for cosmetic surgery during our work as public health doctor in the region. On the other hand, public health policy seems to underscore the benign nature of CL. Therefore, our approach was to get a broader understanding to what extent a population living in a CL endemic area suffers psychologically and/or socially from the consequences of this disease. We started by studying the perception of CL among the general population and carried out Focus Group Discussions (FGDs) (Article 3) [18], in two different areas, endemic respectively for *L. major* and *L. tropica*. We included both people with a modern and a traditional perspective, and stratified groups into those who use modern health care facilities and those who have more confidence in traditional remedies or use traditional healers in a first instance. We took into account local sensitivities between ethnic groups and had separate groups for men and women to reach maximum information and create the best possible conditions for uninhibited talks. The impact of the disease was more felt in the *L. major* area than in the *L. tropica* region, probably this was due to the larger size of *L. major* scars. No consensus prevailed in these communities on the psychological effect of CL. However, as discussed by Deacon et al. (2005), in their study of HIV stigma, there is no need for an explicit consensus for the stigma to be at work [19]. Some respondents in our Focus Groups asked for a quick start of treatment to reduce the size of final scarring, while others shared their fear of injections which they believe could damage their kidneys or just stated that scars became very dark or darker than the rest of the skin because of the use of this medication. According to the respondents, it is a problem that no therapeutic options are proposed because health professionals give the same medication to all patients. The issue of the disfiguring scars came up time and again. Scars were discussed in terms of treatments that can reduce the lesions or make them disappear and in terms of their location and psychological impact. CL scars have become linked to a shared social identity, they are “the label (stamp) of the area”. Some people expressed proud to have CL scars but said to be ashamed to show it once they went abroad. Nonetheless, there was a consensus that CL diminishes the marriage prospects of young women, and many illustrations of stigmatizing behavior towards CL patients were given.

The problem of CL is also perceived quite differently according to age. Adults and the elderly may consider it as a relatively minor problem, while young people, male and female alike, see it as a big problem. In Article 2 [20] we analyzed the psychosocial impact of scars caused by *L. major* in adolescents attending a boarding school in two CL endemic zones. Students were asked to fill in a questionnaire about their knowledge of this disease and related psychological effects. Twenty percent of the boarding school students who were surveyed had been affected by CL in the past. CL scars led to a considerable psychosocial burden in this adolescent age group. Girls seemed to
be more affected than boys, but both genders equally expressed their concerns and demands for scars treatment [20].

We broadened the scope of our analysis by conducting a review of the literature on the psychosocial weight (stigmatization, psychological consequences, and impact on quality of life) of CL in its localized form (LCL) in countries other than Morocco, to establish the external validity of our results. Article 4 [21] is a scoping review of the psychosocial impact of LCL. There are several studies from Latin America and Ethiopia showing the stigmatizing effect of CL in its mucocutaneous and diffuse forms. However, little information on stigma among people affected by the most common form, localized CL (LCL) was found at the beginning of this Ph.D. study. Fortunately, in the last two years, other articles were published. This scoping review showed that the psychosocial impact of CL on the affected person is real and corroborates our results obtained in Morocco. Most of the published work is based on cross-sectional surveys of CL patients that are asked to answer a standardized scaling questionnaire. Few truly qualitative published research are available, except for the work carried out by Reyburn in Afghanistan who documented huge discrimination against and stigma of CL patients, because the disease was thought to be contagious by direct contact. We also concluded that the psychological effect of CL is not so easy to measure in cross-sectional studies, as it may evolve over time, and prospective studies are in fact needed to adequately measure it. We also realized the importance of documenting the context, as confounding factors (i.e. socioeconomic level, the educational level, religious and traditional beliefs) may play an important role.

As explained by Moscovici, the researcher working on social representations should keep in mind the religious, political and cultural context of phenomena that social psychology is supposed to explain [22]. The way how rural poor Moroccan citizens think about the meaning of scars in their everyday life, cannot be understood in isolation from the present sociocultural reality and the desired future. The collective awareness in this society will shape the degree to which CL has or not a psychological impact.

The comparison of CL’s perception in Latin America with North Africa gave rise to the following reflections. As described previously, the mucocutaneous CL form is more stigmatizing than the localized form. However, when comparing LCL in both regions, one should include religion and traditions that influence behavior and women’s power in the society. In Muslim countries, by tradition women cover most of their body, so CL scars on unexposed parts do not matter much. However, when the CL scar cannot be covered, the body image gets seriously damaged and CL scars may have an impact similar to burns. This impact can be profound, affecting spirituality and belief in God [23]. Sometimes patients want doctors to talk with them about spirituality as part of their psychological healing [24]. A study from Iran reported how indelible burn scars and disfigurement led some of the patients to feel so desperate they began doubting the existence of God or His mercy [25]. Religion and spirituality play an important role in the quality of life of
Moroccan patients, and this will affect their coping strategies when confronted with the loss of their body image. Physicians should pay attention to the alarm signs of hidden psychological suffering when patients with LCL explain their dermatological ailments by referring to religion and God’s will.

3. Cutaneous leishmaniasis clinical management

In the absence of effective treatment against CL scars, and the lack of an effective vaccine to prevent infection, we focused on improving the clinical management of patients affected by CL by targeting rapid diagnosis tools at the point of care. The ultimate objective was to ensure, in remote isolated areas in Morocco, an early start of treatment to may avoid the emergence of a larger lesion but also avoid the abusive or inappropriate use of the available first-line treatment (meglumine antimoniate or Glucantime®).

Hence, the assessment of the accuracy of a Rapid Diagnostic Test (RDT) for CL in Morocco in comparison with reference laboratory tests that were included as manuscript n°5 [26]. Such a point-of-care test, if considered effective, is an appropriate solution both for healthcare professionals and patients who work and live far from the provincial laboratories. Geographically, in endemic areas with anthroponotic cutaneous leishmaniasis (ACL), most primary health care centers (in the selected provinces Sefrou, Sidi Kacem and Tinghir) are located more than 50 km away from the nearest provincial laboratory. Our study found that if health professionals follow the recommendations of the Leishmaniasis Control Guide 2010, they will not start treatment on suspicion of ACL before receiving microscopic confirmation about the presence of _Leishmania_ bodies in the skin sample. To get this laboratory confirmation, the smear should be sent from the primary health center to the nearest qualified technician working in a fully equipped laboratory. This information then needs to be sent back to the health center where appropriate treatment can be started. In practice, professionals who follow this procedure will ask the patient or his/her family to take the prepared glass slides to a laboratory, return later to the same laboratory to get the results which they then hand back to the health facility. Many times, they are faced with unpredictable weather conditions and the risk of partial or complete destruction of the glass slides during transport. Furthermore, some laboratories take it upon themselves to re-sample suspicious skin lesion(s) to ensure they have enough skin smear on which to carry out microscopic reading. The result is almost never obtained on the same day the patient brought the sample. In our study, we noted that almost all microscopic analyses took on average at least two working days, even with committed laboratory and health center staff. Moreover, in routine clinical management, most patients, and their families do not have the means to undertake the trip to these laboratories for analysis of their sample and send it by other means. There is, in that case, a high probability that the information has not yet reached the health center before a therapy decision is taken. In case of suspected CL, some
professionals start by giving one or two intra-lesional test injections of Glucantime® often on request of the patient. Then, they wait to see what happens and if the result is positive, the treatment is continued. If the treatment did not give the desired effect, patients are referred to a dermatologist or given prescriptions of other medicines. In the predominantly L. major endemic areas, this presumptive treatment is actively promoted by the MoH. Indeed, the MoH recommends treating based on presumptive clinical signs of CL and using laboratory confirmation only in the event of an outbreak in a new area not previously known as CL endemic.

“...health professionals make a clinical diagnosis and microscopically confirm the first cases of leishmaniasis declared in the suspected outbreak. It is recommended that later cases receive treatment based on a clinical diagnosis in the absence of a microscopic diagnosis.” [10, 27].

Glucantime® also has side-effects which should not be inflicted on patients with skin conditions other than CL. Indeed, as described in paper #5 [26], our study showed that 38% (83/219) of patients with skin lesions that were clinically suggestive of CL were not confirmed by the reference standard. In L. major endemic regions more specifically, this percentage was around 30%. This is a high proportion of patients without laboratory-confirmed CL who risk being treated. At least one diagnostic test for CL should be positive before the start of any antimoniate treatment.

