# The geographical distribution patterns of human *Leishmania* species detected within molecular methods in Iraq: a systematic review and meta-analysis.

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**Abstract**

The aim of this reference study on the spread and distribution of various parasites was to aid in the control and treatment of these parasites and save researchers time and effort by providing them with essential and valuable information. This systematic review includes articles related to the detection of *Leishmania* species through molecular diagnostic methods published in Arabic and English from 2009 to 2021. From five international and local databases Google Scholar, PubMed, Science Direct, Scopus and Iraqi Academic Journals, we extracted 35 papers that satisfied the inclusion criteria and were eligible for systematic review and meta-analysis. This systematic review attempted to answer the following question: Did *Leishmania* species follow a specific pattern in its geographical distribution in Iraq? The systematic review results show that *Leishmania major* is distributed from the northern provinces (the least prevalent) to the southern provinces (the highest prevalence). On the other hand, *L. tropica* follows a heterogeneous distribution pattern, which is the highest in the northern and the lowest in the southern regions. The meta-analysis shows the presence of *L.major* in 1226/2542 (48.23%; 95%CI 09.60-101.21) and *L.tropica* in 1313/2542 (51.65%; 95%CI 29.87-119.56). As a diagnostic method, most articles reported the use of conventional polymerase chain reaction (PCR) or nested-PCR. Most of these used ITS-1 and KDNA as genetic markers. In conclusion, However, the movement of populations (immigration and displacement) among different governorates can sometimes change this distribution curve. The distribution of *Leishmania* species follows a specific pattern influenced by the presence of the parasite vector and climatic conditions. This pattern maintains *L.tropica* prevalence in the northern regions and *L.major* superiority in the southern regions.

**Keywords**

Cutaneous leishmania; visceral leishmania; systematic review; molecular detection; Iraq.

High light

The geographical distribution of Leishmania species is subject to climate factors and the presence of vectors and does not differ from the global geographical distribution.

ACL is prevalent in southern Iraq, while ZCL is more common in central and northern Iraq.

Wasit is the governorate with the most cases of ACL, and Baghdad recorded more cases of ZCL.

The use of molecular detection methods is the best and most appropriate method for parasite diagnosis. Also, the use of ITS1 and kDNA parameters gives more accurate results.

## Introduction

Leishmaniasis is an epidemic that has infected pupils in over 90 countries. The annual infection rate is 1500000 (1). There are now three types of Leishmaniasis: cutaneous (CL), visceral (VL) and mucocutaneous (MCL). In the old world, leishmaniasis resulted from one of three species: *Leishmania major, L.tropica* and *L.infantum* (2). Although humans are the common parasite carriers and reservoir hosts, the parasite can also be transmitted by wild animals such as rodents and dogs, which are more common in rural and forested areas (zoonotic infections) (3). The parasite cycle includes two phases: the infective phase (promastigote), which resides in the stomach and salivary glands of the sand fly (*Phlebotomus* spp.), and the amastigote phase, which resides in the definitive host (human) and is later transmitted to mosquitoes after a blood meal (4).

The epidemic geographical distribution and transmission are associated with several factors, including the vector's (sandfly) presence and the migration of people from rural into suburbs or urban (5). In infected pools, mammals constitute a reservoir of parasite-infected animals (6). Rescue changes in environmental conditions and human migration are factors that lead to advance the spread of the epidemic, leading to spatial and temporal changes (7). For example, 40,000 people were infected in Delhi during the early 1940s, but there are rarely any infections in Delhi today (8).

The prevalence of cutaneous leishmaniasis is often underestimated because most official statistics are obtained through an approximate expected survey based solely on the number of patients who visit official health institutions (9). In addition, multiple factors lead to the diagnosis of a faulty or undiagnosed infection, including limited or absent access to diagnostic services to medical facilities (10), epidemiological expansion and the slow progression of the disease. *L.major* produces ulcers near the bite site only, so the spread of these infections is usually late and only into the adjacent skin (causing accompanying lesions). However, these morphological characteristics must be unique to *L. major*, since *L.tropica* have been reported to cause visceral infections (far from bit site) (11).

The presence of an infection is primarily confirmed via optical and microscopic diagnoses. Serology or cultures are also used to detect parasites (12). Although the presence of the parasite can be revealed by all these methods, they cannot be used to identify the species. Precise geographical and epidemiological maps for these species are thus difficult to establish. However, the diagnosis of species and sub-species has produced a genetic tree for this parasite(13).

This systematic review aims to identify the factors affecting the distribution and transmission of Leishmania species in Iraq that are diagnosed by molecular methods.

## Methods

### Search Methodology:

This systematic review assesses the extent of *Leishmania* spp. distribution in the various provinces of Iraq and attempts to answer the following question: can the geographical location in Iraq influence the distribution of *Leishmania* spp.? We scanned all the articles published in various scientific databases (Google Scholar, PubMed, Science Direct, Scopus and Iraqi Academic Journals) between the years 2009 and 2021. We searched for Medical Subject Heading (MeSH) terms such as Baghdad boil, visceral leishmaniasis, cutaneous leishmaniasis, genotyping, and molecular diagnosis in Iraq using Arabic and English.

### Inclusion Criteria:

Articles discussing Leishmania spp. and meeting the following conditions were included: use of epidemiological and molecular detection methods sole focus on *Leishmania spp*., no discussion of approaches to therapy or pathology, and published from 2009 to 2021. All documents not fulfilling these conditions were excluded (Fig. 1).

**Data Extraction:**

Each of the selected 35 articles was divided into one of two categories: (1 the spread of cutaneous leishmaniasis and (2) the spread of visceral leishmaniasis. From these articles, the following information was extracted: the year of publication, governorate, sample size, infection rate, Leishmania species, the molecular detection method and gene marker.

**Statistical Analysis**

To conduct the meta-analysis, we used GraphPad Prism Version 7.

