

## Using Ecosystem Approach to Address Infection with *Leishmania* Major in Central Tunisia

Hedia Bellali<sup>1,2\*</sup>, Farah Saffar<sup>1</sup>, Nissaf Bouafif Ben Alaya<sup>2,3</sup>, Issam Nouiri<sup>4</sup>, Jamila Ghrab<sup>5</sup>, Mohamed Kouni Chahed<sup>1,2</sup>

<sup>1</sup>Department of Epidemiology and Statistics, Abderrahman Mami Hospital, Tunisia

<sup>2</sup>Department of Epidemiology and Public Health, Tunis-El Manar University, Tunisia

<sup>3</sup>National Observatory of New and Emerging Diseases, Ministry of Health, Tunisia

<sup>4</sup>National Institute of Agricultural of Tunisia, Tunisia

<sup>5</sup>Environnemental Sciences and Technologies Institute, Borj Cedria, Tunisia

\*Corresponding author: Hedia Bellali, Department of Epidemiology and Statistics, Abderrahman Mami Hospital, Tunisia. Tel: +216-70160349; Email: hedia.bellali@gmail.com

**Citation:** Bellali H, Saffar F, Ben Alaya Bouafif N, Nouiri I, Ghrab J, et al. (2019) Using Ecosystem Approach to Address Infection with *Leishmania* Major in Central Tunisia. Arch Epidemiol 3: 128. DOI: 10.29011/2577-2252.100028

**Received Date:** 18 December, 2018; **Accepted Date:** 12 March, 2019; **Published Date:** 18 March, 2019

### Abstract

**Background:** Cutaneous leishmaniasis is endemic in many parts of the world. There are about twenty different species of *Leishmania* capable of infecting humans. The distribution of cutaneous leishmaniasis is very closely related to the geographical characteristics and the ecological specificities of the endemic areas. Zoonotic Cutaneous Leishmaniasis (ZCL) is the most common form of leishmaniasis in Tunisia. We used Eco Health Approach to understand if and how farming practices in two communities in Central Tunisia affect farmers' exposure to *P. papatasi*, the vector of ZCL.

**Methods:** We reviewed irrigation records and conducted site visits and interviews with community members and entomological investigation in 2 rural areas (Hichria and Ouled Mhamed) in Sidi Bouzid, Central Tunisia.

**Results:** In Hichria, relatively intensive farming, combined with inefficient surface irrigation technology and limited irrigation capacity, results in a need for nighttime irrigation activity. In Ouled Mhamed (Bir Badr), farmers used more efficient sprinkler and drip irrigation techniques. The aging infrastructure and limited economic opportunities from farming have discouraged investment in more intensive agriculture. Farming is mostly limited to end-of-season olive production. Local demand for irrigation is easily met in daytime hours. In Hichria, female *P. papatasi* was present in the irrigation zone and in livestock shelters nighttime. *Leishmania* DNA was detected in 20 of 241 females in this area including 8 in the irrigated zone. Farming practices in Hichria require farmers to irrigate at night, increasing their exposure to the ZCL vector and hence their risk of infection. In nearby Ouled Mhamed, because of different farming practices, farmers do not engage in nighttime irrigation and thus do not face this same exposure.

**Conclusion:** Economic strategies (e.g. intensity of agriculture, crop choices, and animal husbandry), technology (e.g. surface vs. drip irrigation) and institutional arrangements (e.g. community responses to meet irrigation needs) may be important human environment determinants of ZCL risk. Interventions targeting these same factors may help reduce risks of ZCL infection, complementary to more conventional vector control and case detection and treatment, where they exist.