The reference standard used in our study was based on the combination of PCR and microscopy. During the training organized for all health professionals participating in our study, a single technique for the collection and preparation of laboratory slides was adopted and explained by an expert from the national leishmaniasis laboratory at INH. All professionals were instructed to take their samples from the center as well as from the edges of the lesion as recommended by the literature [28]. This same laboratory expert had to re-read all the microscopic slides after recoding and anonymization to verify the first microscopic reading by the laboratory technicians of the participating provinces. In this way, we could optimize the sensitivity of the reference standard.

We acknowledge that the conventional PCR technique used in our reference standard has its limitations, that is why we included a repeat PCR in the same lab for one half, and in an international reference laboratory at ITM-Antwerp for the other half of the samples. The selection of slides for this second reading was made randomly. At ITM, real-time PCR was used, which is faster and has a lower contamination risk with fewer manipulations than conventional PCR based on the ITS1 primer [29]. Also, qRT-PCR is the best tool to quantify Leishmania parasites with a high sensitivity and reproducibility [30]. However, for species typing, other methods are better [31, 32]. Standardised protocols of real-time PCR assay to quantify CL parasite load should be recommended in the WHO Eastern Mediterranean Region particularly for multi-centric research studies. This was successfully done in Latin America supported by the DNDI and the WHO initiative [33].

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Another concern is the possible recall bias of the duration of disease (lesion) prior to enrolment as reported by patients and/or their relatives. Olliaro et al. [34] published in 2013 methodological recommendations for doing CL clinical trials in which they explained that the duration of the lesion is partially species-dependent and there was a concern that natural healing as the example of *L. major* may be a confounder. Moreover, until today there is no standardised way to estimate the duration of the disease and the authors for this clinical trial methodology recommended to include patients who reported lesions of a duration between 3 to 6 months [34]; another recent article written by the same first author did not report answers to those questions [35].

So far, we did not perform any cost analysis of any of the laboratory tests used. It is recommended that the diagnostics in development strike a balance between being sensitive enough and remaining cheap, usable and having a quick turnaround of results [36]. As the study project was performed in well-known endemic CL areas, further research is required to understand how the predictive values might change at the lower transmission level, in off-season settings or in settings with declining transmission.

Some recent publications recommend the loop-mediated isothermal amplification (LAMP) as diagnosis tool rather than an RDT [37–39]. We believe that making preparations of 4 to 6 primers needed for LAMP looks like a real laboratory task that Health Centre nurses would not accept to perform. Moreover, even if LAMP allows species identification, this seems not useful in the Moroccan context where the same treatment guidelines apply to the three CL species, depending only on the localization, the size and the number of CL lesions. This is also the case for the stepwise algorithm of CL treatment published in 2014 by EMRO where the treatment decision is based on the risk-benefit ratio of the intervention for each patient [2]. However, species identification could be valuable for travellers and tourists with suspected CL lesions and valuable for epidemiological surveillance [2]. Furthermore, nurses will prefer using an RDT which result stays readable for many months rather than a LAMP that has a limited reading time less than one hour.

How was this test seen by health professionals? This is another question that needs more investigation about the acceptability of this new diagnostic tool. Indeed, as reported in another study from Australia, being able to test and treat on the same day is very valuable, and could reduce delays for treatment, loss of follow up, recall efforts and unnecessary treatments for false positives patients [40].

As currently there is no validated treatment of scars, CL treatment should aim at controlling the size of these scars since smaller scars are more tolerable than the big ones.
To increase the chance of maintaining the size of the scar at a tolerable dimension, an early and easy diagnosis is key. This CL RDT can be a gamechanger as it makes diagnosis more accessible to patients in remote areas. Especially, when the RDT rk 39 for visceral leishmaniasis was found highly accurate, cheap and simple in a recent publication from Morocco [41]. Furthermore, a recent update of a Cochrane review assessing the available interventions for old world cutaneous leishmaniasis found a small data targeting the prevention of CL scarring [42]. One of the choices that could be accepted by the population in Morocco is the use of heat to accelerate the ulcerative lesions cure (normalize epithelialization and reduce scarring), by employing a dressing for the epithelialization stage as reported in this Cochrane review [42].

4. Recommendations and conclusion

Based on our research, we would like to propose a number of recommendations.

4.1. Recommendations for policy and practice

In the Moroccan context, we recommend that MoH policy-makers update the national Leishmaniasis Control Guide, based on documented patients’ and population felt needs, and develop more effective strategies that could be sustained at the community level. Nowadays, the dermatological long-term consequences of CL have become a central theme of discussions in rural communities in Morocco, a subject of jokes and mockery among individuals, and led to strained community relations in regions where ethnic and racial relations were already tense. There is clearly a strong popular demand for better CL and scar care (as well as health care in general).

More specifically, we recommend, therefore:

*MORE COMMUNITY INVOLVEMENT*, i.e. to include citizens as individuals, families, and associations when developing and deploying CL control policies. This includes two dimensions: responding to their strong demand for better CL and scar care, by increasing access to diagnosis and treatment, but also considering the acceptability of any control policy. Indeed, listening to CL patients’ voices means hearing a large poor community living in precarious sanitary and environmental conditions. This is in line with the new Moroccan Constitution (2011) with its emphasis on the need to respond to popular demand for better health and protection against diseases linked to poverty. Secondly, we recommend involving the community before deploying CL control interventions. Guidelines and protocols should be evidence-based. However, the acceptability of those actions remains another step of validation that is too often neglected.
Consultation/involvement of the exposed population is needed to adapt each strategy to the regional and local context.

CL control is not possible without the participation of the fully informed community. However, this should not be used as an excuse for the departments to avoid taking responsibility (fight against poverty, awareness, and education about possible means of prevention, professional behaviors for CL case management on the population).

A number of preventive actions can be taken based on what we know now. However, the MoH must convince all hygiene services in rural endemic communities of the need to carry out permanent insect control interventions negotiated with the communities depending on their sleeping behaviors and their cultural beliefs.

**Increase access to diagnosis and care.** To defend the right of all poor people in Morocco living in remote areas affected by this disease to be detected correctly and quickly before using the existing antimonials treatments, we recommend including the RDT we evaluated in a revised algorithm, that would lead to CL treatment in case of a positive RDT, and to a microscopy smear in case of a negative RDT. We recommend the Moroccan health authorities to ensure its availability in all primary health care facilities in CL endemic areas. We note that at present, although the rapid test for cutaneous leishmaniasis provides only a sensitivity of 68% [95% CI, 61-74], specificity = 94% [95% CI, 91-97], NPV = 64% [95% CI, 58-70], PPV = 95% [95% CI, 92-98], it is very useful in reference or provincial laboratories in remote areas. It is also useful in the absence or lack of trained technicians who can respond promptly to the request for microscopic analysis of samples. RDT should be part of the algorithm for the management of patients with suspected CL lesions. If RDT is positive, then it is advisable to rapidly start the standard treatment. If it remains negative, a laboratory test (microscopy) is necessary at least in the present state of knowledge. Further development is needed to optimize the sensitivity of this RDT as explained in chapter 6.

**Offer psychological support in adolescent health programs.** We also recommend the integration of the psychological follow-up of adolescents enrolled in the national programme promoting health at school and at the university. MoH should introduce this as soon as possible in consultation with the Ministry of Education and Higher Education. Psychological group interventions like the cognitive behavioral therapy can reduce the loss of social support and the social burden of CL stigma as assessed in a recent Cochrane review publication for HIV patients [43]. Moreover, the psychological group interventions could work for other diseases and health conditions that affect the psychological well-being.
We highlight that the “Framework for action on cutaneous leishmaniasis in the Eastern Mediterranean Region 2014–2018” published by WHO\(^{17}\) referred briefly and only one time to ‘stigma’. No word about ‘psycho’ or ‘psychosocial’ or ‘psychology’ was found. We hope that the future framework for action on cutaneous leishmaniasis in the EMR will finally focus on the psychosocial burden of CL and the operational strategies for a psychological support adapted to young affected people with CL scars.