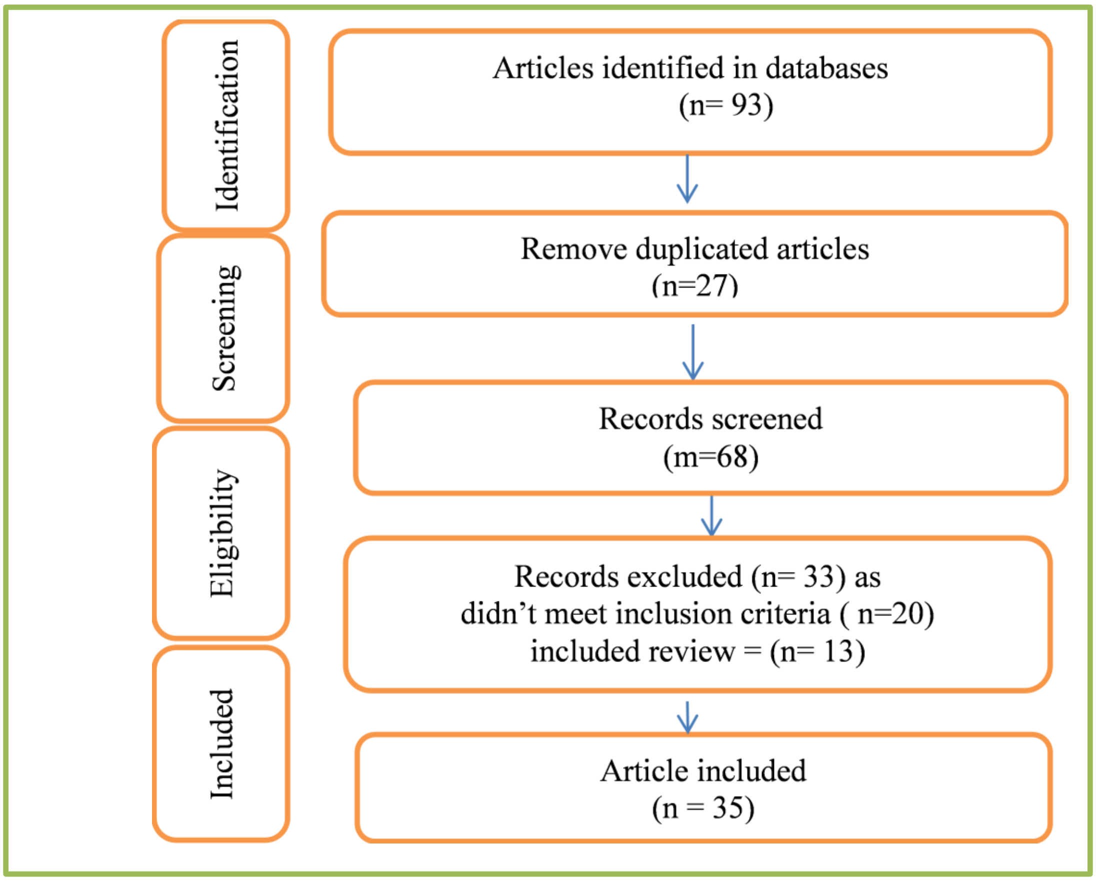


Fig.1 summary diagram of systematic flowing.

**Results**

Scanning five scientific databases yielded 93 research papers and 58 articles not meeting the criteria of the study were excluded. A total of 35 studies were evaluated, 33 of which focused on the prevalence of cutaneous leishmaniasis (CL) and two of which dealt with visceral leishmaniasis only (Fig. 1).

In total, 7,842 subjects were examined. Of these, 2,542/7,205 (35.28%) were confirmed to be infected with cutaneous leishmaniasis (Tab. 1) and 190/637 (29.28%) with visceral leishmaniasis (Tab. 2). In the cutaneous leishmaniasis group, patients infected with *L.tropica* were 1,313/2,542 (51.65% , %95CI: 29.87-119.56) with *L.major* were 1,226/2,542 (48.23%, %95CI: 09.60-101.21) (Tab. 5 , Fig. 2). In the visceral leishmaniasis group, *L. tropica* was found in 148/190 (77.89%) patients, whereas L. *Infantum* was found in 42/190 (22.11%) patients (Tab. 2).

In Baghdad and the middle provinces, the prevalence of *L. tropica* was higher, whereas L. *major* was more common in the southern and middle - Euphrates (Tab. 3). Wasit had the highest *L.* major prevalence at 262/332 (%95 CI: 0.29-0.354), whereas

Baghdad had the highest *L.tropica* prevalence at 303/426 (%95 CI: 0.13-0.18). *L. aethiopica* was only detected in Tikrit province (3/117) (Tab. 3, Fig. 3). The year 2018 recorded the highest prevalence of 1,071/1,400 (76.5%). Of these, 518 (%95 CI 0.37-0.43) were infected with *L. tropica* and 553 (% 95 CI: 0.42-0.47) with *L. major*. (Fig. 4, Tab. 4).

Skin biopsy was the most common detection procedure, whereas blood samples were used as a detection method in only two research papers. Commercial PCR was used in 11 papers (752 patients) (495 were *L. tropica*: 254 were *L. major*), real-time PCR and RFLP were used twice for all one method, and nested-PCR was used in 17 papers (1,457 patients) (648 were *L. tropica*: 827 were L. *major* )(Tab. 1). The genetic marker ITS-1 was used in 11 papers, the KDNA in 18 papers, and the protein kinase in one paper (Tab. 1).

**Discussion**

Leishmaniasis has spread significantly in Iraq in recent years, a total of 8691 cases of infections were reported by the World Health Organization(WHO) in 2020 (14). The reason for this significant increase in infections is the great population growth (15), the presence of vectors and climatic temperature and humidity conditions (16). There are two types of Leishmaniasis (cutaneous and visceral) in Iraq (17). Various methods can be used to detect Leishmania spp. but, it is not possible to differentiate between the types that cause the disease except with the use of the molecular method (18). The goal of this systematic and meta-analysis review was to determine the geographic distribution of *Leishmania* species and to confirm whether this distribution follows a specific pattern.

In Iraq, there have been very few epidemiological studies using molecular methods. The number of papers examined clearly shows this. Perhaps this is because parasitologists depend on the dermatologist's initial diagnosis and rely on cases that reach hospitals without performing a comprehensive survey of the entire region(10). Most of the previous studies aimed to find ways to treat or determine the immunological or physiological effect of the presence of the parasite in the patient's body.

According to the findings of the study, cutaneous leishmaniasis is more common than visceral leishmaniasis in Iraqi provinces. Although the study included 15 out of 18 governorates, the data show that cutaneous leishmaniasis has spread further, while visceral leishmaniasis has been limited because the sand fly (*P. alexandri*) which transmits *L. infantum* is rare in Iraq (19), or the occurrence of the parasite must be transmitted through the blood to infect areas far from the site of the bite for the disease to occur (20). The efforts of the Iraqi Ministry of Health and the World Health Organization, on the other hand, have played a significant role in reducing the prevalence of visceral leishmaniasis (21).

