

Zoonotic cutaneous leishmaniasis and *Leishmania* infection among *Meriones shawi* population in Setif Province, Algeria

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Abstract. Fellahi A, Djirar N, Cherief A, Boudrissa A, Eddaikra N. 2021. Zoonotic cutaneous leishmaniasis and *Leishmania* infection among *Meriones shawi* population in Setif Province, Algeria. *Biodiversitas* 22: 2547-2554. Zoonotic cutaneous leishmaniasis (ZCL) is the most endemic disease in Algeria. *Leishmania major* is the causative agent, and Gerbils *Meriones shawi* and *Psammomys obesus* are the main reservoir hosts. The Province of Setif in Algeria has become a very active focus for this zoonosis. Our study was carried out from January 2017 until January 2019 in ten representative stations of the study area, with an aim to identify the association between zoonosis and reservoir host species. Eighty-six (86) specimens of *M. shawi* and three (3) specimens of *P. obesus* were captured and determined. Superficial lesions were subjected to Giemsa stained smears. In addition, Livers and spleens were tested for *Leishmania* DNA using ITS1 PCR. The results showed the presence of *M. shawi* in almost all communities of Setif Province and the highest population with 50 specimens (58.13%) was in the center and south. The microscopic and molecular detection of *Leishmania* showed high infestation in most specimens of *Meriones* (32/86) with a rate of 37.20 %, however, no infestation in *Psammomys* has been detected. The PCA results showed a negative association between rainfall and ZCL cases; however, positive association was found between temperature and the human CL cases. Also, positive association was demonstrated between ZCL in south and center of Setif Province and *Meriones* captured. Our finding, confirm that *M. shawi* is the principal reservoir host (76.78%) of the ZCL in Setif Province, since *P. obesus* was nearly absent (2.67%).

Keywords: *Meriones shawi*, reservoir host, rodent, zoonotic cutaneous leishmaniasis

INTRODUCTION

Zoonotic Cutaneous leishmaniasis (ZCL) is one of the most important parasitic diseases of mammals and humans (Barkati et al. 2019; Tabbabi 2019). This form represents 75% of all leishmaniasis (de Souza et al. 2015). *Leishmania major* (*L. major*) an obligate intracellular parasite are known to be the causative agent (Aoun and Bouratbine 2014; Chaara et al. 2014). The vector is *Phlebotomus papatasi* (Izri et al. 1992). The main mammalian reservoirs of the parasite *L. major* are specifically wild rodents of Gerbillidae family, including *Meriones shawi* (*M. shawi*) and *Psammomys obesus* (*P. obesus*), which are closely associated with humans (Belazzoug 1986; Reithinger et al. 2007; Aoun and Bouratbine 2014; Akhouni et al. 2016). This rodent species has a significant role in the transmission of disease (Bernard 1977; Tekka et al. 2002; Derbali et al. 2012; Samy et al. 2016). The ZCL disease is a major public health problem, that about 10,000 new cases are reported every year, and about two-thirds of cases occur in Afghanistan, Colombia, Syria, Brazil, Iran, and Algeria (Alvar et al. 2012; Mohamedbeigi et al. 2019; WHO 2019). It is difficult to treat because no vaccine is yet available (Seid et al. 2014). This form of leishmaniasis is endemic in many areas of the tropics and subtropics and the Mediterranean

basin, spanning generally more than 98 countries (Reithinger et al. 2007; Alvar et al. 2012; WHO 2019). In fact, this means that there are about 350 million people at risk of zoonotic CL that has existed for many years and caused by *L. major*, the most common parasite found in northern Africa, where the incidence rate of ZCL largely exceeds thousands of cases per year (Pratlong et al. 2009; Alvar et al. 2012; Eddaikra et al. 2018; WHO 2019).

