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The spatio-temporal distribution of rodent species, potential reservoir hosts of zoonotic cutaneous leishmaniasis in Morocco

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Abstract
Zoonotic Cutaneous Leishmaniasis (ZCL) still remains a major public health problem in Morocco. It's caused by *Leishmania major* and transmitted by *Phlebotomus papatasi* with *Meriones shawi* as the only proven reservoir host. With the aim to investigate the ZCL risk in central Morocco, rodent and sandfly species were collected from 22 localities and their activities were followed during one year study (June 2014-May 2015).
10 rodent species were collected: *Rattus rattus* (30.85%), *Mus musculus* (23.40%), *Rattus norvegicus* (9.57%), *Apodemus sylvaticus* (9.15%), *Mus spretus* (8.51%), *Mastomys erythroleucus* (6.81%), *Meriones shawi* (5.53%), *Gerbillus campestris* (2.34%), *Meriones libycus* (2.13%) and *Lemniscomys barbarus* (1.70%). Their spatio-temporal distributions were discussed according to many ecological factors and according to *P. papatasi* seasonality.
We noted that all potential ZCL reservoirs collected presented a short activity period which overlaps with the summer peak of *P. papatasi* activity. This might provides important data on ZCL risk periods.

Keywords: Zoonotic cutaneous leishmaniasis, Rodent species, spatio-temporal distribution, *Phlebotomus papatasi*, Morocco

Introduction
Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) are worldwide vector-borne diseases caused by protozoan parasites of the genus *Leishmania* (*Kinetoplastida*: *Trypanosomatidae*) and transmitted by female sandflies (Diptera, *Psychodidae*, *Phlebotominae*). Leishmaniasis are emerging diseases that affect humans and other mammal reservoir hosts [1] and closely related to Environment parameters [2].
In Morocco, both CL and VL occur and still remain a public health problem [3]. ZCL is the oldest leishmaniasis form in Morocco. It has been identified since 1914[4] and still considered as the most dominant leishmaniasis form [5, 6]. Currently, it is widespread in the country from the Atlantic coast, south of the Anti-Atlas Mountains to the north-east, across the saharan areas south of the Anti-Atlas and High Atlas mountains, and east of the Middle Atlas Mountains [7]. ZCL form is caused by *Leishmania major*-MON 25, the only zymodeme identified in Morocco up-to-date [8], and transmitted by *Phlebotomus papatasi. Meriones shawi* (*Rodentia*: *Gerbillidae*) is considered as the only proven reservoir host of *L. major* in Morocco [9, 10], while, in the other countries of the Maghreb (i.e. Algeria, Tunisia and Libya), *Psammomys obesus* (*Rodentia*: *Muridae*) is the main reservoir host of this parasite [11, 12]. *Rattus norvegicus* (*Rodentia*: *Muridae*), *Meriones libycus* and *M. crassus* (*Rodentia: Gerbillidae*), largely spread throughout Morocco, are also implicated in *L. major* transmission in other countries, such as Iran, Libya, Tunisia, Egypt and Israel [13].
Central Morocco, area with large distribution of *P. papatasi* [14], is classified by many authors as being at risk of *L. major* transmission for its arid climate [11, 15] and the presence and the important density of *P. papatasi* throughout the year [16] in addition to its proximity to ZCL foci in the south-east region[17]. This study is the first contribution to identify the mammal reservoir species composition across central Morocco and to provide data on the Seasonality and Dynamics of the potential ZCL reservoirs populations in our study area. These results, with local entomological data, can predict the ZCL risk in central Morocco.

2. Material and Methods

2.1. Study area

The study was conducted in four regions of central Morocco (Fig. 1): Al Haouz (31°22'37''N; 7°48'20.2''W), Chichaoua (31°10'35''N; 8°51'10''W), Essaouira (31°34'36''N; 9°32'8''W) and Marrakech (31°42'55.5''N; 8°04'05.8''W). A total of 22 localities were sampled with altitude ranging from 318 to 2579 m.

