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Assessment of Incubation Period of Cutaneous Leishmaniasis due to *Leishmania major* in Tunisia

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Abstract. The period between the infective sandfly bites and appearance of cutaneous leishmaniasis (CL) lesions is still hypothetical and little studied. This work aimed at assessing the incubation time of zoonotic CL (ZCL) due to *Leishmania major* using a standardized methodology. The retrospective analysis used the epidemiological, clinical, and biological information available in the database recording all the CL cases diagnosed at the Parasitology Department of the Pasteur Institute of Tunis during 2015–2019. It allowed for the selection of 92 privileged observations 1) of confirmed CL cases with presentation suggestive of ZCL form 2) living in northern regions free of ZCL 3) with a single infective trip of less than a week to ZCL foci during transmission season and 4) with accurate dates of travel and onset of lesions. Incubation length computed in this population ranged from 1 to 21 weeks, with a median of 5 weeks (interquartile range: 3–8.5 weeks).

Cutaneous leishmaniasis (CL) is caused by protozoa of genus *Leishmania* and transmitted by sandfly bites.[1,2] Three CL forms are endemic in Tunisia.[1,3] The zoonotic CL (ZCL) form caused by *Leishmania* (*L.*) *major* is the most prevalent with about 95% of recorded cases.[1] It prevails in the central and southern parts of the country where it represents an important public health threat.[1] The two other CL forms are the sporadic CL (SCL) due to *Leishmania infantum* restricted to the northern regions and the chronic CL (CCL) caused by *Leishmania tropica* mainly encountered in the southeast.[1,3] The silent CL incubation period corresponds to the time lasting between *Leishmania* inoculation by infected sandflies and the appearance of clinical signs, that is, CL skin lesions.[2] This period was estimated by very few studies and has been largely approached in a hypothetical manner.[2,4,5] To our knowledge, no work studied specifically the ZCL incubation length on a large number of individuals. This was probably because it is not easy to determine the precise contamination date in endemic areas where inhabitants are exposed to sandfly bites for months or even all year round. Furthermore, dates of lesion appearance are often imprecisely reported, making the calculation of incubation times even more difficult. The objective of this study was to assess the incubation period of ZCL lesions in Tunisia on the basis of data collected from privileged CL cases with fairly accurate dates of contamination and onset of lesions.

This retrospective study used epidemiological, clinical, and biological information available from the database recording all CL cases diagnosed at the Parasitology Department of the Pasteur Institute of Tunis. All information have been collected as part of the routine diagnostic process, and no personal data were used in this study. From January 2015 to November 2019, 257 CL cases referred by physicians, mainly dermatologists, were recorded in the database. Diagnosis was carried out on dermal scraping of CL lesions using microscopic examination of Giemsa-stained smears (203 cases) and real-time PCR targeting *Leishmania* kinetoplastic DNA (54 additional cases). All the 257 CL cases were pseudonymized, and information concerning age, gender, district of residence, travel to ZCL endemic areas, dates of travel/onset of lesions/seeking medical advice, number of lesions, their location, and *Leishmania* species identification were reported on an anonymous sheet. To estimate the incubation period, only the CL cases who met the specific inclusion criteria were selected for analysis. These were 1) a residence in the northern governorates of Tunisia, where CL incidence is extremely low and ZCL absent; 2) a single travel to center or southern governorates where ZCL is highly prevalent and where proportion of ZCL cases is much higher than CCL ones; 3) several characteristics of the trip which has to be short (less than a week), took place from June to November (which corresponds to sandfly's activity season in Tunisia), and no more than 6 months before diagnosis (to avoid overlapping transmission seasons); and 4) fairly accurate records concerning dates of travel and onset of lesions. Following these criteria, we selected 92 cases for which we can assume that individuals have probably been infected during their trip to ZCL foci and for whom we can calculate with enough precision the incubation time of CL lesions. Among these 92 patients, 13 had information about the *Leishmania* species identification: five from positive Novy, McNeal, Nicolle cultures by multilocus isoenzyme electrophoresis and eight from molecular analysis by PCR and restriction fragment length polymorphism.[6,7] All the 13 identified strains corresponded to *L. major* species.