### 4.2. Recommendations for research

**Better tools for rapid diagnosis of CL.** Though an RDT is already available and can be used, there is much scope for optimization. Its sensitivity could possibly be increased by e.g. modification of the sampling technique. WHO highlighted that CL RDT based on antigen detection should allow making the difference between *L. tropica* and *L. major* species \(^{[44]}\). More clinical research on CL diagnosis is definitely needed as there is not yet any standardized outcome measure.

**Better tools for vector and reservoir control.** As no vaccine is available, vector or reservoir control seems the only option. Existing interventions for ZCL seem to work, even if no hard-experimental evidence is available to inform the policy.

Our review of the evidence has shown the need for better control interventions that should be evaluated with methodological rigor. The effectiveness of such new interventions should be tested in a logical order, from small-scale proof of concept studies over field challenges that measure the effect on entomological outcomes/or rodent population to finally, large-scale intervention trials that document the impact on CL incidence \(^{[45]}\). Some intense advocacy will be needed, as CL research in this area remains underfunded and neglected.

**Better tools for measuring the CL burden.** The overall control strategies implemented in recent years in high CL burden countries do not bring sustainable solutions as the disease remains present at the same level or with rising yearly incidences. Moreover, the actual indicator assessing CL severity by using only the number of patients undergoing systemic CL treatment by the total number of patients diagnosed is not adequate \(^{[2]}\). An indicator presenting the number of patients suffering psychologically from the CL scars after 6 months to three years of follow up could be introduced. Furthermore, areas with a CL burden could be assessed by a general vulnerability index (a composite index including four vulnerability indices: socio-economical, epidemiological, climatological and psychosocial components) as partially presented in one study from Brazil and Morocco \(^{[46, 47]}\) and more generally discussed in an integrative review which documented the wider existing vulnerability synthetic indices \(^{[48]}\). WHO could lead such a project with the aim to validate an index that would take into account the risk of CL, the

possibility to react and the perception of the psychological CL scars suffering or in contrast, the population resilience. Concomitant coinfections with synergetic or antagonistic interaction between different species of CL or with visceral leishmaniasis for immunocompetent patients or the cutaneous CL forms progress to visceral forms or to malignant disorders (cancers) are other hidden burdens that need further multicentric research by focusing on the new or atypical clinical forms [49–52].

5. Conclusion

In conclusion, both our epidemiological and sociological research have emphasized the importance of the problem of cutaneous leishmaniasis in endemic areas in Morocco. Epidemiological research has revealed that children and adolescents are the most affected age group. Our sociological research has shown that adolescents fear this disease most. The facial lesions or perceived disfigurement due to indelible scars can become a source of stress with an impact on the individual psycho-social well-being. CL was found more devastating than acne and even suicidal ideations were mentioned by some adolescents. Therefore, the psychosocial burden generated by CL should not be neglected. So far, the psychosocial weight of this condition has not yet sufficiently been taken into account by the health authorities.

The high number of CL scars and cases in Morocco should mobilize more public health attention to sustain preventive measures against vectors and animal reservoirs. The clinical management should be reviewed based on current evidence. The introduction of the RDT in its current format—with a moderate sensitivity—seems beneficial for endemic CL areas marked with difficult geographical access. We recommend its progressive introduction under closely monitored operational research conditions, and advocate for further development and optimizing of this kind of diagnostic tools.
References


2. WHO (2014) Manual for case management of cutaneous leishmaniasis in the WHO Eastern Mediterranean Region. 35:


Supporting information
Fig. S1. Maps of the administrative division of districts in Errachidia Province – Kingdom of Morocco.
Table S1. The number of burrows before and after operations to control the density of rodent Meriones Shawi by subdivision in the Errachidia Province

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Table S2. Distribution of cases and incidence of cutaneous leishmaniasis due to *Leishmania major* in Errachidia Province by districts

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* incidence rate per 100,000 inhabitants of all population (in affected area and not)
† Natural decrease in cases of CL before starting the poisoned bait intervention
‡ Natural increase in cases of CL in four districts which was excluded by the poisoned bait intervention in 2010 (starting intervention in 2012)
Supporting information Chapter 3

Additional file 1: English translation of the self-administered questionnaire

Questionnaire about Cutaneous Leishmaniasis (April 2015)

This questionnaire is part of ongoing research about ‘Control of Cutaneous Leishmaniasis in Morocco’ with the support of the National School of Public Health in Rabat and the Institute of Tropical Medicine in Antwerp, Belgium. The principal aim of this study is to understand knowledge, representations and practices of the population about Cutaneous Leishmaniasis.

Your participation in this questionnaire will help to understand your personal experience with this disease. You will not be asked to give your name and your identity. You can decide at any time to stop answering and withdraw from the study, or not participate. 15 minutes will be enough to complete all the questions by selecting the appropriate answer.

Do you agree to participate in this study?

1. Yes, I agree 2. No, I do not agree

Area: 1. Tinejdad/Ferkla High school 2. My Alicherif/Rissani High school

Actual study level: 1st Year common section 2nd Year 3rd Year

Gender: 1. Man 2. Woman

Age of the participant (in years):

Name of the district where your family home is located:

Do you know a disease named cutaneous leishmaniasis? 1. Yes 2. No

Do you know a local name for this disease, if so, which?

Have you personally experienced this disease? 1. Yes 2. No 3. I do not know

If your last response is “Yes”, in which year have you been affected?
Do you know anyone affected by this disease?  
1. Yes  
2. No  

What is your relationship with this/these person(s)?  

This disease is transmitted to humans by:  
1. Dogs  
2. Mosquitoes  
3. Water  
4. Rodents  
5. Garbage  
6. I do not know  

Do you think girls are more exposed to get cutaneous leishmaniasis?  
1. Yes  
2. No  
3. I do not know  

Does this disease leave marks and scars (cicatrices) in the site of its occurrence?  
1. Yes  
2. No  
3. I do not know  

Is it possible to have scars (cicatrices) of this disease on the face?  
1. Yes  
2. No  
3. I do not know  

How long to those marks and scars (cicatrices) remain visible in the skin?  
1. It becomes less visible in the skin within years  
2. It disappears completely  
3. It does not disappear and stays visible on the affected skin  

Do you think that medical treatment of marks and scars (cicatrices) of Cutaneous Leishmaniasis exist?  
1. Yes  
2. No  
3. I do not know  

Is there any traditional remedy that is used to decrease the scars (cicatrices)?  
1. Yes  
2. No  
3. I do not know  

If your answer is “Yes” to the above, write which?
### In your point of view, why did the number of CL cases in your area decrease in the last years? (You can select more than one answer)
1. Due to the hygiene campaigns
2. Due to the interventions against the wild rodents (forest)
3. Due to the interventions against the mosquitoes
4. Due to the individual acquired immunity
5. Due to the natural herd immunity of the population
6. Due to the climate change
7. Due to other factors

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**Do you use, or does your family use at home:**
1. Insecticide spray
2. Bednets
3. Smoke or herbs to repel mosquitoes
4. Nothing of the above

**During the summer, do you and your family frequently sleep:**
1. outside the home or on the terrace
2. inside a room with closed door and windows
3. inside a room with open door and/or windows

**Are you afraid for yourself and your family to be affected by cutaneous leishmaniasis in the future?**
1. Yes, I am very afraid
2. Yes, I am a little bit afraid
3. No, I am not afraid at all

**Is it possible that the marks and scars (cicatrices) of Cutaneous Leishmaniasis influence the psychological state of the person affected?**
1. Yes  
2. Maybe  
3. No influence

**Could you write a small paragraph about the probable psychological state of the person (woman or man) affected by those scars?**

Thank you very much for finishing this questionnaire
Additional file 3: Sample of French translation of participants’ responses to the last question in the questionnaire: Could you write a small paragraph about the probable psychological state of the person (woman or man) affected by those scars?

Réponses écrites des élèves internes des lycées à propos de la dernière question du questionnaire auto-administré.