The current systematic review found that the prevalence of *L. tropica* (ZCL) is greater than that of *L. major* (ACL). This is due to the displacement of many people from the northern governorates and their movement to the southern and central regions after the ISIS invasion of Iraq, with many living in unhygienic conditions in the camps, and the proximity of the transmission foci of *L. tropica* on the Iraq_Iran border, in addition to the presence of parasite vectors (rodents and rats) (22).

The presence of the sandfly is the primary cause of the increase in the rate and spread of the foci of ACL(23). Sandfly species (*P. papatasi, P. sergenti and Sergentomiya sentoni*) have been confirmed in southern Iraq by Al-Mayali and Al-Hassani (24). Moreover, a study by Al-Bajalan *et al*. reported that, in addition to other mammalian hosts and vectors, the propagation of the sand fly species *P. papatasi* is only present in the northern regions (25). All of these factors have influenced the spatial and geographical distribution of cutaneous leishmaniasis in Iraq. The ACL spread is also affected by certain climate factors such as humidity and temperature, which effectively contribute to the vitality and spread rate of the parasite (26).

Even in a single province, the distribution of cutaneous leishmaniasis continues geographically and spatially. The prevalence rate of ACL is higher in each of the southern provinces than that of ZCL, and vice versa in the central and northern provinces. The growing population and the displacement of many residents in other provinces is the cause of the high infection rate in Baghdad (27) and of the surging waves after the ISIS invasion. Wasit province (southeast of Baghdad) exhibits the highest number of ACL infections due to the presence of parasite vectors (28). Most of the governorate's areas border on Iran (29). In addition, studies in this governorate examined large numbers of infected people. The highest prevalence rate of leishmaniasis was in 2018, because we adopted only molecular studies and the number of research papers in this year was more, this finding does not agree with what was published by the World Health Organization (2).

In systemic testing, two genetic markers, ITS1 and kDNA have been reported in the majority of previous studies, with a tendency to use KDNA more often. Previous studies indicated that the sensitivity of kDNA is higher than that of ITS-1and that it can detect fewer than 200 DNA copies. Therefore, the use of either primer gives a better diagnostic result(30).

## Conclusion

The present review concluded that the Leishmania species' spatial and geographical distribution is a result of climate change and parasite vector presence. This distribution does not differ in the prevalence of Leishmania species from the general distribution. The review is a database allowing the number of infections in Iraqi regions over several years to be estimated by researchers and by epidemiological studies interested. It's not new to say that molecular diagnosis is the best approach. The most suitable diagnosis is the use of genetic markers ITS1 and kDNA.

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## References

Al-Naimy AFA. & Al-Waaly ABM. Investigation of Cutaneous Leishmaniasis Cases in Baghdad province, Iraq. Medico Legal Update. (2021); 21(1), 65-69. https://doi.org/10.37506/mlu.v21i1.2282.

Sundar S. Singh OP. Molecular Diagnosis of Visceral Leishmaniasis. Mol Diagn Ther. 2018;22(4):443-457. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6301112/

Alsaad RKA. & Kawan MH. In vitro Cultivation of Leishmania spp. using Blood from Human and Dog in Novy-MacNeal-Nicolle (N.N.N.) Modified Medium. Annals of RSCB. 2021;25(6):17753-62.
https://annalsofrscb.ro/index.php/journal/article/view/9137

Agha NFS. Al-khayat ZAY.Alharmni KIF. incidence of cutaneous leishmaniasis among patients presented to hawler teaching centre for dermatology diseases, Erbil-

Iraq, from January 2015 to December 2017. Polytechnic Journal. 2018;8(3):102-120. https://doi.org/10.25156/ptj.2018.8.3.247.
Mahmood OI. Taha Z. ASHOOR MH. Prevalence of cutaneous leishmaniasis among refugee camps in Salahdeen province, Iraq. Assiut Vet. Med.J. 2019;65 (16) :259-262. https://doi.org/10.21608/avmj.2019.168908.
Echchakery M. Chicharro C. Boussaa S. et al. Molecular detection of Leishmania infantum and Leishmania tropica in rodent species from endemic cutaneous leishmaniasis areas in Morocco. Parasites Vectors 2017;10:454. https://doi.org/10.1186/s13071-017-2398-8.
OryanA. Akbari M. Worldwide risk factors in leishmaniasis. Asian Pacific Journal of Tropical Medicine. 2016; 9(10): 925–932.
http://dx.doi.org/10.1016/j.apjtm.2016.06.021.
Mokni M. Leishmanioses cutanées [Cutaneous leishmaniasis]. Ann Dermatol Venereol. 2019;146(3):232-246. https://doi.org/10.1016/j.annder.2019.02.002.
Naghian A. Oshaghi MA. Moein-Vaziri V. et al. Molecular Identification of Leishmania Species in Phlebotomus alexandri (Diptera: Psychodidae) in Western Iran. J Arthropod Borne Dis. 2020;14(1):8-16. http://doi.org/10.18502/jad.v14i1.2699.
Sharquie KE. Noaimi AA. Saleh BA. Cutaneous Leishmaniasis as Imitator of Skin Diseases and a Diagnostic Challenge. Journal of Cosmetics, Dermatological Sciences and Applications, 2018;8(3):158-177.
https://www.scirp.org/journal/paperinformation.aspx?paperid=87509
Abdulla QB. Shabila NP. Al-Hadithi TS. An An outbreak of cutaneous leishmaniasis in Erbil governorate of Iraqi Kurdistan Region in 2015. The Journal of Infection in Developing Countries. (2018);12(08):600-607. https://doi.org/10.3855/jidc.10306.
Alwan HR. Erzaige ZS. Estimation of immunoglobulin ( IgG and IgM) in sera cutaneous leishmaniasis patients (L.donovani and L. infuntum) in Baghdad city. Al-Kufa University Journal for Biology . 2019 ;11 (1):95-100. https://www.iasj.net/iasj/article/178093.
Shakir OM. Jasim MM. The use of ana antibody in the diagnosis of leishmania. Biochem. Cell. Arch. 2019;19( 1): 2055-2060. http://doi.org/10.35124/bca.2019.19.S1.2055.
WHO. Number of cases of cutaneous leishmaniasis reported Data by country.
https://apps.who.int/gho/data/node.main.NTDLEISHCNUM?lang=en
Al-Hayali HL. Al-Kattan MM. Overview on Epidemiology of Leishmaniasis in Iraq. Rafidain Journal of Science. 2021;30(1): 28-37. https://rsci.mosuljournals.com/article_167680.html
Al-Warid HS. Al-Saqur IM. Al-Tuwaijari SB. AL Zadawi KA. The distribution of cutaneous leishmaniasis in Iraq: demographic and climate aspects. Asian Biomedicine. 2017;11): 255 – 260. https://doi.org/10.5372/1905-7415.1103.556.
Lazar LY. & Abass KS. Morphology, life cycle, pathogenesis and virulence factors of genus leishmania: a review. Plant Archives. 2020; 20 ( 2) : 4057-4060. http://plantarchives.org/20-2/4057-4060%20(6640).pdf.
Rahi AA. Cutaneous Leishmaniasis in Iraq: A clinic-epidemiological descriptive study. Sch. J. App. Med. Sci., 2013; 1(6):1021-1025. http://saspublisher.com/wp-content/uploads/2013/12/SJAMS161021-1025.pdf.
Mody RM. Lakhal-Naouar I. Sherwood JE,. Koles NL. Shaw D. Bigley DP. Co EA. Copeland NK. Jagodzinski LL. Mukbel RM. Smiley RA. Duncan RC. Kamhawi S. Jeronimo SMB. DeFraites RF. Aronson NE. Asymptomatic Visceral Leishmania infantum Infection in US Soldiers Deployed to Iraq. Clin Infect Dis.