## Author Summary

- Research on the ZCL in Tunisia has mostly focused on disease epidemiology, including more recently on its eco-epidemiology and the environmental determinants of risk, and on clinical research. There has been virtually no attention to the human environmental determinants of risk. This paper is the first-ever research work, using Eco Health approach, to study the effect of different agricultural regimes on ZCL exposure in Tunisia. A combination of factors (farming intensity, crop choices, irrigation technology and management) required one community to practice nighttime irrigation, thus increasing their exposure to the lone vector of ZCL, the nocturnal sand fly *P. papatasi*. Understanding of human environmental determinants suggests of new possibilities for ZCL prevention that are cost-effective and practicable in the near-term in Tunisia and other endemic-epidemic regions.

## Introduction

Zoonotic Cutaneous Leishmaniasis (ZCL) is a major and growing public health issue in Tunisia. More than 100,000 cases have been reported since its emergence in the early 1980's [1]. Since then, it has established in several areas in central and southern Tunisia in stable endemic form with epidemic outbreaks every four to seven years [1,2]. The most important foci of disease are in the Sidi Bouzid Governorate in central Tunisia, which reports 25-30% of ZCL cases annually [3]. Unfortunately, there remains no vaccine or effective treatments methods, and no cost-effective methods for reservoir host or vector control [4]. Rural poor populations in endemic regions remain at high risk of ZCL infection. The number of infected populations increases each year, and the health system bear the burden of the disease. Public health professionals are challenging to find alternative preventive measures to reduce ZCL morbidity, meanwhile the production vaccines and effective treatment [5,6].

In Sidi Bouzid, the etiological agent - Leishmaniasis major - is transmitted to humans via a sand fly vector *Phlebotomus papatasi* [7]. The rodent species *Psammomys obesus* serves as the animal reservoir host [8]. Water resources and management are the most important determinants in ZCL disease ecology. Temperature, rainfall and humidity have been implicated in the ZCL transmission cycle via their effects on vector and reservoir host population densities and dynamics [1]. But for a single study that indicated the importance of environmental changes caused by the construction of a dam [9], there has been no research on human environmental determinants of risk of ZCL in Tunisia, and very little elsewhere in the world.

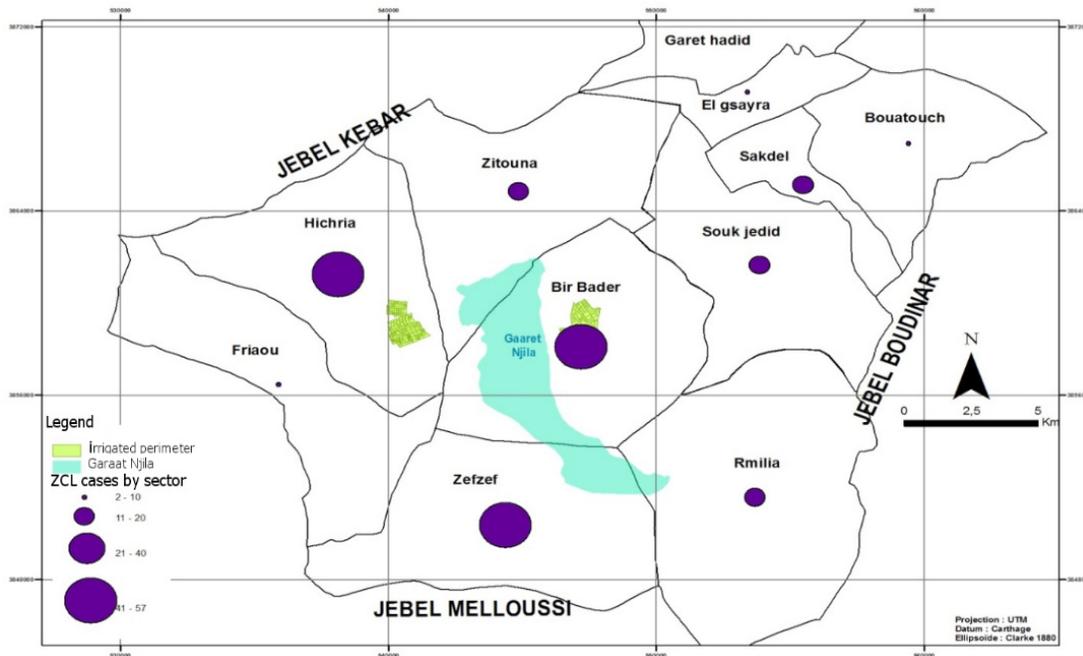
The history of ZCL emergence in Tunisia and in the Sidi Bouzid region demonstrates the significant interaction between humans, vectors, reservoir hosts, pathogen and climate and environment influences. The emergence of ZCL in 1982-1983 was consequent to a major agricultural development project, the construction of the Sidi Saad Dam in Kairouan Governorate in Central Tunisia. Similarly, agricultural development projects in the communities of El Hichria and Ouled Mhamed in Sidi Bouzid Governorate results in environmental changes leading to the spread and the establishment of ZCL in this region. Previously, these areas were pastoral lands without human settlements. After the construction of irrigation infrastructure in the early 1950's, humans occupied the land, developed private wells, expanded agricultural and livestock production, creating favorable ecological conditions to the transmission of ZCL [10,11]. Irrigated agriculture is essential to social and economic development in the region [10]. Irrigated agriculture has already been identified as a factor of ZCL emergence [12]. Irrigation increases soil humidity, which is favorable for the reproduction and the growth of *P. papatasi* [13]. Moreover, the simultaneous presence in irrigated areas of both farmers and the ZCL vector increases the farmers' risk of exposure to infection.

