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PHENOTYPIC AND MOLECULAR DETECTION OF BIO-ECOLOGICAL CUTANEOSIS LEISHMANISIS VECTOR (*DIPTERA: PSYCHODIDAE*), AND IDENTIFICATION OF LEISHMANIA PARASITE IN CUTANEOUS LEISHMANIASIS CASES IN ERBIL PROVINCE

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IRAK'IN ERBİL İLİNDEKİ HASTALARDA KUTANÖZ LEISHMANIASIS TANISI İLE BİYOEKOLOJİK KUTANOZ LEISHMANISIS VEKTÖRÜNÜN (DIPTERA: PSYCHODIDAE) FENOTİPİK VE MOLEKÜLER TESPİTİ

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Flebotomin kum sinekleri, leishmaniasis'in bulaşmasından sorumlu olan tıbbi açıdan önemli bir böcek grubudur. Irak'ın Ocak-Aralık ayları arasında ilk kez Irak'ın Erbil vilayetinde kutanöz leishmaniasis vektörü olarak kum sineği (Al-Harms) türlerinin fenotipik ve genotipik incelemesi ile Hastalarda biyo-Ekolojik Kutanöz Leishmaniasis'in mikroskobik incelemesi yapıldı. Toplanan 2054 örneğin 1137'si (%55,4) erkek, 917'si (%44,6) kadındır. istatistiksel olarak cinsiyet ile kum sineği türleri arasında korelasyon yoktur (P<0.20). Phlepotomus cinsine ait üç tür tanımlanmıştır: P. papatasi, P. sergenti ve P. Alexandria. P. papatasi 1163 (%15.6) ile en sık görülen tür olurken, bunu P. sergenti 598 (%29.1) ve P. alexandri 293 (%14.1) izledi. Kum sineklerinin mevsimsel bolluğunun iki zirvesi vardı, mevsimsel olarak bol olan kutanöz leishmaniasis vektörlerinin ilk zirvesi Mayıs ayında meydana gelirken, ikinci zirve Eylül ayında meydana geldi, istatistiksel olarak dönemler ile kum sineği türleri arasında bir korelasyon var (p<0.00). **Bu çalışmada,** Irak'ın Erbil vilayetindeki kum sineği türlerinin morfolojik tanımlamasının sonuçlarını doğrulamak için mt DNA COI gen bölgesinin dizileme analizi kullanılmıştır. Mevcut çalışma, P. papatasi, P. sergenti ve P. alexandria'yı bulmak ve tanımlamak için PCR-doğrudan dizileme kullandı. Erbil ili endemik CL kökenlidir. Phlebotomus tür standardı NCBI BLAST programı kullanılarak yapılan filogenetik ağaç analizine göre, Erbil ilinin çeşitli yerlerinden yerel Phlebotomus papatasi, Phlebotomus sergenti ve Phlebotomus alexandri izolatları DNA dizilimine tabi tutuldu. Yerel alandan Phlebotomus papatasi, Phlebotomus sergenti ve Phlebotomus alexandri izolatları, bir çoklu hizalama analiz aracı olan M'EGA 6.0 kullanılarak mt DNA COI gen dizisini analiz etmek için kullanıldı. Şehrin sağlık sistemi, kutanöz leishmaniasis hastalığının taşıyıcıları olan kum sineği türlerini kontrol etmek için gerekli önlemleri almalıdır. Kum sineklerini kontrol etmek, (CL) hastalığı kontrol etmenin anahtarı olacaktır. Mikroskobik inceleme, insan hastaların vakalarından elde edilen 135 kişiden 82'si (65.6) CL ile smear örneklerinin boyanmasını takiben yağa daldırma ışık mikroskobu (100X) kullanılarak gerçekleştirildi. Cinsiyetler arasında anlamlı fark vardı (P>0.05) ve bu oran erkeklerde kadınlardan daha yüksekti (84 (%62.2) ve 51 (%37.8). KL önemli bir sıklığa sahipti ve Mahmur bölgesinde en sık görülen endemik hastalıktı. Bu çalışmada 82 hastada (%65.6) laboratuvar ortamında KL enfeksiyonu saptandı. KL enfeksiyonunun genç yaş gruplarını etkileme olasılığı daha yüksektir. Araştırma, bir yaşından küçük çocuklarda iki ciddi ve çok sayıda CL lezyonu vakası keşfetti. 63 vakada (%46.6) KL en sık 1-25 yaş aralığında görüldü.Klinik sonuçlar açısından KL'den en sık etkilenen bölgeler 62/135 hastada (%45.9) üst ekstremiteler, 39/135 hastada (%28.9) alt ekstremiteler ve 28/135 hastada (%20.7) yüz (kulaklar ve burun dahil) idi. Ek olarak, 6/135 hastanın (%4.4) vücudunda KL lezyonlarının bulunduğu birkaç organ vardı. P<0.05 bu sonuçların istatistiksel olarak anlamlı olduğunu göstermektedi. Bulgulara göre, enfeksiyon oranı Şubat, Ocak ve Mart aylarında orta ortalama sıcaklıklarda (Cº) daha yüksekti. Buna karşılık, oran Nisan, Mayıs, Haziran, Temmuz, Ağustos ve Eylül aylarının daha sıcak aylarında azaldı (enfeksiyon oranları sırasıyla% 4.4, % 6.7,% 3.7,% 3.7,% 3.7,% 3.7 ve% 3.7), ancak Ekim ayında sıfırdı. Bu verilerde KL oranında anlamlı farklılıklar görüldü (P<0.05). Ocak, Şubat ve Aralık aylarının kış ayları en yüksek yüzdeye sahipken, yaz ve sonbahar en düşük yüzdeye sahipti. Bu çalışmada kum sinekleri spp. ortaya çıkması ile

kutanöz leishmaniasis olguları arasındaki ilişki araştırılmaya çalışılmıştır. Çalışma bulguları ışığında, çalışmada ortaya çıkan kum sinekleri spp. ile kutanöz leishmaniasis hastalığı arasında güçlü bir ilişki olduğu fark edilmiştir. İstatistiksel analiz sonuçları, aylar arasında ($P<0.05$) önemli farklılıklar olduğunu göstermektedir. Yıl boyunca mevcut CL'nin iki zirvesi vardı, ilk zirve Şubat'ta, ikinci zirve ise Aralık'taydı. Leishmania parazit inkübasyon süresi ile Kum Sinekleri ve kutanöz leishmaniasis olgularının ortaya çıkışı karşılaştırıldığında, istatistiksel analiz ($P<0.05$) noktasında anlamlı farklılıklar ortaya koymaktadır.

ANAHTAR KELIMELER: kum sineği, Psychodidae, leishmaniasis, CL, Erbil-Irak

ABSTRACT

Ph.D. Thesis

PHENOTYPIC AND MOLECULAR DETECTION OF BIO-ECOLOGICAL CUTANEOSIS LEISHMANISIS VECTOR (DIPTERA: PSYCHODIDAE), WITH DIAGNOSIS CUTANEOUS LEISHMANIASIS IN PATIENTS IN ERBIL PROVINCE, IRAQ

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Phlebotomine sandflies are an important group of insects from a medical perspective that are responsible for the transmission of leishmaniasis. The phenotypic and genotypic investigation of species of sand flies (Al-Harms) as the vector of CL, with the microscopic investigation of bio-Ecological CL in Patients was conducted in the Erbil province-Iraq for the first time between January and December 2022. Out of 2054 collected samples 1137 (55.4%) were male and 917 (44.6%) were female. statistically, there is no correlation between gender and sandfly species at (P=0.20). Three species were identified as belonging to the genus *Phlepotomus: P. papatasi, P. sergenti*, and *P. alexandria. P. papatasi* was the most common species, with 1163 (15.6%), followed by *P. sergenti* 598 (29.1%), and *P. alexandri* 293(14.1%). There were two peaks of seasonal abundance of sand flies, the first peak of the seasonally abundant CL vectors occurred in May, while the second peak occurred in September, statistically, there is a correlation between the periods and sand fly species at ($p<0.05$). This study used sequencing analysis of the mtDNA COI gene region to verify the results of morphological identification of sandfly species in Erbil province, Iraq. The current work used PCR-direct sequencing to find and identify *P. papatasi, P. sergenti*, and *P. alexandria.* originated from Erbil province endemic CL. According to a phylogenetic tree analysis using the Phlebotomus species standard NCBI BLAST program, local *Phlebotomus papatasi, Phlebotomus sergenti,* and *Phlebotomus alexandri* isolates from various places in the Erbil province were subjected to DNA sequencing. *Phlebotomus papatasi*, *Phlebotomus sergenti, and Phlebotomus alexandri* isolates from the local area were used to analyze the mtDNA COI gene sequence using M'EGA 6.0, a multiple alignment analysis tool. The city's health system must take the necessary precautions to control sand fly species as the vectors of CL disease. Controlling the sandflies will be a key to controlling (CL) disease. The microscopic investigation was carried out using oil immersion light microscopy (100X) subsequent to the staining of smear samples, due to of getting treatment or contamination the human CL cases: only 82(65.6%) cases were positive CL, out of 135 obtained from human patients' cases. There was significant difference between the genders at ($P>0.05$) in the CL rate, which was higher in males than females 84(62.2%) versus 51 (37.8%). CL had a significant frequency and was the most frequent endemic disease in the Makhmur district. In this investigation, 82patients (65.6%) were shown to have CL infection in a lab setting. A CL infection is more likely to affect younger age groups. The investigation discovered two cases of severe and numerous CL lesions in children younger than a year old. At 63 cases (46.6%), CL was most common in the 1 to 25-year-old age range. In terms of clinical outcomes, the most frequently affected areas by CL were the upper limbs in 62/135 patients (45.9%), the lower limbs in 39/135 patients (28.9%), and the face (including the ears and nose) in 28/135 patients (20.7%). Additionally, 6/135 patients (4.4%) had several organs in their bodies where CL lesions were found. $P<0.05$ indicates that these results are statistically significant. According to the findings, february 2022, January 2022, March 2022, December 2022 and November 2022 all had higher infection rates (31.9%, 20%, 9.6%, 6.7% and 5.9% respectively). Following that, there was a significant decline in the rates. According to the

findings, the rate of infection was highest in the months of February, January, and March at moderate mean temperatures (C°). In contrast, the rate decreased in the hotter months of April, May, June, July, August, and September (infection rates: 4.4%, 6.7%, 3.7%, 3.7%, 3.7%, and 3.7%, respectively), but it was zero in October. Significant variations in the rate of CL were seen in these data at ($P<0.05$). The winter months of January, February, and December had the highest percentage, while the summer and fall had the lowest. The study tries to find out the correlation between emerging of sand flies spp. and CL cases. In the lighting of study findings, the study noticed a strong relationship between emerging sand flies spp. and CL disease. In comparing the Leishmania parasite incubation period to the emergence of Sandflies and CL cases, statistical analysis reveals significant differences at ($P<0.05$). There were two peaks of existing CL. along the year, first peak was in February and the second peak was in December.

KEYWORDS: sandfly, Psychodidae, leishmaniasis, CL, Erbil-Iraq

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LIST OF SYMBOLS AND ABBREVIATIONS

ACL	**Anthroponotic cutaneous leishmaniasis**
AFR	African Region
ASL	Above sea level
ASP	Aspirator
CDC	Centers for Disease Control and Prevention
CL	Cutaneous leishmaniasis
DCL	Disseminated cutaneous leishmaniasis
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease control
EMR	Eastern Mediterranean Region
EUR	European Region
IPCC	Intergovernmental Panel on Climate Change
KOH	Potassium hydroxide
KRG	Kurdistan Regional Government
LR	Leishmaniasis recidivance
LT	Light trap
MCL	Mucocutaneous leishmaniasis
NCBI	National Center for Biotechnology Information
NJ	Neighbor Joining
NNN	Novy, MacNeal-Nicolle
NTD	Neglected Tropical Diseases
NW	New World
OW	Old World
OWCL	Old World cutaneous leishmaniasis
PBS	Phosphate buffer solution
PCR	Polemerase chain reaction
PKDL	post-kala-azar dermal leishmaniasis
RL	Recidivans leishmaniasis
RNA	Ribodeoxyribonucleic acid
SEAR	South-East Asia Region
SP	Species
TV	Tlevision
UK	United Kingdom
USA	United State Amerecan
UV	Ultraviolet transilluminator machine
VBD	Vector-borne diseases
VL	Visceral leishmaniasis
WHO	World Health Organization
ZCL	Zoonotic cutaneous leishmaniasis
EMTM	Evan‟s modified Tobie‟s medium
ELISAs	Enzyme-linked immunosorbent assays
Sb	Sodiumstibogluconate
TB	Tuberculosis

1. INTRODUCTION

1.1. Ecology of Erbil

Due to the city's growing population and businesses engagement in oil extraction and export industries, Erbil City has seen a rapidly growing region since past 20 years. These factors such as oil extraction, construction and growing population have caused the province's temperature, humidity, and precipitation to change, all of which have a significant impact on the number of vectors in the study area (province). A one third of the population of Iraq lives in poverty, which means that they have inadequate sanitation, which contributes to the spread of sand flies in the area. The sandflies (phlebotomine), which are the only vectors for the leishmania parasites that cause leishmaniasis disorders, are the subject of the study. The genotype and phenotype investigation of *Phlebotomine* sandflies in Erbil-Iraq, and the bio-ecology of the disease's vectors, as well as the identification of the leishmania parasite from patient cases were the goals of the study. This section will include a field summary, research issues, goal and objectives, research questions, and study area scope.

1.2.Reasons of Increasing Vectors Borne Disease

The ability of nature to regenerate itself has been exceeded as a result of people's increased use and dominance over the natural world, and the harmony between living things and the environment has started to degrade. Humans, who have been attempting to industrialize primarily with the idea of developing in terms of economy and technology since the past century, have become the architects of a process where the natural environment is contaminated and natural resources are used extravagantly. Industrialization has led to the emergence of environmental issues including the greenhouse effect and global warming, which have a detrimental impact on the ecology of the entire planet.

Climate change is a result of global warming and occurs everywhere. These modifications are anticipated to have an impact on the ecological balance of the ecosphere and local ecosystems. Infectious illnesses are on the rise and spreading due to the world's rapidly growing population as well as conditions including, malnutrition, pollution, careless chemical usage, and a lack of infrastructure. Our nation generates favorable habitats for vector species to survive and reproduce as a result of its geographic position, climatic, geological, and biological qualities. The quantity, density, and dispersion of vectors are all predicted to change as the temperature and humidity rise. In this situation, it is obvious that the distribution of vector-borne illnesses, particularly malaria and oriental boil, would alter. In actuality, vector-borne illnesses are currently the leading cause of public health issues (Alten and Çağlar, 1998).

1.3. Leishmaniasis Vectors

The genus Phlebotomus was originally recognized by Rondani in Rome, Italy, in 1840, while Scopoli first characterized Phlebotomus sandflies in 1786 (Mohsen, 1983; Abul-Hub and Ahmed, 1984; Abul Hub and Al-Hashimi, 1988). Phlebotomine sandflies (Diptera: Psychodidae) are proven vectors responsible for the transmission of different species of Leishmania. Leishmania species, the causative agents of leishmaniasis, are known to be transmitted by phlebotomine sandflies (Diptera: Psychodidae) (Moncaz et al., 2012; Chagas et al., 2018). Sand flies, which comprise roughly 800 species and fall into six main genera (Lutzomyia, Brumptomyia, Warileya, in the New World, while Phlebotomus, Chinius and Sergentomyia, in the Old World), Leishmania is only transmitted by species from the genera Phlebotomus and Lutzomyia, (Bates, 2007). In the developing regions of the world, a sizable category of Neglected Tropical Diseases (NTDs) is known as vector-borne diseases (VBDs). More than a billion individuals are thought to be exposed to these illnesses (Karimian et al., 2018; Valenzuela and Aksoy, 2018). One of the 13 NTDs seen in 88 tropical nations throughout the world is leishmaniasis (Azizi et al., 2011; Oryan et al., 2013). A variety of Leishmania parasites from the Trypanosomatidae family are the cause of this illness (Davami et al., 2014;

Ramezankhani et al., 2017; Parija, 2022). Infectious bites of female phlebotomine sand flies of the Phlebotomine subfamily transmit parasites to humans and other vertebrate hosts (Khosravani et al., 2016; Azizi et al., 2016). Visceral, muco-cutaneous, and cutaneous leishmaniases are the three primary manifestations of leishmaniases. The most prevalent kind of leishmaniasis in the world is cutaneous leishmaniasis (CL). In Iran, both visceral and cutaneous leishmaniases are regarded as native illnesses (Oshaghi et al., 2009; Alipour et al., 2014; Tofighi et al., 2014).

Leishmania is a protozoan with digenetic life cycles that can take one of two morphological forms: either amastigotes inside of mammalian macrophages or flagellated promastigotes inside of a phlebotomine sand fly. (Chappuis et al., 2007). In Iraq, during the late 1940s there was a severe decline in cases of Leishmaniasis, especially in the middle of the 1950s hat decline followed the control of sand flies by insecticides in the malarial eradication scheme and an improvement in general sanitation at the beginning of the 1960s (Guirges, 1971). Poor sanitation, a rise in vector populations and movement of non-immune populations to endemic areas, were the factors behind this surge in leishmaniasis cases. CL Clinical symptoms and serological tests are typically used by doctors to make a diagnosis (WHO, 2014). Nowadays, (sand flies are commonly identified based on morphological features, mostly by internal structures such as the cibarium, pharynx, spermatheca, and terminal genitalia of females and males), (Killick-Kendrick, 1995).

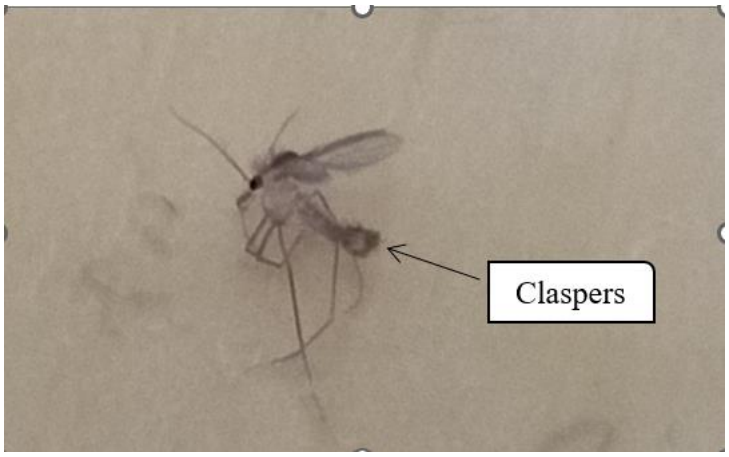


Figure.1. 1. Male of sand fly (Phlebotomus) in Erbil province 2022

1. 4. Cutaneous Leishmaniasis

(CL) is the most common form of leishmaniasis disease that annually affects approximately 1 million people who suffer from skin lesions in different parts of the body (CDC, 2020; WHO, 2020). The disease occurs in 90 countries, highly prevalent in 10 countries including Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran, Iraq, Pakistan, Syria and Tunisia (Gurel et al., 2020). The CL is caused by several Leishmania species depending on geographical distributions including Old-World and New-World species, which are transmitted by bites of infected female sand flies which mainly include Phlebotomus and Lutzomyia genera, respectively (Gurel et al., 2020). Several cases of cutaneous form of leishmaniasis in *L. major*-infected dogs have been recorded in Saudi Arabia, Egypt, and Iraq. Generally, infected canines often exhibit both VL and CL forms, although certain human leishmaniasis lesions (oriental sores) only affect the skin (Mehregan et al., 1999; Perez-Molina et al., 1999). In endemic locations, diseased dogs are a possible major reservoir host for sandflies (Dantas-Torres et al., 2006). Malnutrition, poor hygiene, age, gender, geographic location, and seasonal distribution are all risk factors for this illness (Al-Mayali and Al-Hassani 2017). The parasite can be diagnosed by using a variety of techniques: include histopathology, serology, microscopic examination of direct smears using Giemsa stain or Leishman stain, and specific molecular techniques

based on detecting the parasite DNA using polymerase chain reaction (PCR) to amplify the Leishmania genome (Ekşi et al., 2017).

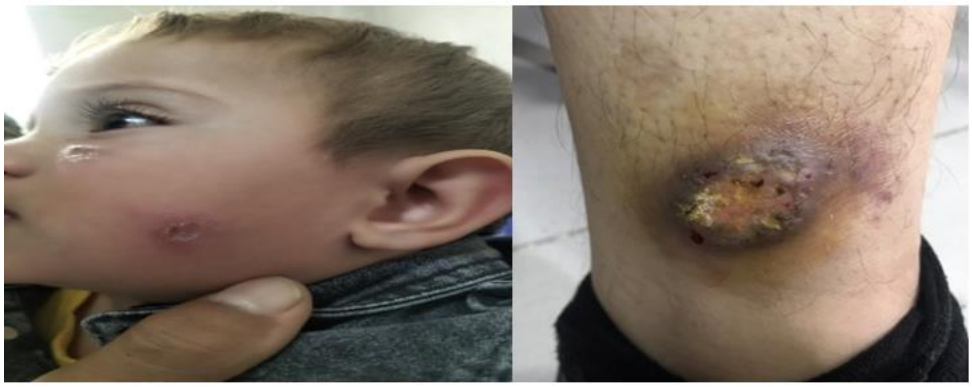


Figure 1.2 Some CL, cases in Erbil province 2022

1.5. Research Problems

While CL is still endemic in some parts of Iraq, the gap of the research is CL vectors, where the phlebotomine sandflies is the only vector to transmit leishmania parasites to humans and animals, which is causing leishmaniasis diseases, especially (CL). This research gap needs to be filled as the sand fly (phlebotomine) transmits leishmania parasite to human and it causes leishmaniasis disease. Some cases of "leishmaniasis" are highly acute, and cause death if left untreated. The Identification of sand fly species (phlebotomine) vectors of leishmania is a key to control the leishmaniasis disease, especially (CL) in the study area.

1.6. Research Objectives

In our country, especially in Erbil province and its surroundings, studies on the leishmaniasis and the distribution, biology, ecology and vector capacity of vector species have been very limited so far. However, the goals of this study are to determine the vector species in the epidemiology of the CL disease, and it is necessary to define leishmania

parasites in patient cases and the epidemiology, bio-ecology of CL vectors, correctly and reveal their distribution in a healthy way. Finding the species of sand fly (phlebotomine) in Erbil province and it is surroundings are the key to control the leishmaniasis disease, especially (CL) diseases. Additionaly, to asses the correlation between sand fly spp. and CL cases in the surrounding areas.

1.7. Research Questions

This research tries to answer the following questions:

1-Will detect and diagnose CL in human cases, does age, sex, districts, and months of the study effect on infection rate?

2- Are there any effect of humidity, precipitation and temperature on occurring sand flies and CL cases rate (Portion) in the study area.

3- How many species of sand fly (Diptera: phlebotomine) exist in Erbil province and its surroundings?

4- Which species of sand fly (Diptera: phlebotomine) is most dominant in Erbil province and its surrounding?

5- The research tries to find out whether there is any relationship between existing sand-fly species and appearance of CL cases in the Erbil province according to Erbil districts and the months of the year.