2019;68(12):2036-2044. http://doi.org/10.1093/cid/ciy811.PMID: 30239631; PMCID: PMC6769235.

Farahani LZ. Mohebali M. Akhoundi B. Saghafipour A. Kakooei Z. Seroepidemiological study on visceral leishmaniasis in an endemic focus of central Iran during 2017. Journal of parasitic diseases : official organ of the Indian Society for Parasitology. 2019; 43(1):22–27. https://doi.org/10.1007/s12639-018-1049-0.

Al-Warid HS. Al-Saqur IM. Kadhem AJ. Al-Tuwaijari SB. Al-Zadawi KM. Gompper ME. Spatial and demographic aspects of kala-azar (visceral leishmaniasis) in Iraq during 2011-2013. Trop Biomed. 2019;36(1):22-34. https://pubmed.ncbi.nlm.nih.gov/33597423/

Al-Obaidi MJ. Abd Al-Hussein MY. Al-Saqur IM. Survey Study on the Prevalence of Cutaneous Leishmaniasis in Iraq. Iraqi Journal of Science. 2016;57(3):2181-2187. https://www.iasj.net/iasj/download/2ab2af313163ccdf.

Alsaad RK. Kawan MH. The effects of some climate factors upon frequency distribution of local sandflies breed in Misan province, Iraq. Iraqi J. Vet. Med. 2021; 45(1): 63-68. https://jcovm.uobaghdad.edu.iq.

Al-Mayali HM. Al-Hassani MK. Molecular Identification of Phlebotominae Sand Flies (Diptera: Psychodidae) in Al-Diwaniyah Region/Iraq. Journal of Global Pharma Technology. 2017; 10(9):424-430.http://doi.org/10.13140/RG.2.2.22053.73446.

Al-Bajalan MMM. Niranji SS. Al-Jaf SM. Kato H. First molecular identification of Leishmania major in Phlebotomus papatasi in an outbreak cutaneous leishmaniasis area in Iraq. Acta Trop. 2021;215:105807. http://doi.org/10.1016/j.actatropica.2020.105807.

Al Subaihawi AJK. Taher TMJ. et al. Descriptive study of cutaneous leishmaniasis in Wasit Province, Iraq in 2018, Ann Trop Med & Public Health. 2020;23 (S5): 479-484. http://doi.org/10.36295/ASRO.2020.23521.

Al-Naimy AFA. & Al-Waaly ABM. Study Molecular and Immunological Techniques for Detection of Leishmania Tropical, Major in Baghdad , Iraq. Al-Qadisiyah Journal of Pure Science. 2021;26(2) : Bio 1–8. https://doi.org/10.29350/jops.2021.26.2.1212.

Rahi AA. Jasim SE. Abed FA. Clinical picture of cutaneous leishmaniasis in Wasit, Iraq. EAS J Orthop Physiother. 2019;1(3): 29-30. http://doi.org/10.36349/easjop.2019.v01i03.002.

Hamzavi Y. Nazari N. Khademi N. Hassani K. Bozorgomid A. Cutaneous leishmaniasis in Qasr-e Shirin, a border area in the west of Iran, Veterinary World. 2018;11(12): 1692-1697. http://doi.org/10.14202/vetworld.2018.1692-1697.

Rasti S. Ghorbanzadeh B. Kheirandish F. Mousavi SG. Pirozmand A. Hooshyar H. Abani B. Comparison of Molecular, Micscopic, and Culture Methods for Diagnosis of Cutaneous Leishmaniasis. J Clin Lab Anal. 2016;30(5):610-5. http://doi.org/10.1002/jcla.21910. Epub 2016 Feb 18. PMID: 26891976; PMCID: PMC6807126.

Hassan ZI. Molecular Characterization of Cutaneous Leishmaniasis Isolated from Human in Erbil Province- Kurdistan Region/ Iraq. ZJPAS (2018);30 (2): 76-85. http://dx.doi.org/10.21271/ZJPAS.30.2.10.

Kadhim IK. And Al-Quraishi. Molecular And Diagnostic Study Of Cutaneous Leishmaniasis Species By NestedPCR Technique In Babylon Province/ Iraq. European Journal of Molecular & Clinical Medicine.2020;7(3):1190-1199. https://ejmcm.com/article_1597.html.

Qader AM. Abood MK. Bakir TY. Identification of leishmania parasites in clinical samples obtained from cutaneous leishmaniasis patients using pcr technique in Iraq.

Iraqi Journal of Science. 2009; 50(1) : 32 – 36. https://www.iasj.net/iasj/download/de4ccaa7a9cec0db.

Younis NN. and Al-Thwani AN. Identification of Leishmania tropica and Leishmania major by REAL-TIME PCR using ITS1 and KDNA genes in Baghdad. Pak. J. Biotechnol. 2018;15(2): 599-602. https://www.pjbt.org/index.php/pjbt/article/view/435.