Algeria is considered one of the ten most affected countries of cutaneous leishmaniasis globally, and it represents the second focus, after Afghanistan, with 10847 cases in 2018 (Eddaikra et al. 2018; WHO 2019). Thus, Zoonotic Cutaneous leishmaniasis is endemo-epidemic in arid and semi-arid Algeria areas. The first identification of the parasite was in the Province of Biskra in 1910 (Sergent et al. 1921). In recent years, there has been an increase in the prevalence of the LCZ and an extension to the north of the country (Harrat et al. 1996; Alvar 2012). The disease is spreading rapidly and creating new and important outbreaks such as those in M'Sila, Batna, Bechar, El Oued, Ghardaia, Bordj Bou Arreridj, Djelfa, Saida, and Setif (Belazzoug 1982; Boudrissa et al. 2012; Benelmouffok et al. 2017). Our interest is particularly focused on the Province of Setif. This province is bordering the epidemiological foci of ZCL, especially those of Hodna which is considered a permanent focus. Until now, no eco-

epidemiological work on ZCL has been done; based on this, an intervention study of the reservoir host is needed to better understand the prevalence of ZCL in Setif. Due to the importance of these Gerbils in the maintenance of *L. major*, This study aimed to identify the relationship between *M. shawi* and ZCL prevalence in Setif area to establish a preventive and successful control strategy based on reservoir host in endemic periods.

MATERIALS AND METHODS

Study area description

Setif Province is situated on a high plain (North East of Algeria), with an estimated area of 6550 km², an altitude of 1100 m, and approximately 1,489,979 inhabitants. It is distant from the capital; Algiers, by 300 km. To the north, it borders Bejaia and Jijel, to the south; it borders M'Sila and Batna, to the east, Mila and to the west, Bordj Bou-Arridj. The relief of Setif is divided into three main areas: northern mountainous zone, central plains spacing, and southern low zone (composed of desert chain). The Setif Province enjoys a semi-arid continental climate with wet and cold winter and hot and dry summer (Figure 1).

Retrospective study of cutaneous Leishmaniasis in Setif

We conducted a retrospective study on the incidence of cutaneous leishmaniasis in our study area (Departement of Sétif), over 11 year period from 2008 to 2018. All data were provided by the Directorate of Health and Population (DSP: Direction de la santé et de la population) from the Annual Epidemiologic Data collection (REM: Relevé épidémiologique mensuel). Among these data: the number of cases of CL recorded per year, and the distribution of cases by age and gender. Positive cases of ZCL were

reported and recorded by health facilities in the study areas during the 2008-2018 period.

Climatic data

To determine the links between the frequency of ZCL and the climatic factors, we collected the temperature and rainfall data recorded during 2017 and 2018, from the National Office of Meteorology (ONM) of Setif in the Setif International Airport (World Meteorological Organization), which were registered daily.

Rodent trapping and capture

Site selection

Two criteria were used to choose the target communities for the study of the relationship between zoonotic cutaneous leishmaniasis and the reservoir host *Meriones shawi* (Figure 1): The first was based on the number of ZCL cases registered and notified in Setif Province (Figure 2). The CL clinical data used in this study were taken from the Directorate of Health and Population (Direction de la Santé et de la Population; DSP- Setif) to find the pattern of cutaneous leishmaniasis and theredistribution points. Villages with more positive ZCL cases during 2017 and 2018 were selected as best sites for the study (critical areas for transmission of ZCL); and the second was related to rodents distribution, their ecological niches, and active burrows number, established based on field observations in search for activity indices and reports from IPVCT-DSA (Directorate of Agricultural Services; DSA -Setif). Besides, related to infestations in agricultural areas by *M. shawi*. The target stations are chosen for the catch of rodents. The coordinates of the selected sites were recorded by GPS (Table 1).