1.8. The Significant (Value) of the Study

The study will be as important as it makes a profile about existing sand fly (phlebotomine) species and CL cases in Erbil province and it is surroundings, it will state the epidemiology, bio-ecology, seasonal distributions of CL cases and (sand fly (phlebotomine) species. These findings will be completely important to the Ministry of Agriculture, because the ministry of agriculture can spread specific repellents against those species of sand fly (phlebotomine that the study will identify), spreading the

repellents cause controlling the CL vectors as it will be a very prominent way to control the leishmaniasis disease especially (CL). Furthermore, the ministry of health will get benefits from the findings of this study, as it will explain the epidemiology of the sand fly (phlebotomine) species in Erbil province, it helps the ministry of health to send their stuffs to those areas for controlling the CL diseases when this vectors in sharp during the year. At the end the findings of these study will useful to the community (population), especially to those people who live in the province, as it shows them when and where CL disease and its vectors are prominent in the province. It assists them to protect themselves and to be more aware about the CL vectors.

2. LITERATURE REVIEW

2.1. Vectors and (Sandfly)

2.1.1. Insects as the Vectors

Estimates the existence of 200 million insects alive per each human at any given point; among them, around 14,000 species feed on blood, some, with potentially severe implications for human health (Lehan, 2005; Alvarez-Hernandez et al., 2020). In fact, diseases associated with arthropod vectors (generally known as vector-borne diseases) account for more than 17% of all infectious diseases, and cause at least 700,000 deaths annually, as per the most recent estimates (Müller et al., 2019; WHO, 2020). Of note, since most of these diseases disproportionally affect individuals in resource-poor countries of the tropics and subtropics, they are considered Neglected Tropical Diseases (NTDs) (Müller et al., 2019; Hotez et al., 2020). High numbers of vectors are the consequence of several human and natural factors such as the looseness of the vector control campaigns, strong urbanization of the rural and suburban areas, and changes in land use that stimulate the expansion or migration of the reservoir hosts all these factors led to the emergence of new **foci** of transmission as reported (Harrat, et al., 2009; Boudrissa, 2012). Thus, synergic effects of globalization, climatic change, and various human activities allow the parasites and their vectors to spread in space and time as it was noticed in Europe (Antoniou, et al., 2013).

2.1.2. Sandfly Description

Phlebotomine sand flies (Diptera: Psychodidae) are important vectors of various human and animal pathogens such as Bartonella bacilliformis, Phlebovirus, and parasitic protozoa of the genus Leishmania, causative agent of leishmaniases that account among most significant vector-borne diseases (Chagas et al., 2018). Phlebotomine sand flies are small insects with nocturnal activity, females being hematophagous and feeding on various vertebrate hosts depending on species (Killick-Kendrick, 1999; Abonnenc, 1972). They live in various habitats, some

species thriving in the vicinity of human dwellings and shelters of domestic animals that provide favorable humidity and temperature conditions and breeding sites as their larval stages are terrestrials, living in microhabitats with organic material (Abonnenc, 1972; Killick-Kendrick, 1999). So far, over 950 described species were classified into several genera, approximately 100 species in both Old and New World are incriminated in transmission of various pathogens including those infecting humans: bacteria *Bartonella bacilliformis*, sand fly–borne viruses, and, most importantly, parasitic protozoa of the genus Leishmania (Munstermann, 2018). Sand fly taxonomy is traditionally based on analysis of decisive morphological characters on the head and genitalia (Lewis DJ, 1982; Munstermann, 2018), but the advent of molecular techniques that deploy mainly sequencing analyses of suitable genetic markers (Depaquit, 2014).

(Perfiliew, 1968; Kashkool, 2009) described the effect of wind speed and sun light on distribution of sand flies that high winds prevent the spread of sand flies and reduce it ability of blood absorbed, which is during several times from 7 PM to 2 AM. The optimal duration of activity and effectiveness of female Harms directly after the sunset, nocturnal activity of sand flies indicated that they were most active early in the evening during the cold months, whereas they were more active in the middle night during the hot months). adult phlebotomine, sand flies are found only during the summer months, with populations) of certain species peaking in late spring whereas others tend to peak later on in summer. (Dinesh, et al., 2001). The seasonal, activity of adult sand flies is affected mainly by temperature, and rainfall (Killick-Kendrick, 1999). Mohsen (1973), reported detection temperature has an effective role and specific effects of air and climate on the activities of the Harms vector of the infection stage disease of leishmania, in Iraq and explained that they are of systematic importance". Determined at, temperature 25-28 C^{o}, which affects the biological and physiological activity of biting sand flies. The males congregate in lakes on or near the host and produce sex pheromones. Vibration of the wings by males can be important in encouraging females to mate (Oliveira et al., 2001). Resting sites are often near to larval breeding sites and consist of cool, humid and dark micro-habitats.

Figure 2. 1 . Male Phlebotomus (Atlen et al., 2016)

2.1.3. The Sandfly Life Cycle

It comprises four major stages: Eggs, larvae, pupae, and adults (Figure 2.2). On average, a female sand fly deposits 30 to 70 eggs in protected places chosen based on humidity and the presence of organic matter (e.g., cracks and holes in the ground, animal burrows/dens, termite mounds, leaf litter), (Killick-Kendrick, 1999; Cambridge University, 2012). Typically, the eggs hatch between four and 20 days after oviposition, although this timing may be extended in cooler weather— eggs may diapause under unfavorable conditions (Killick-Kendrick, 1999; Cambridge University, 2012). There are four larval instars, and larval development is usually completed in 20–30 days, depending on the sand fly species (Volf and Volfova, 2011), as well as on the temperature and availability of food. However, this period may be prolonged to several months in sand fly species that diapause to cope with winter (only full-grown larvae diapause—instar four) (Killick-Kendrick, 1999; Cambridge University 2012). Pupation usually takes from six to 13 days with adults emerging during the hours of darkness, often just before dawn (Cambridge university, 2012). Males usually emerge before females. However, it is known that while males may live only about a week in the wild, females may live longer as they undergo more than one gonotrophic cycle, (Rutledge and Gupta, 2002). Normally, oviposition occurs

between five and eight days after blood-feeding, although some species are known to feed multiple times before successfully developing viable eggs (Rutledge and Gupta, 2002). Of note, most sand fly species are exophagic (feed outside of dwellings), although some are known to be endophagic and endophilic (feeding and resting in human and animal dwellings), commonly referred to as domestic or peri domestic species, (Rutledge and Gupta 2002; Killick-Kendrick, 2001; Cambridge University, 2012).

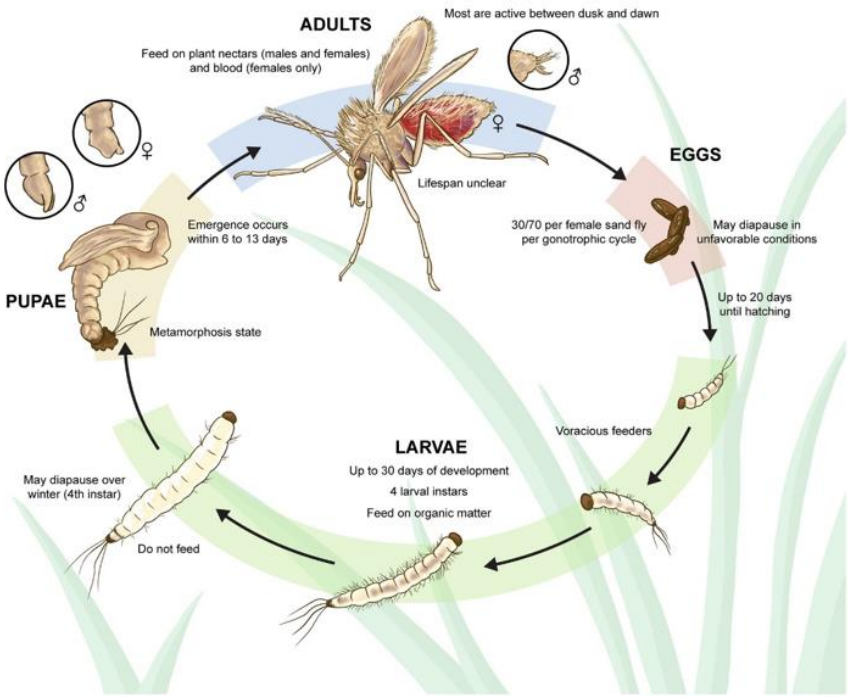


Figure. 2.2. Schematic representation of the sand flies' life cycle. Adapted from (Martin et al 2018)

The sand fly life cycle comprises four major stages: eggs (orange background), larvae (four instars: green background), pupae (yellow background), and adults (blue background). In the latter two stages, different morphological features (highlighted within the circles) can be used to distinguish the gender. The most important characteristics with respect to each stage (sub-stage), are listed near the images, as are the average timings of development. Adapted from (Akhoundi, 2016; Dillon, 2008; Martin-Martin et al., 2018).

The need for blood to give rise to a new sand fly generation (for the perpetuation of the species), associated with the fact that sand flies are weak fliers (reports state that adults usually disperse 100 meters or less from their larval habitats (ECDC, 2014).

2.1.4 Medical Important of Sandflies

2.1.4.1 Sandflies as Vectors of Multiple Diseases

Although sand flies are mostly recognized as Leishmania vectors, they transmit other pathogens, such as bacteria and viruses. Carrion's disease is a sand fly transmitted biphasic illness caused by *Bartonella bacilliformis* bacteria in Central/South America (Minnick, 2014; Pons et al., 2016). It is characterized by either intermittent febrile states (called Oroya fever), sometimes with hepatic involvement that can lead to death in the absence of (or delayed) treatment (when infected individuals are naïve); or by cutaneous lesions called Peruvian warts (when infected individuals were previously exposed to the bacteria), (Pons et al., 2016).

2.1.4.2. Sandflies are a Significant Public Health

Sand flies are a significant public health concern in many parts of the world, where they are known to transmit the agents of several zoonotic diseases to human" (Killick-Kendrick 1999; Ready, 2013), there are some zoonotic diseases which sand fly cause them.

A-Leishmaniasis: "The most common form of the disease is caused by a number of different Leishmania species in different parts of the world. CL is characterized by a skin ulcer at the site of the sand fly bite that may persist for months before healing on its own even without treatment. (Maroli et al., 2013).

B-Bartonellosis: The Sand flies are the suspected vector of the bacterium *Bartonella bacilliformis*, which causes Carrión's disease. The acute phase is a sudden, potentially life-threatening infection associated with high fever, decreased levels of circulating red blood cells, and transient immunosuppression. This infectious disease is rare being

found only at higher elevations in Andean regions of Peru, Ecuador, and Colombia. Infection may result in two clinical phases: an acute hemolytic phase (Oroya fever) and a chronic eruptive phase associated with skin lesions (verruga peruana). (Ergunay et al., 2014).

C- Sand Fly Fevers: "Sand fly fevers also known as Pappataci fevers are febrile viral (Bunyaviridae, Phlebovirus) infections transmitted by sand flies in the genus Phlebotomus. They occur in the subtropical regions of the Eastern Hemisphere. Infections may range from mild febrile illness to severe central nervous system complications (Depaquit et al., 2010).

D-Sand Flies as Nuisance: In the absence of potential disease transmission, sand fly bites can be painful and result in dermal reactions that may become secondarily infected. Unprotected personnel may receive multiple bites while sleeping, especially in locations without air conditioning, causing them to infer that they were attacked by other arthropods e.g., bed bugs, scabies and sucking lice. When sand fly densities are high, and deployed military personnel lack or do not use proper personal protective measures, large numbers of bites can be psychologically demoralizing and result in distracted or poor duty performance (Seth et al., 2015).

2.1.5. Psychodidae Taxonomy and Classification

In 1957, Alder and Theodor investigated taxonomic structures of sandflies, as abdominal hairs, pharynx, buccal cavity, and spermatheca, basically using Sinton's 1929 criteria, Later, sandfly spp. and subspp. from all zoogeographical areas were divided into six genera, i.e., Phlebotomus, Chinius and Sergentomyia in the Old World and Lutzomyia, Brumptomyia, and Warileya in the New World (WHO, 1990). Lutzomyia is not classified as a sub-genus, but there exists a great diversity in the genus (Lewis, 1971). The family Psychodidae is very old and maintains some of the most ancient dipteran characters. Members of the family are distinguished by a dense covering of narrow scales on head, thorax, legs and wing veins. Phlebotomine sand flies of Iraq have been studied by a limited number of Iraqi's such as Abul-Hab who was the first Iraqi entomologists to conduct basic studies on sand flies in Iraq.

(Abul-Hub, 1978) divided family of Psychodidae to three subfamilies": 1. Phlebotominae 2. Psychodinae 3. Trychomyiinae. "Only subfamily Phlebotomine has piercing mouthparts capable of taking blood. Furthermore, three features of phlebotomies are diagnostic to distinguish them from other Psychodidae: (1) when at rest, they characteristically hold their wings at an angle above the abdomen (2) they are hairy; and (3) when alighting to engorge, they typically hop around on the host before settling down to bite.

The sand flies of Iraq were described by (Adler and Theodor, 1929; Pringle, 1952). Most entomologists still follow the classification by (Lewis et al., 1977) have proposed sub division of the phlebotomine sandflies into three genera for Old World species, Phlebotomus, Chinius and Sergentomyia, and three genera for New World species, Lutzomyia, Brumptomyia and Warileya. Currently, the classification of Psychodidae according to (Perfiliew, 1968).

The family of Harms has about 2000 species, divided into three genera Phlebotomus, Sergentomyia and Lutzomyia; the second genus Sergentomyia has no medical importance because it depends on feeding on animals; the third genus, Lutzomyia, is spread only in Americas (New World), while the first genus Phlebotomus attacks humans and other animals and transmits many pathogens (Abul-Hab, 1978). In Iraq, about 17 species of sand fly were identified (Sukkar, 1974). In Baghdad only 7 species of insect vectors were diagnosed (Abul-Hab and AlBaghdadi, 1972a, b). (Sukker et al., 1983) suggested that both *P. papatasi* and *P. alexandri* might be the vector". The classification of sand fly has historically based on phenotypic of morphology, but the subfamily's systematics are improving because of genetic and genomic advances. These advances are rapidly expanding Phlepotominae‟s classification, include many tribe and sub tribe levels with numerous genera (Akhoundi et al., 2011). The classification of Psychodidae according to (Perfiliew, 1968).

Kingdom: Anamilia

Phylum: Arthropoda

Class: Insecta

Subclass: Pterygota

Division: Endopterygota

Order: Diptera

Sub order: Nematocera

Family: Psychodidae

Sub family :1- Phlepotominae, 2- Psychodinae,

3- Trychomyiinae

i-Genus: Phlebotomus

Species: 1-papatasi 2-sergenti 3- alexandri

ii- Genus: Sergentomyia

Species: 1- sintoni

iii- Genus: Lutzomyia

2.1.6. Ecology of Phlebotomine Sandflies

Eggs are banana-shaped and approximately microscopic in size (0.3–0.5 mm). The eggs of sandflies are primarily white or light gray in color then covert to dark brown or black during hours of being oviposited, according to the species. The time to hatching is of high temperature dependent, but in 4 to 10 days the eggs hatch into the next stage (larvae). Sandfly larvae are caterpillar-shaped, with head capsules and small leaf-like antennae. Mainly, they are scavengers, feeding on organic matter such as fungi, semi-rotting vegetation, decaying forest leaves, decomposing bodies of arthropods, and animal feces (Lawyer and Perkins, 2004). At low temperatures, the period of larval maturation can be as short as a month, whereas at high temperatures the duration of the development can be as long as three months (Tesh and Guzman,

1998). Before pupation, sandfly larvae cease feeding, and some species may travel a short distance upward to a drier location. Pupae resemble a small butterfly (Abul-Hab and Ahmed, 1984). After pupation, adults emerge in 4 to 6 days, at the dark hours of the day, often just before dawn. Most adult sandflies are within a size range of 2.5 to 3.5 mm. Depending on the species, the color of adult sandflies varies from silvery gray to nearly black. The overall dimensions of males and females are similar, but the two are readily distinguished. The abdomen of the female is round and robust, whereas the male is slender and the terminal claspers viewed from the side form a "C" shape (Lane and Crosskey, 2012; Habeeb, 2005; Killick-Kendrick, 1999) pointed out three basic features that help to distinguish adult sandflies from other flies. These features are: while sandflies at rest they characteristically carrying their wings at an angle over the abdomen, forming a "V" shape; their body and wings are entirely covered by hair.

2.1.7. Distribution of Sandflies

2.1.7.1. Sand Flies Worldwide

In Serbia, (Vaselek et al., 2017) recorded genus Phlebotomus with species *P. Larroussius, P. perfiliewi and P. neglectus.* In South America (Zorrilla et al., 2017) study the distribution and abundance of Lutzomyia of the genus Lutzomyia (58 species) and Brumptomyia (2 species) and identify sand flies' species naturally infected with Leishmania". In Iran, (Yaghoobi-Ershadi et al., 2015) showed that the number of sand flies species include 26 Phlebotomus species of 6 subgenera and 18 Sergentomyia species of 6 subgenera. Phlebotomus sergenti and P. sergenti similis. Similarty in Iran a study done by (Sofizadeh et al., 2018) collected sand flies that were identified as belonging to 18 species. *Phlebotomus wenyoni* was reported for the first time from the area villages. "The frequency of sand flies in the villages located in northeast of the Golestan province. In Turkey, (Ozbel, 2013) among 22 species of sand flies recorded by him, 7 are proven or suspected vectors of human leishmaniasis and phlebovirus infections. In the Amazon region of Brazil, (Pereira et al., 2014) collected a total of 456 sand flies, comprising 256 females and 200 males.

In Syria, (Bakdash et al., 2012) collected 879 sand flies (Phlebotomus) from some regions of Homs Governorate; specimens were represented by 447 male (51.2 %) and 432 female (48.2%) which contained two genus: Phlebotumus (93 %) and Sergentomyia (7%), and four subgenus Phlebotomus (78%), *P. papatasi* was the dominant species in the study area). In Saudi Arabia, a study by (Al-Ajmi et al., 2015) also identified sand flies into five species; three of them belong to genus Phlebotomus (*P. papatasi, P. bergeroti, P. sergenti*), and two belong to genus Sergentomyia (*S. antennata* and *S. clydei*). In Sudan, (Adam et al., 2017) recorded 10 species of sandflies were three Phlebotomus species and seven Sergentomyia species of these sandflies, *P. rodhaini* and *P. orientalis*). "In Egypt, (Ali et al., 2016) collected 143 of *P. papatasi* the highest prevalence was 44.8% in Al Hawareya, while Marakya was free of sand flies, with male to female sex ratio 1:1.6 and two peaks of abundance in both July and September. while the lowest monthly abundance was in November. In Palestin, (Sawalha et al., 2017) collected sand flies from different districts (Phlebotomus and Sergentomyia) the genera Phlebotomus and Sergentomyia are represented by 13 and nine species and subspecies, respectively.

2.1.7.2. Distribution of Sandflies in Iraq

Phlebotomine sand flies are of widespread importance in the transmission of pathogens. Although CL (Baghdad Boil) are widespread in Iraq, sand flies occur in a wide range of habitats and individual species often have very specific habitat requirements, depending on locally occurring environmental factors such as frequency of precipitation, temperature, the distribution and abundance of vertebrate hosts and rainfall can affect the relative abundance of sand flies over the seasons, is usually found in sub-tropical to temperate climates where the sand fly season is often associated with the warmer months" (Young and Arias ,1992). "Many studies have examined the temporal and geographic distribution of sand flies throughout the country. The earliest published studies on the sand flies of Iraq were conducted by (Newstead, 1920) and (Adler and Theodor, 1929). Sand flies have been collected and described from a number of locations throughout Iraq; In 1952, Pringle published results of the first comprehensive survey of the sand flies of Iraq, collected from around Baghdad total, 12 species 70% of the specimens were. *P. papatasi* was the

most commonly found species in 54 of the 57 sites; P. *sergenti* and *P. alexandri* were relatively uncommon. (Pringle, 1956) subsequently conducted a limited survey for sand flies in the Zagros mountains and the central plains of Iraq, only 155 phlebotomine sand flies were collected in the mountains, of which 28% each were *P. papatasi* and *P. sergenti.*

in AL-Najaf province by (AL-Tufaily, 2003) who observed three species, *Phlebotomus papatasi, P. sergenti* and *P. alexandri, P. papatasi* was the most abundant in the rural regions while *P. sergenti* was found to be mostly abundant in the urban regions, *P. alexandri* was very rare. There were two peaks of seasonal abundant of the vectors, the first during May and the second during October. In Al-Diwaniya province, (Al-Mayali, 2004) recorded five species *Phlebotomus papatasi, P. sergenti, P. alexandri*, *Sergentomiya sintoni* and *Ser.* Squampilerius and there were two peaks for this density, one in May and a nother in September. Sand fly with different distribution along year, but with high density in February, May and decrease in the hot months. “In Al-Diwaniya province,

2.1.8. Morphological Analysis of Phlibotominae

Genus Phlebotomus

Morphological characters keys (e.g., pharynx and Spermathecae of females and terminalia of males) were considered during the preparation of the classification, these characters were reported in the keys based on by (Lewis, 1978, 1982; Abul-Hab and Ahmed, 1984; Singh and Philips-Singh, 2010**)** who classified the family of Al-Harms in Iraq (Diptera: Psychodidae). Morphological characters including:

2.1.5.1 Genus Phlebotomus

A. Females of Phlebotomus According to:

A1. "Buccal (mouth) cavity:

“Cebarium” contains many vertically scattered cibarial teeth surrounded by some of the spines in. It relates with Pharynx which is narrow in the fore ground then takes expanding back to the related with esophagus so take the form of a similar Lamp

Glass, covers part of the expanding spines which may be small or large and take the one form or several forms gives, called this region Pharyngeal Armature".

A2. Spermatheca:

"Consist of capsule a multi-shaped that may be divided or undivided accorded to genus and associated with a narrow duct of varying lengths" (Abul-Hab and Ahmed, 1984).

B. Males of Phlebotomus: According to terminalia

B1. Superior Claspers: "Consist of two pieces, the first container of bristles called coxite and the second ends called style with 5 short spines: 3 apical and 2 external spines in its apical third. Paramere with 2 dorsal ramifications.

B2. Intermediate Appendages:"They were was located in the ventral region of the superior claspers and (Paramere) as well as a pair of copulatory valves and the penis (Aedeagus), which connect with genital pump".

B3.) Interior claspers: It consist of one-piece, cylindrical structure, sometimes called a lateral lobe.

B4. Anal Cerci:) They are thin sheets located at the base of the interior claspers and are sometimes called submediant plates.

(Al-Mayali, 2004) explained the classification structures of common sand fly's species in Iraq:

2.1.8.1. Phlebotomus *Papatasi,* Scopoli, 1786

Scopoli described Phlebotomus sandflies in 1786 for the first time in the world and the genus Phlebotomus was mentioned by Rondani (1840) in Rome, Italy (Mohsen, 1983; Abul-Hub and Ahmed, 1984).

Species *Phlebotomus papatasi,*

It is one of most studied species due to its large geographical area of occurrence and its medical importance as it is the principal vector of the zoonotic cutaneous leishmaniasis (ZCL) in high- land caused by *Leishmania major* (Izri et al., 1992). It has a vast distribution from northern to the southern part of the Maghreb region (Algeria, Morocco, Tunisia, and Libya) with a high abundance in the highlands and north of Sahara between 27˚/35˚ N and −4˚/58˚ E worldwide (Karmaoui, 2020).

A. Male "Style with 5 short spines: 3 apical and 2 external spines in its third apical and basal prosses in superior claspers" (Figure 2.3).