In this study area, the localities are representative of all the major bioclimatic environments of Morocco. The climate is arid to semi-arid in the plain and humid in the Atlas Mountain and coast area. The mean temperature is below 22°C, from November to April, which corresponds to the wet period, while it is greater than 25 °C in the dry period, from May to October.

The total population of the study area was 3 576 643 in 2014 [18] with 1 491 243 urban populations (42%) and according to region, 573 128 registered inhabitants in Al Haouz, 369,955 inhabitants in Chichaoua, 450,527 inhabitants in Essaouira and 1 330 468 inhabitants in Marrakesh [18].

For rodent and sandfly seasonality studies, investigations were carried out in the land-fill site (31°42'10.0''N; 8°03'48.9''W; 393m) of Marrakesh region (S6) because of the presence of the most collected rodent species (Fig. 1).

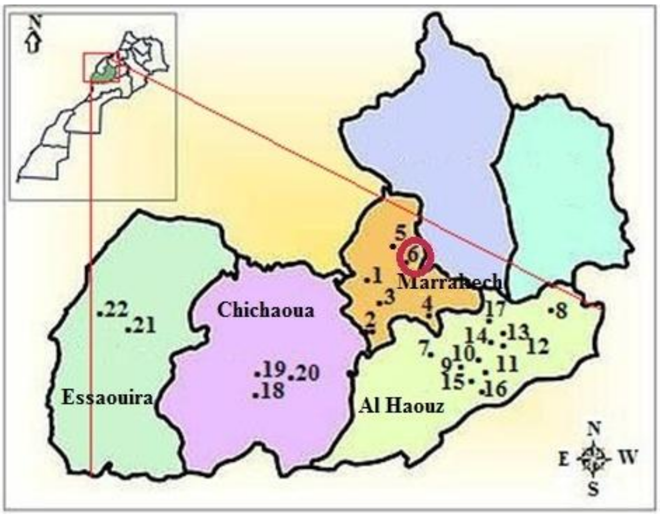

Fig 1: Regional and local map showing study area with the 22 trapped localities. Seasonality study was conducted in land-fill site (S6).

2.2. Reservoir data

2.2.1. Rodent trapping

Specimen capture was conducted by random live trapping using metal traps (Sharmann) placed at burrow active entrances (Fig. 2). The presence of rodents was guided by indirect methods including traces, the active burrows, the cut stems and fresh droppings (Fig. 3).

The 22 localities were sampled twice in June 2014 according to transect method. Different baited traps (bread with olive oil, tomatoes, potatoes and dates) were placed in the sampling sites in the afternoon and recovered early the next morning.

Then, S6 locality was selected for seasonality study and 40 traps were used each time. The site monitoring was carried out twice monthly (every 15 days) from June 2014 to May 2015.

Fig 2: Indicators marking the active and inactive burrows

Fig 3: Traps used in capturing rodents in study area

2.2.2. Specimen treatment and species identification

Samples were transported to the laboratory for processing. After anesthesia with ether, weighing and sex determination, morphometric measurements were performed (ear length, head and body lengths, hind foot length and tail length) for species identification [19].

2.2.3. Ethical guidelines

Our specimens were treated according to European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1st February 2013, NOR: AGRG1238767A. Thus, all efforts were made to minimize the number and suffering of animals used.

2.3. Entomological data

Phlebotomine sandflies were collected using sticky traps as shown by Boussaa *et al* [16]. Traps were placed around burrow active entrances, twice a month, from June 2014 to May 2015. 30 sticky traps were set at 6 pm and collected around 6 am of the next day.

In the laboratory, the specimens caught were cleared in potash 20% and Marc-André solution, and then they were mounted in Hoyer medium. The species identification was made by examining the morphology of male and female genitalia and pharynges [14, 16].