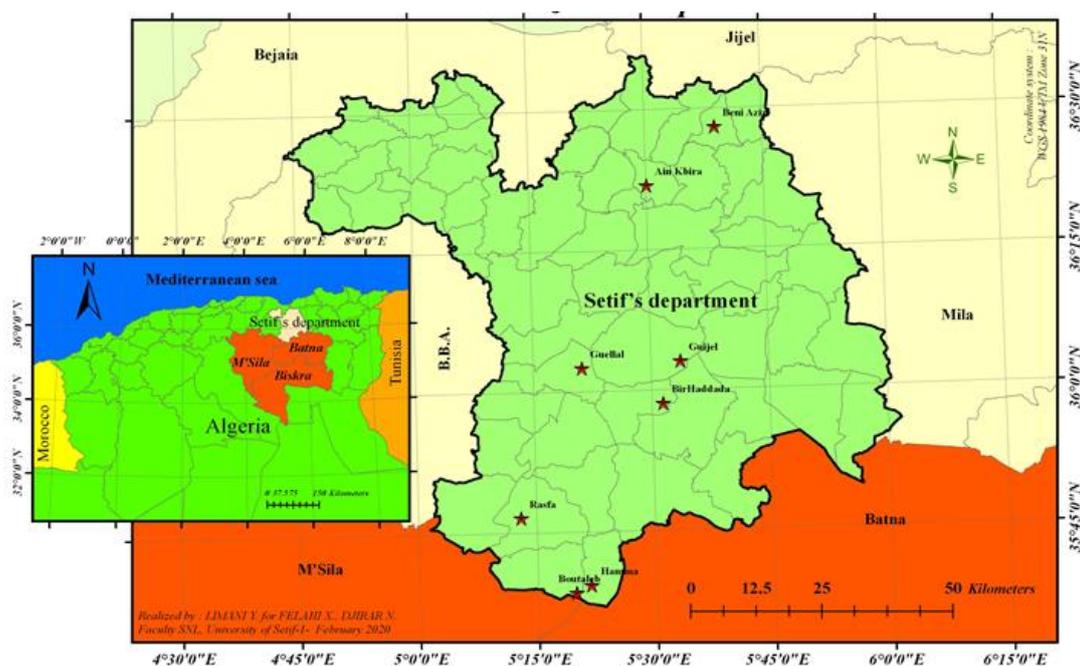


Figure 1. Location of Setif Province in Algeria indicating the sampling sites of rodents and its borders with epidemiological foci of ZCL

Table 1. Coordinates of rodents trapping sites, Setif Province, Algeria

Site	Latitude	Longitude	Alt. (m)
Beni Aziz	36°28' 0" N	5°39'0" E	775
Ain Kbir	36°21' 53" N	5°30'7" E	1024
Bougaa	36°19' 57" N	5°05'19" E	1050
Guijel	36°3'3.81" N	5°33'47.33"E	969
Guellal	36°2'31.16"N	5°21'14.69"E	910
Bir Haddada	35°58'34.63"N	5°31'28.04"E	951
El Eulma	36°9' 37.082" N	5° 41' 54.232"E	959
Rasfa	35°46'37.86"N	5°13'1.55"E	833
Hamma	35°39'18.04"N	5°21'45.61"E	833
Boutaleb	35°38'31.45"N	5°19'51.74"E	795

Ethics statement

All experiments were carried out in compliance with the Federation of European Laboratory Animal Science Associations (FELASA) guidelines and approved by the Ethical Committee of the Pasteur Institute in Algiers.

Rodents catching and identification

The rodent catching period was from January 2017 to January 2019 (two years), using two types of traps, Sherman and wire Mesh cages. Eight (8) traps were placed once time per month in each of the ten selected sites in the afternoon and collected the morning of the following day, which makes (80) traps in total. The aerated wire-mesh traps have been equipped with food (dates) to limit the stress of the trapped animals before transferring them to the laboratory. Trapping sessions were conducted in winter, spring, summer and autumn. The collected rodent specimens were brought to the Laboratory of Institute Pasteur of Algeria (IPA)/ Branch of M'sila. Each specimen was killed with ether, and then identified according to the determination keys described by Bernard 1977 and Etemad 1978. Four external measurements were taken from each specimen in mm by using a digital micrometer caliper (Fisherbrand): head and body length (HBL), ear length (EL), tail length (TL), and hindfoot length (HFL). The animals were also weighed (gr).

Smear of lesions

CL lesions were found mainly on rodents ears and noses. All superficial lesions were subjected to dermal smears stained with MGG May Grunewald Giemsa. Slides were MGG stained for microscopic observation of *Leishmania* amastigotes (Gx1000).