In this study, we explored if and how farming practices and irrigation practices in particular, may increase risk of exposure to infection with *Leishmania* in central Tunisia.

## Methods

### Study Sites: Hichria and Ouled Mhamed

- The study was conducted in the governate of Sidi Bouzid in central Tunisia, mainly in the rural communities of Hichria and Ouled Mhamed (Bir Badr). **The field studies did not involve endangered or protected species and no specific permissions are required for these research activities.** This is an arid region, with average annual rainfall of less than 200 mm, and considerable year-to-year variability. Agriculture is the principal economic activity in both communities, although agricultural practices vary. Both communities have public irrigation infrastructure built to promote settlement and agricultural development in the region. Hichria was the site of the first major public irrigation project in Sidi Bouzid region in 1956. A surface irrigation system now provides water across a zone of 163 ha. In Ouled Mhamed, a drip and sprinkler system provide irrigation across 76 ha. The two communities are situated on either side of the salt pan (Garat Njila, sebhka), a saline land that homes a large population of *Psammomys obesus*, the rodent reservoir host. It plays a critical role in sustaining the stable endemic-epidemic pattern of disease here. The irrigation zones in each community are within a kilometre, the flying range of *P. papatasi* [14], of the salt pan (Figure 1).



**Figure 1:** Main study sites: Hichria and Ouled Mhamed (Bir Badr).

### Farming Systems and Irrigation Practices

- Members of the project team met repeatedly with farmers and representatives of the Agricultural Development Group (GDA) in each community to learn more about local farming practices. The GDAs manage farmers' access to the irrigation system. From their records, we constructed for each irrigation zone an hourly schedule of farmers' irrigation activity for the months of July and August 2010. Previous studies have shown peak transmissions to occur during the period July-September [15,16]. In order to evaluate the risk of exposure of farmers, as well as those who work with them, to *Phlebotomus papatasi*, a "Risk" parameter was calculated which reflects the importance of the length of time that farmers and the vector of ZCL are together simultaneously in each irrigated area. Equation (1) expresses this risk parameter:

$$Risk = \sum_{t=1}^{t=T_{max}} \sum_{h=1}^{24} \left( \frac{d(t, h)}{d_{max}} \right) \times \frac{N_{ai}(t, h)}{N_{a_{max}}} \quad (1)$$

Where:

- $T_{max}$  : The calculation period of the risk parameter.
- $d_{(t,h)}$  : The density of the vector of ZCL during the day "t" at time "h".
- $d_{max}$  : The maximum density of the vector of ZCL in the study area.
- $N_{ai(t,h)}$  : The number of farmers irrigating during the day "t" at time "h".
- $N_{a_{max}}$  : The maximum number of farmers who can irrigate at the same time.