2.4. Geographic and Climatic data

Geographic locations of study sites were identified with GPS and elevation data were obtained from the Shuttle Radar Topography Mission (http://srtm.usgs.gov/).

Monthly temperature and precipitation data were extracted from the data base available at: http://en.tutiempo.net/climate/morocco.html, corroborating with NDM (National Directorate of Meteorology) data, for the study period (June 2014 - May 2015).

2.5. Data analysis

Various ecological parameters and indexes were calculated to characterize the rodent populations in the different prospected sites:

Specific richness (S): the number of species in the sample [20];

Shannon Diversity Index (H'): to evaluate the rodent species diversity.

$$H' = -\sum_{i=1}^{s} pi.\,Log_2\,(pi)$$

Where *pi* = **ni / N,** is the proportion of each species in the sample;

The value of Shannon Diversity Index usually falls between 1.5 and 3.5, only rarely it surpasses 4.5. A value near 4.6 would indicate that the numbers of individuals are evenly distributed between all the species [21].

Shannon's equitability (EH): measures the distribution of individuals within species, regardless of species richness [22].

EH= H'/H'max

With H'max = $Log_2(S)$ Equitability assumes a value between 0 and 1 with 1 being complete evenness.

3. Results

3.1. Rodent diversity

During this investigation, a total of 470 specimens belonging to 10 species and 7 genera were collected (Table 1). *Rattus rattus* was the most abundant species (30.85%) followed by *Mus musculus* (23.40%), *Rattus norvegicus* (9.57%), *Apodemus sylvaticus* (9.15%), *Mus spretus* (8.51%), *Mastomys erythroleucus* (6.81%), *Meriones shawi* (5.53%), *Gerbillus campestris* (2.34%), *Meriones libycus* (2.13%) and *Lemniscomys barbarus* (1.70%). For all the species, except *M. shawi* and *L. barbarus*, the sex ratio favors female.

According to regions, nine species were collected in Marrakesh (6 sites) while only two species were identified in Chichaoua (3 sites). According to sites, only *M. musculus* and *R. rattus* were collected in the 22 localities (Fig. 4).

Ecological indexes were calculated for different localities (Table 2). The greatest specific richness (S) was detected in S6 (in Marrakesh), where the least specific richness was two species and it was noted in many localities (S11, S15, S16, S17, S18, S19 and S20).

Shannon Diversity Index (H') ranged between 0.918 (in S17) and 2.424 (in S2 and S7), while, Shannon's equitability (EH) ranged between 0.501 (S13) and 0.994 (S16).

According to altitude, five species (*R. rattus, M. musculus, M. spretus, A. sylvaticus* and *M. shawi)* were collected between 300 and 2600 m altitude.

R. norvegicus and *M. erythroleucus* were collected only in low altitude (300-599m), while *M. libycus, G. campestris* and *L. barbarus* were collected from 600m (Table 3).

3.2. Sandfly diversity

A total of 345 specimens were collected belonging to five species (Table 4). *Phlebotomus papatasi* was the most abundant species (58.55%) followed by *P. sergenti* (22.61%), *S. minuta* (11.59%), *P. longicuspis* (4.06%) and *S. fallax* (3.19%). The sex-ratio was in favour of the males for all species.

3.4. Rodent & sandfly seasonalities

Fig. 5 shows the evolution of total rodent individual activity, conducted in S6 during 1-year-study. In this arid area, rodents were active throughout the year but their activity was strongly marked between April and September. This period corresponds to the higher temperature and lower precipitation (dry season).

Fig. 6 shows the yearly activity evolution of all rodent species and *P. papatasi* collected in Marrakech (S6). Two rodent groups were distinguished:

- Rodent species presenting a short period of activity: *M. erythroleucus. M. shawi* and *R. norvegicus.*
- Rodent species with a long period of activity: *R. rattus. M. musculus*. *A. sylvaticus* and *M. spretus.*

Pearson correlation coefficients between abundance of these rodent species with mean temperature and precipitation were calculated. The seven species activities showed negative correlation with precipitation and positive correlation with temperature. *M. spretus* was the most sensitive species to temperature (r=0.76), followed by *M. musculus (r=0.70), A. sylvaticus* (r=0.51), *M. erythroleucus* (r=0.41), *R. rattus* (r=0.40), *M. shawi* (r=0.39) and *R. norvegicus* (r=0.19).