DNA extraction and electrophoresis

Spleen and liver samples were obtained from all rodent specimens. After collection, the specimens were stored in 70% ethanol until DNA extraction. To perform Phenol/chloroform-based DNA extraction method: 10 mg of spleen tissue was homogenized in 200µL of lysis Solution (100 mM NaCl, 10 mM Tris-HCl, and 0.5% SDS, pH 8). To digest the protein, 20 µL proteinase K (20 mg/mL) was added, and then, the samples were incubated at 56°C for 12 h. A phenol chloroform extraction was

performed (Sidorova et al. 2012). DNA was detected after amplification of the ribosomal internal transcribed spacer 1 (ITS1 F: CTGGATCATTTTCCGATG; ITS1 R: TGATACCACTTATCGCACTTA) was monitored as described by Schönian et al. (2003), using the primers LITSR and L5.8S. The PCR mix (25 µL) contained 2.5 µL of DNA, 10x buffer, 300 µM MgCl₂, 200 µM dNTP, 500 nM of each primer, and 2U of Taq DNA polymerase. PCR amplification products being obtained were separated on a 1% agarose gel at 75 V for 35 min and were visualized after stained with ethidium bromide (EtBr). After electrophoresis, DNA results were visualized via gel documentation (gel doc) to trace DNA fragment migration using a UV transilluminator.

Statistical analysis

The Quantum GIS (Arc GIS 10.6) was used to present spatial and geographic distribution of rodents trapping sites, and the bordering of Setif with epidemiological foci of ZCL. To study the link between climatic variables (temperature and rainfall), the cutaneous leishmaniasis and *Meriones* captured in Setif Province, we used the Excel stat 2014.5.03 for the Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

Cutaneous leishmaniasis incidence

In the last decade (2008-2018), 676 cases of human cutaneous leishmaniasis were registered in the Province of Setif. The zoonotic CL disease was spread in most parts of the Setif Province with a higher prevalence in the southern zone, where 609 (90.08%) cases were recorded during this period, 55 (8.13%) cases in the central zone and only 12 (1.77%) cases in the northern zone. This may be due to climatic factors and rodents as a reservoir host existed in the Province of Setif. Addedly, the migrations of infected *Meriones shawi* population can catalyze a pandemic's spread from endemic regions to non-endemic regions.

Regarding the ZCL annual distribution in Province of Setif from 2008 to 2018, we have noted a 600% increase (from 30 cases in 2008 to over 140 cases in 2018). The highest yearly recorded cases occurred in 2010, 2011, and 2018 with 123, 116, and 139 cases, respectively. The annual incidence rate in Province of Setif was 9.46 cases per 100,000 inhabitants in 2010, 8.85 cases in 2011 and 9.34 cases in 2018. The evolution of ZCL incidence in Setif between 2008 and 2014 is similar to the country incidence and from 2014 to 2018 became inversely proportional. The Evolution of the incidence of Human ZCL in Algeria from 2008 to 2018, show that several peaks have occurred in 2009, 2011, and 2017, however, in Province of Setif higher the incidence rate was 9.46 cases per 100,000 inhabitants in 2010, 8.85 cases in 2011 and 9.34 cases in 2018 (Figure 2).

Concerning the gender of affected patients, the males are the most affected with 451 (66.71%), however, 225 (33.28%) females were registered. Zoonotic cutaneous leishmaniasis affects all ages from children to the elderly. Concerning the age distribution of ZCL, Figure 3 shows that young adults [16-25] and adults [26-50] are the most affected.

Relationship between ZCL and rainfall

The principal component analysis applied on the variables annual rainfall and the cases of the LCZ recorded during the period 2008-2018, gives information on the relationship that may exist between the two variables. Indeed we notice that factor 1 and factor 2 contain 100% of the inertia of the variation. Bartlett's test of sphericity informs the absence of correlation between the variables ($P_{value}=0.45$). We also note that the number of cases of the LCZ and rainfall are negatively correlated with respect to axis 1, which contains 62.75% of the inertia (Figure 4). Rainfall is an important factor in the activity of the reservoir host and vector, but the occurrence of LCZ depends on other factors (Ramezankhani et al.2017). Negative association was demonstrated between rainfall and ZCL incidence, where no significant relationship was

found between the annual mean rainfall and CL incidence, consistent with previous work in Nikonahad et al. (2017). The effect of rainfall was found to be significant ($P_{value}=0.45$). Our findings are quite convincing with those obtained from other Ramezankhani et al. (2018) studies, where rainfall exerted inverse effects on ZCL. Other results were also reported by Ghatee et al. (Ghatee et al. 2018). Hence, Zoonotic CL is an emerging disease linked to the affected environment and climatic factors (Nikonahad et al. 2017; Mohammadbeigi et al. 2021). Hence, it can be reasonably concluded that the permanence of endemicity depends generally on the climatic factors, that CL is more prevalent in dry and semidry areas with low precipitation (Mokhtari et al. 2016). Also, the appearance of the ZCL depends on other factors (Benelmouffok et al. 2017; Inceboz 2019).