B. Female: "Spermatheca divided to 8–12 rings (apical segment was short). Pharyngeal armature is not extending beyond its third posterior, with scaly teeth arranged into a wide-meshed network" (Figure 2.4).

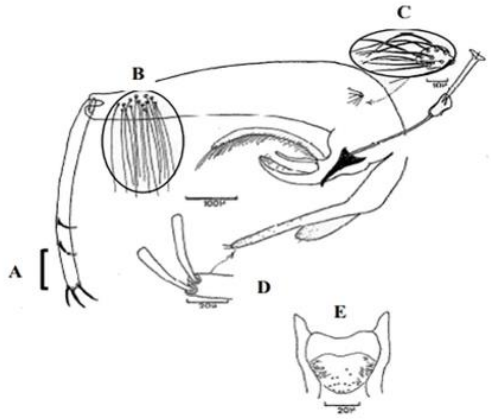


Figure. 2. 3. *Phlebotomus papatasi* ♂ (Abonnence, 1972). (A) Spine's position. (B) Coxite setae. (C) Tuft of seta. (D) Setae of the lateral lobe. (E) Cibarium

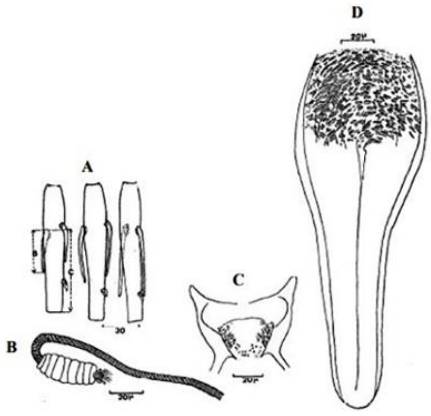


Figure. 2.4. *Phlebotomus papatasi* ♀ (Abonnence, 1972). (A) Fourth antenna segment. (B) Spermathecae. (C) Cibarium. (D) Pharynx

2.1.8.2. Phlebotomus *sergenti* Parrot, 1917

Whereas in 1917, Parrot divided sandflies as Newsteadia and Phlebotomus depending on the external genitalia of male flies (Habeeb, 20057; Boubidi et al., 2011; Tabbabi et al., 2011) suspected vector of L. killicki MON-306, recently reported in the East of Algeria (Mansouri et al., 2012). This species is collected both inside houses and outdoors, reported in almost regions of the Iraq from the north to the southestern, occurring in the humid and arid bioclimatic zones.

Species Phlebotomus *Sergenti,*

A.Male) Style is short with 4 long spines: 2 apical and the internal more basal than the external spine. Paramere without dorsal ramification. Surstyle (=lateral lobe) without short distal spines (Figure 2.6).

B.Female) "Spermatheca consists of 4–5 rings. (Figure 2.5). Pharyngeal armature not extending. beyond its posterior third, with scaly teeth arranged into a wide-meshed" (Figure 2.5).

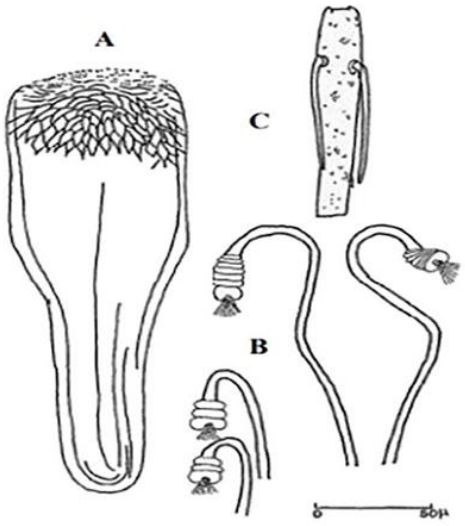


Figure. 2.5. Phlebotomus sergenti ♀. (Abonnence, 1972). (A) Pharynx. (B) Spermathecae. (C) Fourth antenna segment

Figure. 2.6. *Phlebotomus sergenti* ♂ (chamkhi et al., 1966)]. General genitalia

2.1.8.3. Phlebotomus *alexandri* Sinton, 1929

It suspected its role in leishmaniasis cycle transmission due to its abundancy in dry and rocky cavities) where rodents especially reptiles occur abound (Dedt, 1984). In most of the studies, P. alexandri had less abundance in many parts of the country. Its ecology, feeding, resting and bionomics are far from known. In addition, the vectorial status of this species is not clearly established despite its being a proven vector of L. infantum in China (Hanafi et al., 1996), and a suspected vector of L. tropica in some Arabian countries (Maroli et al., 2013).

*This species is easily identified from the rest of the subgenera of Phlebotomus.

Males of the subgenus are characterized by having four spines (two terminal and two median) on the style and a process (apophysis) or lobe with a tuft of hair on the coxite (Figure. 2.8). Whereas females of the group can easily be recognized by the large and backwardly directed pharyngeal teeth and the usually fully segmented spermathecae that expand distally with an apical segment lacking a terminal process (Figure.2.7).

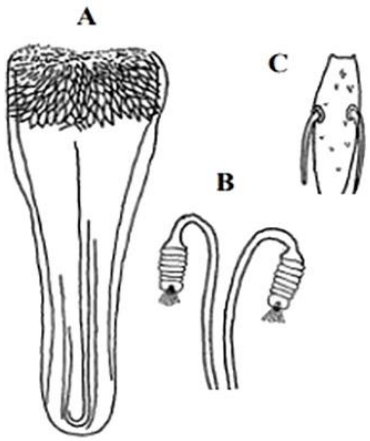


Figure.2.7. *Phlebotomus alexandri* ♀ (Abonnenc, 1972). (A) Pharynx. (B) Spermathecae. (C) Fourth antenna segment

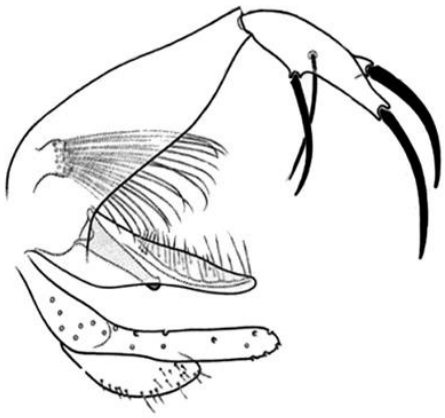


Figure.2.8. *Phlebotomus alexandri* ♂ (Chamkhi et al., 1966) General genitalia

2.1.9 Molecular Analysis of Phlibotominae

Currently, the polymerase chain reaction (PCR) methods represent the main molecular diagnostic approaches for sand fly spp. identification, and the protocols have been growing-up to diagnose phlibotominae DNA in different clinical samples (Abd El-Salam et al., 2014). PCR-based methods can be utilized to amplify sand fly-specific DNA, and they have highly sensitive and specific values, (Pereira et al., 2017). According to the type of clinical samples, sample storage, the extraction of DNA protocol, the sequences choosing targeted in the sand fly spp. genome, the use of variable primer pairs, and the PCR methodology, the protocols can vary extremely in sensitivity and specificity (Reithinger and Dujardin, 2007).

2.2. Leishmaniasis

2.2. 1 Historical background

A neglected vector-borne disease, leishmaniasis is a serious problem with an increasing number of cases (Strelkova et al., 2015; Mustanov and Nematov 2019; Suvonkulov et al., 2020; Yurchenko et al., 2023). It is caused by Leishmania spp. (Kinetoplastea: Trypanosomatidae) (Bruschi and Gradoni 2018; Kostygov et al., 2024). The protozoa Leishmania have digenetic lifecycles and can exist in two morphological forms; either as (amastigotes inside the immune cells) (macrophages) of mammals, or as flagellated promastigotes within the gut of a phlebotomine sand fly (Chappuis et al., 2007; Gradoni and Gramiccia, 2017). CL was highly incidence in troops deployed to the Arabian Gulf region (Qasmi et al., 2008).

William Leishman and Charles Donovan first mentioned the protozoan parasite in the spleen of patients complained from a malaria-like illness, separately but simultaneously in 1903 (Awasthi et al., 2004). Leishmania was established by Rogers in 1904 and Nicole in 1908, when they described the promastigote stage in vitro culture (Beaver, 1984). In the early 1940s, Swaminath, Shortt, and Anderson in India, and Adler and Ber in Palestine elucidated the transmission of L. donovani and L.

tropica (probably L. major) by Phlebotomine sandflies (WHO, 2011b). The parasite was also observed in cutaneous lesions in India in 1885 by David Cunningham and then in 1889 by Peter Borovsky. The genus Leishmania was described by James Wright in 1903 (Vannier-Santos et al., 2002).

CL had been recognized for many hundreds of years, as one of the first clinical descriptions was made in 1756 by Alexander Russell, who named it Aleppo boil. It is known as tropical sore, oriental sore, and chiclero ulcer (Calvopiña et al., 2013), and it is the most common form of leishmaniasis affecting human (James et al., 2019). In Iraq in the late 1940s, there was a major decline in cases of leishmaniasis, especially by the middle of the 1950s.

Old World cutaneous leishmaniasis (OWCL), or oriental sore, has been described in texts dating back to 1500-2500 BC. First descriptions of which can be traced back to the 9th century (Balkh sore). Arab physicians provided more detailed descriptions in the 10th century (Desjeux, 2001). As for the new world, evidence of the cutaneous form of the disease was found in Ecuador and Peru in pre-Inca potteries depicting skin lesions and deformed faces dating back to the first century AD. 15th and 16th century texts from the Inca period and from Spanish Colonials mention "valley sickness", "Andean sickness", or "white leprosy", which are likely to be CL.

2.2.2 Leishmaniasis Description

Leishmaniasis is a complex disease caused by protozoan intracellular parasites, belonging to the genus Leishmania, order Kinetoplastida, family Trypanosomatidae. The infection transmitted through female Phlebotomine sand flies of the genus Lutzomyia in New World (America) further to Phlebotomus in the Old World (Asia, Africa and Europe) (Garrido-Jareno et al., 2020). There are 12-15 million cases of leishmaniasis around the world, one to two million new cases are recorded annually. Visceral leishmaniasis (VL), is estimated in 500 thousand new cases annually and that 70 thousand of these injuries lead to death (Iddawela et al., 2018). Currently, the World Health Organization (WHO) recognizes leishmaniasis amongst the nine most

important tropical and subtropical diseases occurring in all continents: Africa, Americas, Asia, and Europe (Torres-Guerrero et al., 2017).

Leishmaniasis is becoming more common worldwide because of urbanization and vector distribution. Recently, some studies discussed the crucial role of traveling between endemic and non-endemic areas and the possibility of production of new Leishmania hybrid (Sabzevari et al., 2020). There are 4 main forms of the disease: (VL, also known as kala-azar), post-kala-azar dermal leishmaniasis (PKDL), (CL) and mucocutaneous leishmaniasis (MCL). While CL is the most common form of the disease, VL is the most serious and is almost always fatal if untreated (WHO, 2021). Humans are an incidental host and become infected when bitten by a female vector in searching for a blood (Ovalle-Bracho et al., 2019). The characteristics of the infecting parasite species and the host's immune status determine the clinical features of the disease. Based on this, there are three main clinical forms of the disease: visceral or kala-azar leishmaniasis, mucocutaneous and cutaneous (disseminated or localized) (Torres-Guerrero, et al., 2017).

2.2.3. Forms of Leishmaniasis

2.1.3.1. Visceral Leishmaniasis (VL) (or Kala-azar)

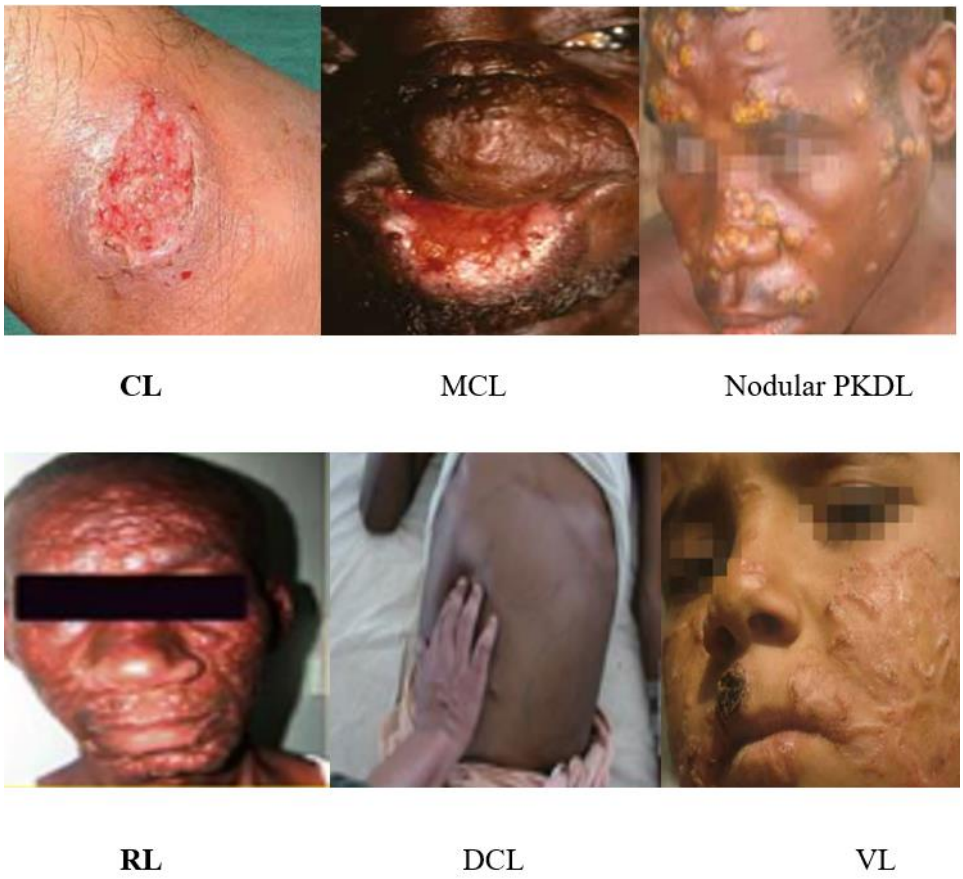
It is a systemic, severe form of this infectious disease and is caused by L. donovani, L. infantum, L. chagasi, L. amazonensis and L. tropica. Development of the disease can take months to years and infected macrophages disseminate through the reticuloendothelial system. Fever, weight loss, anorexia, pallor, diarrhea, epistaxis, hepatosplenomegaly and lymphadenopathy are the 3 common symptoms of VL. This disease can cause death within two years if left untreated (Kevric et al., 2015). Post-kala-azar dermal leishmaniasis (PKLD) is a complication observed in half of the patients successfully treated for VL and is characterized by diffuse hypopigmented macules, malar rash, papules and nodules all around the body. PKLD is caused by L. donovani and it is almost exclusively found in India and East Africa (Berman, 1997). The type of leishmaniasis they cause is Summarized in (Figure 2.9).

2.1.3.2. Mucocutaneous Leishmaniasis (MCL)

It is another form of leishmaniasis in which the nasopharyngeal mucosa is invaded and destroyed by L. braziliensis, L. guyanensis, or L. panamensis, which are the most common species that cause MCL (Davies et al., 2000). Progression of the disease usually takes places through the nasal mucosa to oral and pharynx mucosa and to the skin of the nose and lips. Atrophy of the nasal turbinates and cartilaginous septum destruction can cause death in extreme cases (Lessa et al., 2007).

2.1.3.3. Cutaneous leishmaniasis (CL)

Generally, manifests as a localized skin lesion, characterized by reddish brown infiltrative plaques and hard erythematous nodules on the skin (Bailey & Lockwood, 2007). However, in rare cases lesions can also disseminate through blood and lymph to various anatomical regions, including the face, chest and upper limbs (disseminated cutaneous leishmaniasis; DCL). Specific to Leishmania tropica infections, recidivans leishmaniasis (RL) is a recurrent form of CL, appearing at the original skin ulcer site, generally within 2 years and often around the edge of the scar (Alvar and Arana, 2018).



* (MCL, mucocutaneous leishmaniasis; PKDL, post-kala-azar-dermal leishmaniasis; CL, cutaneous leishmaniasis; RL, recidivans leishmaniasis; VL, visceral leishmaniasis; DCL, disseminated cutaneous leishmaniasis).

Figure 2.9. clinical forms of leishmaniasis Adopted from Alvar & Arana, (2018)

2.3. Cutaneous Leishmaniasis

(CL) is a worldwide public health and a social problem in many developing countries. It can affect the skin and mucous membranes, and is caused by different Leishmania species widely spread in the New and Old World. Old World cutaneous leishmaniasis (OWCL) is present in many endemic areas in North Africa, Mediterranean, Middle East, Indian subcontinent and Central Asia. The species responsible for OWCL are mainly L. major and L. tropica. L. infantum and L. donovani can also cause localized CL but are observed less frequently in the Mediterranean areas. Diffuse CL is uncommon and is caused by L. aethiopica in Africa, with regard to transmission (Hayden, 2014). They located at the site of the bite of the sandflies vector, due to replication of organism in the skin dermis (Alvar et al., 201; Desbois et al., 2014).

2.3.1. Forms of Cutaneous Leishmaniasis

2.3.1.1. Anthroponotic Cutaneous Leishmaniasis (ACL)

ACL is caused by L. tropica and occur in Iran, Syria, Afghanistan, Iraq, Pakistan, Morocco, and Saudi Arabia (Jalouk et al., 2007). It is transmitted by Ph. sergenti, which is a sandfly that live equally well in the vicinity of human habitation or far from people. It serves on to occur in towns and villages with dense human populations (Reithinger et al., 2010; Antoniou et al., 2013; Gramiccia and Gradoni, 2007; WHO, 2010).

2.3.1.2. Zoonotic cutaneous leishmaniasis (ZCL)

Leishmania major is the main cause of ZCL in a region that stretches from India through Central Asia, the Middle East, to North and West Africa (WHO, 2010). L. major has been identified in internal organs of North African hedgehogs (Atelerix algirus) collected in North-Western Tunisia and Algeria (Chemkhi et al., 2015). CL due to this Leishmania species, which is transmitted by different Phlebotomus species of Phlebotomus and Paraphlebotomus sub-genera (Akhoundi et al., 2016), is widely spreading in rural regions and figured an epidemic pattern of seasonal appearance of cases (Aoun and Bouratbine, 2011; Elbihari et al., 1987; Binhazim et al., 1987).

2.3.1.3. New-World CL

The clinical lesion is localized CL, but diffused CL has been recorded. Besides, about 5% of cases develop a severe mucocutaneous infection (WHO, 2010; Alvar et al., 2012). Domestic animals like dogs, mules, donkeys, horses, and cats, may act as blood sources to *Phlebotomine sandflies* but might also participate in the cycle of transmission (Santaella et al., 2011; Truppel et al., 2014). Several Lutzomyia species are involved in CL transmission (Akhoundi et al., 2016). As causese ulcer in different tissues as skin heart, liver, and spleen (Berzunza- Cruz et al., 2015; Alvar et al., 2012).

2.3.1.4. Old World CL

Human CL cases, and to less extent DCL or MCL, occur mostly in rural villages built on river banks or rock hills, correlated with proximity to hyrax colonies. They have been recorded in and near urban centres, (Lemma et al., 2009). Different Phlebotomine sandflies of the Larrossius sub-genus are definite vectors of L. infantum, while dogs are the main reservoir hosts for human CL (Moriconi et al., 2017; Molina, 2012; Jiménez et al., 2014).

2.3.2. Clinical forms of CL

CL has been also categorized into different clinical forms (Blum et al., 2012).

2.3.2.1. Localized

In the localized form the parasite is confined to the skin. After an incubation period of 1- 12 weeks a papule or bump develops at the site of the insect bite. The papule grows and turns into an ulcer. Most people with CL have one or two lesions varying in size from 0.5 -3 cm in diameter, usually occur on exposed parts of the body such as the face, arms or legs (Figure 2.10-6) (Blum et al., 2012).

2.3. 2..2. Diffuse Leishmaniasis

Diffuse Leishmaniasis affects only the skin but with generalized skin lesions. It is seen mainly in Africa transmitted by L. aethiopica (Hayden, 2014). Post kala-azar dermal leishmaniasis, is a form of diffuse CL and a sequel of VL that may appear in affected individuals up to 20 years after treated (Figure 2.10-7) (Pace, 2014).

2.3.2.3. Recidivans

In Leishmaniasis recidivance (LR) which is a relapsing form of oriental sore. Its frequency is about 1% of all L. tropica lesions. The lesions continue to spread over a period of many years with partial healing" (Figure 2.10-4) (WHO, 2010).

2.3.2. 4. Mucosal leishmaniasis

(In mucosal leishmaniasis the parasite may spread to the mucous membranes, especially those of the nose, mouth and throat, and cause extensive damage and disfiguration) (Figure 2.10-5). “It is mainly seen in South America but it can also be caused by species from Old World countries including L. tropica, L. major and L. infantum. Infections are acquired in the warmer months when sand flies are active (WHO, 2015).

2.3.2.5. Rural Leishmaniasis

(Wet type) “It is caused by L. major, which has wet fumes tend to ulcers very early as the incubation period for the parasite varies from 1-3 weeks and it is automatically heals within a period of two months to a year leaving a low colored scar with permanent immunity(Schmidt et al., 2005) it is the most common in Iraq and it is common for rural areas when the parasite is transmitted by sand fly from dogs, rodents, or gerbils to humans (Figure 2.10-1) (WHO, 1984).

2.3.2. 6. Urban Leishmaniasis

*(*Dry type) “Caused by L. tropica is found in urban areas in the Mediterranean, Middle East, Pakistan and parts of India (Schmidt et al., 2005). Dry ulcer is a small, slow-developed, non-invasive, usually non-permanent form. It has a long incubation period of 2-8 months. (Figure 2.10-2).

2.3.2.7. Acute Cutaneous Leishmaniasis

This is the most common type of leishmaniasis and is caused by any type of CL. The infection occurs within one year, in a different form of scarring that is not ulcerated and is similar to eczema” (Figure 2.10-3) (Tan et al., 2000).

* Aleppo evil" and "Dehli boil" were names used to describe some cases of CL in the Indian subcontinent and the Middle East respectively, when lesion biopsies were located to contain protozoa. CL lesions later known by different names, such as Baghdad sore, Uta, Chiclero's ulcer, Rose of Jericho, and forest yaws (Grevelink and Lemer, 1996).