Rasheed ZK, Mohammed NS, Turki SG, Abed ZAS. The genetic sequence of Leishmania tropica among patients with Cuotaneous leishmaniasis in Iraqi patients. International Journal of Drug Delivery Technology. 2020;10(4):577-580. http://impactfactor.org/PDF/IJDDT/10/IJDDT,Vol10,Issue4,Article12.pdf.

Yousif WT. Al-kaysi AM. Selman MO. Aljuboori KK. Zuhdi A. Molecular and biochemical studies on the effect of the infection by cutaneous leishmaniasis in Iraqi people. 2020; 8(8) : 403-410. http://doi.org/10.5281/zenodo.3996694.

Younis NN. Muhammed S. Clinical Isolation and Molecular Diagnosis of Cutaneous Leishmaniasis using RFLIP for ITS1 Gene in some Baghdad Hospitals. Annals of the Romanian Society for Cell Biology. 2021; 25(6): 774-781. https://www.annalsofrscb.ro/index.php/journal/article/view/5493.

Noori N. Al-Thwani AN. Aboody BA. Clinical Isolation and Molecular Diagnosis of Cutaneous Leishmaniasis by using KDNA Gene PCR at some Baghdad Hospitals. Journal of Kerbala for Agricultural Sciences. 2017, ;4(5): 307-315. http://journals.uokerbala.edu.iq/index.php/Agriculture/article/view/715.

Hawas MR. Al-Zubaidy IA. Mohamed NS. Molecular identification of leishmaniaspp.in cutaneous leishmaniasis. Plant Archives.2020; 20(2): 6951-6958. http://plantarchives.org/20-2/6951-6958%20(6487).pdf.

Al-Jubori MI. Al-taae AA. Al-Faham M A. Detection of Cutaneouse Leishmaniasis species via PCR in Salah Adeen and Baghdad provences. Tikrit Journal of Pure Science, 2019;24(1): 57-61. http://dx.doi.org/10.25130/tjps.24.2019.009.

Al-Ghabban MJ. Al-Bayati NY. Al-Mayah QS. Molecular Typing and Phylogenetic Analysis of Cutaneous Leishmania from Iraqi Patients. Medico Legal Update. 2020; 20(4):349-355. https://doi.org/10.37506/mlu.v20i4.1834.

Seaad AA, Abdulah SR. Kelef S F. Epidemiological prevalence of cutaneous Leishmaniasis In immigration people in Al-Qadissyia province. Al-Anbar Journal of Veterinary Sciences. 2017;10(1): 77-83. https://www.iasj.net/iasj/article/134919.

Al-Difaie RS. Jasim GA. Detection the effect of the treatment of pentostam on LPG, CPA, and PPG virulence factors of cutaneous Leishmania by real time-PCR. QJVMS. 2018; 17(2): 9-17. https://doi.org/10.29079/vol17iss2art496.

Mohammad FI. Hmood KA. Detection of leishmania species by nested-pcr and virulence factors gipls, gp63 in *L. major* by conventional-pcr. Biochem. Cell. Arch. 2018;18( 2) 2255-2259. www.connectjournals.com/bca.

Abdlkadhim SJ. Epidemiological And Molecular Characteristics Of Cutaneous Leishmaniasis In Al-Diwanyiah hospital. 2018; 14:71-80. https://qmed.iraqjournals.com.

Jabbar EK. AL-Aboody BA. Jarullah BA. Noori N. Isolation and Molecular Diagnosis of Leishmania major and Study Activity of Aqueous Extract of Plant Nigella sativa Against the Parasite In Vitro. ijpqa. 2019;10(1):47-50. http://doi.org/10.25258/ijpqa.10.1.7.

Flaih1 MH. Al-Abady FA. Hussein KR. Detection of Leishmania tropica Using Nested-PCR and Some of Their Virulence Factors in Thi-Qar Province, Iraq. Baghdad

Science Journal. 2021; 18 (1): 700-708. http://dx.doi.org/10.21123/bsj.2021.18.1(Suppl.).0700.

Al-Fahdawi HA. Al-Ani SF. AlKubaisi TA.. Detection of Cutaneous Leishmaniasis Based on ITS1 Gene by PCR-RFLP Technique. Egypt. Acad. J. Biolog. Sci.2018; 10 (2): 1 – 13. https://eajbse.journals.ekb.eg/issue_2847_4308_.html.

Al-Bajalan MMM. Al-Jaf SMA. Niranji SS. Abdulkareem D. Al-Kayali K. Kato H. An outbreak of Leishmania major from an endemic to a non-endemic region posed a public health threat in Iraq from 2014-2017: Epidemiological, molecular and phylogenetic studies. PLoS Negl Trop Dis. 2018; 12(3): e0006255. https://doi.org/10.1371/journal.pntd.0006255.

Hamza, D. M., Obayes, L. H., & Zghair, F. S. Molecular Diagnosis and Phylogenetic Analysis of 5.8 s rDNA Gene of Cutaneous Leishmaniasis Species in Holy Karbala/Iraq. Indian Journal of Public Health Research & Development. 2019; 10(4). 414-419. http://doi.org/10.5958/0976-5506.2019.00729.0.

Hassan HF. Salman MM. Mohammed BM. Evaluation of the PCR Method for the Diagnosis of Cutaneous Leishmaniasis in AL Hawija District, Kirkuk, Iraq. Indian Journal of Forensic Medicine & Toxicology, January-March 2020; 14(1): 729-733. Http://doi.org/10.37506/v14/i1/2020/ijfmt/192989.

Al-Lamy SHA. Al-Abady FAM. Molecular study to diagnose and identify Leishmania spp. that causes Cutaneous leishmaniasis in humans in Maysan Governorate, Iraq. Annals of the Romanian Society for Cell Biology. 2021;25(4):14135-14143. https://www.annalsofrscb.ro/index.php/journal/article/view/4560.

AL- Hucheimi SN. Sultan BA. Al-Dhalimi MA. Mahmood TA. Tracking of Ceotaneous Leishmaniasis by Parasitological, Molecular and Biochemical Analysis. KJNS. 2015;5(1): 243-253. https://journals.uokufa.edu.iq/index.php/kjns/article/view/3977.

AL- Hucheimi SN. Sultan BA. Al-Dhalimi MA. A comparative study of the diagnosis of Old World cutaneous PCR diagnosis of cutaneous leishmaniasis in Iraq by polymerase chain reaction and microbiologic and histopathologic methods. International Journal of Dermatology. 2009; 48:404–408.