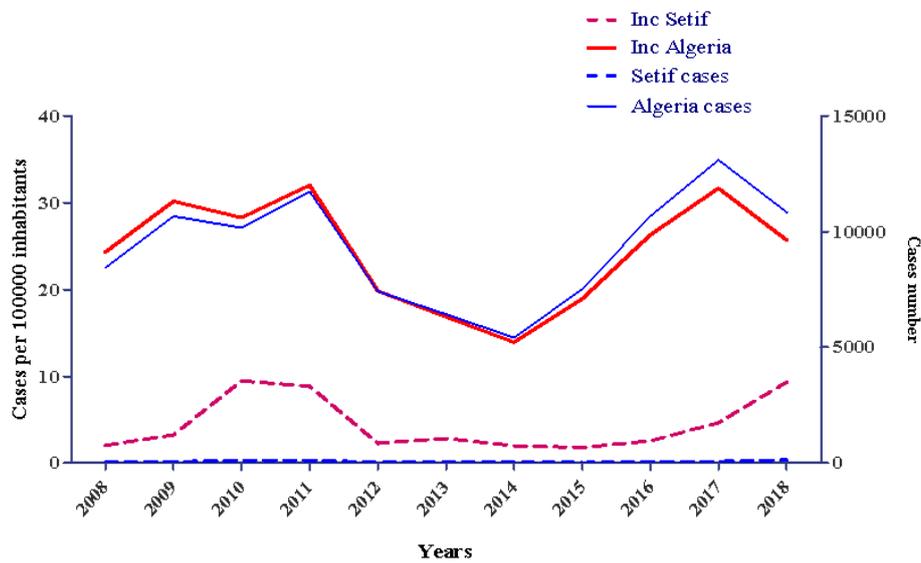


Figure 2. Evolution of the case number and the incidence of Human ZCL in Algeria and the Province of Setif from 2008 to 2018.

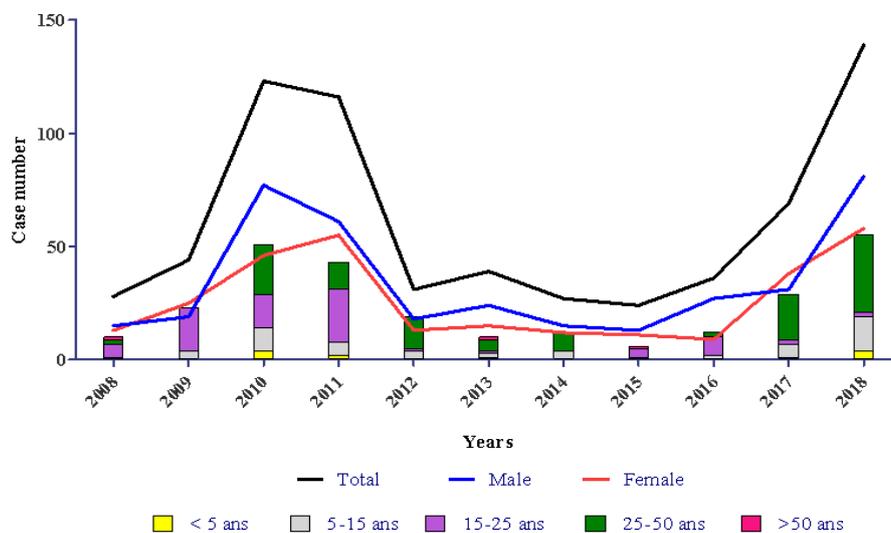


Figure 3. Number of ZCL cases reported in Setif by gender and age, during 2008-2018 period