La question : est-ce que l’apparence visuelle des cicatrices de la leishmaniose cutanée peut influencer l’état psychologique de la personne touchée : 1-Oui il y a un effet 2-c’est probable 3-non aucun effet. Elle a été suivie par la question ouverte suivante :

Est-ce que vous pouvez écrire une petite phrase sur l’état psychologique probable de la personne portant la cicatrice de la leishmaniose cutanée

Voici les réponses des 454 participants :
FR Répondant Féminin du lycée de Rissani Moulay ali cherif province Errachidia
MR Répondant Masculin du lycée de Rissani Moulay ali cherif province Errachidia
FT Répondant Féminin du lycée de Tinejdad Ferkla province Errachidia
MT Répondant Masculin du lycée de Tinejdad Ferkla province Errachidia

FR001 Selon mon point de vu, la maladie de la leishmaniose donne un état psychologique compliqué chez la personne touchée et surtout chez la femme. Car les femmes ont plus peur sur leurs visages, peur que le jeune homme qui va se présenter pour demander sa main disparait après avoir vu ces taches.

FR002 Peut être que l’état psychologique de la personne touchée sera très compliquée et critique.

+FR003 Selon mon point de vu, la personne touchée par cette maladie va sentir un état psychologique faible et va perdre confiance en sa beauté

FR004 Elle laisse des marques visibles sur la personne touchée. Mais je ne sais pas si elle influence ou non son état psychologique c’est à vous de nous le dire

FR005 la personne reste très gênée, car les cicatrices et les effets de cette maladie apparaissent sur son corps et surtout au niveau du visage
l’état psychologique probable de la personne touchée par ces cicatrices est très mauvais. Absolument insupportable

l’état psychologique de la personne touchée est qualifié par la gêne et l’embarrât. Surtout si la maladie se trouve dans une zone qui ne peut être cachée
/il va sentir la gêne à cause de l’apparition de ses malformations surtout au niveau du visage

Elle m’influence chaque fois que je rencontre mes amies, elles regardent ma cicatrice, cette dernière diminue ma valeur devant les gens.

la personne touchée se dit pourquoi je suis malade par cette maladie et les autres gens ne le sont pas et ne diminue pas de leurs personnes

la sensation de peur et d’inquiétude de ne pas trouver un traitement à cette maladie est ce qui affecte la personne touchée par cette maladie

la personne touchée par la maladie de la leishmaniose souffre de plusieurs maladies psychologiques dont les plus importantes sont la tristesse, la dépression et l’inquiétude permanente

elle a honte à cause des effets de cette maladie et elle a une sensation d’être une étrangère.

 selon mon point de vu je dis que cette maladie influence l’état psychologique de la personne touchée. Car quand vous êtes touché par cette maladie il reste des effets de cette maladie. Et le grand problème est que ces marques restent sur ton visage. Tu perds ton autoconfiance et tu te dis j’ai perdu mon visage. Le visage est trop sensible.

vide

vide

le touché se trouve dans un état très mauvais, car cette maladie apporte la honte et il peut ne plus être dans un bon état. Cette maladie déforme la peau sur n’importe partie touchée.
L’état psychologique de la personne touchée par ces cicatrices est mauvaise un petit peu. Car elles déforment la beauté (dans le cas de la femme) car la femme s’occupe beaucoup de son apparence extérieure. Et dans le cas que ces cicatrices apparaissent au niveau du visage, la fille a honte de monter son visage ou même les cicatrices peuvent l’empêcher de sortir de la maison ce qui augmente sa souffrance psychologique.

La personne touchée par cette maladie a un psychisme effondré à cause de la peur de la déformation et de l’effet que laisse la maladie quand il saura que les marques ne vont pas disparaître.

La personne touchée par cette maladie ne sens pas la paix de l’esprit car elle a un sentiment de peur et de manque de réconfort.

Je peux dire que l’état psychologique de la personne touchée homme ou femme par ces cicatrices va conduire à des troubles psychologiques qui vont l’empêcher de sortir à la rue. Et surtout si ces cicatrices sont sur le visage des filles.

Il se peut que l’état psychologique de la personne touchée soit affaibli. Il se peut qu’il sente la déformation de son visage et qu’il ait honte de sortir de la maison ou quelque chose de semblable. Et ceci est représenté comme une maladie psychologique.

Oui la personne touchée par la maladie de la leishmaniose souffre énormément car il est touché au niveau du visage. Comme vous le savez ce fut une fille dans l’âge des fleurs elle veut être normale mais elle est exposée à une grande gêne et on l’oblige à mettre des crèmes tout le temps.

FR038 l’état psychologique de la personne touchée par leishmaniose à travers ses cicatrices est un peu difficile surtout si les tâches trouvent sur les visages ce qui crée un sentiment de gêne car les cicatrices ne disparaissent pas avec le temps et restent visible sur la peau.

FR039 il se peut que la personne touchée par ses cicatrices à un état psychologique détériorée à cause de cette maladie. Mais ce n’est pas d’une manière systématique. Car plusieurs causes peuvent créer ses lésions au niveau de son corps laissant les cicatrices et des tâches. Est-ce que vous pouvez nous proposer le traitement des médicaments pour cette maladie est merci beaucoup.
FR 040 oui ses cicatrices peuvent influencer la personne touchée qui ne pourra plus supporter de voir ces déformations sur son visage où son corps en général. Les gens peuvent se moquer de lui et il peut paraître ses qualités de beauté. Donc il peut influencer l'état psychologique de la personne. C'est juste mon avis.

FR 041 si la maladie de la leishmaniose se trouve au niveau du visage cela peut retentir sur son état psychologique. Il va voir que les autres ont des visages normaux alors que son visage et cicatrisé.

FR 042 états psychologiques propres de personnes touchées par ces cicatrices vont les rendre insatisfait et tristes surtout les filles.

*FR 043 cette maladie n'affecte pas que la peau mais également la psychologie des personnes touchées et leurs proches. La question qui reste posée qu'elle est la solution pour cette maladie

FR 044 ce que je peux dire sur l'état psychologique probable de la personne touchée par ses cicatrices c'est qu'il sent une déformation au niveau de son corps avec un sentiment de déception à cause de cette maladie qui laisse des marques sur son visage. Parfois des personnes touchées finissent par se détester eux-mêmes.

+FR 045 L'état Psychologique de la personne touchée par ses cicatrices l'influence car elle laisse des traces sur sa peau visibles, comme c'est le cas chez Les filles qui ont des visages défigurés à cause de ses cicatrices source de méprise de la part des gens.

FR 046 je peux dire que les personnes touchées par ces phénomènes quand les symptômes apparaissent il doit aller chercher la solution à l'hôpital.

FR 047 case vide

FR 048 je peux dire que les gens touchés par cette maladie de la Leishmaniose doivent partir à l'hôpital immédiatement avant que la maladie se transmette à d'autres personnes. Il faut réaliser des compagnes de propreté pour un environnement sain de toute maladie.

FR 049 souvent les cicatrices chez l'homme touché et surtout chez la femme touchée sont un obstacle lors du mariage car sa cicatrice est visible, chose que ne tolère pas le fiancé.
FR 050 il se peut que la situation de la personne touchée féminine soit psychologiquement instable et complexée à cause de la présence des cicatrices et des tâches sur son visage ceci va laisser un trouble psychologique à chaque fois qu'elle voit des gens-là regardant par méprise et l'évitent dans ce cas et sera seule est complexée.

FR 051 il peut sentir qu'il porte un poids sur son corps et il va sentir la fatigue et l'épuisement et la non satisfaction psychologique et corporelle. Il va sentir une douleur dans la zone touchée.

+FR 068 L'état psychologique de la personne touchée atteint la dépression à cause de l'inexistence de moyens pour faire disparaître les traces de la leishmaniose. Puisque je suis une personne touchée par cette maladie et je souffre de ses conséquences et ce qu'il existe une solution pour guérir les cicatrices ? Merci.

FR 069 case vide

FR 070 exemple chez les femmes et les filles touchées par cette maladie qu'on peut dire que c'est une cause est un obstacle qui les empêche de se marier.

MR 071 Il sera dans un état psychologique difficile à cause de la longueur de la maladie et de la perte de plusieurs privilèges de bonne santé surtout lors de l'apparition de cicatrices au niveau des visages chez les personnes de sexe féminin.

+MR 072 il sent un peu de honte et un malaise. Cette maladie influence de manière très grande sur la psychologie du malade et également à l'égard de ses sentiments négatifs car il n'accepte pas qu’il ait ces taches et ces cicatrices.

MR 089 L’état psychologique de la personne touchée n’est pas bonne, car cette maladie influence beaucoup son état psychologique.