Data concerning dates were homogenized for all cases as follows. Each month was divided into 4 weeks (day [D]1–7, D8–14, D15–22, and D23–30/31) beginning from July 2014 until November 2019. Each recorded date was assigned by the corresponding week. When the period of the trip overlapped 2 weeks, the week for which the patient stayed the longest was retained. The incubation period was calculated by subtracting the week allocated to the trip from that allocated to the lesion onset and the consultation delay by subtracting the week allocated to the lesion onset from that allocated to the medical consultation. Statistical analysis was performed using IBM SPSS (statistics 23) software (IBM Corp., Armonk, NY). Qualitative variables were described by percentages, and quantitative variables were expressed by means and SD or by the medians and interquartile range (IQR). We compared

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TABLE 1
Main characteristics of the 92 CL cases

Gender ratio (Male/Female)	1.56 (56/36)
Age (years)	Mean ± SD: 31 ± 22 Range: 1.33–86
Purpose of travel to endemic zoonotic CL foci	Eid el Kebir day (28.2%), summer holidays (25%), and work (17.3%)
Number of CL lesions	Mean ± SD: 3 ± 2.5
Location of lesions	83.6% on the limbs
Incubation time (weeks)	Median: 5 Range: 1–21

CL = cutaneous leishmaniasis.

means using the Student's independent *t*-test. The Pearson correlation test was applied to compare two quantitative variables. The significance level was set to 5% for all tests.

Among the 92 CL patients, 56 were males and 36 females, the mean age was 31 ± 22 years, with a range from 16 months to 86 years (Table 1). All were resident in northern part of the country, with 85.8% of them living in the Great Tunis region (Figure 1). Twenty-three (25%) traveled to Gafsa (Southwest), 21 (22.8%) to Tataouine (Southeast), and 19 (20.7%) to Kairouan (center) (Figure 1). These three governorates are among the most endemic for ZCL in Tunisia.[3,4] Most patients moved during August (n = 48, 52.2%) and September (n = 24, 26%) (Figure 2). The motive of the travel was celebration of Eid el Kebir day (n = 26, 28.2%), summer holidays (n = 23, 25%), and work (n = 16, 17.3%) (Table 1). The majority of CL lesions (85.8%) appeared between August and November, whereas medical consultations took place mainly between October and January (84.7%) (Figure 2). Average consultation delay was 7.8 ± 4.5 weeks (range: 1–22 weeks). The mean number of CL lesions per patient was 3 ± 2.5, and limbs were the most affected (77 patients, 83.6%) (Table 1). Incubation times ranged from 1 to 21 weeks, with a median of 5 weeks (IQR: 3–8.5) (Figure 2) and an average of 6.5 weeks ± 4.5. No significant difference in incubation time was observed with respect to gender, age, month of travel, location, or number of lesions.

Incubation time during leishmaniasis remains little studied. Interesting study designs are those monitoring naive not exposed subjects who travel to endemic areas. Such situations concern workers who are displaced to build dams and roads or to exploit mining or forest resources as well as scouts in holiday camps and army-deployed personnel.[8–10] However, the usual long stay duration may leave uncertainties about the effective date of the sandfly's infecting bites.[8,10] To avoid this