- The monitoring of farmer behavior in the two irrigated areas was carried out simultaneously during the months of July and August 2009, 2011 and 2013 when the irrigation and the vector activity are at the highest levels.

### Entomological Survey

- We conducted an entomological survey in El Hichria in order to determine the spatial-temporal distribution and infection of *P. papatasi*, the lone vector of ZCL in the region. The survey was conducted over a five-day period in August 2010. Modified CDC miniature light traps and CDC with rotator, segregating collects by hour were used to capture sand flies in five local ecotopes: Irrigated Zone (IZ); Intra Habitation (IH); Extra Habitation (EH) and Livestock Shelters (LS) described in Table 1. The traps were set from sundown to sunrise. Species and sex were confirmed through morphologic identification.

| Ecotope | Description   | Mean nb CDC/Night | <i>P. papatasi</i> Female/Male | % of Female | Infected/Screened Females |
|---------|---|-------------------|--------------------------------|-------------|---------------------------|
| IZ      | Irrigated zone: Open irrigated field with principally olive trees   | 1                 | 23/6                           | 79,3%       | 8/8                       |
| IH      | Intra-habitation: Bedroom with cemented and painted walls   | 2                 | 34/33                          | 50,7%       | 6/6                       |
| EH      | Extra-habitation: open area (yard) where human commonly rest and sleep at night   | 1                 | 22/13                          | 62,8%       | -                         |
| LS      | Livestock shelters: Animal shelters (sheep, goat), walls built with stones and the roof with trunks of shrubs, situated nearby the house in the peridomestic area | 2                 | 79/22                          | 78,2%       | 6/6                       |
| RB      | Rodent borrow: Active rodent borrows situated in Garat Njila (sebkha)   | 2                 | 83/138                         | 53,2%       | -                         |

**Table 1:** Results of real-time PCR investigation on 20 females of *P. papatasi* by ecotopes.

Detection of *Leishmania* (L.) was performed by real-time PCR from captured females of *P. papatasi* in 3 different ecotopes: IH, RB and IZ. DNA extraction was performed as described by Esseghir, et al. [17], in 20µl elution buffer. Real-time PCR was performed using *Leishmania* specific probe and primers targeting kinetoplasmic DNA (kDNA) according to Esseghir, et al. [17]. One µl of DNA of each sample was used in a final reaction volume 25 µl. There was no night time irrigation activity in the second irrigation zone, thus no need to investigate vector activity there.

### Principles of an Ecosystem Approach to Health

- Eco health research is an approach linking the environmental and social determinants of health with those of ecology and systems thinking in an action-research framework applied mostly within a context of social and economic development. Eco health approach concentrate on the interactions between the ecological and socio-economic dimensions of a given condition, and their influence on human health, as well as how people use or impact ecosystems, the implications for the quality of ecosystems, the provision of ecosystem services, and sustainability. This approach is based on six principles: Systems Thinking, Transdisciplinary Research, Participation, Sustainability, Gender and Social Equity and Knowledge to Action [18].