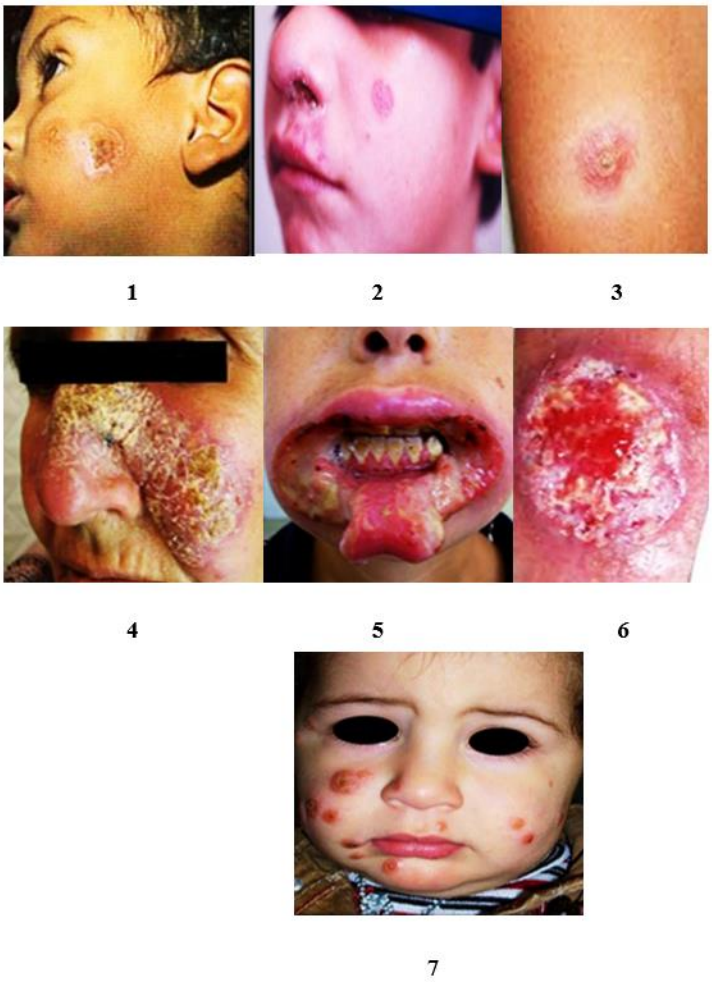


Figure 2.10. Clinical forms of Cutaneous Leishmaniasis (Hayani et al., 2015)

2.4 Leishmania parasite

Genus Leishmania belongs to sub-kingdom Protozoa, order Kinetoplastida and family of Trypanosomatidae. Leishmania genus is further divided into two subgenera as Leishmania and Viannia, based on their location in the vector's intestine (Bañuls et al., 2007).

2.4.1. Classification and Taxonomy of Leishmania

Classified parasite Leishmania depending on the sequence of nitrogenous bases of DNA and DNA homologous enzymes Isoenzyme. Primarily, species classification was depended on various extrinsic properties as clinical, biological and geographical characteristics. For example, L. infantum (isolated from a child in Tunisia), L. peruviana (isolated in Peru), L. guyanensis (isolated in Guyana), and L. gerbilli (isolated from gerbils) (Chappuis et al., 2007). Generally, 30 species are known and approximately 20 are pathogens, based on the scheme published by WHO (WHO, 2009), for human (These species generally present different epidemiological and clinical features correlated to different genetic and phenotypic profiles). All members of the Leishmania genus are parasites of mammals, and the two sub-genera, Viannia and Leishmania are separated on the basis of their situation in the vector's intestine, by using iso-enzyme analysis to define species complexes within sub-genera (Lainson and Shaw, 2005; Rioux et al., 1999).

* Classified parasite Leishmania depending on the sequence of nitrogenous bases of DNA and DNA homologous enzymes Isoenzyme (Figure 2.11), (Chappuis et al., 2006):

Kingdom: Protista

Subkingdom: Protozoa

phylum: Sarcomastigophora

Subphylum: Mastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Suborder: Trypanosomatina

Family: Trypanosomatidae

Genus: Leishmania

Species: L. major, L. tropica, etc.

2.4.2. Features of leishmania

Leishmania parasite are sand fly-vector parasites that appear as intracellular amastigotes multiplies inside the phagolysosomes of mammalian and as extracellular flagellated promastigotes either in the culture or in the gut of the sand fly (Rahi et al., 2013). There are a lot of species of Leishmania recognized to cause disease in human beings; they are very similar morphologically but produce strikingly different pathological responses. Leishmania species pathogenic for humans, (Solbach and Laskay, 2000). In the Eastern Hemisphere, Old World (OW) leishmaniasis exists and is endemic in Africa, southern Europe and Asia. In the Western Hemisphere, New World (NW) leishmaniasis exists and is endemic in south central Texas to Central and

South America except for Chile and Uruguay, leishmania Parasite species that cause CL are listed in (Table 2.1.) (Kevric et al., 2015).

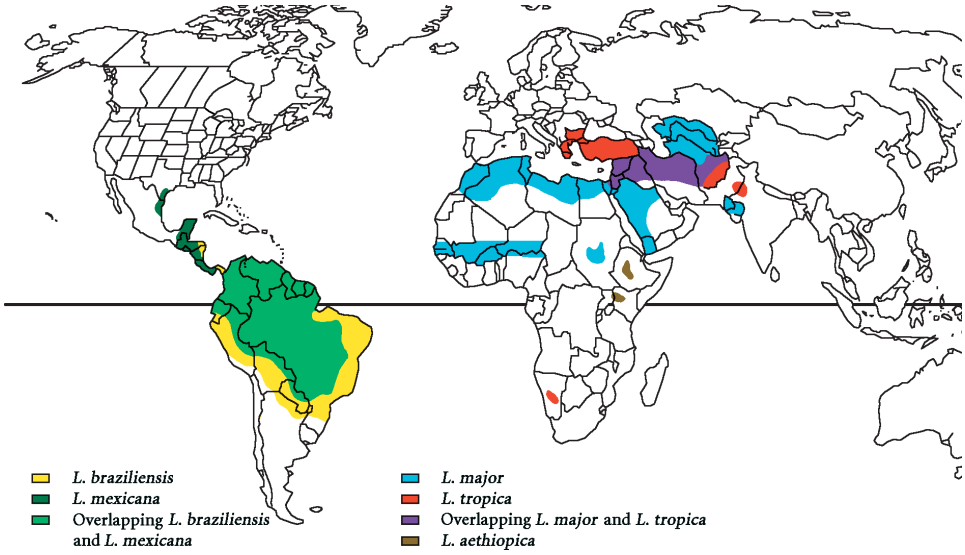


Figure 2.11. Geographical distribution of Leishmania sp worldwide. (Alan et al., 2019)

Table 2.1. Taxonomy of Leishmania parasites

Region Complex	Species	Clinical Manifestation
Old World		
Leishmania donovani	L. donovani	CL, VL, PKLD, MCL
	L. infantum	CL, VL, PKLD, MCL
	L. chagasi	CL, VL, PKLD, MCL
Leishmania tropica	L. tropica	CL, MCL, VL (rare)
New World		
Leishmania Mexicana	L. mexicana	CL, DCL (rare)
	L. amazonensis	CL, DCL, ML, VL
	L. venezuelensis	CL, DCL
Leishmania (Viannia)	L. braziliensis	CL, MCL, VL
(Braziliensis)	L. guyanensis	CL, MCL
	L. panamensis	CL, MCL
	L. peruviana	CL

2.4.3. Morphology of Leishmania

Morphology of Leishmania (Leishmania parasite is a protozoan unicellular obligative, which has two forms: the first is round or semi-circular untreated amastigote grows inside the macrophages of the mammalian hosts, and the second is promastigotes in the gut of female sand fly's genus phlebotomus is about 20 microns long (Akopyants et al., 2009).

2.4.3.1. Amastigotes

Amastigote Leishmania exists as an obligate intracellular organism within the phagolysosome of the mammalian host macrophages or other phagocytic host cells; the amastigotes are round or oval non-flagellated cells, 2-3 µm length 2-6 µm in diameter (Dedet et al., 1999). The flagellum is internalized and the kinetoplast and nucleus are also easily visible in cytological stains. The flagellum does not come out of the cell freely. In all Leishmania species. The cytoplasm contains a single mitochondrium, the Golgi apparatus and lysosomes. With their various enzyme activities, they help the parasite to feed (Schönfeld, 1980), the kinetoplast is often distinguishable from the other structures due to its kidney shape and is characteristically used for microscopic diagnosis. It stains intensely on giemsa stain due to the high concentration of DNA, the amastigote form can be obtained sequential in specific media with decrease of pH and increase in temperature; it is called axenic amastigote (Figure 2.12B) (Ready, 2013).

2.4.3.2. Promastigotes

Promastigotes are slender cells about 15-20 Mµ in length, 1.5-3.5 Mµ in diameter, with a flagellum that is approximately 15-28 Mµ. Promastigote is an extracellular stage within the alimentary canal of invertebrate host phlebotomine sand fly and in the culture, The nucleus is central while, the characteristic kinetoplast is located adjacent to the base of the flagellum (Dedet et al., 1999). In culture, the flagella will often attach to each other forming rosette structures or large clumps promastigotes classified as two types: procyclic and metacyclic promastigotes; the procyclic

promastigote is multiply in the mid gut of the sand fly, while the metacyclic promastigote found in the mouth parts and anterior gut of sand fly, it is not divided and considered as the infective stage) (Figure 2.12A) (Oliviera et al., 2005).

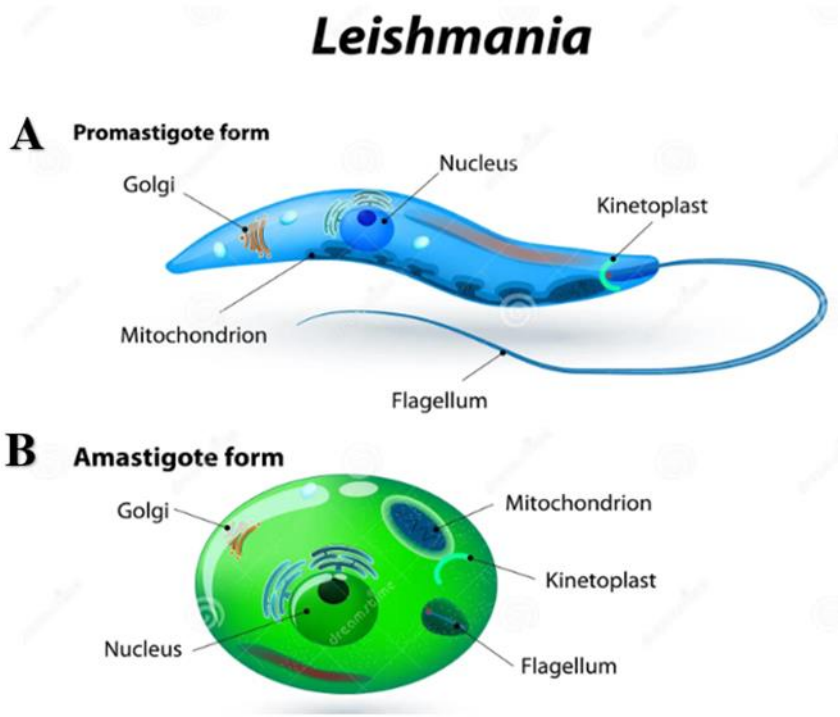


Figure 2.12. Morphology of Leishmania stages (Rodriguez et al., 2018)

2.4.4. Life cycle of Leishmania

The life cycle starts when a parasitized female sandfly takes a blood meal from a vertebrate host as the sandfly feeds. Leishmania parasites have a digenetic life cycle which requires an insect vector and a mammalian host. Female sandflies (Phlebotomus spp. and Lutzomyia spp.) carry the flagellated and motile promastigotes which are the extracellular form of Leishmania. Within the insect mid-gut, the parasites undergo several developmental changes and differentiate into this infectious form. Infective metacyclic promastigotes forms enter inside the mamalian host through the insect's proboscis. The promastigotes are then phagocytosed quickly by macrophages, following their internalization by phagocytosis, inside which they metamorphose into amastigote forms and reproduce by binary fission. Their number

increase until the cell lastly burst and they then infect another phagocytic cell to continue their cycle (Khyatti et al., 2014). During their complex life cycle, Leishmania parasites are exposed to several extra- and intra-cellular environmental conditions, showed in (Figure 2.13).

Inside the sand fly, amastigotes go to the middle or far end of the fly‟s gut, where they transform into a larger stage, the promastigote, and attach to the gut wall. Promastigotes are more than three times as long as the amastigotes and they have a long whip-like flagellum attached at one anterior end. The Promastigotes multiply by reproducing their nuclear material and simply dividing in to, a process called longitudinal binary fission (Khyatti et al., 2014). In this way, though the sand fly may have only ingested a small number of parasites, it multiplies soon to large numbers in the sand fly‟s gut. In less than a week, the promastigotes move forward again, lodging in the sand fly‟s esophagus. When the infected sand flies next feeds on a mammalian host, it transfers the promastigotes to the host along with saliva into the bite wound (Figure 2.13) (Santos et al., 2014).

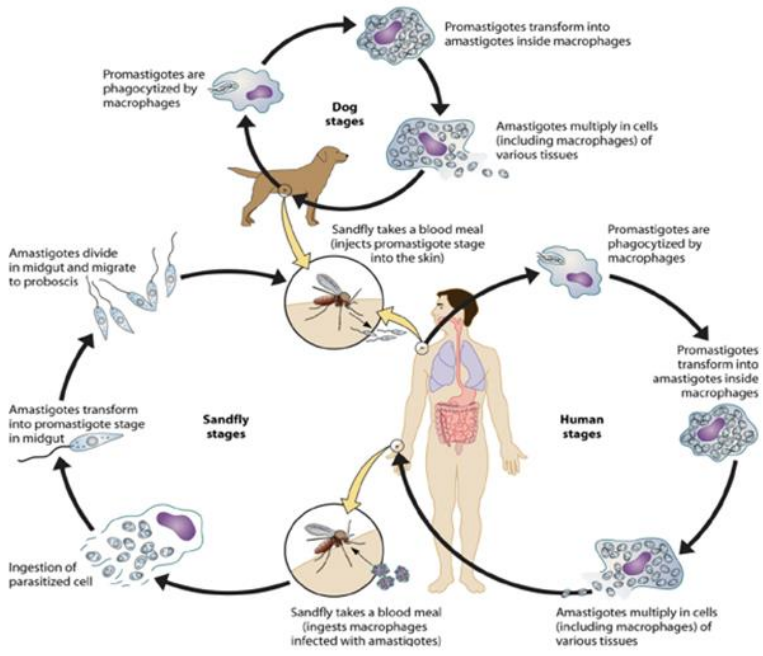


Figure 2.13. Adopted from (Chappuis et al., 2007). The Figure summarizes the stages of Leishmania lifecycle in sand fly and mammalian host cells

2.4.5. Hosts of Leishmania

2.4.5.1. Dogs

Dogs are reservoirs or accidental hosts for Leishmania spp. It is also found that dogs are naturally infected by several species of Leishmania (Gavgani et al., 2002). (Dereure et al. 2003) documented that infection by these species in dogs was accidentalm Canine leishmaniasis (CanL) is largely an outcome of high infection rates in human; the highest sero- prevalence reported in dogs was noticed during an outbreak of human VL. Canine Leishmania serves out to occur in villages and towns with abundant human populations. Although the disease was detected in domestic dogs, and it is believed that dogs are an incidental host, and that the significant reservoir host is human (Mackenstedt et al., 2015).

2.4.6. Transmission

The blood-sucking sandflies involved in CL spreading belong to the family Psychodidae; at least 70 known species are having ability of transmitting CL (Croft et al., 2006). The role of sandflies in the leishmaniasis transmission have focused on the function of maxadilan, which is a vasodilator peptide same as in structure to the calcitonin gene-related peptide (Rogers, 2012). An accidental mode of transmission is contact with an infected vector in the laboratory. This can result from exposure to animals' samples or via wounds caused by contaminated needles or people infected with contaminated blood or through pre- existing skin scraping, and inadequate management of crop pests (Delgado et al., 1996). The transmission of leishmaniasis is increasing at high rates in several regions of the world; this increase is a sequel of locations that boost the probability of being exposed to sandflies, as the establishment of new settlements in high-risk endemic or wild regions, where zoonotic infection may happen; disturbance in social and economic conditions in the poorer suburbs of some towns; and increment migration toward urban regions by populations that live in rural areas (WHO, 2016).

2.4.7. Incubation Period of Leishmaniasis

The initial lesions appear at the site of insect biting. The incubation period last from two weeks to three months. Primarily, lesion is a small, itchy, erythematous papule or nodule that may be led to enlarge of the draining lymph node. Spontaneously, wound may heal; alternatively, it may be evolved after few weeks to patent disease with different clinical manifestations (Jirmanus et al., 2012). Again, (Machado et al., 2002) estimated the time between appearance of the lesion and identification ranged from 8 to 20 days. Variable clinical features record, ranging from a single ulcerative form that is sometimes self-healing to more severe diffuse CL, for which the treatment is a very challenge (Barral-Netto et al., 1997). Even in the regions of endemicity, detection is rarely made early in the infection, and most patients diagnosed within one to two months after the presence of a lesion, at which point an ulcer is quite evident (Machado and Carvalho, 2012).

2.4.8. Diagnosis of Cutaneous Leishmaniasis

Differential diagnosis is important because diseases of other causes but with a similar clinical spectrum to leishmaniasis e.g. leprosy, skin cancers, tuberculosis, cutaneous mycoses, are common in leishmaniasis endemic areas (Paniz et al., 2013). Diagnosis of CL is depended on clinical and parasitological examination. Parasitological diagnosis remains the gold standard in CL diagnosis, because of its high specificity. Amastigotes can be diagnosed by microscopic examination of lesion smear samples after staining with Leishman's or Giemsa stain. Promastigotes can be growing and isolating in culture (N.N.N medium) from different samples, leading to more sensitive findings (Elmahallawy et al., 2014; Thakur et al., 2020).

2.4.8.1. Clinical Diagnosis

This type of diagnosis on the distinctive appearance of prominent ulcers, which appear as a blister or lesion covered by a crust with high edges can be ulcers and secondary infection of other organisms such as: Staphylococcus aureus or fungi, especially areas where the disease is settled" (Reithinger and Dujardin, 2007).

*Parasitological diagnosis. The classical way used for the direct diagnoses of Leishmania include the visualization of amastigotes by:

2.4.8.2. Microscopy

Amastigote visualization was form via light microscopic by examine of aspirates of cutaneous lesions, which is the classical confirmatory test. The technique is advantageous as it is having the ability to directly diagnose the organism with low cost. The specificity of this technique is high, beside its sensitivity (WHO, 2010). Samples are smeared directly on the slide, then stained with of Giemsa or Leishman's stain to visualize the amastigote by direct examination of the slide under oil immersion. The efficiency depends on the clinical forms and, in the case of CL, on the causative Leishmania spp. and the cutaneous lesion age (Srivastava et al., 2011).

2.4.8.3. Parasitic Culture

There are different culture media which used for culturing the Leishmania spp. pepton yeast extract, Novy-MacNeil-Nicole medium, Evan‟s modified Tobie‟s medium (EMTM), Grace‟s medium and Schneider‟s Drosophila medium (Orenstein, W.A. and Committee on Infectious Diseases, 2015). The ulcer fluid in tubes containing Novy-MacNeal-Nicolle medium from suspected lesions is difficult, requires significant technical expertise, and is prone to contamination. It is incubated at a temperature of 26 Cº for 5 days to a week. A drop of the center medium is then taken and the test is examined. The test is positive when the phase is seen, (Boggild et al., 2008). The sensitivity of culture tends to be low and highly variable. The animal inoculation into hamsters may also be valuable, especially for adaptation of the parasite (De Vries et al., 2015).

2.4.8.4 Histopathology

Tissues biopsy was processed according to (Ul Bari and Rahman, 2006). Briefly, the tissue from the skin collected and placed in 10% formalin for histopathological

studies, histopathology lesion can increase the likelihood of detecting the organism when few parasites are present (Ul Bari and Rahman, 2006).

2.4.8.5 Serological Diagnosis

Enzyme-linked immunosorbent assays (ELISAs) and indirect fluorescent antibody tests have been most successful serological tests but both show cross-reactivity with kalaazar (Boelaert et al., 2004).

2.4.8.6. Molecular Diagnosis

PCR-based methods can be utilized to amplify Leishmania-specific DNA or RNA, and they have highly sensitive and specific values, but they require cost and expensive equipment and materials (Pereira et al., 2017). According to the type of clinical samples, sample storage, the extraction of DNA protocol, the sequences choosing targeted in the Leishmania genome, the use of variable primer pairs, and the PCR methodology, the protocols can vary extremely in sensitivity and specificity (Reithinger and Dujardin, 2007).

2.4.9 Treatment

Several drugs are available for leishmaniasis treatment, and localizing the optimal drug depend on gain of regional efficacy (based on the Leishmania spp. and strains), available resources, and risk-advantages estimation. sodium stibogluconate (Sb) or Pentavalent antimony (pentostam) has been considered as the gold standard for leishmaniasis treatment for many decade (Frézard et al., 2009). Because of most cases of CL are self-resolving, and management are not without side effects, the decision whether to recommend a specific drug according to the gravity of the lesion forms, their sites, and the immune system status (Reithinger and Dujardin, 2007). Generally patient compliance to the current drugs due to the source of description and the high risky effects, necessitates the growing-up of new therapies (Singh and Sundar, 2015).

2.4.10. Control and Prevention

There is a no form of passive (immunoglobulin) or active (vaccine) immunoprophylaxis that is safe and sufficient against leishmaniasis (Foroughi-Parvar and Hatam, 2014). Without any prophylactic vaccine, only individual protection methods against sandflies bites and community-based efforts aimed at decreasing sandflies habitats and maturation can prevent the spreading of disease (Stockdale and Newton, 2013). Leishmanization is an ancient practice, resampling to cow-pox immunization, and predates modern vaccinations. It is widely used in the Central Asia and Middle East (Saljoughian et al., 2014). Prevention of leishmaniasis requires a binding of interventional strategies because transmission occurrence in a complex biological mode including the human or animal hosts, parasites, and sandflies vectors (WHO, 2020). WHO postulated key strategies for the prevention of leishmaniasis, which are initial detection and prompt, effective management to reduce the leishmaniasis prevalence and prevent dysfunction and death, vector control to decrease the number of sandflies by using insecticide as a spray (Davies et al., 2003).

2.4.11. Epidemiology and Distribution of Leishmaniasis

2.4.11.1. Distribution of CL Worldwide

There are more than one billion people at risk of exposure to this disease, Globally, leishmaniasis is one of the three major neglected tropical diseases after malaria and filariasis (Wamai et al., 2018). There are more than twelve million cases of leishmaniasis around the world, and one to two million new cases are recorded annually (Iddawela et al., 2018; WHO, 2018). This is because of several different factors that may be related to the environment and/or be man-made, including: demographics, poverty, large migrations, deforestation, lack of public health awareness, immunosuppression, urbanization, vector distribution, traveling between endemic and non-endemic areas, and the possibility of the production of new Leishmania hybrids (Al-Hayali and Al-Kattan, 2021). A schematic approach objected at targeting epidemiological cycles with preferable control measures was suggested by WHO in 1990 and reiterated in 2010 (WHO, 2010). Regarding, Iraqi neighboring

countries, such as Syria, there is an epidemic of CL among the displaced, especially Halp (Halp boil), which had spread to the rest of Syria (Rehman et al., 2018). Turkey also has an epidemic of CL, especially in the south (Eksi et al., 2017).

Iran, especially the South, is considered to have an epidemic of this disease (Khademvatan et al., 2012). Saudi Arabia also has CL (Zakai, 2014), as does Jordan (Hijjawi et al., 2016). Six years ago, WHO (2018) noted that more than 70% of CL cases worldwide were recorded in Eastern Mediterranean countries. The eco-epidemiological complexity interactions among reservoir hosts, sandflies vectors, and their environment make it difficult to performance national programs to handling Leishmania transmission control. According to WHO, the disease is endemic in 88 countries, with probability a total of 350 million people may be at risk (Khan and Muneeb, 2005). In Africa, VL, CL, and MCL are highly endemic in Algeria and other countries in East Africa, whereas in the Americas, the epidemiology is very complex, with variations in life cycles transmission, reservoir hosts, sandflies vectors, clinical features and therapy curative, and multiple circulating Leishmania species in the same geographical regions (WHO, 2020). In 2020, WHO published a report about the distribution of endemic countries for CL from 2014 to 2018. The report divided the world into six territories: Europe (53 countries), Americas (36 countries), Africa (47 countries), South-East Asia (11 countries), Eastern Mediterranean (22 countries), and Western Pacific (31 countries). Seven countries represented eco-epidemiological “hotspots” for leishmaniasis: Syrian Arab Republic, Iran (Islamic Republic of), Algeria, Afghanistan, Brazil, Iraq, and Pakistan (WHO, 2019).