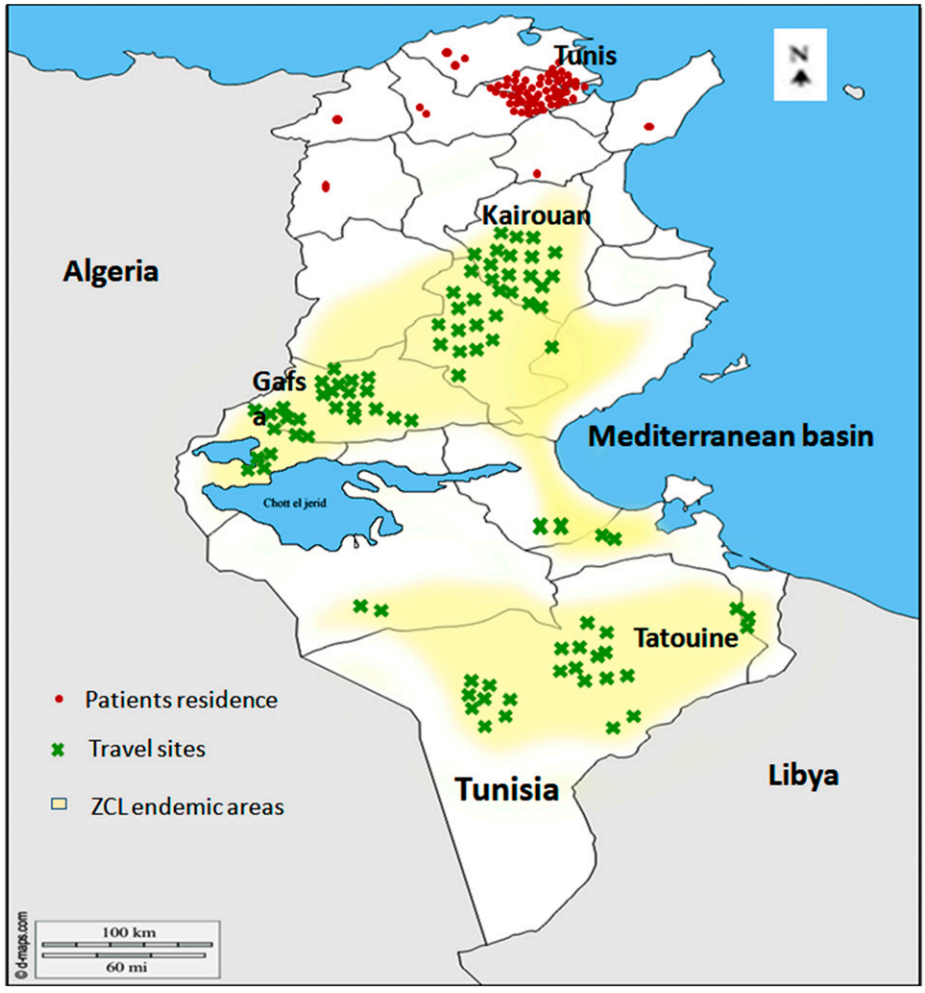

FIGURE 1. Geographical locations of residences and travels of cutaneous leishmaniasis (CL) cases. On this map of Tunisia, zoonotic CL (ZCL) foci are colored in yellow. All CL cases lived in the northern zones outside the geographic distribution of ZCL and moved during their trip to central and southern hot spots of the disease. This figure appears in color at www.ajtmh.org.