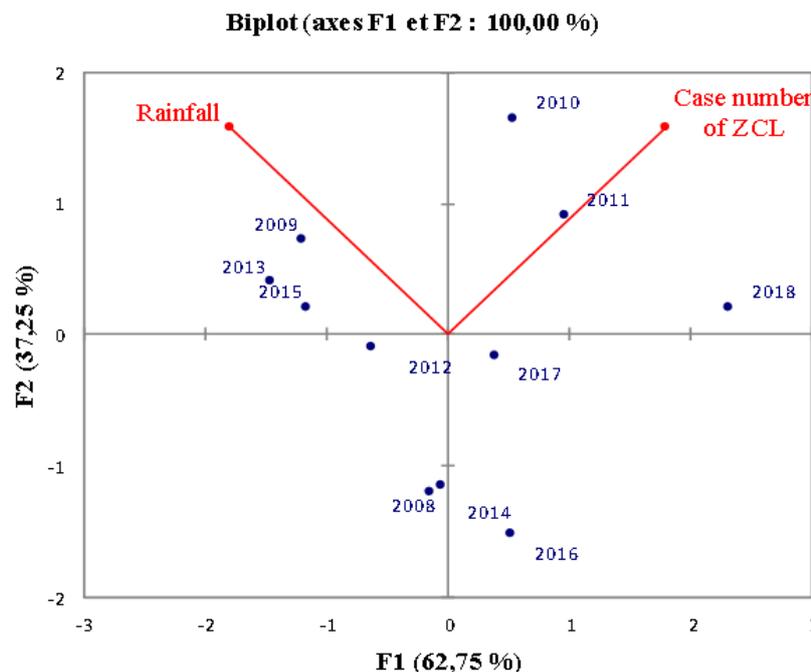


Figure 4. Principal component analysis of variation of cases number and rainfall among ten years in Setif Province (2008 to 2018) The number of cases of ZCL and rainfall are negatively correlated

Rodent identification and dispersion

For determination of reservoir hosts of the disease, altogether 112 rodents were captured and identified. They were *M. shawi* (86/112) (76.78 %), *Psammomys obesus* (3/112) (2.67 %), *Mus musculus* (5/112) (4.46 %), *Ratus ratus* (12/112) (10.71 %), *Gerbilus gerbilus* (4/112) (3.57 %) and *Duprasi* sp. (2/112) (1.78 %). *M. shawi* has been the most abundant species. The lowest number was found in the north with five specimens (5.81%), whereas, the highest number of 50 specimens (58.13%) was observed in the south (Table 2). Also, 47 (54.65 %) male and 39 (45.34 %) female of this species were registered (Table 4). *P. obesus* was nearly absent. It prefers presaharienne area according to Boubekri and Gernigon 2013, while a semi-arid climate characterizes the Province of Setif.

Although all collected animals were examined by direct examen and PCR for parasite, only *M. shawi* were found to be infected by *L. major*. Thus, the results showed a remarkable prevalence of *Leishmania* in *Meriones*. These findings are also consistent with the findings of other studies carried out in Iran by Foroutane et al. (2017) and the largest number of infection cases (32/86) was found (Table 3). Smears were taken from 32 specimens that showed lesions. Cutaneous Leishmaniasis lesions of ears and noses were found mainly on 28 specimens. Although *Leishmania* amastigotes were found after microscopic observation in 87.5 % of smear. These findings are also consistent with the findings of other studies in Algeria,

where, the first *M. shawi* was found to be naturally infested by *L. major* in M'sila outbreak and the second, *M. shawi* Ksar chellala outbreak (Belazzoug 1982; Belazzoug 1986).

The most important infected animals were reported in 10 females and 22 males (an overall prevalence of 37%) (Table 4). Similarly, the treatment of specimens captured in two years (Figure 5) confirmed that areas located in center (43.75%) and south (53.12%) of Setif Province had the highest potential to harbor this species than northern area (3.12%). The all infested individuals came from the sites of Beni Aziz (1/32), Guellal (2/32), Eulma (9/32), Bir Haddada (3/32), Hamma (5/32), Rasfa (4/32) and Boutaleb (8/32); however non-infested specimens were found in Aain Kbira, Bougaa, and Guedjel (Table 3). The prevalence of *L. major* infection in *Meriones* living in the south and center of Setif Province (Table 3) can be explained by the capacity of these rodents to harbor the parasite as good reservoir hosts. Thus, this area is probably considered as the best ecological niche, where the risk of transmission was high. Addedly, the highest reported CL cases incidence were noticed in sites with an altitude lower than 800 m, on the other hand, the lowest number of cases were found in areas with an altitude of over 900m. Our result is in line with Research by Ramezankhani et al. (2018), showing that CL is more prevalent in areas with low altitude. Also, the migrations of infected *M. shawi* population can catalyze a pandemic's spread from endemic regions to non-endemic regions.