*MR 090 Il faut qu’un traitement à cette maladie soit disponible au niveau de l’ensemble des dispensaires urbains et surtout ruraux où cette grave maladie est fréquente.

MR 091 Quand cette maladie touche la personne, elle lui fera naître des effets psychologiques
MR 092 Non elle n’a pas d’effet sur la personne touchée. Il reste juste sa trace pendant une semaine. Cette maladie n’est pas importante et elle ne crée pas de Paine car on est habituée sur cette dernière.

MR 093 Peut être que cette maladie laisse un trouble psychologique chez la personne touchée. C’est pour cela que la commission concernée ou le gouvernement en général doit trouver une solution pour cette maladie et non pas le peuple.

MR 094 il se sent être mépris devant ses amis et avec soi-même, ce qui le rend marginaliser dans la société.

MR 095 la personne touchée sent la peur et la honte à cause de ces cicatrices et ceci dans sa famille et avec ses amis. Il a également peur d’avoir des effets négatifs à cause de ces cicatrices.

MR 096 il se peut que la personne touchée par ces cicatrices sente un peu de peur lors des premières étapes de l’apparition de cette maladie. Sauf qu’il va s’habituer sur ces cicatrices avec le temps pour que tout ceci redevienne une chose normale.

+MR 097 pour la personne est touchée par cette maladie, il se peut qu’il y est un peu de peur que ces cicatrices restent durant toute la vie sur sa peau et de ne pas guérir.

MR 098 la personne touchée sent la peur et la faiblessé à cause des cicatrices et elle a peur pour sa santé.

MR 099 il se peut que les cicatrices influence sont état psychologique et créer chez la personne touchée la sensation de l’isolement des autres à cause de ces effets sur la peau et surtout s’ils sont sur le visage ce qui rend la personne touchée très honteuse de s’intégrer dans la société. Et ceci selon mon point de vue va influencer son état psychologique.

+MR 100 dans le cas de l’atteinte par cette maladie il y aura l’apparition d’une fièvre comme pour toute les autres maladies, en plus qu’il va être exposé à au moins trois piqûres et qu’il ne développera pas cette maladie une deuxième fois selon ce que j’ai entendu.

MR 113 la peur de la mort

+MR 114 Il peut être exposé à la mort lente
MR 115 Il se peut qu’elle sente un peu de faiblesse dans sa personnalité. Mais avec le temps il commence à oublier tout cela même avec la permanence de la cicatrice sur sa peau.

+MR 116 L’état psychologique de la personne touchée peut être influencé après le traitement car le problème est que les cicatrices ne disparaissent jamais.

+MR 117 il est inquiet et perturbé à cause de cette maladie car cette dernière influence son état psychologique et laisse des traces sur sa peau.

*MR 118 il faut que toute personne touchée par cette maladie d’être patient et prier Dieu de l’aider. Et de visiter le médecin régulièrement et suivre ce que dit le médecin. Pour les effets psychologiques que peut avoir le malade est de ne pas pouvoir affronter sa société et ne pas être capable de sortir et il va s’isoler totalement à cause de sa honte due aux cicatrices sur sa peau.

* MR 119 oui ces cicatrices influencent la santé psychique de la personne touchée et qui doit être prudent pour se prévenir de cette maladie.

MR 120 le sentiment de la solitude et l’absence de la stabilité dans la vie quotidienne à cause du regard négatif de la société vers patient.

*MR 121 quand la maladie de la leishmaniose touche la personne elle doit aller voir le médecin, car c’est une maladie grave et qui influence les nerfs et l’immunité va s’effondrer. Egalement il cause chez le patient des effets psychologiques et dermatologiques pendant une durée très longue ce qui nécessite la visite d’un psychiatre.

+MR 122 case vide

MR 123 Cette maladie influence souvent quelques personnes car elle laisse depuis le début des cicatrices qui conduisent la personne jusqu’au suicide

MR 124 le remède est le Coran en plus la personne peut utiliser TAMAGHOT qui se trouve à Merzouga.

*MR 125 il ne doit pas sentir la peine car pour toute maladie il y a un remède soit maintenant soit dans le futur.
+MR 141 dans la période où la personne est touché par cette maladie il sent la peur ce qui influence son psychique.

MR 142 on retient que les rats et les moustiques sont l’origine de l’atteinte de la personne par la leishmaniose cutanée.

MR 143 case vide

MR 144 cette maladie ne donne aucun effet sur la psychologie de la personne touchée, selon mon point de vue

MR 145 il faut faire attention des moustiques car ils sont à l’origine de l’apparition de cette grave maladie et aussi faire attention des rats.

MR 146 Dans le cas de l’atteinte par la leishmaniose il se peut que l’état psychologique soit influencé de la personne touchée et ceci apparait dans la peur et les troubles psychiques

+MR 147 en ce qui concerne l’état psychologique probable est qu’il n’y a aucun effet sur l’état psychologique même après que les cicatrices restent à cause de cette maladie car il n’y a pas de grandes peurs

+MR 148 on peut dire que la maladie de la leishmaniose peut être guérie mais après traitement il reste les cicatrices pour toujours et il faut acheter un traitement “cicatrisol”*

MR 149 dans le cas de la présence des cicatrices sur le visage de la personne touché il y aura bien évidement un effet psychologique et la personne va sentir la honte.

*M 150 cette personne touchée n’a pas le pouvoir de dire qu’il est atteint par cette maladie par peur que les gens s’éloignent de lui.

MR 151 si la personne est touché au niveau du visage, il se peut que la maladie affecte son état psychique avec l’apparition de la honte de montrer son visage et surtout devant les filles si la personne touchée est de sexe masculin et il va sentir également une laideur ce qui influence son état psychologique négativement.
+MR 152 pour toute maladie il y a un traitement
MR 174 il faut soigner rapidement cette maladie pour qu’il n’y ait pas d’effet sur l’état psychologique de la personne touchée

MR 175 cette maladie ne donne aucun effet sur l’état psychologique car ce n’est pas une chose essentielle. On est habitué à voir cette maladie. Et il faut que le gouvernement trouve une solution à cette maladie.

FT176 on peut dire que l’état psychologique de la personne touché par ces cicatrices est un très mauvais état et surtout chez les filles car cela conduit à la diminution de leurs beautés.

+FT177 quand la personne voit les cicatrices il aura un état psychologique lamentable surtout il va plonger dans la souffrance quand il se rend compte qu’il n’y a pas de traitement.

+FT178 on peut dire que l’état psychologique de la personne touché par ces cicatrices est trop difficile et surtout chez les filles car cela va faire diminuer leurs beautés

FT179 on peut dire que la personne touché par cette maladie souffre de cette dernière dans tous les temps, par exemple vous ne voulez pas qu’une autre personne soit touchée par cette maladie car elle fait souffrir beaucoup.

FT180 son état psychologique est affecté un peu car il a peur de contaminer ses proches et sa famille

+FT181 l’état psychologique de la personne touché par ces cicatrices est mauvais car il conduit à l’infériorité chez le porteur de ces cicatrices.

FT182 la réponse sera que la personne ne va absolument pas supporter cela car il conduit à une perte de performance dans les études et à la démotivation.

FT183 l’état psychologique probable de la personne touché par ces cicatrices sera dangereux ou bien cela ne va absolument pas l’affecté

*FT184 je prie Dieu qu’il guéri toute personne touché par cette maladie. Amen.
FT185 il se peut que l’aspect des cicatrices et les taches de la leishmaniose cutanée qu’elles influencent l’état psychologique et surtout celui des filles. Car elles vont sentir l’infériorité et la peur qu’elles apparaissent sur cet aspect devant leurs amis. Elles vont s’auto-détester quand elles vont se voir dans le miroir à cause des cicatrices et des taches de cette maladie.

FT186 il se peut que l’apparence des cicatrices et des taches de la leishmaniose cutanée qu’elles influencent l’état psychologique de la personne touchée. Elle va se sentir trop faible pour paraître devant les amies avec ces cicatrices et surtout si ces taches sont sur le visage. Car la personne pense que les gens sont entrain de jeter un regard de méprise. La personne touchée va se dégouter en se voyant dans le miroir.

