Control of cutaneous leishmaniasis caused by *Leishmania major* in south-eastern Morocco

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**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 intersectoral response measures including vector and reservoir control. The aim of this article was 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 analysed routine surveillance data and other information using the data of the CL control programme.

**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, 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.

**Conclusion** Errachidia Province was the epicentre 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.

**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 article. In North African countries, *L. major* belongs almost exclusively to the zymodeme Mon 25 [4]. The sand fly *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 (*Rhabdomys opimus*) [5]. *Psammomys 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]. In 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 ulcer-healing. ZCL lesions usually present as multiple ulcerated and often superinfected lesions. 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 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].

Zoonotic CL cases usually fluctuate in an epidemic cycle with 5- to 10-year 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 immunoprotective 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 do not fully understand the factors that regulate the induction and maintenance of immunity against *Leishmania*. Understanding these factors is critical for the design of an 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 article 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 Ghris Valley in the south-east 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 (Fig. S1). 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 inter-
ventions 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 centres.

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. Technical staff of the MoA distribute 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 burrows 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 intersectoral, 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 peri-domestic 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 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.

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 and 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 analysed routine reports on the control programme’s standard preventive measures, as well as anonymised data from the epidemiological surveillance system and reports at the provincial and national level. We consulted the central database to cross-check 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 analysed 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 centres 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 and period of diagnosis for each case, which were subsequently anonymised. Data entry was performed using Microsoft Office Excel 2013 and analysed 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.

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 centres or hospitals, but 237 cases were parasitologically confirmed (of a total of 303 smear examinations performed) (Table 1).

The outbreak was first recognised as such in 2007 in the area of Merzouga in the south-east 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 3 years, 1287 kg of strychnine-poisoned wheat was used. The total number of active burrows observed before and after these operations is provided as additional information (Table S1). 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 neigh-
bouring 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 (Table S2).

After the rodent control, intervention was equally applied throughout this area in 2012, and the number of annual cases decreased to 12.

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- to 20-year-olds with 28% of cases, followed by the age group 0- to 10-year-olds (26% of cases) (Table 1). Women were more often affected than men 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).

### 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 intersectorial 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 [11, 31]. 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 3 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).

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

<table>
<thead>
<tr>
<th>Total Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups*</td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>1854</td>
</tr>
<tr>
<td>11-20</td>
<td>2038</td>
</tr>
<tr>
<td>21-30</td>
<td>971</td>
</tr>
<tr>
<td>31-40</td>
<td>782</td>
</tr>
<tr>
<td>41-50</td>
<td>634</td>
</tr>
<tr>
<td>51-60</td>
<td>471</td>
</tr>
<tr>
<td>&gt;60</td>
<td>348</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4184</td>
</tr>
<tr>
<td>Male</td>
<td>2915</td>
</tr>
<tr>
<td>Area</td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>5940</td>
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<tr>
<td>Urban</td>
<td>1159</td>
</tr>
<tr>
<td>Diagnosis trimester</td>
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</tr>
<tr>
<td>Fall</td>
<td>3822</td>
</tr>
<tr>
<td>Winter</td>
<td>2453</td>
</tr>
<tr>
<td>Spring</td>
<td>304</td>
</tr>
<tr>
<td>Summer</td>
<td>520</td>
</tr>
<tr>
<td>Diagnosis confirmation†</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>5250</td>
</tr>
<tr>
<td>Laboratory</td>
<td>303</td>
</tr>
</tbody>
</table>

*One missing data.
†Data available from 2009.
reservoir [35]. If less sandflies have an infected blood meal, it is expected that less will accidentally contaminate humans. 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 to our study was that cases were mainly ascertained clinically, although 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 [11].

**Conclusion**

It appears that the intersectorial ZCL control strategy adopted by the province of Errachidia since 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.

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**References**

I. Benn et al. Cutaneous leishmaniasis control in Morocco


I. Benni et al. Cutaneous leishmaniasis in Morocco


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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