Ali L. Al-Hadraawy, SK. Detection of cutaneous leishmaniasis species using nested polymerase chain reaction (nested-PCR) technique and phylogenetic tree in Al-Najaf province/Iraq. Drug Invention Today. 2019; 11(8): 1777-1780.

Ali MA. Khamesipour A. Rahi AA. Mohebali M. Akhavan A. Firooz A. Keshavarz HV. Epidemiological study of cutaneous leishmaniasis in some Iraqi provinces. 2018; 14(4);18-24. http://doi.org/10.22374/1875-6859.14.4.4.

Rahi AA. Nsaif S. Hassoni JJ.. Ali MA. Hamza HA. Comparison of diagnostic methods in Cutaneous Leishmaniasis in Iraq. American Journal of BioScience. 2013; 1(1) : 1-5. http://doi.org/10.11648/j.ajbio.20130101.11.

Rahi AA. Identification of leishmania species causing cutaneous leishmaniasis using REAL-TIME PCR in Iraq. International Journal of Development Research. 2014; 4(8): 1459-1462. http://journalijdr.com/archive/201408.

Rahi A A. Genetic characterization of Leishmania species causing cutaneous leishmaniasis in Iraq. Int Res J Eng Technol. 2015; 2(3):1057-1062. https://www.irjet.net/archives/V2/i3/Irjet-v2i3155.pdf.

Al-Tamemy AA. Al-Qurashi NN. Identification and Sequences of Two Leishmania Species Isolated From Human Cutaneous Lesion in Iraq. IOSR-JPBS. 2017;1(4):53-57. http://doi.org/10.9790/3008-1204075357.

Al-Khanaq MN. Detection of Leishmania species by using nested PCR in Al-Kut city. Journal of Entomology and Zoology Studies. 2018; 6(2): 2815-2819. https://www.entomoljournal.com/search/?q=May+Naji+Al-Khanaq.

RahiAA. Jasim SE. Abed F A. Clinical picture of cutaneous leishmaniasis in Wasit, Iraq. EAS. J. Orthop. Physiother. 2019; 1(3), 29-32.

Faieq ZA. Detection of Visceral leishmaniosis by RT-PCR technique in Wasit Province of Iraq. Wasit Journal for Science & Medicine. 2019; 12(1):68-78. https://www.iasj.net/iasj/article/169743.

AL-Mishry MK. Al-Shimmary MN. Mahdi NK. Molecular diagnosis of visceral leishmaniasis in children of Basrah governorate, Iraq. 2013; 4(1): 54-59. https://jsci.utq.edu.iq/index.php/main/article/view/582.

Al-Hussaini RM. Al-Tufaili RN. Hussein R A. Molecular Study of Pediatric Visceral Leishmaniasis in Mid-Euphrates Area, Iraq. Inter. J. Sci. Engineer. Res. 2017: 8(8): 148-152. https://www.ijser.org/research-paper-publishing-august-2017.aspx.

Tab.1 Characteristics of the cutaneous leishmania species identified in systematic review from 2009 – 20121.

| Author | Year | Province | type of sample | total | infected | *L. tropica* | *L. major* | *L. aethiopica* | method | Gene markers |
|---|---|---|---|---|---|---|---|---|---|---|
| Hassan Z. I. (31) | 2018 | Erbil | Skin biopsy | 58 | 58 | 58 | 0 | 0 | PCR | ITS-1 |
| Kadhim and Al-Quraishi(32) | 2020 | Babylon | Skin biopsy | 215 | 178 | 141 | 37 | 0 | Nested-PCR | ITS-2 |
| Qader, etal. (33) | 2009 | Baghdad | Skin biopsy | 27 | 2 | 0 | 2 | 0 | PCR | kDNA |
| Younis and Al-Thwani (34) | 2018 | Baghdad | Skin biopsy | 75 | 68 | 42 | 26 | 0 | Real-Time PCR | kDNA |
| Rasheed et al. (35) | 2018 | Baghdad | Blood samples | 150 | 150 | 120 | 30 | 0 | PCR | KDNA |
| Yousif, et al., (36) | 2020 | Baghdad | Skin biopsy | 90 | 82 | 63 | 19 | 0 | Nested-PCR | Random |
| Younis and Muhammed (37) | 2021 | Baghdad | Skin biopsy | 88 | 82 | 57 | 25 | 0 | PCR , RFLIP | ITS-1 |
| Noori, et al., (38) | 2017 | Baghdad | Skin biopsy | 44 | 42 | 30 | 12 | 0 | Nested-PCR | KDNA |
| Hawas, et al., (39) | 2020 | Baghdad (displaced)* | Skin biopsy | 50 | 40 | 26 | 14 | 0 | Nested-PCR | ITS-1 |
| Al-Jubori, et al., (40) | 2019 | Baghdad and Tikrit | Skin biopsy | 117 | 117 | 87 | 27 | 3 | PCR | ITS-1 |
| Al-Ghabban, et al., (41) | 2020 | Diyala | Skin biopsy | 30 | 30 | 17 | 13 | 0 | Nested-PCR | ITS-1 |
| Seaad, et al., (42) | 2017 | Qadisiyah (displaced) | Skin biopsy | 4007 | 62 | 41 | 21 | 0 | PCR | Random |
| Al-Difaie and Jasim (43) | 2017 | Qadisiyah | Blood samples | 55 | 49 | 22 | 27 | 0 | PCR | KDNA |
| Mohammad and Hmood (44) | 2018 | Qadisiyah | Skin biopsy | 50 | 42 | 7 | 35 | 0 | Nested-PCR | Random |
| abdlkadhim, S.J. (45) | 2018 | Qadisiyah | Skin biopsy | 145 | 38 | 10 | 28 | 0 | Nested-PCR | KDNA |
| Jabbar, et al., (46) | 2019 | Thi Qar | Skin biopsy | 50 | 48 | 0 | 48 | 0 | Nested-PCR | KDNA |
| Flaih, et al., (47) | 2021 | Thi Qar | Skin biopsy | 80 | 65 | 46 | 19 | 0 | Nested-PCR | KDNA |
| Al-Fahdawi, et al., (48) | 2018 | Anbar | Skin biopsy | 122 | 62 | 42 | 20 | 0 | RFLP | ITS-1 |
| Al-Bajalan, et al., (49) | 2018 | Sulaimaniyah | Skin biopsy | 30 | 15 | 0 | 15 | 0 | PCR | ITS-1 |
| Hamza, et al., (50) | 2019 | Karbala | Skin biopsy | 92 | 92 | 0 | 92 | 0 | PCR | ITS-2 |
| Hassan, et al., (51) | 2020 | Kirkuk | Skin biopsy | 200 | 143 | 143 | 0 | 0 | PCR | KDNA |
| Al-Lamy and Al-Abady (52) | 2021 | Maysan | Skin biopsy | 80 | 40 | 25 | 15 | 0 | Nested-PCR | KDNA |
| AL- Hucheimi, et al., (53) | 2015 | Najaf | Skin biopsy | 83 | 52 | 7 | 45 | 0 | Nested-PCR | KDNA |
| AL- Hucheimi, et al., (54) | 2009 | Najaf | Skin biopsy | 57 | 37 | 16 | 21 | 0 | Nested-PCR | KDNA |
| Ali and Al-Hadraawy (55) | 2019 | Najaf | Skin biopsy | 60 | 33 | 10 | 23 | 0 | Nested-PCR | KDNA |
| Ali, et al., (56) | 2018 | Different** | Skin biopsy | 700 | 583 | 233 | 350 | 0 | Nested-PCR | ITS-1 |
| Rahi, et al., (57) | 2013 | Wasit | Skin biopsy | 64 | 44 | 16 | 28 | 0 | PCR | ITS-1 |
| Rahi, AA. (58) | 2014 | Wasit | Skin biopsy | 60 | 53 | 11 | 42 | 0 | Real-Time PCR | KDNA |
| Rahi, AA. (59) | 2015 | Wasit | Skin biopsy | 46 | 20 | 8 | 12 | 0 | PCR | KDNA |
| Al-Tamemy and Al- | 2017 | Wasit | Skin biopsy | 80 | 68 | 7 | 61 | 0 | Nested- | KDNA |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Qurashi (60) | | | | | | | | | PCR | |
| Al-Khanaq, MN (61) | 2018 | Wasit | Skin biopsy | 70 | 55 | 6 | 49 | 0 | Nested-PCR | KDNA |
| Rahi, et al., (62) | 2019 | Wasit | Skin biopsy | 60 | 42 | 4 | 38 | 0 | Nested-PCR | KDNA |
| Faieq, ZA(63) | 2019 | Wasit | Skin biopsy | 70 | 50 | 18 | 32 | 0 | RT-PCR | KDNA |