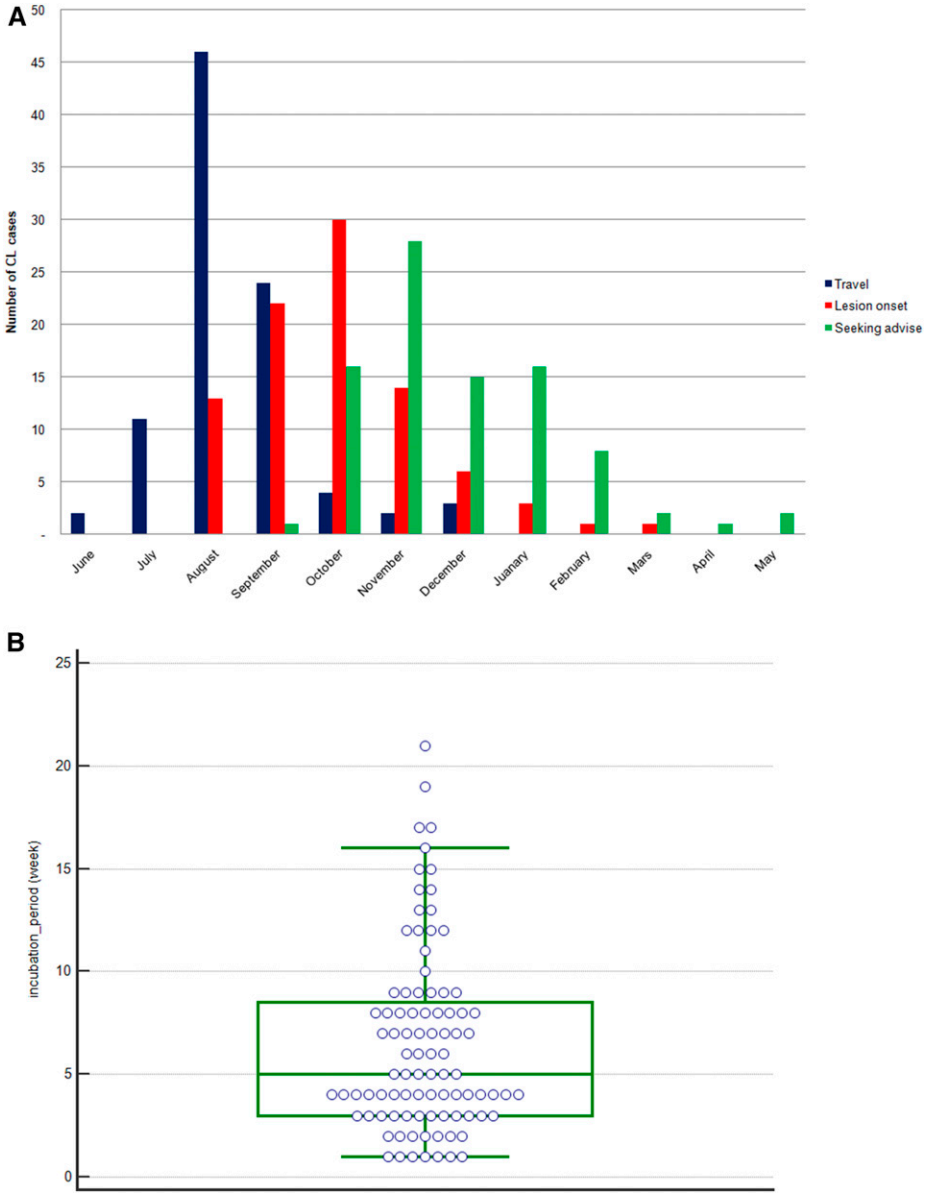

FIGURE 2. Estimation of the incubation period of cutaneous leishmaniasis (CL). (**A**) Distribution of the CL case number according to the month of travel to endemic zoonotic CL foci, onset of lesions, and medical-seeking advice. This graph shows the gap between the likely contamination period (mainly August–September) and lesion onset (mainly September–October) and first medical consultations (mainly October–January). (**B**) Incubation periods of CL cases. In this graph, estimated incubation periods are reported by week and plotted for all CL cases. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum value, excluding outside and far out values which are displayed as separate plots. This figure appears in color at www.ajtmh.org.

bias, we selected only very short trip to endemic areas. Most of our patients traveled during August and September which correspond to the peak of density of *L. major* vector, that is, *Phlebotomus papatasi*.[11] Although several reasons for travel were reported, our study has taken benefit from the fact that Eid el Kebir celebration day, depending on the lunar calendar, has matched over 2014–2019 with the summer period of intensive *L. major* transmission.[1,11] This important annual religious event for Muslims is associated with a 3-day holiday and is a usual reason for Tunisian people to visit their families in native endemic regions. Most CL patients sought medical advice between October and January for CL lesions mainly multiple and preferentially affecting limbs. These epidemiological and clinical presentations were highly suggestive of ZCL in Tunisia and corroborate results of *Leishmania* species identification performed on several cases isolates.[1,3] The median incubation period of *L. major* lesions was estimated to be 5 weeks (IQR: 3–8.5) which was fairly short compared with that of *Leishmania braziliensis* CL reported by Oré in Peru.[11] This short period could be explained by the high virulence of *L. major*.[12] This estimated incubation period of *L. major* showed, however, a large variation from 1 to 21 weeks. Such large intervals have been reported by many authors.[2,4,5,10] It could be explained by the huge pattern of virulence of *L. major* infective isolates already suggested by the great polymorphism of ZCL lesions and shown in mouse models and by genome sequencing of strains.[13–16] Several other factors such as the individual genetic susceptibility and immunity of the

human host,[13] the number of inoculated promastigotes,[17] the sandfly's saliva composition, and the skin microbiota could also play a role.[18,19]

Cutaneous leishmaniasis incubation times are interesting data that can be useful in routine practice. They could weaken or strengthen CL suspicions by analyzing the interval between a risky travel and the date of lesion appearance. Thus, it would be unlikely that lesions appearing a few days or more than 4 months after a visit to an endemic zone could correspond to ZCL. However, vigilance must be kept because seven (7.6%) patients have reported the emergence of their lesions only 1 week after their displacement. Such short delays have been reported by some authors.[5,6,20] The incubation periods may also be used to estimate the age of lesion and consequently adapt the therapeutic option.[4] Antimonials are the first-line CL treatment in Tunisia. Abstention could be recommended for old lesions of the limbs that have started healing. Finally, incubation time estimation would be helpful for ZCL control by identifying the parasite's high transmission period. Such data will guide and optimize the implementation of preventive and struggle measures, especially those directed against sandflies.