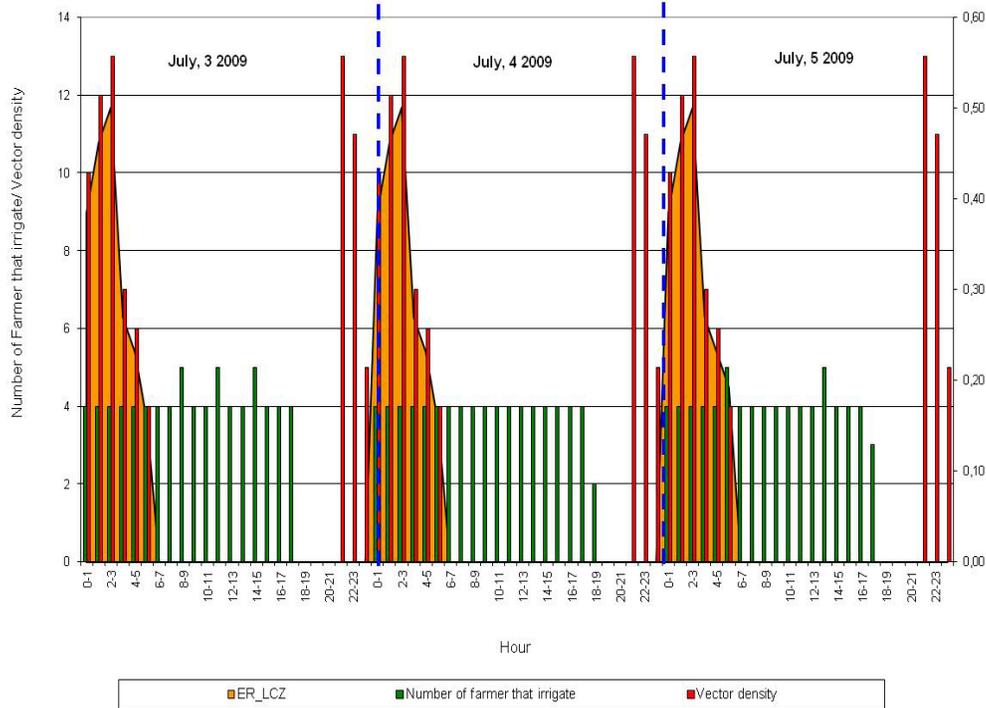
## Results

### Hichria

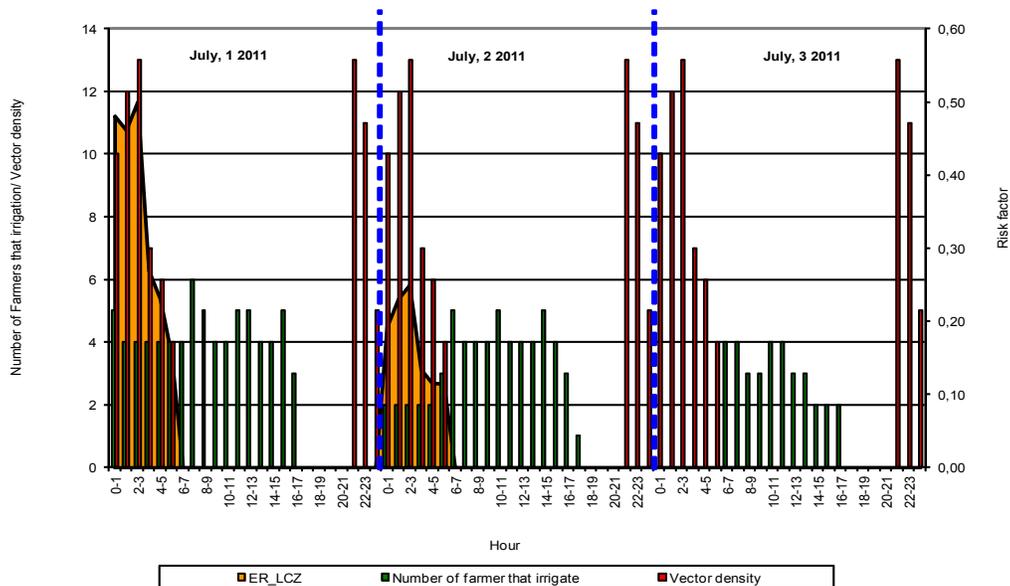
In Hichria, relatively intense agriculture in the irrigation zone results in a high-water demand. The public irrigation zone covers 163 ha and is used by 163 farmers. There are large quantities of olive and pomegranate trees in production that require irrigation during the hot summer months (from June to September). A large part of land in production is outside of the public irrigation zone and is served by private wells as well as by pipes exporting water from the IZ. There has since been no significant expansion of the public irrigation zone; private well construction has continued, however. Seasonal truck farming is common both inside and outside of the public irrigation zone. The additional production increases demand on water resources in the summer months.