2.4.11.2. Distribution of CL in Arabian Countries

Bahrain, Oman, UAE, and Qatar, no data were available about CL according to the reports, whereas Kuwait reported a few cases of CL, ranging between 2 and 7. Moreover, Saudi Arabia was estimated to have 2190 cases of CL in 2014 and 921 cases in 2018. Furthermore, there was an increase in CL cases documented in Yemen each year from 2014 to 2018, the lowest was in 2015 (4063 cases) and the highest was in 2016 (9120 cases) (WHO, 2019).

All Arabian countries on the African continent are endemic for CL. In 2018, Algeria recorded 10,847 cases, representing the highest rate of CL, followed by Tunisia (7,467 cases), Libya (2,977 cases), and Egypt (1,161 cases) (WHO, 2019). The most endemic areas before 1991 were central Iraq and the greater Baghdad area (WHO, 2010).

2.4.11.3. Distribution of CL in Iraq

Leishmaniasis is considered as one of the important health uncertainties in Iraq because of it had high endemicity and the high annual expenses during an epidemic. The study included analysis of available database from Iraqi CDC to determine the distribution of CL cases for the period (2008-2015 years) in Iraq, found total reported cases (for this period were 17001 (range 2.9-10.5 per 100,000 individuals). Highest reported cases were recorded in the year 2015 (4000 cases), cases of the disease were increase during the September and October months, reaching a maximum during the months of January and February, and decreases in March and reach the lowest levels in June and August) (WHO, 2019). (CL is endemic in Iraq, Iraq with a population about 32 million, where 23% are living below the national poorness line, has seen much struggle and combating in the past 25 years (Al-Samarai and AlObaidi, 2009). According to reports of) WHO (1996), Thi-Qar province was recorded the highest number of infections (1348 cases), the lowest of which was in Al-Muthanna province (110 cases) between periods (1971-1997).

In the year 2012 the Ministry also recorded the number of cases CL, the highest in Diyala province 508 cases followed by the of Nynwa 190, Salah al-Din province 326, Baghdad province 119, Diwaniyah province 35, Karbala 209, Wasit 212, Al-Muthanna 29 and Najaf 62 cases respectively" (Ministry of Health ,2012). "In 2012, Kadhum recorded 7200 cases of CL in Diyala province in 2011. CDC recorded 18,200 cases between 2008 and 2016 in Al-Tuz, Iraq (Hassan, 2017). The presence of wars and bad conditions, poor sanitation, and bad situation for people who were exposed to the displacing and lived in camps, the presence of swamps near the camps that are

very important for sandfly reproduction, all lead to a marked increase in the percentage of infection (Hawas et al., 2020).

Al-Azzawi (2015), recorded in Diyala province from the period 2013 to 2014 ,115 cases of CL. In Al-Qadisiya province (Al-Hassani, 2016) recored 1445 cases of CL during of study". (In Thi-Qar province, (Atshan, 2014) recored 91 cases of CL. In the same province also (Al-Obaidi et al., 2016) found 125 patients of CL from the begnning of October, 2013 to the end in May 2014. So, (AL- A'dhami, 2017) recorded 370 case of CL during the period 2016-2017. In Baghdad, (Younis, 2018) found 75 patients with CL in Department of Dermatology in AL-Yarmook, AL-Karamaa Al-Kadhimiya, Al-Kindi, Teaching Hospitals in Baghdad, during the period) between April / 2016 to April / 2017.

Previous reports showed that cutaneous infection was indeed endemic in the northern parts of Iraq, such as Kirkuk, Salah-Eldin, next to Baghdad, Diyala, and Wasit in central Iraq, in addition to the southern provinces Misan, Basrah, and Thi-Qar (Al-Hayali and Al-Kattan, 2021). There were also thousands of cutaneous cases recorded in American soldiers in Iraq after the war (Coleman et al., 2007; Aronso Neafie, 2005; Al-Hayali and Al- Kattan, 2021). Additionally, (Hussein et al., 2019) recorded 1482 cases of CL in Mosul city during 2016. (Ali et al., 2018), revealed that the prevalence of CL in Iraq was very high (83.3%).

3. MATERIALS AND METHODS

3.1. Study Area

This research was conducted in the province of Erbil between January 2022 and the December of the same year. The province serves as the administrative center for Iraq's Kurdistan Region (Mojarradgandoukmolla and Akan, 2023) . This independent metropolis, which has a total size of 14,873.68 $km^{2,}$ while the Centre district of Erbil, has an area of 1131.44 km^2 and is located 350 km north of Baghdad, is the third-largest city in Iraq after Baghdad and Mosul (Kurdistan Regional Government, 2022). Erbil Governorate had a population of 2,009,637 million people in 2015, while the Centre district had a population of 930,389 people. The northeast and east of Erbil are primarily hilly, whilst the Center district is generally flat. In addition, a lowland area, which is an agricultural region populated by rural residents, is located south of the city (Mustafa et al., 2019). Seven separate sample sites were selected, then each district divided into some separate regions for the research, each one reflecting a different environment and human activity across the province's whole urban and rural communities. Coordinates, elevations and numbers of population in each sampling habitats of Erbil districts are shown in (Table 3.1). (Figure 3.1) illustrates geographic locations where sandflies were collected during this study. Samples were collected by using Aspirators, light traps and sticky papers in the selected areas in Erbil province, Iraq. Furthermore, samples were obtained from Makhmur, Khaba, Gwer, Koya, Soran, Shaqlawa and City Center districts.

Table.3.1. Shows the coordinates, elevations, and numbers of population in each sampling habitat in the Erbil province of Iraq in 2022

No	District	Province	Country	Coordinate (N-E)	EL (m)
1	Makhmr	Erbil	Iraq	35 o 46'- 43 o 34'	264
2	Khabat	Erbil	Iraq	36 o 26'- 43 o 52'	252
3	Gwer	Erbil	Iraq	35 o 36'- 43 o 44'	273
4	Koya	Erbil	Iraq	36 o 5.08'- 44 o37'	600
5	Soran	Erbil	Iraq	36 o 92'- 44 o 68'	680
6	Shaqlawa	Erbil	Iraq	36 o 40'- 44 o 23'	975
7	Center	Erbil	Iraq	36 o 19'- 43 o 99'	390

Figure 3.1. Map of Erbil province showing the geographic locations of the study area where sandflies collected along this study (Kurdistan Regional Government. 2016)

3.1.1. General Characteristics of Sampling Regions

3.1.1.1. Makhmur Region

Makhmur is a district and town in Iraq's Erbil Governorate. Only a tiny eastern portion of the city is still under Kurdistan Regional Government (KRG) sovereignty, while a portion of the district is now under federal Iraqi control. Makhmur has a population of 173,801 as of 2014 (Kurdistan Region Statistics Office). Almost everyone in a household in Khabat district uses the same room for sleeping and sitting. Animal shelters and residential areas can be found in several regions of the district which are very close to each other. Four zones were chosen as the sampling area. Samples were collected from four areas with 3 different methods. The climate of this region is similar to the khabat region. The number of collected sand fly in Makhmur district are Shown in (Table 3.2).

Table.3.2. Collecting samples of sand fly in Makhmur District in Erbil province according to the months of the year between January-December 2022 by using aspirators, light traps and sticky papers

No.	Tools	Ja	Fe	Mar	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	L. T	0	45	90	206	264	0	0	1	0	0	0	0	606
2.	S. P	0	32	28	7	0	0	0	0	0	0	0	0	67
3.	ASP	0	2	8	0	0	0	0	0	3	0	0	0	13
T.		0	79	126	213	264	0	0	1	3	0	0	0	686

3.1.1.2 Khabat Region

It is a district in Iraq's Erbil Governorate's western region. It contains 64 villages and three subdistricts: Rizgary, Kewrgosk, and Darashekran. Khabat has a population of around 93,442. On the main route between Erbil and Mosul, the district is located 37 kilometers to the west of the city of Erbil (Erbil Official Website, 2015). In this region, 3 zones were chosen as sampling sites. The number of collected sand fly in Khabat district are Shown in (Table 3.3).

Table.3.3. Collecting samples of sand fly in Khabat district in Erbil province according to the months of the year between January-December 2022 by using aspirators, light traps and sticky papers

No.	Tools	Ja	Fe	Mar	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	L. T	0	45	90	206	264	0	0	1	0	0	0	0	606
2.	S. P	0	32	28	7	0	0	0	0	0	0	0	0	67
3.	ASP	0	2	8	0	0	0	0	0	3	0	0	0	13
T.		0	79	126	213	264	0	0	1	3	0	0	0	686

3.1.1.3. Gwer Region

Gwer is a town in Erbil Governorate. The Gwer area is home to roughly 65,000 inhabitants (Erbil Official Website, 2015). Three tools were used to collect sand fly samples in two different zones monthly, between January-December 2022. The number of collected sand fly in Gwer region are Shown in (Table 3.4).

Table.3.4. Collecting samples of sand fly in Gwer region in Erbil province City Center of Erbil province according to the months of the year between January-December 2022 by using aspirators, light traps and sticky papers

No.	Tool	Ja	Fe	Ma	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	L. T	0	0	2	4	143	3	4	38	89	0	0	0	283
2.	S. P	0	0	0	0	24	2	0	18	10	2	0	0	56
3.	ASP	0	0	0	0	0	0	0	0	0	0	0	0	0
T.		0	0	2	4	167	5	4	56	99	2	0	0	339

3.1.1.4. Koya Region

It is a district located in Iraq's Kurdistan Region. The district includes the four Sub-Districts of Shorash, Ashti, Segrdkan, and Taqtaq. The Koya area is home to roughly 95,550 inhabitants. The Little Zab River separates the district from the governorates of Kirkuk and Suleimaniyah on its east and south borders. It is also bordered to the north and east by Mount Haibat Sultan and to the west by Mount Bawage (Erbil Official Website, 2015). Sand fly insects are suspected to be very common in this region. Three sampling areas were selected and samples were collected with three sampling methods. The number of collected sand fly in Koya district are Shown in (Table 3.7).

Table.3.5. Collecting samples of sand fly in Koya District in Erbil province according to the months of the year between January-December 2022 by using aspirators, light traps and sticky papers

No.	Tool	Ja	Fe	Ma	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	L. T	0	0	2	3	28	6	7	32	46	3	0	0	127
2.	S. P	0	0	2	9	17	0	0	7	13	0	0	0	48
3.	ASP	0	0	0	0	0	0	0	0	0	0	0	0	0
	T.	0	0	4	12	45	6	7	39	59	3	0	0	175

3.1.1.5. Soran Region

This city in the Erbil Governorate is called Soran or Diana. One of the biggest cities in the Kurdistan Region is Soran, which has a population of over 225000. Diana serves as the capital of the Soran District, which is comparable to a county. According to (Carlson, 2014), Soran was divided into five sub-districts: Mergasur, Diyana, Khalifan, Rawandiz, and Sidekan. However, Rawandiz just separated into its own district. This region is between the borders of Turkey and Iran. Three areas were chosen in Soran district to collect sand fly samples by utilyzing light traps, sticky papares and Aspirator tools between January-December2022. The number of collected sand fly in Soran district are Shown in (Table 3.6).

Table.3.6. Collecting samples of sand fly in Soran district in Erbil province according to the months of the year between January-December 2022 by using aspirators, light traps and sticky papers

No	Tool	Ja	Fe	Ma	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	**L. T**	0	0	9	9	13	3	4	11	18	9	0	0	76
2.	**S. P**	0	0	0	6	14	0	0	6	16	0	0	0	42
3.	**ASP**	0	0	0	0	0	0	0	0	0	0	0	0	0
T.		0	0	9	15	27	3	4	17	34	9	0	0	118

3.1.1.6 Shaqlawa Region

Shaqlawa is a historic city and a hill station in the Erbil Governorate in the Kurdistan Region of Iraq. The district, which is located 51 kilometers northeast of Erbil at the base of Safeen Mountain, is home to over 145,000 people (Ibrahim, 2015). Between Safeen Mountain and Sork Mountain, Shaqlawa is located at 975 meters above sea level. According to (Carlson, 2014), the city is renowned for its waterfalls, trees, and vegetation. Three sampling sites were selected for collecting CL vectors, depending on the incidence rates, and samples were collected by using three sampling tools. The number of collected sand fly in Shaqlawa district are Shown in (Table 3.7).

Table.3.7. Collecting samples of sand fly in Shaqlawa district in Erbil province according to the months of the year

No.	Tool	Ja	Fe	Ma	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	**L. T**	0	0	0	10	29	0	0	5	25	0	0	0	73
2.	**S. P**	0	0	0	1	4	0	0	0	3	0	0	0	8
3.	**ASP**	0	0	0	0	0	0	0	0	0	0	0	0	0
T.		0	0	4	11	33	0	0	5	28	0	0	0	81

3.1.1.7. Central Region

This area, which serves as the Kurdistan region of Iraq's capital. There are roughly 930,801 people living in this district. (Official Erbil website, 2015). The City Center of the Erbil serves as a tourist destination because of its historical heritage. The tourists consider it to be the core. As a result, it is regarded as a location where population migrations are visible, *(*Kurdistan Regional Government, 2012*)*. In the central district, two sampling area was selected: Bnaslawa and Qushtapa to collect sand

fly samples by using three different tools. The number of collected sand fly in City Center district are Shown in (Table 3.8).

Table.3.8. Collecting samples of sand fly in City Center of Erbil province according to the months of the year between January-December 2022 by using aspirators, light traps and sticky papers

No.	Tool	Ja	Fe	Ma	Ap	Ma	Ju	Jul	Au	Se	Oc	No	De	T.
1.	L. T	0	1	17	28	32	0	0	8	32	21	10	0	149
2.	S. P	0	0	3	7	13	0	0	9	12	5	3	0	52
3.	ASP	0	0	0	3	6	0	0	0	3	4	0	0	16
T.		0	1	20	38	51	0	0	17	47	30	13	0	217

3.2. Climate Factors

3.2.1. The Climate in Iraq and KRG

Iraq has a subtropical climate, making it significantly drier and warmer than much of the US or Central Europe. The intensity of the rain is only somewhat higher during a few humid months each year. The climate is semi-arid continental, with hot, dry summers and chilly, rainy winters. Compared to the center and southern regions of Iraq, the northern area is colder, (Iraqi atmospheric institute report, 2007). The summers are hot and dry, with highs averaging 35 °C (95 °F) in the colder northernmost regions to a scorching 40 °C (104 °F) in the southwest, and lows between 21 °C (70 °F) and 24 °C (75 °F). The winter months are significantly colder than the rest of Iraq, with highs ranging between 9 °C (48 °F) and 11 °C (52 °F) and lows averaging between 3 °C (37 °F) and 0 °C (32 °F) on average, (Brohan et al., 2006).

3.2.2. Climate in the Province of Erbil

Due to air temperature, the geography and topography of the region have a considerable influence on the geographical distribution of precipitation. Compared to low elevations, the likelihood of precipitation is higher at higher altitudes. The geomorphology of the Erbil Province is depicted in (Figure 3.2, which demonstrates that Erbil's north and northeast have high elevations above sea level (ASL)). It averages

3000 m in certain locations, while the height drops by roughly 2500 m in the Centre district. As a result, less precipitation falls as well. As a result, the Centre district of Erbil experiences different amounts and types of precipitation than other areas (Gaznayee et al., 2023). The amount of rainfall varied between stations. For instance, in Soran district, a mountainous region, the amount of 96.2 mm was recorded at an altitude of 1204 m (ASL). In addition, in Qushtapa sub-district, south of Erbil, which is on more or less flat terrain, the amount of 22.3 mm was recorded at an altitude of 398 m ASL. The Erbil meteorological station reports that 67 mm of rain fell in the Center district, based on the Erbil City Center's automated station of the Erbil Directorate of Irrigation, (Mustafa et al., 2019).

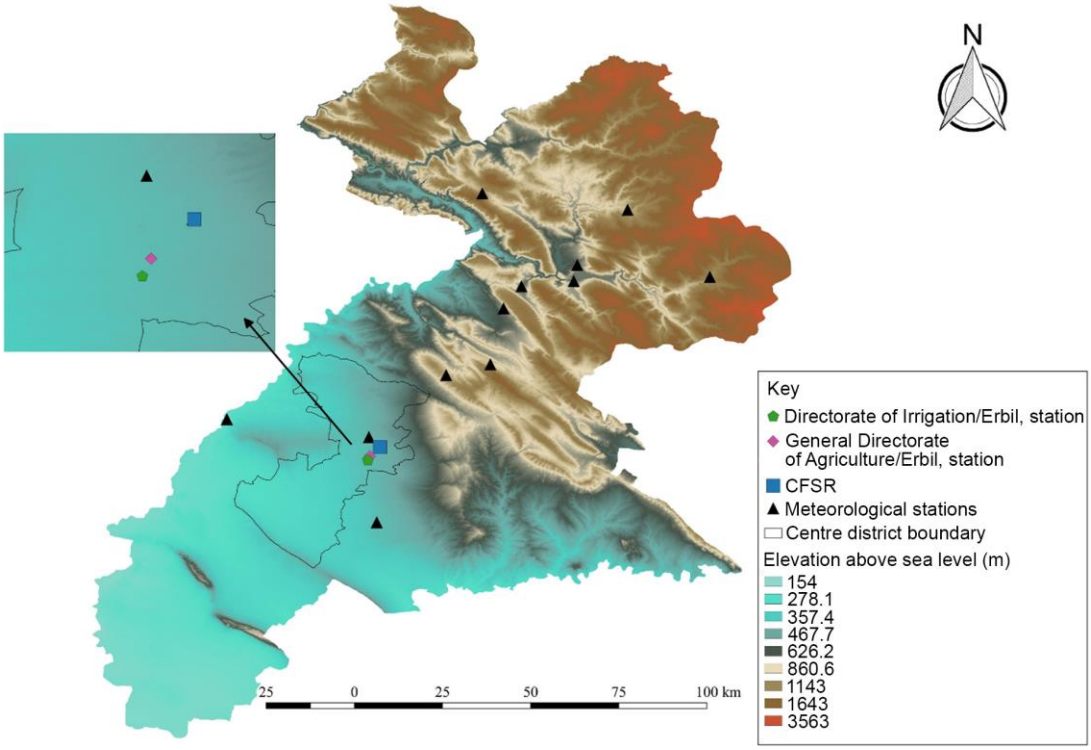


Figure 3. 2. The geography (topography) of Erbil and the locations of weather stations (Mustafa et al., 2019)

3.2.3 Climate of Erbil City

Mediterranean-style summer heat and moderate winters characterize Erbil's climate. Between June and September, there is little to no precipitation, making the summer months particularly dry. Typically, January is the wettest month of the winter,

which is also the most humid. A warm to hot summer and a chilly to agreeably moderate winter define the climate. The city seldom experiences subzero weather, however nighttime temperatures may be low. Summertime air temperatures can exceed 50°C during the warmest portion of the day. The majority of Erbil's annual rainfall, which ranges from 300 to 400 millimeters, falls between the months of October and April *Erbil* (Weather Forecast and Climate Information, 2013). Erbil Weather Forecast and Climate Information (2013), shows that Erbil's annual relative humidity is typically about 35%, the average air temperature for each month of the year in Erbil, and the average amount of rainfall for each month in 2022 (Table 3.9).

Although still debatable, it seems that climatic variables and climate change are related to the establishment of vector-borne illnesses. Climate has an impact on every part of nature, and global warming is a fact. The effects of climate change are quite important in the twenty-first century. According to some earlier research, average global temperatures will have increased by 1.0 to 3.5°C, (IPCC, 2007) increasing the likelihood that numerous vector-borne illnesses would become more prevalent. Changes in the climate and weather have an immediate influence on disease vectors and their patterns of transmission. Phlebotomine sand flies play an important role in disease transmission. Although Cutaneous Leishmanaisis (Baghdad Boil) is common in Iraq, sand flies occur in a variety of habitats, and individual species often have very specific habitat requirements, depending on locally occurring environmental factors such as precipitation frequency, temperature, physical barriers, habitat availability, and the distribution abundance of vertebrate hosts and rainfall can affect the relative abundance of sand flies over the seasons, Sand flies are usually found in sub-tropical to temperate climates where the sand fly season is often associated with the warmer months (Young and Arias, 1992).

Table 3.9. Temperature records for Erbil and the surrounding areas for 2022 for maximum, lowest, relative humidity, real precipitation by month, (Hemn Abdulkhaleq et al 2023) cited in (Copyright © 2023 Weather and Climate - The Global Historical Weather and Climate Data)

Month	Record high °C (°F)	Average high °C (°F)	Daily mean °C (°F)	Average low °C (°F)	Record low °C (°F)	Average precipitation mm (inches)	Average precipitation days (≥ 1.0 mm)	Average relative humidity (%)	Mean monthly sunshine hours
Jan	21.0(69.8)	12.29(54.12)	9.21(48.58)	5.57(42.03)	-1.0(30.2)	133.95(5.27)	10.64	57.18	7.44
Feb	24.0(75.2)	14.53(58.15)	10.99(51.78)	6.38(43.48)	-8.0(17.6)	97.71(3.85)	10	55.42	7.84
Mar	30.0(86.0)	18.8(65.84)	14.93(58.87)	9.27(48.69)	-3.0(26.6)	144.76(5.7)	13.45	54.34	10.68
Apr	36.0(96.8)	24.91(76.84)	20.69(69.24)	13.52(56.34)	4.0(39.2)	91.52(3.6)	10.09	46.46	12.83
May	41.0(105.8)	31.77(89.19)	27.99(82.38)	20.53(68.95)	7.0(44.6)	45.42(1.79)	7.27	31.07	13.99
Jun	46.0(114.8)	38.74(101.73)	34.77(94.59)	26.2(79.16)	18.0(64.4)	2.63(0.1)	0.73	17.58	14.47
Jul	49.0(120.2)	42.7(108.86)	38.35(101.03)	28.68(83.62)	20.0(68.0)	0.37(0.01)	0.09	14.21	14.3
Aug	48.0(118.4)	42.46(108.43)	37.75(99.95)	28.3(82.94)	21.0(69.8)	0.19(0.01)	0.09	14.64	13.43
Sep	45.0(113.0)	37.52(99.54)	32.68(90.82)	23.83(74.89)	16.0(60.8)	3.0(0.12)	0.55	18.03	12.43
Oct	39.0(102.2)	29.49(85.08)	25.37(77.67)	18.91(66.04)	12.0(53.6)	57.46(2.26)	6	29.17	8.79
No	30.0(86.0)	20.23(68.41)	16.89(62.4)	12.31(54.16)	2.0(35.6)	77.69(3.06)	6.82	41.88	8.16
Dec	27.0(80.6)	14.37(57.87)	11.42(52.56)	7.72(45.9)	2.0(35.6)	103.45(4.07)	8.82	52.98	7.57
Year	49.0(120.2)	27.32(81.18)	23.42(74.16)	16.77(62.19)	-8.0(17.6)	63.18(2.49)	6.21	36.08	10.99

3.3. Methods

3.3.1. Sand Flies Collection

Sand flies were gathered monthly between January 2022 and December of the same year by using tools, such as (L.T), (ASP) and (S.P) according to the nature of the site in urban and rural zones and collection of samples made of two nights per one sampling station using 12 (L.T). A total of 2054 sand flies were collected from those

regions, 1137 of which were male and 917 of which were female, and all were utilized for morphological identification, population densities, and monthly dispersion.