FT187 au niveau du visage et surtout chez certaines filles, les cicatrices peuvent représenter un problème psychologique à cause de l’aspect visuel horrible et la longue période que cela prend pour commencer à disparaître de la peau.

FT194 surtout chez les filles quand la maladie laisse une tache sur le visage par exemple, et la fille va penser que c’est dangereux sur son niveau de beauté ce qui va influencer son état psychologique. Surtout dans notre société traditionnelle qui n’est absolument pas clémente à l’égard de ceux qui ont des taches sur le visage car ils pensent que c’est héréditaire.


*FT196 oui cette maladie de la leishmaniose influence l’état psychologique de la personne touchée car lors de l’atteinte par cette maladie tu vas sentir par exemple que les gens sont en train de s’éloigner de vous et de vous éviter. Et quand la personne touchée par cette maladie voit les cicatrices sur sa propre peau au niveau des mains et du visage elle a un sentiment de peur et de dépression. Peur que cette maladie l’influence plus et devienne mortelle.

*FT197 case vide

*FT198 je demande à Dieu qu’il guéri toute personne touchée par cette maladie dans les plus brèves délais et qu’il épargne ma famille et tous les musulmans
+FT199 pour moi je ne souffre d’aucun problème psychologique à cause de cette maladie même si j’ai une cicatrice mais je n’ai pas souffert à cause d’elle.

FT207 selon ce que j’ai pu constater auprès des personnes touchées que je connaisse, est qu’elles sont influencées par ces cicatrices. Surtout chez les filles qui ont une atteinte au niveau du visage ce qui leurs fait perdre leurs beautés. Et cela les poussent à s’isoler et de ne vouloir entendre aucune remarque ou rumeur sur sa beauté.

FT208 je prie pour la personne touchée par ces cicatrices qu’elle guérisse. Qu’elle utilise des traitements médicaux ou traditionnelles c’est pareil pour moi, il faut juste qu’il guérisse et de ne pas développer un problème psychologique.

FT209 Je prie Dieu qu’il guérisse vite les personnes touchées par ces cicatrices et qu’il éloigne de nous cette maladie de la leishmaniose cutanée.

FT210 il doit suivre quelques injections et les conseils du médecin pour diminuer l’effet de ces cicatrices

FT211 selon mon point de vue, il se peut qu’il y soit une influence sur l’état psychologique de la personne touchée. Mais cela dépend de l’évaluation donnée par l’infirmier et l’ampleur de la dangerosité de la maladie.

FT212 selon ce que j’ai vu et j’ai entendu chez la plupart des filles touchées par cette maladie, elles sentent qu’elles ont une malédiction et ces cicatrices font obstacle devant leurs beautés.

FT213 Oui il est possible que les cicatrices influencent l’état psychologique de la personne touchée et surtout si la maladie guérie et laisse des séquelles sur le visage plus précisément.

FT214 Son état psychologique sera très affecté du fait que les cicatrices vont rester sur la peau surtout au niveau du visage des filles

FT215 je peux dire que l’état psychologique peut être affecté chez les personnes touchées par cette maladie. Ces taches et cicatrices peuvent représenter un obstacle pour elles. Si les malformations se situent sur les parties extérieures visibles, ces personnes vont sentir de l’infériorité et un déséquilibre dans leurs beautés ce qui va menacer l’état psychologique.

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FT264 la personne touchée par ces cicatrices est probable qu’elle a un état psychologique perturbé à cause de son sentiment permanent d’isolement et d’anxiété par rapport aux autres, ce qui conduit obligatoirement vers la dépression.

+FT265 tout à fait, les séquelles laissées par la leishmaniose influencent négativement l’état de la personne touchée. Il y aura un complexe psychologique et elle va avoir honte de paraître devant ses amis car c’est une marque de honte et de mépris.

FT266 on souhaite la guérison de cette maladie et que Dieu avec sa puissance le guérisse.

FT283 toujours la personne sent la peur et la honte surtout si la maladie est localisée sur le visage. Il va penser qu’il est isolé et qu’il va sentir l’anxiété.

+FT284 on vous demande de pouvoir éviter cette maladie, car plusieurs personnes n’avaient pas les moyens pour acheter un traitement pour cette maladie dangereuse. Et je souhaite la guérison à tous ceux qui en souffrent.

FT285 quand la personne est touchée par cette maladie il doit se protéger de plusieurs autres maladies qui seront causées par cette première maladie.

FT286 quand la personne est touchée par cette maladie il voit qu’il est devenu défiguré dans son corps à cause de la présence de ces marques cicatricielles sur sa peau, ce qui lui pousse à sentir la honte et la panique. Et je suis pour la réalisation d’une campagne de sensibilisation contre cette maladie.

FT287 la leishmaniose cutanée influence l’état psychologique de la personne touchée car elle lui pousse à avoir peur et même à la mort. C’est pour cela qu’il faut se protéger et protéger nos familles. Et en cas d’apparition de cette maladie il faut aller voir le médecin spécialiste.

FT288 il est probable que la personne touchée par cette maladie a un état psychologique perturbé.
FT289 la leishmaniose cutanée influence l’état psychologique et conduit à la mort. Et pour diminuer l’effet de cette maladie contagieuse il faut prévoir assez de traitements médicaux ou traditionnels.

FT290 la maladie de la leishmaniose est un phénomène dangereux et très répondu surtout dans les villes. Et cette maladie influence l’état psychologique de la personne touchée car elle conduit à la peur. Il faut réaliser des compagnes de sensibilisation sur l’intérêt de l’hygiène. Et on souhaite la guérison aux personnes atteintes.

FT291 il est probable que la personne touchée par cette maladie a un état psychologique perturbé.

FT292 l’état psychologique de la personne touché est marqué par le fait qu’il devient paniqué avec une grande peur, et il n’aime plus rencontrer les gens car ils commencent à l’éviter et ceci le rend triste.

FT293 quand la personne est énervée ou ignorante elle peut avoir des problèmes psychologiques, on demande à Dieu le pardon et la santé.

FT294 la leishmaniose cutanée influence l’état psychologique et conduit à la peur. La peur même de la mort. D’où il faut organiser des compagnes de sensibilisation sur l’importance de l’hygiène pour lutter contre les rats sauvages et les moustiques. Et il faut qu’on se protège de la leishmaniose cutanée et protéger nos familles. Et je souhaite la guérison rapide aux personnes touchées par cette maladie.

FT295 la personne touchée aura un état psychologique très effondré à cause du milieu où il vit et le regard de la société à son égard d’autre part. Il va sentir la dépression et il ne va plus vouloir sortir avec les amis et les proches et il va s’auto-emprisonner dans la maison.

MT337 en regard que la maladie de la leishmaniose cutanée est difficilement guérissable et laisse des cicatrices et des taches permanentes sur le corps et qui influencent l’apparence normale connue de la personne. Ainsi cette maladie provoque beaucoup de problèmes psychologiques car elle diminue la beauté de son apparence extérieure et cause l’irritation chez lui car la société n’aperçoit que les défauts et pense que la personne touchée est une personne négligente qui est non propre et qui ne prend pas soins de son état corporel.
+MT338 on peut dire que la lutte contre cette maladie nécessite l’utilisation des insecticides et les compagnes de chirurgie esthétique.

MT339 il se peut que ces cicatrices causent de nombreuses maladies psychologiques comme la dépression.

MT340 il se peut que ces cicatrices influencent l’état psychologique des personnes touchées par cette maladie et conduit à des perturbations psychologiques multiples dans la vie quotidienne et également sentimentale.

MT358 la personne touchée va souffrir et va avoir une crise psychologique surtout si les cicatrices de cette maladie restent sur le visage par exemple.

MT359 l’impuissance de dormir et ne pas cesser de penser à sa maladie. Et la peur d’être touché par cette dernière.

MT360 il est probable qu’il sera atteint par un peu de peur et de honte devant ses amis. Et il ne va pas pouvoir partager les repas avec sa famille.

*MT361 Je peux dire que cette maladie n’est pas fréquente dans la société et il y a plusieurs sociétés où cette maladie n’existe pas car il n’y a pas la présence de certains animaux porteurs de cette maladie et qui le transmettent à l’humain.