* from Ninawa and salahalddin provinces.
** Qadisiyah 88, Wasit 85, Najaf 79, Thi-Qar76, Basrah 67, Baghdad 65, Diyala 63, and Tikrit 60.

Tab.2. Characteristics of the vescieral leishmania species identified in systematic review.

| Author | Year | Province | type of sample | total | Infected | *L. tropica* | *L. infantum* | method | Gene markers |
|---|---|---|---|---|---|---|---|---|---|
| Al-Mishry, et al. (64) | 2013 | Basrah | Blood | 50 | 42 | 0 | 42 | PCR | KDNA |
| Al-Hussaini, et al. (65) | 2017 | Najaf | Blood | 587 | 148 | 148 | 0 | Nested-PCR | KDNA |

Tab.3 Meta-analysis of cutaneous leishmania species in different provinces.

| Province | Total | infected | *L. tropica* | %95 CI | *L. major* | 595 CI |
|---|---|---|---|---|---|---|
| Erbil | 58 | 58 | 58 | 0.05 - 0.083 | 0 | 0 – 0.004 |
| Babylon | 215 | 178 | 141 | 0.134 - 0.18 | 37 | 0.033 - 0.062 |
| Baghdad | 474 | 426 | 303 | 0.307 - 0.37 | 114 | 0.117 - 0.165 |
| Diyala | 30 | 30 | 17 | 0.011 - 0.03 | 13 | 0.009 - 0.027 |
| Qadisiyah | 250 | 129 | 19 | 0.013 - 0.033 | 90 | 0.09 - 0.134 |
| Thi Qar | 130 | 113 | 46 | 0.038 - 0.067 | 67 | 0.065 - 0.103 |
| Anbar | 122 | 62 | 42 | 0.034 - 0.063 | 20 | 0.015 - 0.037 |
| Sulaimaniyah | 30 | 15 | 0 | 0 - 0.004 | 15 | 0.011 - 0.030 |
| Karbala | 92 | 92 | 0 | 0 - 0.004 | 92 | 0.093 - 0.136 |
| Kirkuk | 200 | 143 | 143 | 0.136 - 0.184 | 0 | 0 - 0.005 |
| Maysan | 80 | 40 | 25 | 0.018 - 0.040 | 15 | 0.011 - 0.030 |
| Najaf | 200 | 122 | 33 | 0.026 - 0.051 | 89 | 0.089 - 0.132 |
| Wasit | 450 | 332 | 70 | 0.062 - 0.097 | 262 | 0.290 - 0.354 |
| | | | P.valu<0.001 | 1.256 - 1.167 | P.valu<0.001 | 0.857 - 0.796 |

Tab. 4. Meta-analysis of cutaneous leishmania species among study years.

| Year | Total | infected | *L.tropica* | %95CI | *L. major* | %95CI |
|---|---|---|---|---|---|---|
| 2009 | 84 | 39 | 16 | 0.007 - 0.0201 | 23 | 0.012 - 0.028 |
| 2013 | 64 | 44 | 16 | 0.007 - 0.0201 | 28 | 0.015 - 0.032 |
| 2014 | 60 | 53 | 11 | 0.004 - 0.0152 | 42 | 0.025 - 0.045 |
| 2015 | 129 | 72 | 15 | 0.007 - 0.0191 | 57 | 0.036 - 0.059 |
| 2017 | 4186 | 221 | 80 | 0.050 - 0.0768 | 121 | 0.083 - 0.116 |
| 2018 | 1400 | 1071 | 518 | 0.376 - 0.4305 | 553 | 0.423 - 0.479 |
| 2019 | 449 | 382 | 119 | 0.078 - 0.1097 | 260 | 0.190 - 0.235 |
| 2020 | 585 | 473 | 381 | 0.272 - 0.3222 | 83 | 0.054 - 0.083 |
| 2021 | 248 | 187 | 128 | 0.084 - 0.1172 | 59 | 0.037 - 0.061 |
| | | | P. value < 0.0001 | 2.61 - 2.67 | P.value <0.0001 | 1.023 - 1.044 |

Tab.5 Meta-analysis publication bias of cutaneous leishmania species.