A surface irrigation system provides water across the public irrigation zone. Water is pumped from the deep aquifer into a holding tank and then distributed across the zone via surface canals. Up to eight farmers can irrigate simultaneously. Water is lost from surface irrigation systems due to infiltration and evaporation and route from the tank to the irrigated plots. Water loss is exacerbated in Hichria, due to the state of disrepair and generally poor maintenance of the surface canals. These losses decrease water

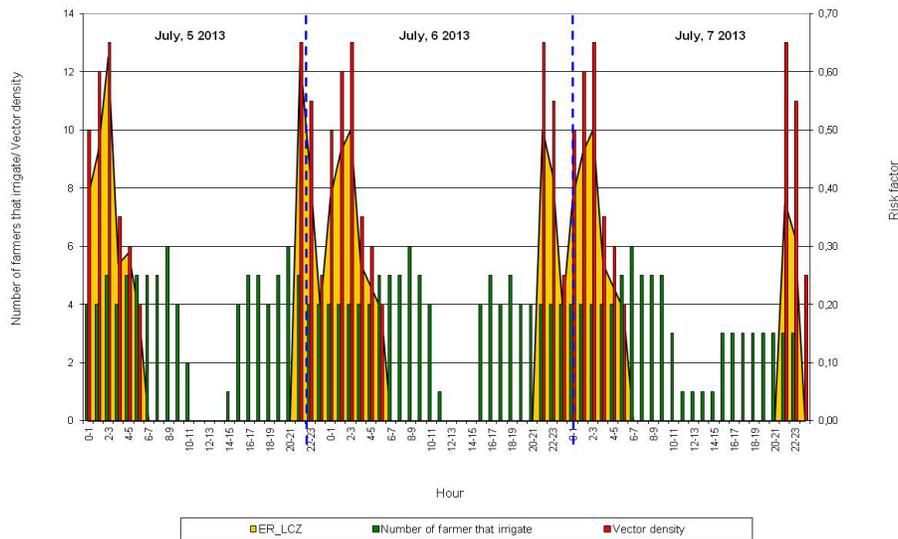
use efficiency and increase the total time required to satisfy demand for irrigation. The demand for irrigation water exceeds the system’s daytime distribution capacity. The GDA has responded by scheduling nighttime irrigation activity. Records from the local GDA indicate that, on most days, irrigation begins at midnight and continues until 19hours. At any time, between three and six farmers are active in the irrigation zone. This schedule is consistent over the study period. Figure 2, 3 and 4 present the farmers and the vectors behaviours in the irrigated area as well as the quantification of the risk factor for peak days in 2009, 2011 and 2013, respectively.



**Figure 2:** Farmer activity in the Hichria irrigation zone and the risk of exposure to *Phlebotomus*, July 3,4 and 5 2009.



**Figure 3:** Farmer activity in the Hichria irrigation zone and the risk of exposure to *Phlebotomus* for July, 1, 2 and 3 2011.



**Figure 4:** Farmer activity in the Hichria irrigation zone and the risk of exposure to *Phlebotomus* for July, 5, 6 and 7 2013.