Table 2. Number of cases of zoonotic cutaneous leishmaniasis reported in Setif during 2008-2018 period

Region	ZCL Number	Percentage (%)
North	12	1.77
Center	55	8.13
South	609	90.08

Table 4. *Meriones shawi* captured and specimens infected with *leishmania* by gender, during 2017-2018 period

Region	Gender of <i>Meriones shawi</i> captured		Gender of <i>Meriones shawi</i> infected	
	M	F	M	F
North	01	03	00	00
Center	19	15	10	04
South	27	21	12	06
Total	47	39	22	10

Table 3. Number of *Meriones shawi* captured and specimens infected with leishmania during 2017-2018 period

Region		Number of specimens captured	Percentage (%)	Number of specimens infected	Percentage (%)
North	Beni Aziz	02	5.81	01	3.12
	Ain Kbir	01		00	
	Bougaa	02		00	
Center	Guidjel	05	36.04	00	43.75
	Guellal	05		02	
	Bir Haddada	08		03	
	El Eulma	13		09	
South	Rasfa	09	58.13	04	53.12
	Hamma	19		05	
	Boutaleb	22		08	

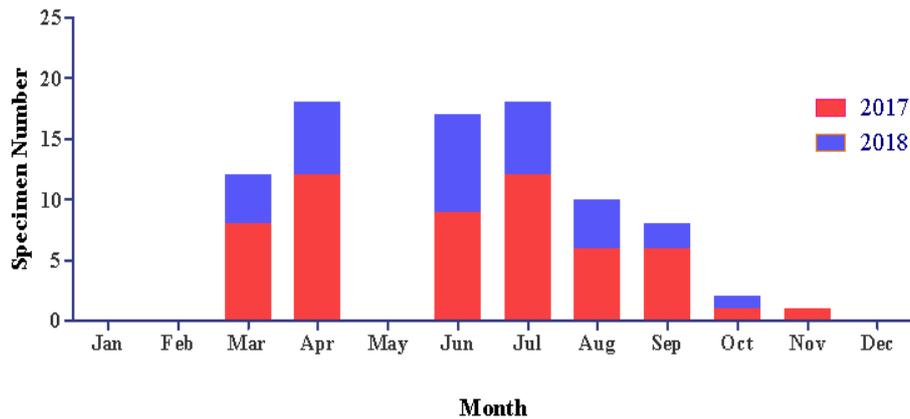


Figure 5. Number of *Meriones shawi* specimens captured during 2017-18 period

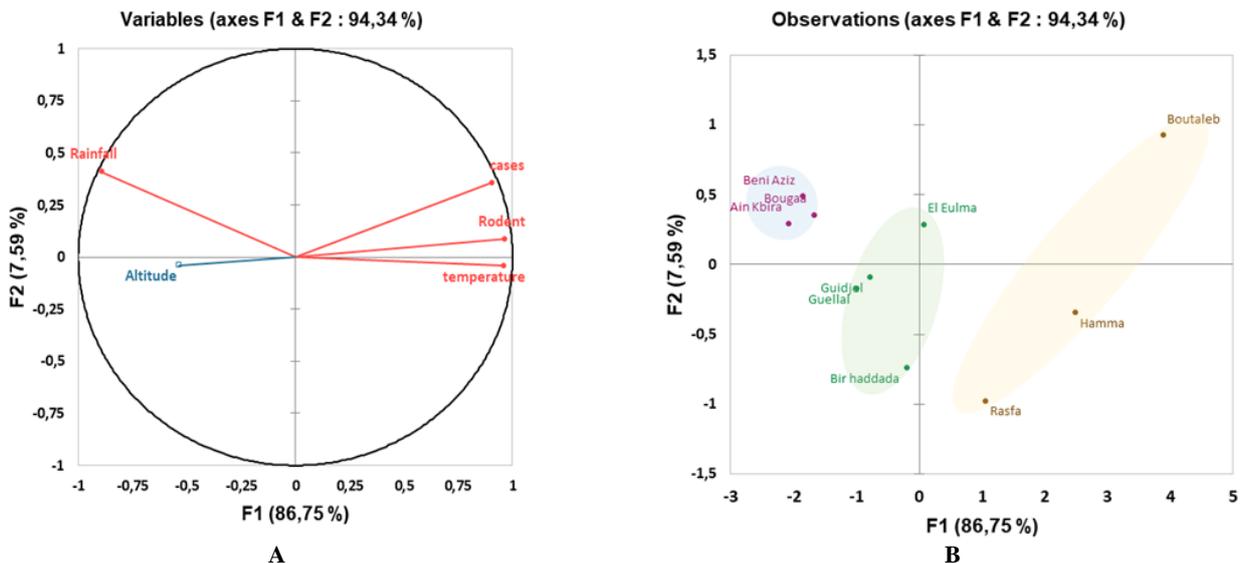


Figure 6. Principal component analysis of variation of cases number, temperature and rainfall among the specimens captured three regions of Setif Province. A. Variables, B. Observations and regions clusters (yellow is south, green is center and blue is north)

To study Rodent dispersion factors in Setif Province, we applied the principal component analysis on the variables annual rainfall and temperature, cases of the LCZ, rodent number recorded during the period 2017-2018 in 10 sites of rodent catching in Setif Province, to identify the information on the relationship that may exist between these variables. Indeed we notice that factor 1 and factor 2 contain 94,34 % of the inertia of the variation. Bartlett's test of sphericity informs the correlation between the variables (P -value < 0.0001). The Pearson Matrices of correlation showed a positive link between Rodent and ZCL cases ($p= 0.861$), Rodent and Temperature ($p= 0.929$), ZCL cases and Temperature ($p= 0.809$) (figure 6B). We also note that the number of cases of the LCZ and specimens of *Meriones* captured and infected and Temperature are positively correlated to axis 1, which contains 86.75% of the inertia (Figure 6.A).