All species activities showed low negative correlation with precipitation: *M. musculus (r=*-0.21*), A. sylvaticus* (r=-0.37), *M. erythroleucus* (r=-0.32), *R. rattus* (r=-0.43), *M. shawi* (r=-0.30), *R. norvegicus (r=* -0.27) and *M. spretus (r=*-0.42).

For *P. papatasi* activity, two peaks were identified: summer peak (June-July) and autumn peak (November-December). Summer peak of *P. papatasi* activity coincides with all local rodent species, while, autumn peak coincides with low activity of only *Rattus rattus* and *Mus musculus* (Fig. 6).

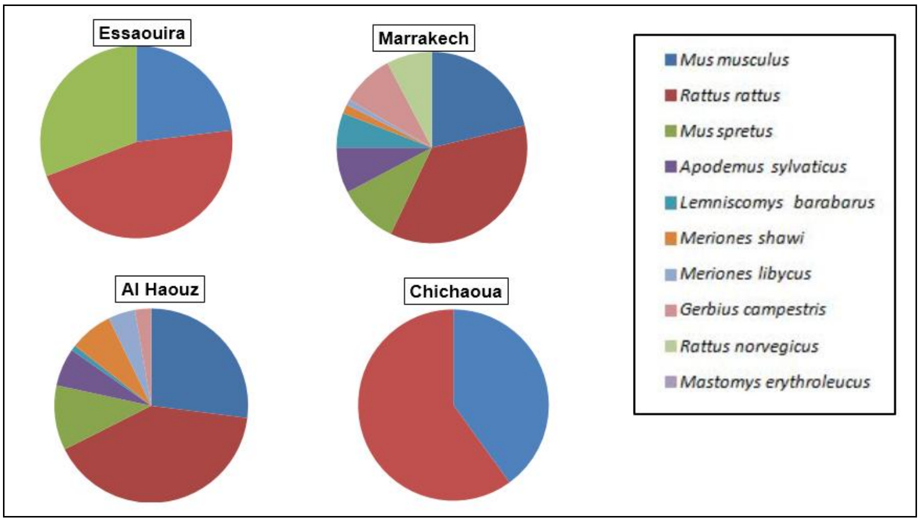

Fig 4: Abundance of rodent species by region

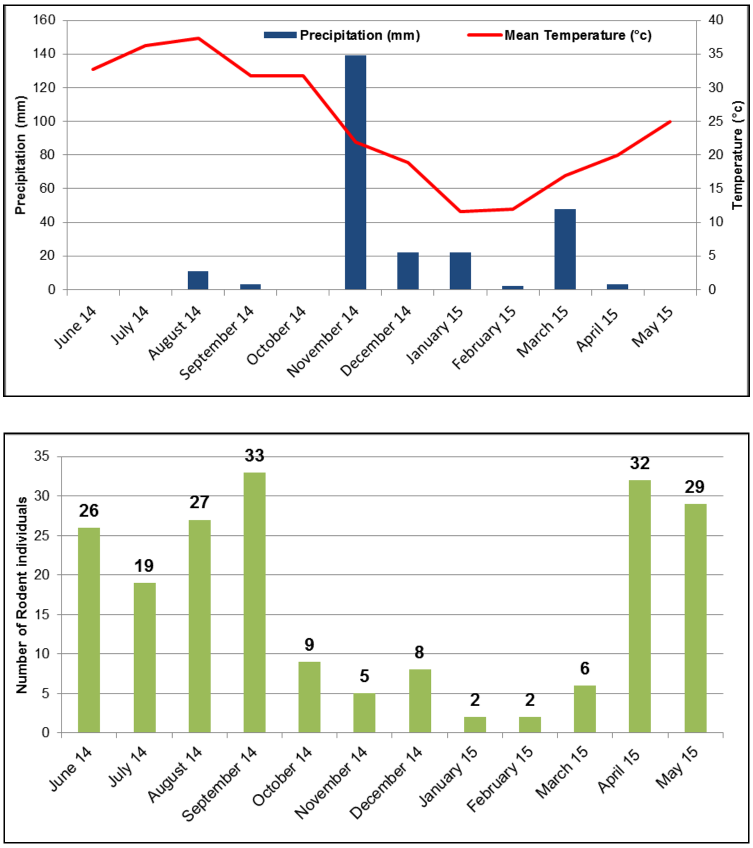

Fig 5: Total of rodent individuals collected by month and variation of the monthly mean temperature and precipitation in Marrakech (S6).

Fig 6: Evolution of Rodent species (Histograms) and *Phlebotomus papatasi* (curve) activities in Marrakech area.

Table 1: Number of male, female and relative abundance of Rodent species collected in study area

Species	Male	Female	Total	%
Rattus rattus	65	80	145	30.85
Mus musculus	47	63	110	23.40
Rattus norvegicus	22	23	45	9.57
Apodemus sylvaticus	18	25	43	9.15
Mus spretus	19	21	40	8.51
Mastomys erythroleucus	13	19	32	6.81
Meriones shawi	14	12	26	5.53
Gerbillus campestris	5	6	11	2.34
Meriones libycus	4	6	10	2.13
Lemniscomys barbarus	5	3	8	1.70
Total	212	258	470	100

Table 2: Ecological Indexes in different localities

Locality*	S	H'max	H'	EH
S1	4	2	1.924	0.962
S2	6	2.585	2.424	0.938
S3	3	1.585	1.002	0.632
S4	3	1.585	0.993	0.626
S5	3	1.585	1.061	0.669
S6	7	2.807	1.485	0.529
S7	6	2.585	2.424	0.937
S8	5	2.322	1.458	0.628
S9	3	1.585	0.924	0.583
S10	3	1.585	0.931	0.587