### Ouled Mhamed

Farming in Ouled Mhamed is less intensive. Most farmers have only olive orchards. There is some end-of-season farming, but seasonal truck farming is not well developed. Farmers indicated less interest in more intensive farming due to the aging irrigation infrastructure, which dates to the early 1970's, and limited income opportunity from farming. A drip and sprinkler irrigation system supply water across the irrigation zone. Water is pumped from a deep aquifer into a holding tank, and then distributed through pressurized underground water pipes to the irrigation terminals. Compared to surface irrigation, this system produces less water loss from evaporation and infiltration. Up to 12 farmers can irrigate simultaneously. As a result of the lower demand for water from less intensive farming and the more efficient irrigation system, demand for irrigation is met during daytime hours, from 06 hours to 18 hours on most days in July and from 05 hours to 16 hours on most days in August. As consequence, there is no exposure risk associated to irrigation in this irrigated area, as computed by the proposed risk factor.

### Entomological Survey

Among the 710 collected sand flies, 453 (63.8%) were identified as *P. papatasi*. It is the most abundant sand fly species in El Hichria. It is distributed across all five biotypes, including in the Irrigation Zone (IZ) and the Livestock Shelter (LS). Spatial distribution differed by sex: females were predominant in each of the irrigation zone (79.3%), extra-habitat (62.8%) and livestock shelter (78.2%) biotypes (Table 1). The predominance of females in these areas suggests that they could be sites for ZCL transmission. The presence of animal shelters, which in El Hichria are always

located adjacent to the homes, could also be an important risk factor for ZCL. The flies were active from 22-23 hours to 05-06 hours each night, with peak activity at 00-01 hour for the species and between 00-03h for females (Figure 1).

- Because of insufficient material, the real-time PCR was carried out only on 20 (8.3%) out of the 241 captured females of *P. papatasi* in 3 ecotopes including IZ and were found to be infected by *Leishmania* (Table 1).

### Discussion

This study explored if and how farming practices in central Tunisia may affect farmers' exposure to infection with ZCL. A comparison of two case communities reveals that different agricultural regimes may produce different exposures of farmers to *P. papatasi*, the vector of ZCL in the area. In El Hichria, due to relatively intensive farming, inefficient surface irrigation technology and limited irrigation capacity, the demand for irrigation exceeds the systems daytime supply capacity. The local GDA, which regulates farmers' access to the irrigation system, responded by scheduling nighttime irrigation activity. Despite having to irrigate through the night, many farmers opt for higher-value irrigated production, over much lower rain-fed production. An entomological survey confirmed the nighttime presence of infected female *P. papatasi*, the lone vector of ZCL, in the irrigation zone. Farmers engaged in nighttime irrigation have an exposure to *Leishmania* during the night time farming activity. The graphs in Figures 2,3 and 4 clearly show that when irrigation is scheduled by night, there is a simultaneous presence of the farmer and the CLZ vector, a positive exposure risk is computed.