Figure 3.3. Sand flies collected by using Light traps, Aspirators and sticky papers in Erbil province between January-December 2022

3.3.1.1 Sand flies Collection Methods:

The sand flies were collected by using three tools according to the nature of the site to collect insects from urban and rural zones, methods were as follow:

3.3.1.1.1. Light Traps:

These traps are widely used in sand fly field research. Sandflies were captured using light traps and collected from household and agricultural fields. Traps were installed 1.5-2 m above ground, ideally at least 10 m distant from any external light (Lewis.DJ, 1978; Rioux et al., 2013). During the hours of 19.00 and 7.00, seven light traps were deployed in the sample zones, throughout the research period, light traps were sited once a month, (Figure 3.4).

Figure.3.4. shows light trap while using it for collecting sand fly in one of the zones in Erbil Districts between January-December 2022

3.3.1.1.2 Aspirators:

It is a common method for catching sand fly individuals who hide during the day or emerge from their hiding places while active at night. Sandflies were gathered here and transferred to the breeding bowl (Killick-Kendrick, 1990). They were delivered to the laboratory by immersing them in 70% alcohol in a sample vessel, (figure 3.5) illustrates the aspirator trap while using it for catching sand fly samples.

Figure 3.5. while using aspirator tool for collecting sand fly in Erbil districts between January-December 2022

3.3.1.1.3. Sticky Oil Papers:

Sticky paper traps represent a method for collecting sand fly samples by intercepting them instead of the attraction process. These traps are usually inexpensive and simple to use in large quantities. Sticky traps are frequently utilized to gather sand fly from indoors, nine sticky papers (20x20cm) dipped in castor (Castrol) oil were placed above 1m high in each household as sandfly baits (Rioux et al., 2013). Traps were set one hour before sunset in the evening and gathered when the sun came up the next morning (Figure3.6). The sandflies were retrieved from the traps with tiny forceps, washed with weak detergent, and stored in 70% alcohol in Eppendorf tubes labeled appropriately until treatment and subsequent laboratory testing.

Figure 3.6. Shows numbers of sticky oil papers while using them for collecting sand fly in different zones in Erbil Districts between January-December 2022

3.3.2 Laboratory Examination

The sandflies were kept in 70% ethanol and add few drops from glycerine stored at room temperature until further examination.

3.3.2.1. Dehydration and Dissection:

To get rid of castor oil and extra hairs, the sandflies underwent two vigorous washes with distilled water. To avoid stiffing of the sample and drying from alcohol, sand-fly samples were kept in 1.5 mL Eppendorf tubes containing 70% ethanol and

drops of glycerine. Labels stating the location and day of the sampling were then fastened. Samples must be preserved for a brief time to avoid deterioration. Then the captured specimens were kept in 5% KOH (Figure 3.10), for 2-3 hours for males and 6 hours for females to become transparent. Transparency is important to see the spermatheca clearly, especially in females. Berlese's Gum Chloral Solution was used for continuous preparation (Topraq, 2005) cited in (Lewis, 1973). After that, the samples were put on glass slides. The samples should be properly positioned to enable observation of their morphological and taxonomic traits. On the basis of the sand fly mounted on each slide, external morphological examinations of the several species were conducted. Drops of mounting material (Berlese's Gum Chloral) were used to mount and prevent the creation of air bubbles. The sample was then allowed to dry before the coverslip as gently placed on top of it. performed morphological categorization under a microscope using the keys of the family of sand flies in Iraq, based on internal and exterior morphological features. A light and dissecting microscope were used to perform internal morphological analyses of various organ structures. (Lewis, 1978, 1982; Abul-Hab and Ahmed, 1984; Singh and Philips-Singh, 2010), used the keys the family of sand flies to classify the morphology of the insects based on internal and external morphological characteristics.

3.3.2.2. Keys of Identification

A-Male:

The anatomy of the sexual organs attached to the last segment of the abdomen and the quantity and arrangement of the teeth at the base of the pharynx in the head were used to identify species in male individuals. The following structures of the male genital organs have been researched: the Coxit Structure, Style's number of thorns, the size and design of the genital pump, the Aedagus and form and the Paramer Structure.

B- Female:

In contrast to males, females' genital organs are concealed in the final two abdominal segments. To prepare for this, the final two segments were positioned such that the spermatheca could be viewed clearly. Additionally, the head half was placed

on the opposite slide since the pharynx teeth and antenna structures could be seen obviously. The spermatheca, whether or not it is segmented, the existence of the terminal cap, and the anatomy of the base of the spermatheca were all evaluated in the female genital organ.

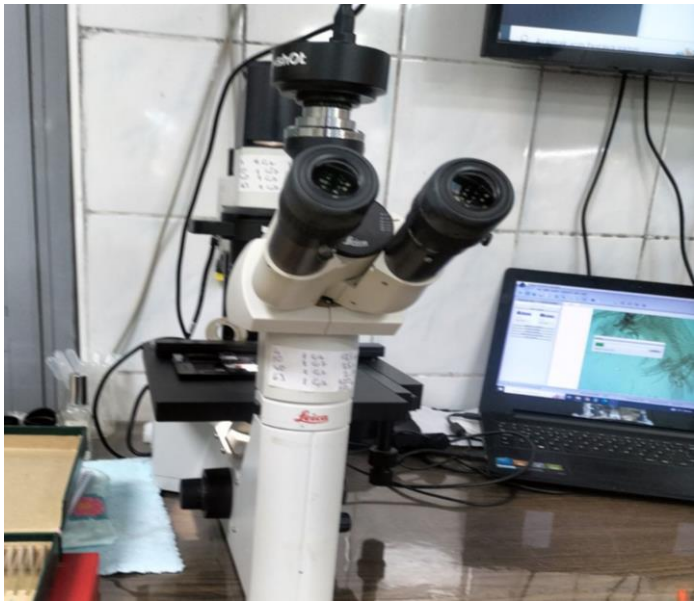


Figure 3.7. Light microscope which was used to identify sand fly species morphologically in this study

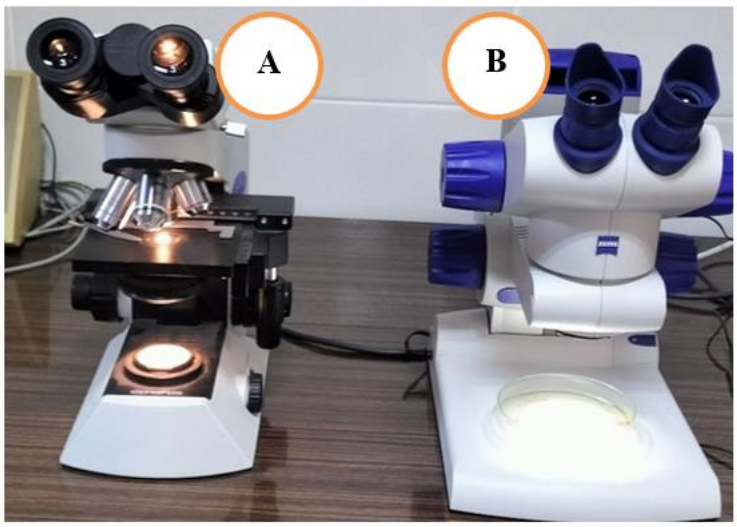


Figure 3.8. Different types of microscope equipment which were used in this study, A) Light microscope, B) Dissection microscope

Figure 3.9. identifying sand fly species under Dissection microscope

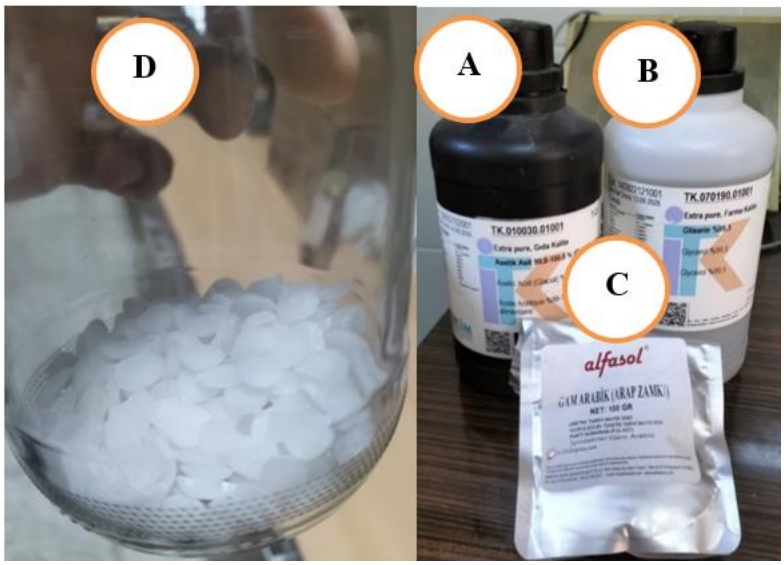


Figure 3.10. Different types of chemicals which were used in this study, A) acetic acid, B) Glycerin, C) Arabic Gam, D) potassium hydroxide (KOH)

3.3.2.3. Berlese's Gum Chloral Solution:

It is very important solution for morphological identification of sand fly species, and (Table 3.10), illustrates how Berlese's Gum Chloral Solution was prepared in this study, as (Topraq, 2005) cited in (Lewis, 1973).

Table 3.10. Components of preparation of Berlese's Gum Chloral Solution

No.	Chemicals	Ml
1.	Arabic Gam	50 gr
2.	Acetic acid	3 ml
3.	Glycerine	40 ml
4.	Chloride hydrate	20 ml
5.	D. W.	50 ml

3.4. Molecular Experiments

The body of seventy collected sand flies' samples, ten in each zone were randomly selected and kept them in (frige) at -4˚C until use.

3.4.1 Primers

Primers which were used for the molecular identification of sand fly samples are stated in (Table 3.11).

Table 3.11. Primers used for molecular identification of sand fly in this study

Primer	Sequence (5'-3')
LCO1490 foreword	F(5'-GGTCAACAAATCATAAAGATATTGG-3')
LCO1490 reverse	R (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')

3.4.2. Primer preparation

One handred microlitre of fordword and reverse primers were prepared separatly by placing 10 microlitre of stock of LCO1490 fordword and reverse primers separately to 90 microlitre of distill water separately, finaley 100 microlitre of both primers were prepared separately.

3.4.3. Dna Extractıon

In order to extract DNA from the sand fly samples, individual ethanol-fixed specimens were homogenized and lysed using a DNA extraction kit made by Thermo Scientific called the Isolation Kit GeneJET Genomic DNA Purification Kit. The PCR gene amplification process was then applied to 8µl portions of the DNA genomic samples.

Kit protocol:

1)Added 200 µl of PBS to a 1.5 ml microcentrifuge tube. Transfered the body of sandfly samples into PBS solution, left the specimen at room temperature for 3 hours.

2)At the end of the holding period, added 20 µl of proteinase K to a new tube. Transfer the sandfly samples to this new tube. Add 200 µl of lysis buffer (buffer AL) to it. The samples were Crushed well in this solution.

3)Centrifuged the tubes at 3000 rpm for 5 minutes.

4)Spin the tubes for 15 seconds. Then placed the tubes in heat block at 56°C overnight.

5)Added 200 µl of ethanol (96–100%) the next day. Mixed thoroughly by vortexing.

6)Pipetted the mixture onto the (filtre tube) QIAamp Mini spin column (in a 2 ml collection tube) and centrifuge at 8000 rpm for 1 minute. Discarded the collection tube.

7)Placed the (filtre tube) QIAamp Mini spin column in a new 2 ml collection tube and added 500 µl of Buffer AW1 (wash buffer 1). Centrifuged for 1 minute at 8000 rpm. Discarded the collection tube.

8)Placed the (filtre tube) QIAamp Mini spin column in a new 2 ml collection tube and added 500 µl of Buffer AW2 (wash buffer 2). Centrifuged at full speed 14,000 rpm for 3 minutes. Discarded the collection tube.

9)Placed the (filtre tube) QIAamp Mini spin column in a new 1.5 ml microcentrifuge tube, added 200 µl of Buffer AE (elution buffer) and wait 1 minute at room temperature. Centrifuge for 1 minute 8000 rpm to separate the DNA.

10)Finally the DNA will be ready to put in to the Electrophoresis equipment and then read the bands wheather was the DNA extracted or not, the bands will appear or not?

Figure 3.11. Preparing Agarose gel electrophoresis for DNA in this study

Figure 3.12. Adding chemical solutions to the fragmented sand fly tissue samples

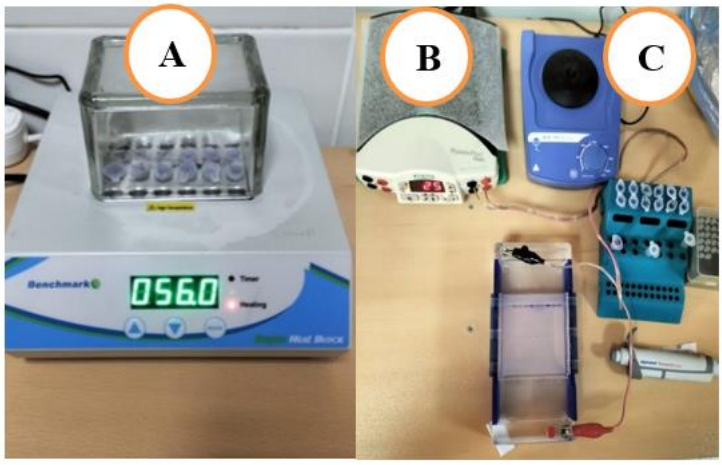


Figure 3.13. The devices which used in this study for DNA extraction of sand fly, (A) heating block device at 56°C (B) electrophoresis device, and (C) vortex device

Figure 3.14. Shows two devices which used in this research, (A) microcenterfuge, (B) spin device

3.4.4 Agarose Gele Electroforesis Preparation

1)- Agarose powder (1 gr) was prepared in using 100µL (TAE 0.5 x) ellectrophoresis buffer soluttion.

2)- After adding (1 gr TAE powder), then heated the solution homogeneously in the microwave. We don't let it boil too much because the percentage should not be disturbed, after that left to cool to 50°C.

3)- Then 5µL of (gel stain) sybr green (safe view, safe green) stain were added into a garose gel solution, to eliminate contamination risk of glassware or gel running tank.

4)- Agarose gel solution was poured in a tray after fixing the comb in proper position after that, left to solidified for 25 minutes at room temperature.

5)- then the comb was removed gently from the tray and 3µl DNA with 3µl of loading dye were added in to each comb well.

6)- The gel tray was fixed in electrophoresis chamber and filled by 0.5 TAE buffer.

3.4.5. Loading and Running DNA in the Agarose Gel

3µl DNA was mixed with 3µl bromophenol blue (loading dye) and loaded into wells of the 0.8-1% agarose gel. The gel was run at 100 V for 30 minutes, and then DNA extracred were examined and visualized by using ultraviolet trans-illuminator as shown in (Figure 3.20).

Figure 3.15. Loading 3µl DNA with 3µl bromophenol blue (loading dye) into wells of the 0.8-1% agarose gel

3.5. PCR Technique

PCR technique was performed for detection sand flies *Phlebotomus papatasi, Phlebotomus sergenti* and *Phlebotomus alexandri* were designed in this study.

3.5.1. PCR Master Mix Preparation

PCR master mix was prepared by using Maxime PCR Pre Mix and done according to company instructions as following (Table 3.12). The PCR master mix reaction components were then added to standard PCR tubes containing the PCR PreMix along with the other materials including the components listed in table (3.12) required for the PCR reaction. To mix all the components, the tube was then inserted into a vortex. It was then put into a PCR thermocycler.

Table 3.12. Standard PCR reaction for each specimen

PCR master mix	Volume
Genomic DNA	8µL
Buffer	2.5µL
$MgCl_2$	2µL
Taq DNA polymerase	0.1µL
dNTP	0.5µL
reverse primers (10pmol)	1µL
forward primers (10pmol)	1µL
D.D. water	9.9 µL
Total	25µL

3.5.2. External Thermocycler Reaction Conditions

PCR Thermocycler conditions was done by using (Optimase protocol writer) online application and based on methods described (table 3.13).

Table 3.13. The Conditions of PCR Thermocycler

PCR cycle	Repeat	Temp.	Time
Initial denaturation	1	95C	3min
Denaturation	40	95C	30sec.
Annealing		48C	30sec
Extension		72C	45sec
Final extension	1	72C	5min

Note: All sand flies species were done at same PCR Thermocycler conditions

Figure 3.16. thermocycler device while running PCR reaction in this study

Figure 3.17. Shows some materials which were used in this study, A) pipetts in different sizes, B) Ependorf tubes 1.5 µl and 0.5 µl, C) PCR tubes 0.2 µl, D) PCR tubes and cups, E) filter tubes 2 µl (kit tubes)

A B

C D

Figure 3.18. Illustrates materials that used in this study, A) PBS solutions, B) agarose powder, C): 1- gel stain, 2- electrophoresis chamber, 3- 100 µl 0.5 TAE electrophores gel, and D) DNA extracted from sand fly

Figure 3.19. Explains the microwave instrument which was used during preparating of agarose gel in this study

3.5.3. Agarose Gele Electroforesis Preparation for PCR DNA Product

1)- Agarose powder (1 gr) was prepared in using 100µL (TAE 0.5 x) ellectrophoresis buffer soluttion

2)- After adding (1 gr TAE powder), then heated the solution homogeneously in the microwave. We don't let it boil too much because the percentage should not be disturbed., after that left to cool to 50°C.

3)- Then 5µL of (gel stain) sybr green (safe view, safe green) stain were added into a garose gel solution, to eliminate contamination risk of glassware or gel running tank

4)- Agarose gel solution was poured in a tray after fixing the comb in proper position after that, left to solidified for 25 minutes at room temperature,

5)- then the comb was removed gently from the tray and 3µl of DNA product with 3µl of loading dye were added in to each comb well. and 5µl of in one well.

6)- The gel tray was fixed in electrophoresis chamber and filled by 0.5 TAE buffer.

3.5.4. Loading and Running PCR Product in the Agarose Gel

3µl PCR product was mixed with 3µl bromophenol blue (loading dye) and loaded into wells of the 0.8-1% agarose gel. The gel was run at 100 V for 30 minutes in electrophoresis device and then PCR products were examined and visualized by using ultraviolet trans-illuminator (figure 3.20).

Figure 3.20. Illustrates Ultraviolet (UV) transilluminator machine which used in this study

3.6.Identification of Leishmania Parasite from CL Cases

3.6.1. CL Cases

Out of the 135 patients who visited the Shadi Center for Skin Diseases in the central part of Erbil city between the beginning of the study period and the end, 135 skin lesion samples (84 males and 51 females) were taken from cutaneous leishmanasis cases, some of them were taken treatement and had sore skin infections, Whole suspected CL cases had been clinically diagnosed by demonstration of the lesions.

3.6.2. History and İnformation Gathering

Each case's history was obtained using a questionnaire form **(Appendix 1).** The information included sex, age, address, months of detection, locations of lesions, severity of the leision, and other associated symptoms, history of prior CL, home type (regarding building), features near the house (such as river, branches, mini-branches, marshes, and settlement), family history of CL, number of family members infected, prior infections with CL and with recoring some other CL features while taking samples.

3.6.3 Clinical Dermatological Examination

All patients with suspected CL underwent a dermatological examination that looked at the locations, types, quantities, consistency, severity, and other accompanying symptoms of their lesions.

3.6.4. Microscopic Examination and Culturing on NNN Media

Sterilized the ulcer 3-5 times with 70% in a circular motion, with a dry cotton wiped the alcohol dry, used a lancet to make 1cm incision around the edge of the ulcer to ooze out tissue and blood. Lancet punctures were made at the edges of skin lesions after cleaning them with 70% ethanol. Skin lesion aspirate was applied on a clean slide, air dried. The slide was stained with Leishman stain for 5-10 minutes, rinsed with tap water, and allowed to air dry. The slides were examined with an oil immersion lens under a microscope to look for amastigotes (Al-jawbareh et al 2003 and Younis, 2018).

By using syringe containg 0.5 normal saline aspirated arround 0.5 ml of blood tissue from the incision of patients, then inoculated into NNN media, (transfered the content of the syringe into the NNN tube and close tightly, then incubated at 22-28 degrees centegrate), monitered the growth of promastigotes, (AL-Jawabreh et al., 2004).

3.7. Preparation of Media, Stains and Solutions

3.7.1. Preparation of (NNN Medium, Novy, Macneal-Nicolle), (Appendix 3)

The genera Leishmania and Trypanosoma, which are flagellates that live in human blood and tissues, are members of the protozoan family Trypanosomatidae. (Novy and McNeal 1804), created NNN Medium, and Nicolle (1908) made modifications to it. The two phases of the NNN Modified Medium are blood agar (Part A) and Lockes solution (Part B), which are modifications of the original medium (Cruickshank and et al,.1975,). According to (Taylor 1978 and Collee et al. 1996), this modified medium is frequently employed for diagnostic work. Blood agar is the base of this medium, and an overlay medium is added on top. Leishmania and

Trypanosoma, two species that are picky about their growth, can flourish in the richly nutrient-rich blood agar basis. Following incubation, the specimens are injected into the diphasic medium's liquid phase. In the insect vector, this promotes the growth of organisms, (Collee et al. 1996).

Part A of solid blood agar that it comprises of the following

Table 3.14. components of solid blood agar part (A) of NNN media

Ingredients	**Gms / Litre**
Meat extract	3 g
Peptone	5 g
Sodium chloride	8 g
Agar	15 g
D.W	1000 ml

3.7.1.1. Procedure of Solid Blood Agar Part A of NNN Media

This is how the solid phase was made:

Part A: Dissolve 31 grams in 1000 milliliters of purified water. For the medium to be fully dissolved, heat to boiling. Autoclave for 15 minutes at 15 pounds of pressure (121°C) to sterilize. Aseptically add 10% of sterile human blood after cooling to 45–50°C and inactivating at 56°C for 30 minutes. After thoroughly mixing, pour out 5 ml into test tubes or 25 ml into flasks. Let the tubed media cool while tilted.

B). part (B) of solid blood agar that it comprises of the following:

Table 3.15. components of liquid blood agar part of NNN media

Ingredients	Gms / Litre
Sodium chloride	8 g
Potassium chloride	0.2 g
Calcium chloride	0.2 g
Monopotassium dihydrogen phosphate	0.3 g
Dextrose	2.5 g
D. W	1000 ml

3.7.1.2. Procedure of Liquid Blood Agar Part of NNN Media

The liquid phase was made in the following manner:

Part B: Dissolve 11.2 grams of Part B in 1000 milliliters of purified water. To fully dissolve the medium, bring the temperature to a boil. Autoclave at 15 pounds pressure (121°C) for 15 minutes to sterilize. After cooling, pour about 2 ml into tubes or 10–15 ml into flasks over the Part A medium that has solidified.