| Parameters | Total | Rate (%95 CI) | Heterogeneity | | | | Egger's test | |
|---|---|---|---|---|---|---|---|---|
| | | | df | P. value | Cochran Q | I2 | E. bias | p. value |
| L. tropica | 1313 | 51.65%(29.87-119.56) | 32 | 1.00 | .00 | 0.00% | 2.574 | < 0.0001 |
| L. major | 1226 | 48.23%(09.60-101.21) | 32 | 1.00 | 0.00 | 0.00% | 18.6 | < 0.0001 |

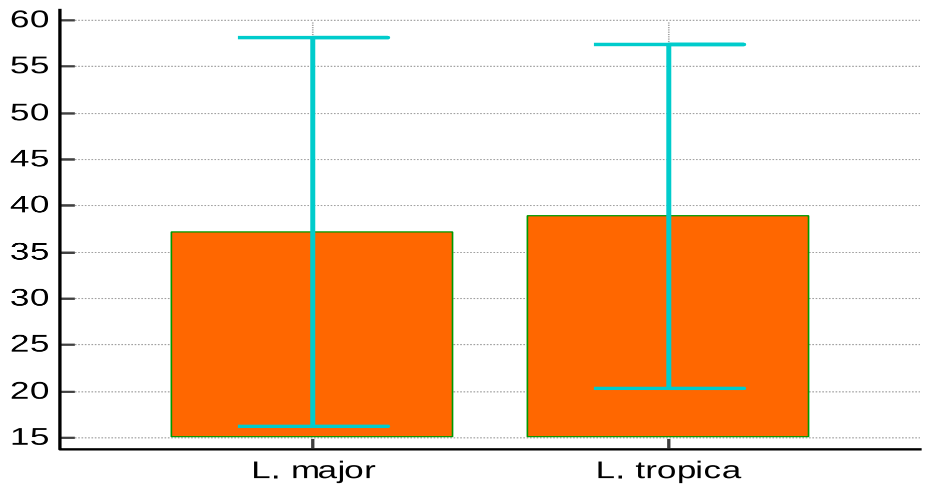


Fig.2. diagram of cutaneous leishmania species prevalence.


URL: http://www.uokufa.edu.iq/journals/index.php/ajb/index
http://iasj.net/iasj?func=issues&jId=129&uiLanguage=en
Email: biomgzn.sci@uokufa.edu.iq

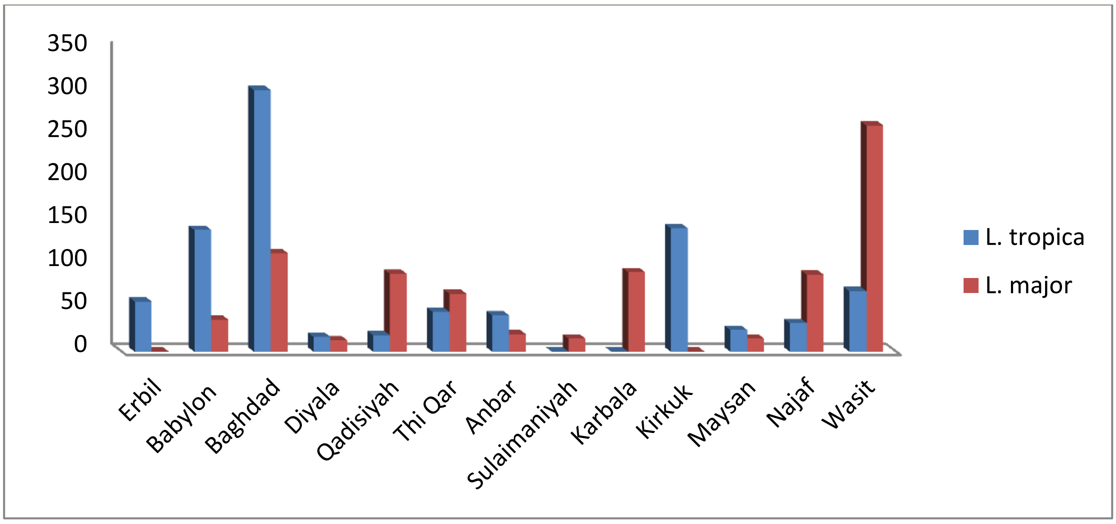


Fig. 3 Distribution of Leishmania species in different provinces.

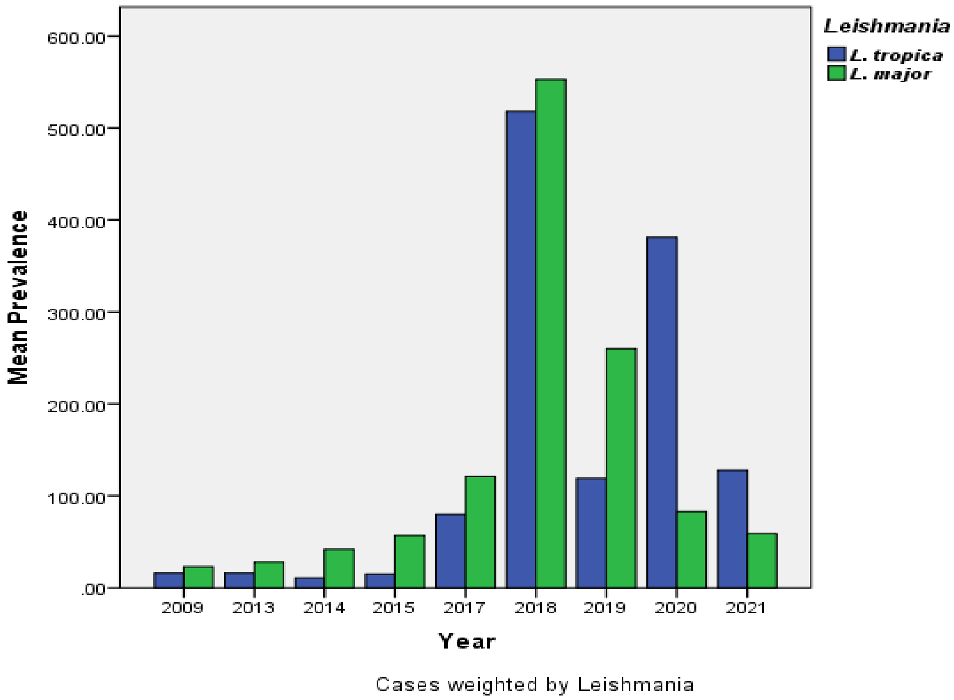


Fig. 4. Prevalence of Leishmania species among study years.