MT362 Il se peut que les cicatrices l’influencent si elles sont présentes dans des zones sensibles et découvertes et qu’elles ne guérissent pas par les traitements médicaux modernes. On demande à Dieu le pardon et la santé.

MT363 l’état psychologique de cette personne est un peu difficile. Mais il se peut qu’il y ait une personne qui peut développer des troubles psychologiques et une autre qui reste normale.

+MT364 la personne touchée par cette maladie peut souffrir s’elle est touchée dans une zone sensible comme au niveau du visage ce qui le rend embarrassé devant les amis et les collègues et la société en gros ce qui le pousse à se ralentir dans ses études et son travail.

MT376 la vie devient dure à cause de l’état psychologique de la personne touchée. Les cicatrices influencent sur sa vie psychologique. Heureusement que cette maladie a commencé à diminuer par rapport auparavant.
MT395 lors de l’atteinte par cette maladie il faut se rendre à l’hôpital pour faire la vaccination. Car cette maladie est très dangereuse et laisse place à des cicatrices sur les zones touchées de la peau. Et vive le Roi.

MT396 Pour la femme, ces cicatrices va lui créer un obstacle avec ces amies et son travail et d’autres choses comme ça.

*MT397 Je souhaite aux personnes touchées par cette maladie la guérison rapide. Il faut faire des compagnes de propreté et de lutte contre les rats sauvages (de la forêt). J’espère que je ne serai pas touché ni moi ni les autres par cette maladie qui est dangereuse et qui provoque beaucoup d’autres maladies.

*MT398 je pense que cette maladie est très forte et elle fait beaucoup de problèmes dans le monde. Je veux dire tout simplement que je veux souhaiter à l’homme qui a cette maladie (leishmaniose) la grande santé et merci à tous.

MT413 Oui si un parmi les jeunes personnes est touché par cette maladie au niveau du visage, il sera incapable d’affronter la société par peur de la discrimination sociale et la méprise et par peur d’autres facteurs qui rendent sa psychologie épuisée.

MT414 je ne pense pas pouvoir connaitre l’état psychologique de la personne touchée par ces cicatrices

*MT451 la personne touchée va chercher le médicament idéal et les médecins doivent chercher les moyens pour combattre cette maladie

+MT452 l’état psychologique est marquée par la complexité psychologique car il n’y a pas de traitement pour ces cicatrices et ils vont rester pour toujours. Et il voudra juste comment faire disparaître ces cicatrices.

MT453 case vide

*MT454 moi je ne connais pas cette maladie pour pouvoir parler à propos de cela. Je m’excuse d’être ignorant pour cette maladie. « Tout ce que je sais est que je ne sais rien ».
Supporting information Chapter 4

Consolidated criteria for reporting qualitative studies (COREQ): 32-item checklist

Developed from:

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<td></td>
<td></td>
<td>Loubna Belaid - anthropo sociologist</td>
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<td></td>
<td></td>
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<td>Vincent De Brouwere - MD PhD Professor</td>
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<td>Hind Filali psychosociologist Professor</td>
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<td>Hamid Sahibi - Veterinary doctor PhD Professor</td>
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<td>Marleen Boelaert - MD PhD Professor</td>
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<td>3.</td>
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<td>6. Relationship established</td>
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<td>7. Participant knowledge of the interviewer</td>
<td>What did the participants know about the researcher? e.g. personal goals, reasons for doing the research</td>
<td>Participant information sheet and Consent Form</td>
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<td>8. Interviewer characteristics</td>
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<td>Theoretical framework</td>
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<td>9. Methodological orientation and Theory</td>
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<td>Participant selection</td>
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<td>10. Sampling</td>
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<td>11. Method of approach</td>
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<td>12. Sample size</td>
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<td>15. Presence of nonparticipants</td>
<td>Was anyone else present besides the participants and researchers?</td>
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<td>16. Description of sample</td>
<td>What are the important characteristics of the sample? e.g. demographic data, date</td>
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<td>22. Data saturation</td>
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<td>Software</td>
<td>What software, if applicable, was used to manage the data?</td>
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<td>Participant checking</td>
<td>Did participants provide feedback on the findings?</td>
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<td>32</td>
<td>Clarity of minor themes</td>
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**Supporting information:** Translated FGD topic guide

This Focus Group is a part of a study for preparation of a PhD titled "Control of cutaneous leishmaniasis in Morocco" at the National School of Public Health in Rabat and the Institute of Tropical Medicine in Antwerp.

The main objectives of this study are to identify the knowledge, attitudes, perceptions and practices of the population around cutaneous leishmaniasis.
I request your consent to participate in this discussion group that will help us to understand your experiences related to this disease. The anonymity and freedom of expression is guaranteed.

You can decide at any time to stop the discussion and to withdraw, or else not to participate in a given theme. 60 minutes will be sufficient to complete this entire discussion.

**Familiarity with cutaneous leishmaniasis**

The knowledge of the existence of CL in this geographical area
How can we have scars on the skin?
Do you know a disease called leishmaniasis? Do you use other names for this disease?
How long this disease exists in your community? Is this a new disease that appeared recently?

The presence of family antecedents
Did you suffer from this disease before?
Do you know someone who has suffered from this disease in your surroundings?
Is this disease common in this village? Who are the people most affected?

CL Knowledge
How we can recognize this disease? How do you know you are sick with it?
How is this disease transmitted to humans?
On what part of the body do we find lesions? May we have several lesions at once?
What do you think about the origin of these lesions?
How can we avoid this disease? May we develop several times this disease even after healing?
How long can this disease last before healing?

Therapeutic possibilities followed
How can we heal? Is there a cure?
What kind of treatment exists? Where can we find it?
What are the traditional treatments that can be used?
How much does it cost to get treatment?
Perception of this disease

*The disease severity*
Is this a serious condition? Is it fatal? Are we afraid for our children to have this disease? What is the impact on daily life?
Is it necessary to treat? Would it be better to prevent? How?

*The aesthetic appearance of the lesion or scar*
Did this disease leave scars? How did they appear? On what body parts?
How can we make them disappear?

*The isolation and stigma*
What are the activities that we cannot do when we are affected by this disease?
How we behave in general with those affected by this disease?
(Working together, living together, taking common meal together, walk with, let the children play with the affected person, can cook for your family, possibility of marriage with him or her).

Preventive practices
What are the preventive measures you take personally to avoid getting sick? (Mosquito bed-nets, insecticides ...)
What measures does the community take to prevent disease in the village? (Waste collection, sanitation ...)

Service use (Question for formal health users)
Do you know some traditional healers in the area? Have you visited them before?
Even if you have never been to a healer, what do you know about that? Do you know someone who’s going? Why and for what health problems?

Reasons for not using health facilities for care (Question for Traditional users)
What are reasons for visiting traditional healer rather than health providers?
For what health problem healers are effective? For what disease?

Thank you
Greetings, I am Essam Beniss, a researcher at the National School of Public Health in Rabat, under the Ministry of Health.

I invite you to participate in the round table discussion that will last approximately an hour and a half, with seven others to talk about the skin disease known as Skin Leishmaniasis.

This disease has affected many people in the area over the past five years.

Your participation should be voluntary and no financial compensation will be given. You have full freedom to refuse participation for whatever reason.

For better understanding, I ask you to listen to the information contained in this memorandum, which you will keep in case you want to ask further questions or require clarification.

The study aims to better understand the viewpoints of the citizens and their way of dealing with skin Leishmaniasis.

In this study, there are more than 150 participants from 10 different groups in the region of Rissani / Tinghir.

The questions to be asked are general and do not require a special effort to understand them.

I ask for your consent to participate in one of the focus groups separately for men and women, which will be chosen in the same way and in the same place.

This conversation will last up to an hour and will take place in the building next to the health center ( ).

Procedure of the study

If you agree to participate in this study, you will be asked to go to that room in the building next door. We will start the session after noon at 1:00 PM and all conversations will be recorded by camera or voice recorder. All recordings will be used for the purpose of recall at both ends. They will be reviewed later.

There will be no display of the recorded tape anywhere.

Your freedom of expression and recording all your answers in an anonymous way without mentioning your name finally. You have the right to stop participating temporarily or permanently without notice.

You also have the right not to answer all or any part of the discussion.

Personal information about you will not be collected. Your name will not be requested later for any biological or sample analysis.