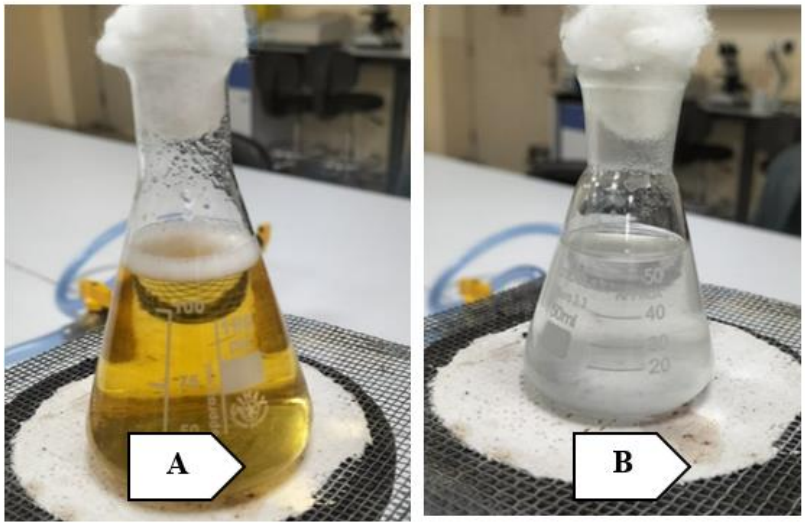


Figure 3.21. Solid Part (part A), and liquid part (part B) of NNN media.

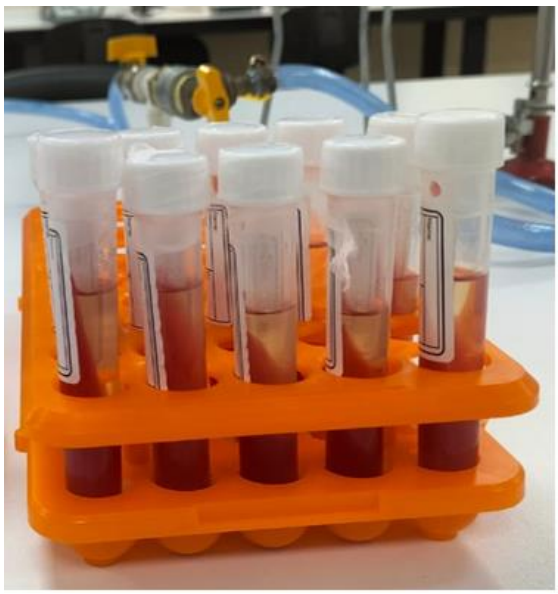


Figure 3.22. NNN media

3.7.2. Preparation of Stains

3.7.2.1. Leishman Stain Preparation, Storage and Stability, (Appendix 3).

Leishman stain solution is stable up to the stated expiry date when stored at 15-25 C.

Table 3.16. Leishman stain compositions.

Contents	Weight
Leishman powder	0.15 g (%)
Methanol	100 ml (70%)

3.7.2.2. Procedure of Leishman Stain

The steps are as follows:

1. Flood air dried smears with stain for one minute on slide rack.
2. Dillute stain gently 1:3 with buffered water (PH :6.6-6.8) or distilled water. Leave slide for 5-10 minutes

3. Wash of the slid with buffered water (PH :6.6-6.8) or distilled water until the slide appear pink to the naked eye and allow to dry.

4. After washing the soiled slide with buffered water or disstiled water, examine under oil immersion lense of microscope.

3.7.3 Preparation of Solutions

3.7.3.1. Physiological Phosphate Buffer Solution (PBS)

The ingredients listed in (table 3.17) were dissolved in 500 ml of D.W. (pH 7.2). After that, it was autoclaved for 15 minutes at 121C and 15 IBS/inch to sanitize it, (Rasha, 2021) cited in (Hay et al., 2002).

Table 3.17. PBS Compositions

Contents	Weight
Na2HPO4	1.15 g
KH2PO4	0.2 g
KCl	0.2 g
NaCl	8 g

3.8. Statistical Analysis

The data were imported into SPSS version 22, and a P value of 0.05 or less was deemed statistically significant. To compare the distribution of discrete independent variables among the current study groups, chi-square (X2) was employed. In the study area, X^2 test was applied in order to determine the change in population density of sandfly species according to Months, the role of trapping efficiency in catching sandflies and the trap preferences of sexes. One-way and two-way analysis of variance was used to determine whether there is a difference between the time intervals in terms of trapping sandflies.

Results of sequencing of DNA analyzed by using some special programs: Different techniques employed in the study of mtDNA COI findings to establish the genetic separation between species, and Neighbor Joining (NJ) joining diagrams created in accordance with these techniques. To graphically represent genetic distances. The Chromas-Pro computer application was used to convert these data into base sequences. The Bioedit computer tool was used to compare this sequence to the reference sequence downloaded from the gene bank. The evolutionary distances were calculated using the MEGA 6.0 version of the phylogenetic UPGMA tree type.

4. RESULTS and DISCUSSIONS

4.1. Morphological Identification of Sandfly Species

In the course of the investigation in the province of Erbil between January and December 2022, 2054 sand flies were collected from 7 main districts, each zone subdivided into some minor areas. Following the gathering of sand flies with the aid of (ASP), (S.P.), and (L.T.) traps, specimens were kept in 70% ethanol. After dissecting each fly into two sections, the head and terminalia were utilized to identify the species morphologically, using the morphological taxonomic keys of (Lewis 1978, 1982; Abul-Hab and Ahmed, 1984; Singh and Philips-Singh, 2010). As for the other body part, it was utilized for molecular identification and kept in (frige) at -4˚C. The morphological analysis of all 2054 (100%) sand fly samples collected was conducted. However, only seventy randomly selected sand fly samples were subjected to a genetic analysis for the purpose of verifying the morphological findings.

According to morphological keys, at least eight characters for the males and four for the females were analyzed. **Male:** The anatomy of the sexual organs attached to the last segment of the abdomen and the quantity and arrangement of the teeth at the base of the pharynx in the head were used to identify species in male individuals. The following structures of the male genital organs have been researched: the Coxit Structure, Style's number of thorns, the size and design of the genital pump, the Aedagus and the Paramer Structure. **Female**: In contrast to males, females' genital organs are concealed in the final two abdominal segments. To prepare for this, the final two segments were positioned such that the spermatheca could be viewed clearly. Additionally, the head was placed on the slide since the pharynx teeth and antenna structures could be seen obviously. The spermatheca, whether or not it is segmented and the anatomy of the base of the spermatheca were all evaluated in the female genital organ.

The Morphological Classification of Sand Flies keys for the Psychodidae family in Iraq (Lewis, 1978, 1982; Abul-Hab and Ahmed, 1984; Singh and Philips-Singh, 2010) were used to morphologically identify each sand fly species. "*P. papatasi, P. sergenti, and P. alexandri*, three species of the genus Phlebotomus were identified. One of the most disregarded tropical diseases is (CL). The principal pathogens found in all endemic foci across the nation are *Leishmania major and L. tropica*. Approved that the three most common sand fly species, *Phlebotomus papatasi, P. sergenti, and alexandri* transmit these two etiological agents (Killick-Kendrick et al., 1999; Volf, & Volfova, 2011).

4.1.1. Phlebotomus (*Phlebotomus papatasi)*, Scopoli, 1786

It is one of the most researched species because of its wide geographic range of occurrence and its medicinal significance as the main vector of the Leishmania major-caused zoonotic cutaneous leishmaniasis (ZCL) in high-land areas (Izri et al., 1992). From humid to extremely hyperarid bioclimatic zones, it is present. It is one of the widespread species that belongs to the genus Phlebotomus; it is mainly found in this research and it has the potential to be a major Leishmania carrier in the study area.

A- Males

The cebarium and pharynx in males resemble those exist in the classification keys (Figure 4.1, A&B). The coxite is a spire bit, containing a set of thick capillaries with a dark color. (Figure 4.1, C&D) shows a male style with 5 small spines: 3 apical and 2 exterior spines. "Paramere have been trilobites, the ventral lobe is short and curved nearly to the top with a small fork short on top, Aedeagus is a short and the funnel-shaped curvature. Has short ejaculatory duct with a funnel shaped.

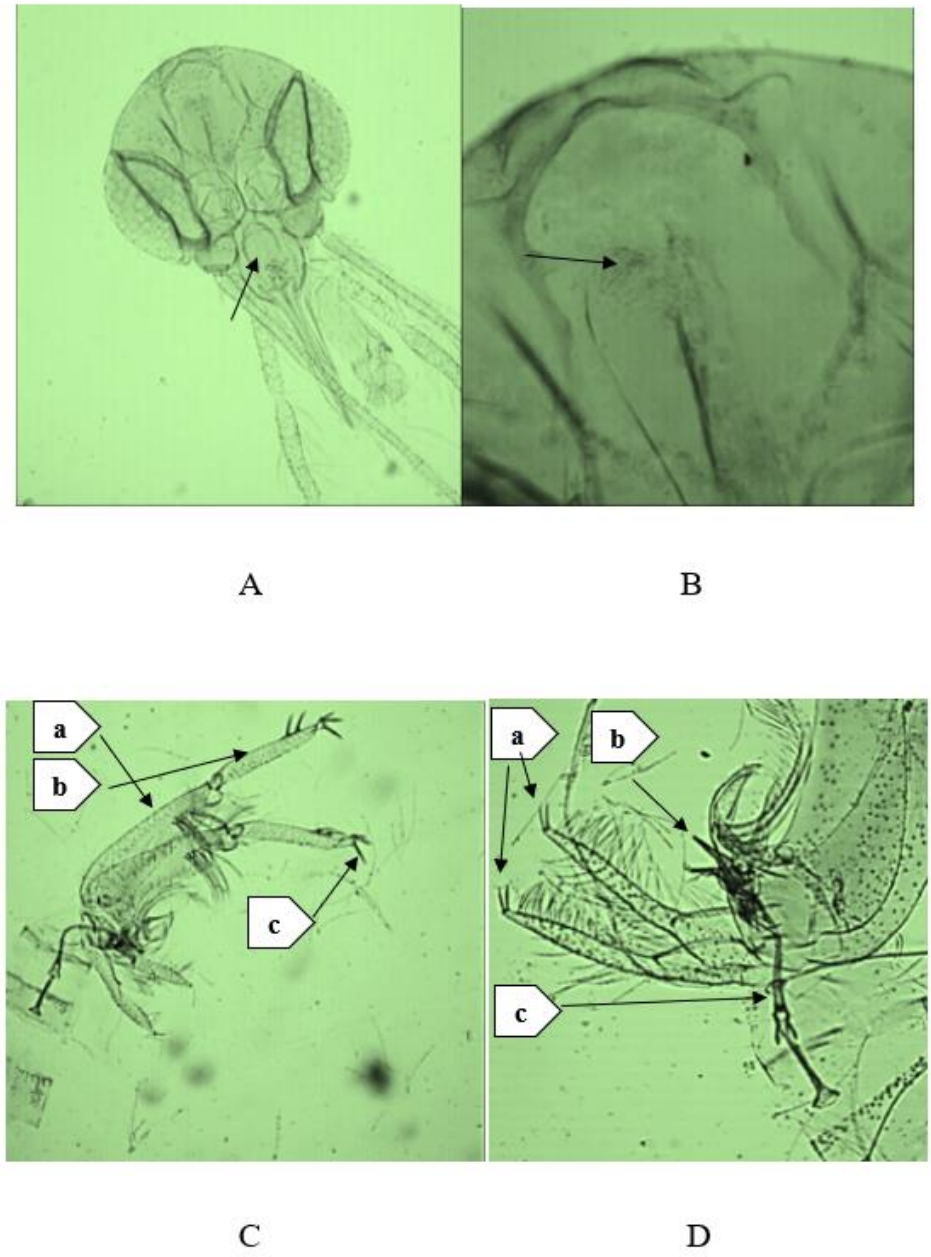


Figure 4.1. (A), Head of male *P. papatasi* (cebarium), (100X). (B), Pharynx of male *P. papatasi* (400X). (C), Male (gonostyle) gentalia of *P. papatasi*, (a= coxite, b= style, c=spines). (100X). (D), enlarged male genital of *P. papatasi* (a= paramers. b= aedeagus, c= edjucatory pump, (400X)

B- Females

The female can be identified by the numerous cibarial teeth that are dispersed vertically as well as on a number of lateral spicules in the buccal cavity, or "Cebarium" (Figure 4.2.A). Pharynx is bottled shaped with armature on the basal part. (Figure 4.2.A). Spermatheca, which have a cylindrical shape and are separated into 8–12 rings (with a short apical segment), are a characteristic of females (Figure 4.2.D).

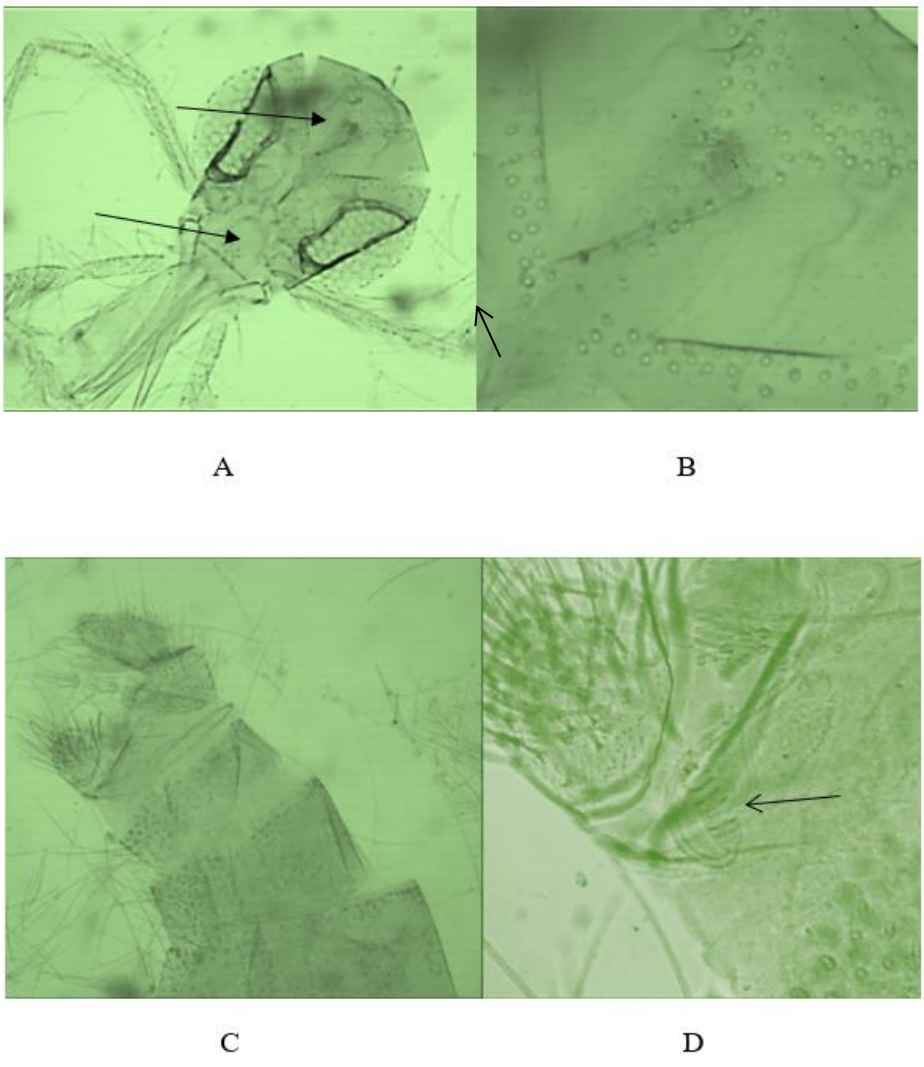


Figure 4.2. (A) Head of female *P. papatasi* shows cebarium and pharynx (100X). (B) Lateral view of Pharynx of female of *P. papatasi* (400X). (C) Female genital (100X). (D) Spermatheca of female of *P. papatasi* (400X)

4.1.2 *Phlebotomus sergenti* (Parrat, 1917)

It is primarily connected to residential habitats in urban and peri-urban areas. Except for few foci from East Africa and Namibia, it is thought to be the most likely vector in all the CL foci caused by L. tropica (Killick-Kendrick et al., 1995; Volf et al., 2002).

A- Males

Clearly flask-like pharynx, (Figure 4.3. B). Coxite is short in length, has a small, elongated head, and a slightly curved abdomen. The style is about shorter than coixite, half as long as coxite, and much narower than coxite, but it has four spicules. Two are apical and straight, while the other two interal (Figure 4.4). The long ejaculatory pump has an oval, curving upper surface, Aedeagus had a top that was curled and some how long (Figure 4.4).

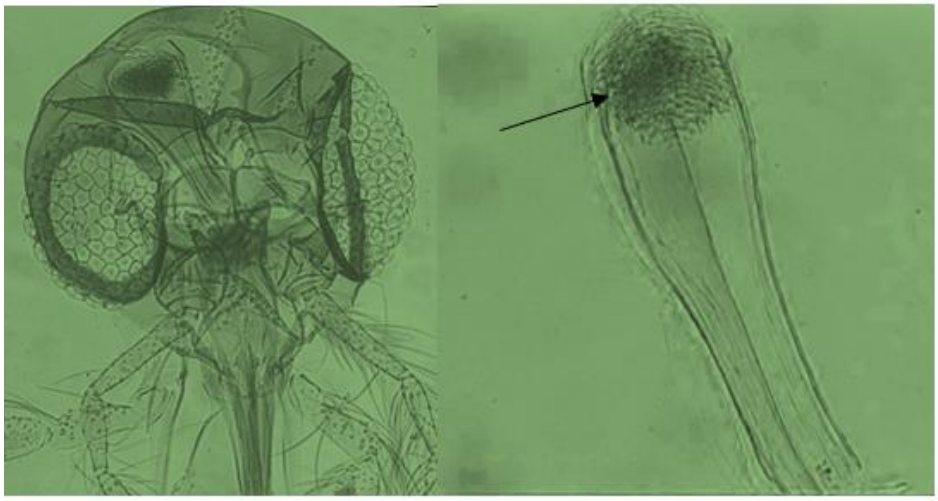


Figure 4.3. (A) Head of male P. *sergent*i (100X). (B) pharynx of male *P. sergenti* (400X)

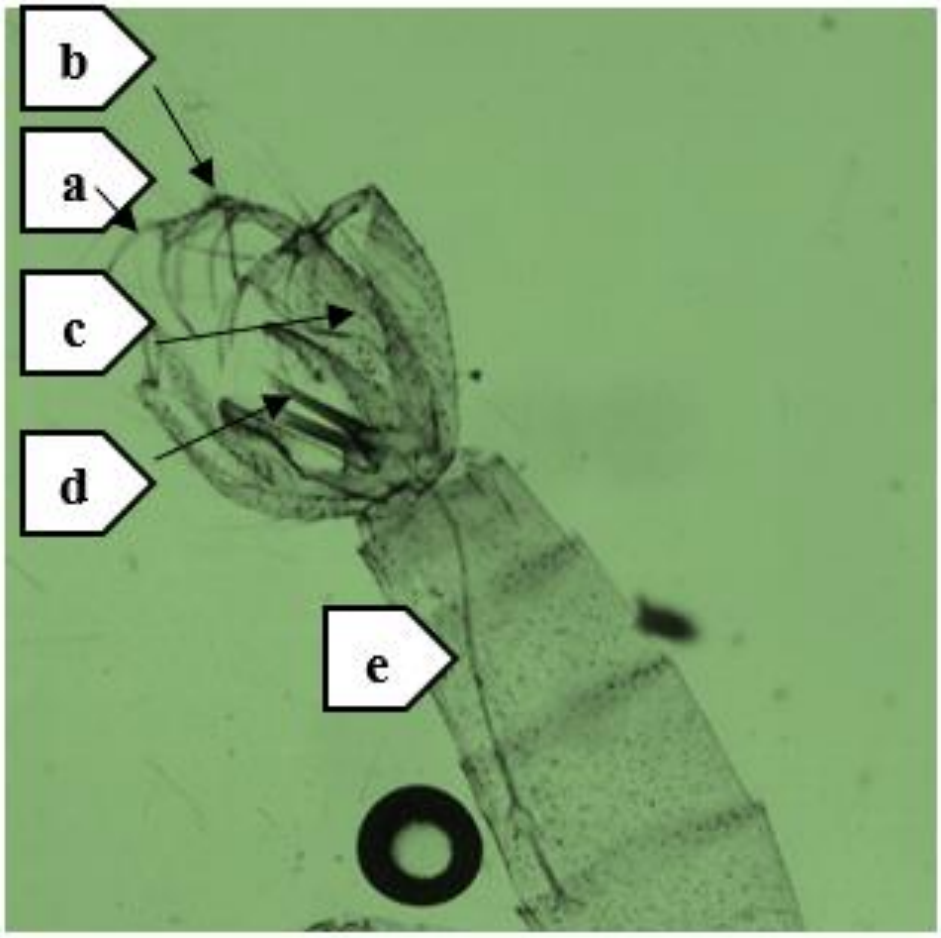


Figure 4.4. The terminal (gonostyle) of male *P. sergenti* (a= spines, b= style, c= coxite, d= aedeagus, And e= long ejucatory pump), (100X)

B- Females

The pharynx is structured like a flask and has an armature that is made of chitin. It is large, elongated. The spermatheca contains a brief capsule made up of 4-6 enlarged terminal rings and has a long apical spicule, and the duct that extends from it is often thick and planned (Figure 4.5).

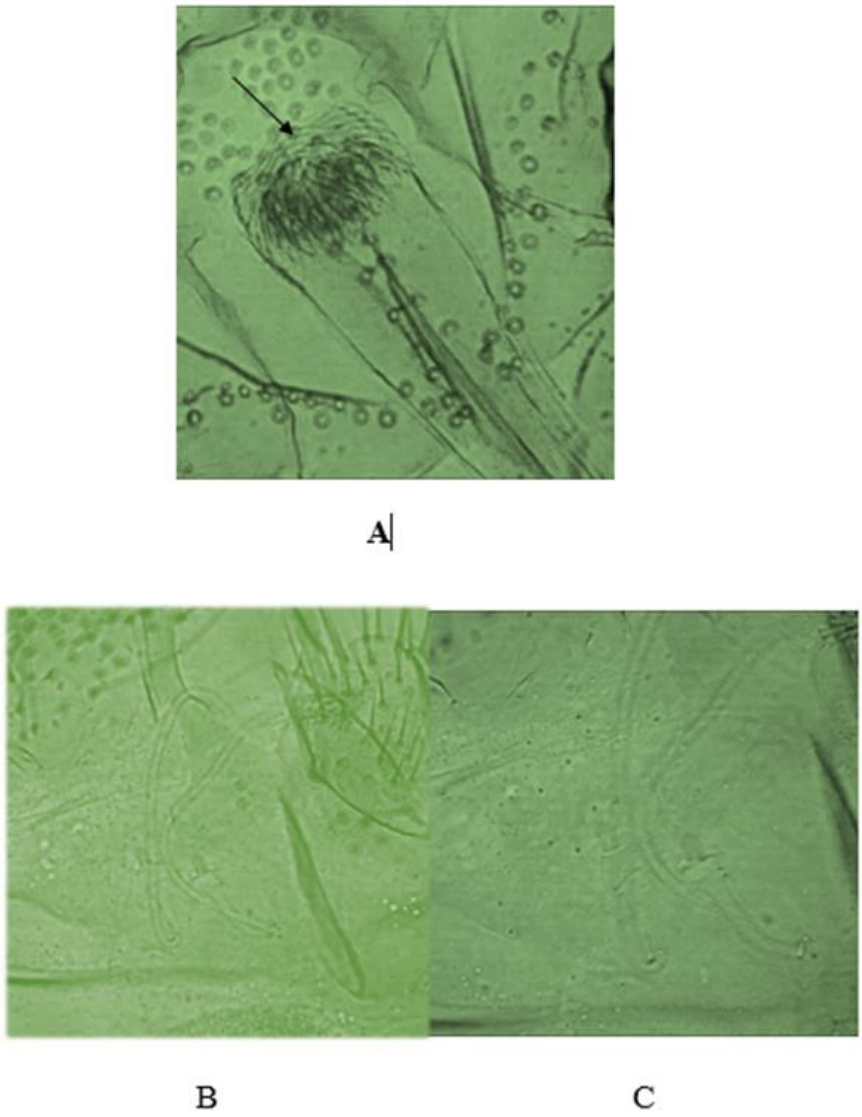


Figure 4. 5. (A) Pharynx of female P. sergenti (400X). (B) Spermatheca of female of P. sergenti (100X), (C) enlarged Spermatheca of female of P. sergenti (400X)

4.1.3 *Phlebotomus alexandri* (Sinton, 1929)

This species is easily identified from the rest of the subgenera of Phlebotomus. There were fewer P. alexandri in several regions of the nation. We know very little about its ecology, nutrition, resting, and bionomics. the vectorial status of this species is also unclear. Comparing this species to the others in the Phlebotomus subgenus is simple.