Positive association was demonstrated between ZCL incidence in south and center areas of Setif Province and *Meriones* captured and infected, where a significant relationship was found, and this is consistent with previous work in previous study in Biskra by Guaouaoui et al. (2017). The activity of *M. shawi* in the southern areas of Setif is therefore probably due to several waves of rodent species migration from the south known endemic regions, such as Batna and M'Sila and conditioned by the climatic oscillations that caused the extension or retraction of the Sahara as reported by Belazzoug (1982); Belazzoug (1986) and more recently by Bounoua et al. (2013). Furthermore, Sedaghat and Salahi-Moghadam (2010); Gholamrezaei et al. (2016) noted that the distribution of rodents is due to the climatic shift. In addition, Boudrissa et al. (2012) showed the spread of cutaneous leishmaniasis from the arid areas to semi-arid areas of Algeria. Benelmouffok et al. (2017) mentioned that cereal-producing provinces were the most affected by ZCL.

Thus, the development of agriculture in the region increases the risk of ZCL transmission, through the agricultural products, which attracts new *Meriones* to build typically their perfect habitats and establish optimal conditions for transmission of this zoonosis between individual hosts of the same species and via *M. shawi* host species to humans. The Altitude was an additional variable studied by the PCA with a positive link with the rainfall ($p= 0,467$). Our results were similar to those of Ramezankhani et al. (2018), where the highest CL cases incidence were found in sites with an altitude lower than 800 m. Besides a small number of cases were found in regions with an altitude over 900 m, and this may be due to the type of vector. Our results were in line with previous studies showing that CL is more prevalent in areas with low precipitation (Mokhtari et al. 2016).

To conclude, this research paper reported the presence of *M. shawi* as potential reservoirs of ZCL in Province of Setif. Our finding suggests that *M. shawi* is probably the principal reservoir host of ZCL and the only source of human infection in Province of Setif. This study, confirms that this Gerbillid plays a significant role in transmission of this zoonose and the incubation of *L. major* as a good and major reservoir host in the study area, since *P. obesus* was

nearly absent. To establish an eco-epidemiological study in a new focus of ZCL, the reservoir host must be taken into consideration. The study findings can be therefore used in proper mapping for endemiological surveillance.

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