S11	2	1	0.971	0.971
S12	3	1.585	1.054	0.665
S13	4	2	1.003	0.501
S14	3	1.585	0.977	0.617
S15	2	1	0.954	0.954
S16	2	1	0.994	0.994
S17	2	1	0.918	0.918
S18	2	1	0.954	0.954
S19	2	1	0.985	0.985
S20	2	1	0.971	0.971
S21	3	1.585	0.931	0.5873
S22	3	1.585	1.040	0.656

*As shown in Figure 1.

Table 3: Rodent species distribution according to altitude in study area

Species	300-599 m (7 sites)	600-899 m (9 sites)	900-2600 m (6 sites)
Rattus rattus	119	35	27
Mus musculus	70	24	16
Mus spretus	33	9	3
Apodemus sylvaticus	23	4	3
Meriones shawi	12	7	8
Rattus norvegicus	33	0	0
Mastomys erythroleucus	25	0	0
Meriones libycus	0	5	5
Gerbillus campestris	0	3	3
Lemniscomys barbarus	0	3	0
Total	315	90	65

Table 4: Number of male, female and relative abundance of sandfly species

Species	Males	Females	Total	%
Phlebotomus papatasi	154	48	202	58,55
P. sergenti	46	32	78	22,61
P. longicuspis	10	4	14	4,06
Sergentomyia minuta	24	16	40	11,59
S. falax	8	3	11	3,19
Total	88	55	345	100

4. Discussion

Leishmania major transmission is complex cycle which needs the implication of a rodent reservoir, such as *Meriones spp*, *Psammomys obesus* or *Rhombomys opimus* [23]. In Morocco, the first and the only study on *L. major* reservoirs was performed, in Tata region (South of Morocco): Among of 28 specimens of *M. shawi*, two specimens were infected with *L. major* MON-25. However, to date no study was conducted to provide the clear information on the potential reservoir role of rodents other than *M. shawi* in Morocco [10]. In contrast, ZCL by *L. major* is the most frequent form of leishmaniasis and the order of *Rodentia* is numerically the largest in Morocco (represented by 7 families and 32 species) [13].