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REFERENCES

1. Aoun K, Bouratbine A, 2014. Cutaneous leishmaniasis in North Africa: a review. *Parasite 21:* 1–9.
2. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S, 2007. Cutaneous leishmaniasis. *Lancet Infect Dis 7:* 581–596.
3. Aoun K, Ben Abda I, Bousslimi N, Bettaieb J, Siala E, Ben Abdallah R, Benmously R, Bouratbine A, 2012. Comparative characterization of skin lesions observed in the three endemic varieties of cutaneous leishmaniasis in Tunisia. *Ann Dermatol Venereol 139:* 452–458.
4. Gradoni L, López-Vélez R, Mokni M, 2017. *Manual on Case Management and Surveillance of the Leishmaniases in the WHO European Region*. Copenhagen, Denmark: WHO Regional Office for Europe.
5. Nacher M, Carme B, Sainte Marie D, Couppié P, Clyti E, Guibert P, Pradinaud R, 2001. Seasonal fluctuations of incubation, healing delays, and clinical presentation of cutaneous leishmaniasis in French Guiana. *J Parasitol 87:* 1495–1498.
6. Rioux JA, Lanotte G, Serres E, Pratlong F, Bastien P, Perieres J, 1990. Taxonomy of *Leishmania*. Use of isoenzymes. Suggestion for a new classification. *Ann Parasit Hum Comp 65:* 111–115.
7. Schönian G, Schnur L, El Fari M, Oskam L, Kolesnikov AA, Sokolowska-Köhler W, Presber W, 2001. Genetic heterogeneity in the species *Leishmania tropica* revealed by different PCR-based methods. *Trans R Soc Trop Med Hyg 95:* 217–224.
8. World Health Organization, 2020. *Leishmaniasis*. Geneva, Switzerland: WHO. Available at: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed March 2, 2020.
9. Felinto de Brito ME, Andrade MS, Lima de Almeida E, Medeiros AC, Pereira Werkhäuser R, Freitas de Araújo AI, Brandão-Filho S, de Almeida AM, Gomes Rodrigues EH, 2012. Occupationally acquired American cutaneous leishmaniasis. *Case Rep Dermatol Med 2012:* 279517.
10. Oré M et al., 2015. Outbreak of cutaneous leishmaniasis in Peruvian military personnel undertaking training activities in the Amazon basin, 2010. *Am J Trop Med Hyg 93:* 340–346.
11. Chelbi I, Derbali M, Al-Ahmadi Z, Zaafouri B, El Fahem A, Zhioua E, 2007. Phenology of *Phlebotomus* papatasi (diptera: psychodidae) relative to the seasonal prevalence of zoonotic cutaneous leishmaniasis in central Tunisia. *J Med Entomol 44:* 385–388.
12. Launois P, Conceicao-Silva F, Himmerlich H, Parra-Lopez C, Tacchini-Cottier F, Louis JA, 1998. Setting in motion the immune mechanisms underlying genetically determined resistance and susceptibility to infection with *Leishmania major*. *Parasite Immunol 20:* 223–230.
13. Chang KP, Mc Gwiere BS, 2002. Molecular determinants and regulation of *Leishmania* virulence. *Kinetoplastid Biol Dis 1:* 1–7.
14. Darabi S, Khaze V, Riazi-Rad F, Darabi H, Bahrami F, Ajdary S, Alimohammadian MH, 2015. *Leishmania major* strains isolated from distinct endemic areas show diverse cytokine mRNA expression levels in C57BL/6 mice: toward selecting an ideal strain for the vaccine studies. *Cytokine 76:* 303–308.
15. Kébaïer C, Louzir H, Chenik M, Ben Salah A, Dellagi K, 2001. Heterogeneity of wild *Leishmania major* isolates in experimental murine pathogenicity and specific immune response. *Infect Immun 69:* 4906–4915.
16. Ghouila A, Guerfali FZ, Atri C, Bali A, Attia H, Sghaier RM, Mkannez G, Dickens NJ, Laouini D, 2017. Comparative genomics of Tunisian *Leishmania major* isolates causing human cutaneous leishmaniasis with contrasting clinical severity. *Infect Genet Evol 50:* 110–120.
17. Jude E, Uzonna JE, Joyce KL, Scott P, 2004. Low dose *Leishmania major* promotes a transient T helper cell type 2 response that is down-regulated by interferon-producing CD8 T cells. *J Exp Med 199:* 1559–1566.
18. Titus RG, Ribeiro JM, 1988. Salivary gland lysates of the sandfly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. *Science 239:* 1306–1308.
19. Gimblet C et al., 2017. Cutaneous leishmaniasis induces a transmissible dysbiotic skin microbiota that promotes skin inflammation. *Cell Host Microbe 22:* 13–24.e4.
20. Aoun K, Chetoui A, Rhaiem A, Ghrab J, Dani R, Bouratbine A, 2005. Early cutaneous leishmaniasis in a 7-day-old newborn. *Med Trop 65:* 394–395.