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الفوائد

هذه هي واحدة من أولى الدراسات بشأن التصورات والمعرفة والمواقف والممارسات من طرف السكان الذين يعيشون في المنطقة المتضررة من داء الليشمانيا الجلدية في المغرب. النتائج المنتظرة ستكون لها فوائد على الصحة العامة للمجتمع.

لجنة الأخلاقيات

تمت الموافقة على إجراء هذه الدراسة من قبل لجنة الأخلاقيات بمعهد الطب الاستوائي في أنتورب، ولجنة الأخلاقيات بالرباط.

بالإضافة إلى تراخيص من وزارة الصحة ممثلة في المدرسة الوطنية للصحة العمومية في الرباط، وقسم علم الأمراض.

المشاركة الطوعية

يجب أن تكون مشاركتك في هذه الدراسة تطوعية تماما. إنه اختيارك إذا كنت ترغب في المشاركة أم لا. لديك أيضا الحق في التوقف عن المشاركة في أي وقت، دون حاجة لشرح السبب.

هل لديك أسئلة حول مجموعة التركيز؟

هل أنت على استعداد للمشاركة؟

سوف يتم تسجيل الموافقة الشفهية الخاصة بك في بداية الجلسة.

إذا كانت لديك أسئلة أخرى حول البحث أو في حال كنت تعتقد أنك عانيت من نتائج هذه الدراسة، يمكنك الاتصال بالباحث الرئيسي للدراسة: عصام بنيس.

العنوان: المدرسة الوطنية للصحة العمومية، شارع المفضل الشرقاوي، مدينة العرفان، صندوق البريد 6329 الرباط المغرب.
**Supporting information Chapter 5**

Additional File 1 Logbook, search history, excluded articles, included articles and Data extraction labels of the main conceptual categories

**Log book:**
Strategy search performed in the end of September 2016 and updated in first September 2017

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<td>R17</td>
<td>PsycINFO</td>
<td>Skin Disorders OR Wounds AND Any Field: Stigma</td>
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<td>PsycINFO</td>
<td>Measurement AND Any Field: stigma</td>
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<td>64</td>
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<td>R20</td>
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<td>wounds AND Index Terms: Life Changes OR Life Satisfaction OR Lifestyle Changes OR Measurement OR Mental Health OR Positive Psychology OR Quality of Life OR Wellbeing OR Work-Life Balance</td>
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<td>R21</td>
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<td>301</td>
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<td>R22</td>
<td>Web of Knowledge</td>
<td>Search query listed within the article</td>
<td>282</td>
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<tr>
<td>R23</td>
<td>Global Health</td>
<td>((Leishmaniasis OR Dermal Leishmaniasis OR Cutaneous Leishmaniasis OR Mucocutaneous Leishmaniasis OR Diffuse Cutaneous Leishmaniasis NOT Visceral Leishmaniasis) AND (((Stigma) OR (Public stigma) OR (Social Stigma) OR (community stigma) OR (Enacted stigma) OR (Self-stigma) OR (Self stigma) OR (Experienced stigma) OR (Perceived stigma) OR (imagined stigma) OR (Anticipated stigma) OR (Felt stigma) OR (Self-report) OR (internalized stigma) OR (Health related stigma) OR (Gender Identity ) OR (Quality of Life) OR (Perception) OR (Rejection) OR (Discrimination)) OR ((Social problems) OR (Public opinion) OR (Social discrimination ) OR (Social isolation) OR (Social exclusion) OR (Social distance) OR (Stereotyping) OR (Social perception) OR (Social conditions) OR (Social adjustment) OR (Social behavior) OR (Social behaviour) OR (Social behavior disorders) OR (Social environment) OR (Social support) OR (Social marketing) OR (Cost of illness)))</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>
Search history and chart flow text description

**Step 1** - Identification of studies in 08 databases: sold with 2485 references, as shown in the table above.

**Step 2** - Identification of duplicates: From the initial references treated together, we found 260 duplications for all 08 databases.

**Step 3** - Screening results: For the 2225 references screened for title or title and abstract, we excluded 2154 and kept 71.

**Step 4** - Articles included from hand searching sold with 07 additional references.

**Step 5** - After reading the full text of the 78 references defined in step 3 and, we excluded 63 references due to not corresponding to our inclusion criteria.

**Step 6** - 15 Articles included for synthesis after step 5.

**Step 7** - Synthesis of the definitely included articles reported within the flow chart of this study.

**Excluded articles after full reading text – Final Results 31th August 2017 (n=63)**

**No or too limited regarding CL stigma or CL psychosocial burden (n=33)**


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No primary study (n=16)


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**Not a Localised CL form (n=06)**


**VL or PKDL or Diffuse form of CL (n=04)**


**Full text not available (n=02)**


**No leishmaniasis article (n=02)**


**All 15 included final articles:**


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Data extraction labels of the main conceptual categories used in NVivo 11

Country of the study

Author names, year, journal

Study design (cross sectional, case control ...) and type (Quantitative, Qualitative)

Population experiencing CL (characteristics)

Sample size (number of participants, gender, age)

Ratio Men/women CL affected

Cutaneous Leishmaniasis species

Dermatological condition involved (CL lesions, CL scars)

Types of stigma (Cite the exact names and types used by those authors)

Types of psychological consequences

Reasons for stigma related to the dermatological condition

Notion of time change of the perception of stigma

Notion of time change of the perception of psychological consequences

Comparison between CL lesions and CL scars

Comparison between CL and other NTD

Comparison between CL and other (No NTD) dermatological diseases

Coping strategies targeting CL outcomes

Measurement scale of stigma

Measurement of psychological consequences

Main finding
<table>
<thead>
<tr>
<th>Mic 1 = S1</th>
<th>Mic 2 = S2</th>
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<tbody>
<tr>
<td>first sample by scraping</td>
<td>second sample by scraping</td>
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**Dental broach sample for RDT**

<table>
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<tr>
<th>S3</th>
<th>S4</th>
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<tr>
<th>Microscopy reading of S1 and S2</th>
<th>FIRST PCR on S4: (ITS1) (Casablanca)</th>
<th>SECOND PCR on S3: half ITS1 (Casablanca) and half kDNA (Antwerp)</th>
</tr>
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<tbody>
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</table>

**Or**

<table>
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<tr>
<th>S3</th>
<th>S4</th>
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<tr>
<th>Microscopy reading of S1 and S2</th>
<th>FIRST PCR on S3: (ITS1) (Casablanca)</th>
<th>SECOND PCR on S4: half ITS1 (Casablanca) and half kDNA (Antwerp)</th>
</tr>
</thead>
</table>
My sincere gratitude to Prof. Dr. Marleen Boelaert my promoter for your human qualities, your vast scientific knowledge, and your unlimited generosity. Your guidance, advice, and reviews have made the thesis a reality. Working with you and with your team was a real pleasure.

Special words of gratitude to my promoter Prof. Dr. Vincent De Brouwere; for the motivating discussions and learning trajectory. He was the best support for me in my home institute to help me finish this thesis.

I am equally grateful to Prof. Dr. Jean Claude Dujardin and Prof. Dr. Hamid Sahibi, my Belgian and Moroccan promoters, for their supervision of my Ph.D. work.

I would like to thank all my other co-authors with whom I had the privilege to collaborate during the past years. For the enjoyable field work and generous feedback and suggestions on the writing process.

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About the author

Birthday August 8th, 1976 in Oujda town in Eastern Morocco. Baccalaureate in Mathematical Sciences in Oujda. Medical studies at the Faculty of Medicine and Pharmacy in Rabat. Worked since 2003 with the Ministry of Health (MoH) as a general practitioner. Then, followed a specialization in Public Health at the National Institute of Health Administration in Rabat.

Worked since 2010 as a representative of the MoH in Southeastern Morocco to manage the public health organizations. There, he was confronted with an epidemic of cutaneous leishmaniasis motivating him to start a Ph.D. study on the topic since 2014 (Unit of Epidemiology and Disease Control at the Institute of Tropical Medicine in Antwerp and the Faculty of Pharmaceutical, Biomedical and Veterinary Sciences - University of Antwerp). Similarly, he was appointed as a Medical Researcher at the National School of Public Health in Rabat, Morocco. He succeeded to stay single without children during all these years of studies.