In central Morocco, the entomological status is well established [14, 24]. Therefore, its ZCL epidemiological risk is linked to the knowledge about reservoir status. In our study area, 10 rodent species were collected. In addition of *M. shawi*, the proven vector of *L. major* in Morocco, five other species were implicated in *Leishmania* species cycle elsewhere [13]. In southern Europe (Greece, Italy, Portugal and Spain), *R. rattus*, *R. norvegicus*, *M. musculus*, *M. spretus* and *A. sylvaticus* were found infected naturally by *Leishmania infantum* [25-32], while, *R. norvegicus, M. erythroleucus* and *M. libycus* played a reservoir role in *L. major* transmission in other countries of North Africa [33-36].

Geographical distribution of reservoir species can predict the ZCL risk area. In our study, three groups of rodent species can be distinguished according to altitude:

- ubiquitous species were collected from 300 m up to 2600 m: *R. rattus, M. musculus, M. spretus, A. sylvaticus* and *M. shawi*
- species were collected specially in intermediate altitude (from 600 m): *M. libycus, G. campestris* and *L. barbarus*
- species were collected only in low altitude (300-599 m): *R. norvegicus* and *M. erythroleucus*

This altitudinal structuring of the rodent species can be explained by the special climatic requirements and consequently their trophic exigencies. It is noted that the weather altered also the rodent activity patterns [37]. At 393 m altitude (S6), the rodent population dynamics showed amono-modal annual pattern for seven species (*R. rattus, M. musculus, M. spretus, A. sylvaticus, R. norvegicus, M. erythroleucus* and *M. shawi*), and it was marked in the dry season, when the mean temperature ranged between 20°c and 37°c and rainfall ranged between 3 and 11 mm. *R. rattus* was active throughout the year and it did not diapause in this region. In contrast, *M. shawi* had the shorter activity period, May-June-July.

Ecological conditions, such as food availability, predator pressures, competition, social biology, and temperature and moisture, interact to modify the daily and seasonal patterns of individual mammalian species [38]. Our species showed positive and negative correlation with, respectively, temperature and precipitation. Significant positive correlation with temperature was noted only for *M. spretus*, *M. musculus* and *A. sylvaticus* (r=0.76, *r=0.70* and r=0.51, respectively). Temperature synchronizes rodent activity less effectively than light in most species [39, 40], which may explain also the high activity of our species during the summer (dry season) when the day period is longer. According to species, *R. rattus, M. musculus*, *A. sylvaticus* and *M. spretus* activities peaked in September (31.8°c in the summer); *M. erythroleucus* and *M. shawi* peaked in June (32.7°c in the summer), while, only *R. norvegicus* peaked in April (20°c in the spring).

Correlation between our rodent species activity and *P.*

papatasi seasonality is an important tool to detect the risk periods of ZCL in this study area. All potential ZCL reservoirs collected in Marrakesh area (*M. erythroleucus, M. shawi* and *R. norvegicus*) presented a short activity period (April-August) which overlaps with the summer peak of *P. papatasi* activity in the same area. This might explain the absence of ZCL cases in the study area up-to-date. However, it provides important data on ZCL risk periods in Marrakesh area.

In conclusion, the study area hosts many rodent species implicated in many *Leishmania* species cycles worldwide. These rodent species are active, at least, in dry season, overlapping with sandfly season in Morocco, including the study area [14, 16]. The data gathered suggests the urgent need of a continuous surveillance in order to prevent leishmaniasis incidence risks.

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