It is important to underline that the daily risk factor, computed by equation 1, demonstrated that there is an increase of the exposure risk over time during the study period. Indeed, it is evaluated to 2.00, for the three days of 2009. In 2011, records lead to a risk parameter of about 2.10, 1.04 and 0.00 for 1, 2 and 3 July, respectively. Last values are explained by a reduced irrigation activity in the IZ. The exposure risk factor was estimated to 3.46, 3.15 and 2.73 for 5, 6 and 7 July 2013, respectively, thus and average value of 3.11. Therefore, the average risk factor has increased by 55% between 2009 and 2013. The proposed exposure risk factor allowed the evaluation of the interaction between a technical activity (irrigation), a management behavior (scheduling) and a health constraint (CLZ vector). It can be considered as a sensor of the management performances of the GDA in term of irrigation scheduling. The farming regime in nearby Ouled Mhamed varies considerably. Farmers use more efficient sprinkler and drip irrigation techniques. The aging infrastructure and limited economic opportunities from farming have discouraged investment in more intensive agriculture. Farming is mostly limited to end-of-season olive production. Local demand for irrigation is easily met in daytime hours. Farmers are not in their fields at night, and thus do not have the same exposure. A combination of factors shaped by economic strategies (e.g. farming intensity, crop choices, animal husbandry), technology (e.g. surface vs. drip irrigation) and institutional arrangements (e.g. scheduling of nighttime irrigation) result in a farming practice (nighttime irrigation activity) with which is associated an exposure to ZCL infection. Interventions that target these human environmental determinants of risk may reduce risks of ZCL infection. The focus on irrigation practices is especially salient in the context of observed and anticipated climate warming. Irrigation is already essential for market agriculture in the region, and its economic impact is substantial: the 7% of total productive agricultural land that is irrigated accounts for 35% of total agricultural production. Ben Hamida [11] showed climate warming in Sidi Bouzid over the period 1979-2010 and declining rainfall in the area over the period 1972-2008. Regional climate change scenarios indicate that “Warming and drying tends could continue in coming decades, potentially exacerbating current water scarcity and putting more stress on agriculture” [17]. Drier conditions will increase the demand for irrigation, but will also encourage new policy responses and investment to increase irrigation system efficiency. Changes in irrigation coverage and practices may have implications for ZCL epidemiology and, as demonstrated in this study, exposures. In a review of the socio-ecology of zoonotic infections, Cascio, et al. [18] highlight the need for pre-emptive studies on the effects of massive or smaller development projects on local animal fauna and zoonotic reservoirs. Such studies might also consider the individual human practices - in this case, the implications of irrigation development on farming practices - that may influence disease epidemiology.

- Additional research is required to further specify and quantify these and related human environmental determinants of risk of ZCL in central Tunisia. While this study has demonstrated that nighttime irrigation activity in the Hichria public irrigation zone puts farmers in the vicinity of the infected ZCL vector, thus creating a particular exposure to infection for farmers irrigating at night, it has not quantified the risk of infection. The focus on nighttime irrigation is to the exclusion of attention on other farming tasks, such as threshing, hoeing and application of pesticide treatments, which generally start early in the morning, end after sunset, and involve a greater number of people. A more complete and precise understanding of risk of ZCL exposure and infection attributable to various farming practices may inform the design of targeted risk-reducing interventions. An update of the formulated exposure risk factor in this contribution will be required to cover all aspects of agricultural activities.
- Results from the entomological survey showed that the 20 sand flies screened for *Leishmania* were positive. Sensitivity and specificity of real-time PCR is currently reported to be the highest of conventional techniques commonly used [19,20]. This PCR can detect 0.0005 parasite per 1 µl of DNA extract [19]. Moreover, the study was performed in September where the infection rate is the highest. Absent a vaccine, effective and practicable vector or reservoir host control method, or effective methods for treating the disease [21-24], public health professionals are challenged to find alternative preventive measures to reduce ZCL morbidity amongst at-risk populations. In this study, we showed how, due to a combination of factors, farmers in one community opted to practice nighttime irrigation, creating for them a particular exposure to ZCL infection. In a nearby community, because of different farming practices, farmers do not engage in nighttime irrigation and thus do not face this same exposure. Understanding of these human environmental determinants through an Eco Health approach suggests of new possibilities for ZCL risk reduction, such as changing of types of farming and irrigation practices, which reduce exposure to the ZCL vector during its active hours.

## Ethics Statement

The “Ethic committee” approved the study since we didn’t need any clinic or biological investigation from all subjects with the guarantee from the research team about confidentiality and the respect of individual information.

## Acknowledgment

The authors would like to thank N Achour, National Observatory of New and Emerging Diseases, Z Ahmadi, Sidi Bouzid Departmental Health Authority, M Mhamedi and T Jelali,

Departmental Agriculture Authority and N Bchir and N Karoui, Tunisia Agency for Technical Assistance and Cooperation, for their logistical support and participation that in this research. Thanks also to B Zaafouri, who facilitated all field activities, and to the Agricultural Development Groups and all farmers for their participation. Special thanks to David Noble, 2degreesC Inc., for his writing and editorial support in preparing this paper for publication.

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