A- Males

Males have four thick spines (two terminal and two median) on the style and the process (apophysis) or lobe with a tuft of hair on the coxite. It has definetly short ejaculatory pump, and median aedeagus of the males of the subgenus are distinctive features (Fig. 4.5).

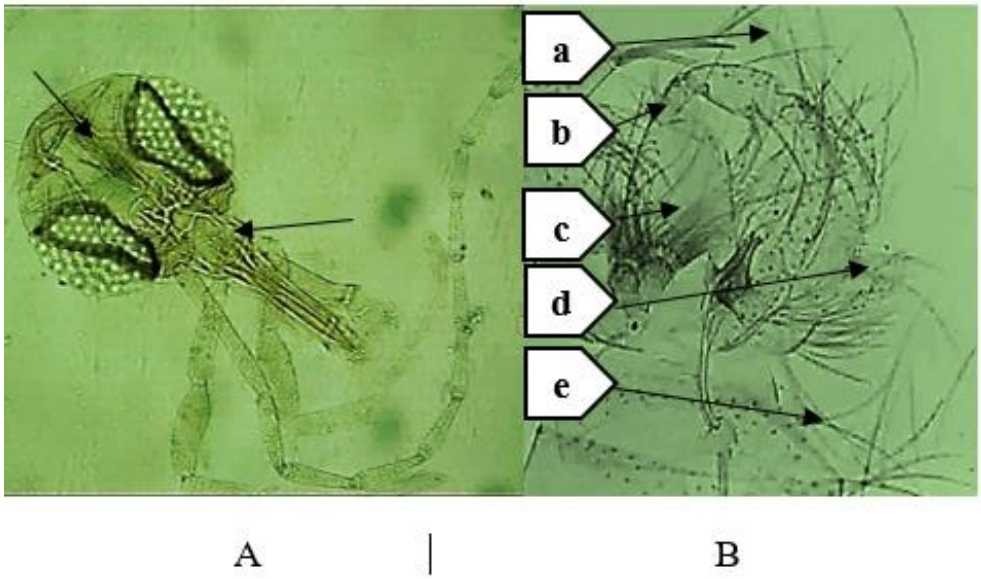


Figure.4.6. A). (A) Male head of P. alexandri shows pharynx and cebarium(100X). (B) Male Gonostyle with four thick spines of P. alexandri, (a= spines. b= Style, c= Coxite, d= aedeagus curved at apex, and e= edujucatory pump(100X)

B- Female

The massive, backward-facing pharyngeal teeth of the group's females resembles to those in males, as well as their often thick and fully segmented spermathecae (9-12 segments) that grow distally with an apical segment devoid (empty) of a terminal process, make them easy to identify (Fig. 4.6).

Figure 4.6. B). (A) pharynx of female p. alexandri (400X), (B) spermatheca of female p. alexandri (400X)

The morphological characteristics of *P. sergenti, P. papatasi, and P. alexandri* matched the Diptera classification key and those described by (Lewis 1978, 1982; Abul-Hab and Ahmed, 1984; Singh and Philips-Singh, 2010): According to forward descriptions of the morphology of both gendes, the Psychodidae family is widespread in Iraq and, due to their abundance and expanding range, are able to spread a variety of diseases, including leishmaniasis. Sand flies occur in a wide range of habitats and individual species often have very specific habitat requirements, depending on locally occurring environmental factors such as frequency of precipitation, temperature, the distribution and abundance of vertebrate hosts and rainfall can affect the relative abundance of sand flies over the seasons, is usually found in sub-tropical to temperate climates where the sand fly season is often associated with the warmer months" (Young and Arias, 1992).

This study is agreement with some other Previous Study: Iraqi sand flies were the subject of the earliest published research by (Newstead, 1920). (Sukkar et al., 1982) collected six species of phlebotomine sand flies from six locations within 35 km^2 of Baghdad, similar to (Pringle, 1956), who had previously conducted a limited survey for

sand flies in the Zagros mountains and the central plains of Iraq, then conducted a small-scale survey for sand flies in the Zagros mountains and the central plains of Iraq. In the mountains, only 155 phlebotomine sand flies were found, of which 28% belonged to *P. papatasi* and 28% to *P. sergenti*.

The current study's findings are consistent with those of (AL-Abady, 2010), who collected 872 sand flies and found that P. papatasi had the highest incidence (81.30%) in the province of Thi-Qar. The findings corroborate those of (Atshan, 2014), who identified two species of Phlepotomus sand flies—*P. papatasi and P. sergenti*—as carriers of Leishmaniasis in the same province. Three different species of sand flies were collected from various areas in Al-Qadisyia province by (Al-Hassani, 2016). Of these, two species belong to the Phlebotomus genus (*P. papatasi and P. sergenti*), while one species is from the Sergentomiya genus (S. sintoni). It was observed that P. papatasi is the most prevalent species at a rate of 53.4%, followed by S. sentoni (28%) and P. sergenti (18.6%).

The current study's findings are in line with the study that conducted by (AL-Tufaily, 2003) in AL-Najaf province who observed three species, *Phlebotomus papatasi, P. sergenti* and *P. alexandri, P. papatasi* was the most abundant in the rural regions while *P. sergenti* was found to be mostly abundant in the urban regions, *P. alexandri* was very rare. There were two peaks of seasonal abundant of the vectors, the first during May and the second during October. In Al-Diwaniya province, (Al-Mayali, 2004) recorded five species *Phlebotomus papatasi, P. sergenti, P. alexandri*, *Sergentomiya sintoni* and *Ser.* Squampilerius and there were two peaks for this density, one in May and a nother in September. Sand fly with different distribution along year, but with high density in February, May and decrease in the hot months.

This study is inagreement with a study which conducted by (Al-Abbas et al., 2018), between January and December 2016, which identified 1376 male and 1102 female sandflies were gathered from five distinct collection regions. The findings indicate that the number of sandflies declined throughout the winter's cold months (December, January,

and February) and reached 0%. In contrast, the insect became more active during the warmer months, particularly in August and September, when its percentages were 16.10 and 13.95 percent, respectively.

Also, this study is ingreement with a study that carried out by (Al-Awadi, 2019) carried out a second study in the province of Thi-Qar to look into the species of sand flies that are present there and act as the disease's vector. A total of 6527 sand flies were collected using aspirators, oil traps (sticky papers), and light traps. Of these, 3064 females and 3463 males were distributed. The two species, *P. papaatasi* and *P. sergenti*, belonged to the same genus, Phlepotomus. The most frequent species found were *P. sergenti*, which reached 2056 (31.5%), and *P. papatasi*, which reached 4471 (68.5%). The density of sand flies has two peaks: the first one occurred in May, and the second one occurred in September.

Also, this study is ingreement with some international stud thiesat carried out In Serbia, (Vaselek et al., 2017) recorded genus Phlebotomus with species *P. Larroussius, P. perfiliewi and P. neglectus.* In South America (Zorrilla et al., 2017) study the distribution and abundance of Lutzomyia of the genus Lutzomyia (58 species) and Brumptomyia (2 species) and identify sand flies' species naturally infected with Leishmania". In Iran, (Yaghoobi-Ershadi et al., 2015) showed that the number of sand flies species include 26 Phlebotomus species of 6 subgenera and 18 Sergentomyia species of 6 subgenera. Phlebotomus sergenti and P. sergenti similis. Similarty in Iran a study done by (Sofizadeh et al., 2018) collected sand flies that were identified as belonging to 18 species. *Phlebotomus wenyoni* was reported for the first time from the area villages. "The frequency of sand flies in the villages located in northeast of the Golestan province. In Turkey, (Ozbel, 2013) among 22 species of sand flies recorded by him, 7 are proven or suspected vectors of human leishmaniasis and phlebovirus infections. In the Amazon region of Brazil, (Pereira et al., 2014) collected a total of 456 sand flies, comprising 256 females and 200 males.

4.2. Sand Fly Distribution According the Followings:

4.2.1 Phlebotomine Geographic Distribution in the Study Areas

Three distinct species of sandflies, all of which are members of the genus Phlebotomus, were identified in the research region, the province of Erbil, between June 2000 and May 2002, utilizing a variety of traps, including light traps, oil paper traps, and aspirators. 2054 sandflies of this species in all, 1137 males and 917 females, were gathered. (Table 4.1, Figure 4.7 and Figure 4.8) all show the distribution of sandfly species gathered in the sampling areas. This study made clear the variations in sandfly populations throughout a number of province regions (Table 4.1). Sandflies were most prevalent (32.46%) in the Makhmur region, with a significant difference ($P< 0.05$).

Genus: Phlebotomus Rondani, 1840

Phlebotomus papatasi (Scopoll, 1786)

Phlebotomus sergenti (Parrat, 1917)

*Phlebotomus alexandri (*Sinton, 1929)

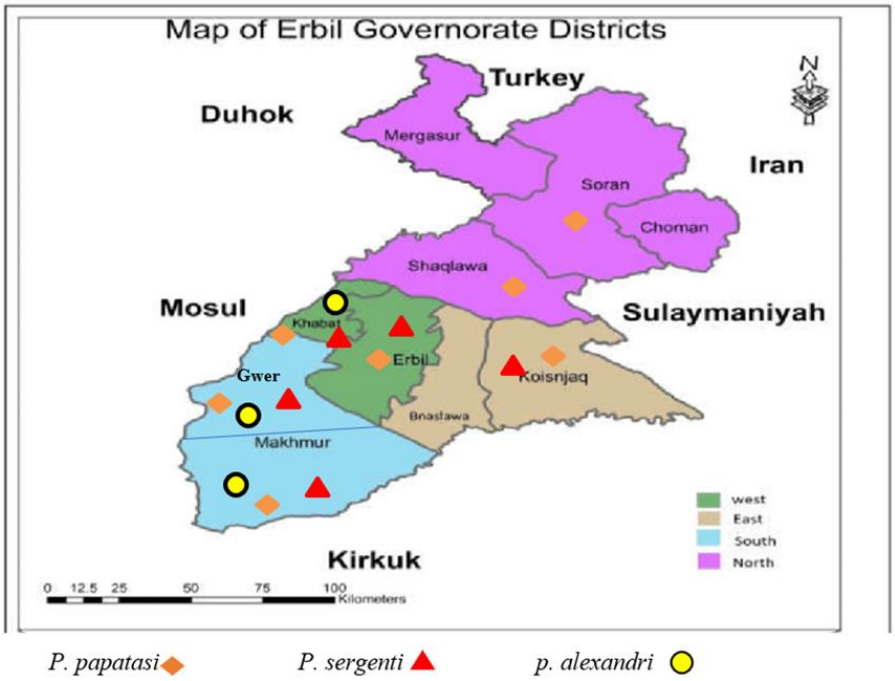


Figure 4.7. Map of Distribution of sandfly species belonging to Phlebotomus genera in Erbil province according to sampling stations between January-December 2022

Table 4.1 Shows the sand fly species abundance (found) and counted in the study area between January-December 2022

Makhmur (686) (33%)	**Khabat (438) (21.3%)**	**Gwer (339) (16.5%)**
p. papatasi (310)	*p. papatasi (229)*	*p. papatasi (136)*
p. sergenti (225)	*p. sergenti (149)*	*p. sergenti (121)*
p. alexandri (151)	*p. alexandri (60)*	*p. alexandri (82)*
Koya (175) (8.4%)	**Soran (118) (5.7%)**	**Shaqlawa (81) (3.9%)**
p. papatasi (95)	*p. papatasi (118)*	*p. papatasi (81)*
p. sergenti (80)	*p. sergenti (0)*	*p. sergenti (0)*
p. alexandri (0)	*p. alexandri (0)*	*p. alexandri (0)*
City center (217) (10.5%)		
p. papatasi (194)		
p. sergenti (23)		
p. alexandri (0)		

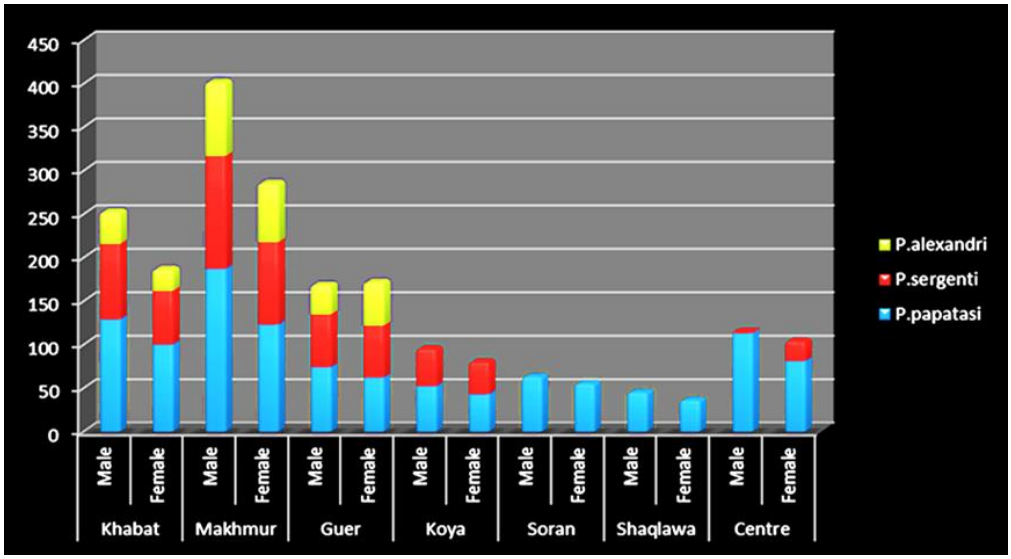


Figure 4.8. Distributions of sand fly species in the sampling stations in Erbil province, Iraq, in January-December 2022

The frequency of sandfly species in various habitats, including the habitat of the town, revealed differences in the sand fly's geographic distribution depending on the type analyzed. The study also clearly demonstrated the impact of the study's areas on the number of species in the group that may be considered rural. A total of all specimens was collected. Phlebotomus papatasi was the most common species sampled in each habitat. Phlebotomus papatasi, constituted (56.6 %) of the total flies collected throughout the study in order of abundance, it was identified in all districts of the province, while p.sergenti and p.alexandri were not identified in all districts in the province, (p.sergenti was identified in all districts except Soran and Shaqlawa districts, while p.alexandri was identified only in Makhmur, Khabat and Gwer areas), statistically there are significant differences between sandfly species and districts at ($P<0.00$). The distribution of the species among the sampling stations can be seen in (Figure 4.7). The majority of the sandflies were identified in rural zones reached (75.8%), while the least sandflies were identified in urban zones reached (25.2%), statistically there is no significant difference between the distirbution of sandflies and zones (rural and urban) at ($P<0.7$). The distribution of sandfly species collected in the sampling areas is given in (Figure 4.7, Figure 4.8 and Table 4.1).

4.2.2 Distribution of Fly Species in Each Sampling Region:

4.2.2.1 Makhmur Region,

In this area, there were three Phlebotomus species were found. With a total of 310 (45%) specimens spread throughout the research area, P. papatasi is the most collected species when compared to the other species' distribution. Then, P. sergenti 225 (33%) was the species that was most prevalent in this area. Finally, 151 (22%) sand flies were p. alexandri (Figure 4.9, Figure 4.10 and Table 4.2).

Table 4.2 Distributions of collected sandfly species in Makhmur region between January-December 2022

Makhmur	Light Trap		Sticky paper		Aspirator		Total		Total
	M	F	M	F	M	F	M	F	
p. papatasi	165	106	20	13	4	2	187	123	310
p. sergenti	109	91	17	5	3	0	129	96	225
p. alexandri	75	60	7	5	1	3	83	68	151
Total	606		67		13		401	275	686

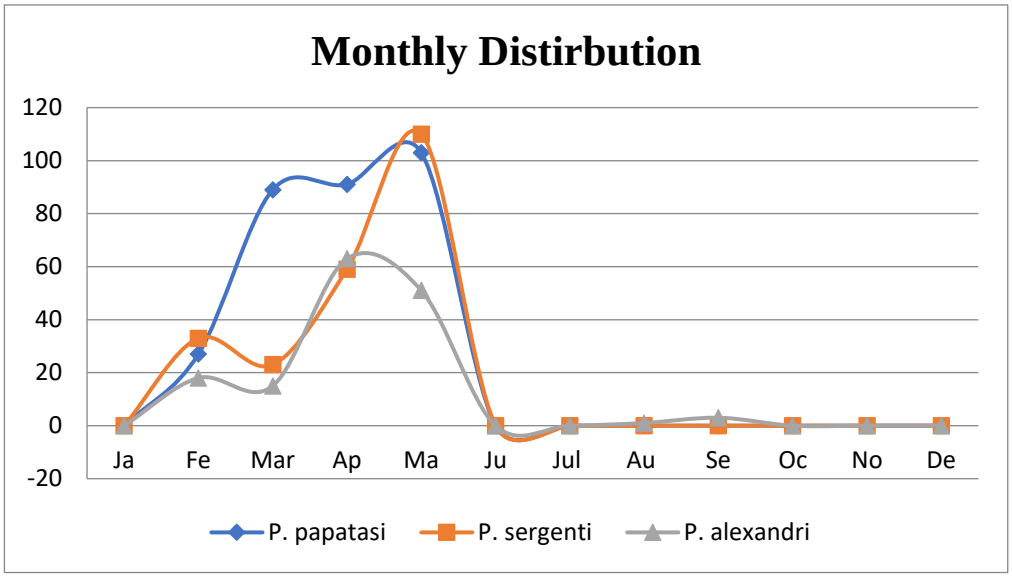


Figure 4.9. Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in Makhmur region between January-December 2022

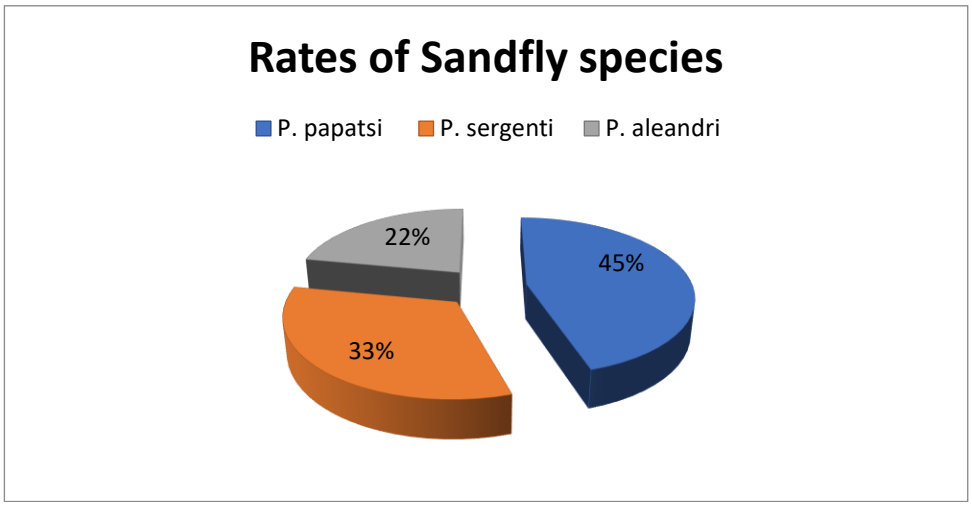


Figure 4.10. The rate of collected sand fly species in Makhmur region between January-December 2022

Phlebotomus papatasi,

With 310 individuals is the most prevalent species in the Makmur region, *P. papatasi*, was caught at varying rates in various areas. The (L.T) trap was used to capture 271 *P. papatasi* sandflies (165 males and 106 females) in this area. Out of the 33 which came to the oil paper trap (ST), 13 were female and 20 were male. Four out the six which were aspirator-collected (ASP) were men and two were women. *P. Papatasi* makes up 45% of the species overall and 27.6% of the males and 17.4% of the females among the sand fly species collected in the Makhmur region (Tables 4.2 and Figures 4.9, 4.10). According to research, *P. papatasi* first appeared in the Makhmur region in February, peaked in May, swiftly declined in June and July, and didn't appear during the remaining months of the year (Figure 4.10).

Phlebotomus Sergenti,

With 225 individuals, 129 males, and 96 females, *P. sergenti* was the second most prevalent species in the Makhmur region. According to the traps, 88% of *P. sergenti* was found in the (L.T), 9% in the (S.T), and 3% in the (ASP). According to the traps, the gender distribution of *P. sergenti* was found to be only 3 males for (ASP). 17 males and 91 females

for (L.T), 17 males and 5 females for (S.T), and 109 males and 91 females for (L.T), (Table 4.2 and Figures 4.9). According to research, P. sergenti first appeared in the Makhmur region in February, peaked in May, declined quickly in June and July, and didn't appear during the remaining months of the year (Figure 4.9, Figure 4.10).

***Alexandrian phlebotomus*,**

P. alexandri, which had a total of 151 individuals, 83 males and 68 females ranked third in terms of species abundance. The percentage of sand fly caught in traps was 89% for (L.T), 7% for (ST), and 4% for (ASP). According to the traps, P. sergenti had a sex distribution of 75 males and 60 females for the (L.T) population, 7 males and 5 females for the (ST) population, and 1 male and 3 female for the (ASP) population (Table 4.2 and Figure 4.9). According to research, P. alexandri first appeared in the Makhmur region in February, peaked in April, and then withdrew in June and July before being absent for the remaining month of the year (Figure 4.9, Figure 4.10).

4.2.2.2 Khabat Region

Three species belonging to the genus Phlebotomus were found in the Khabat region. The most prevalent species was *P. papatasi* 229 (52%) in total, followed by *P. sergenti* 149 (34%) individuals. In the research region between January and December 2022 in Khabat district, *P. alexandri* 60 (14%) individuals were the least frequent species, (Figure 4.11, Figure 4.12 and Table 4.3).

Table 4.3. Distributions of collected sandfly species in Khabat region by using light trap, oily sticky paper and aspirator between January-December 2022

Khabat	Light Trap		Sticky paper		Aspirator		Total		Total
	M	F	M	F	M	F	M	F	
p. papatasi	118	80	10	7	1	13	129	100	229
p. sergenti	78	56	7	2	2	4	87	62	149
p. alexandri	33	19	3	5	0	0	36	24	60
Total	384		34		20		252	186	438

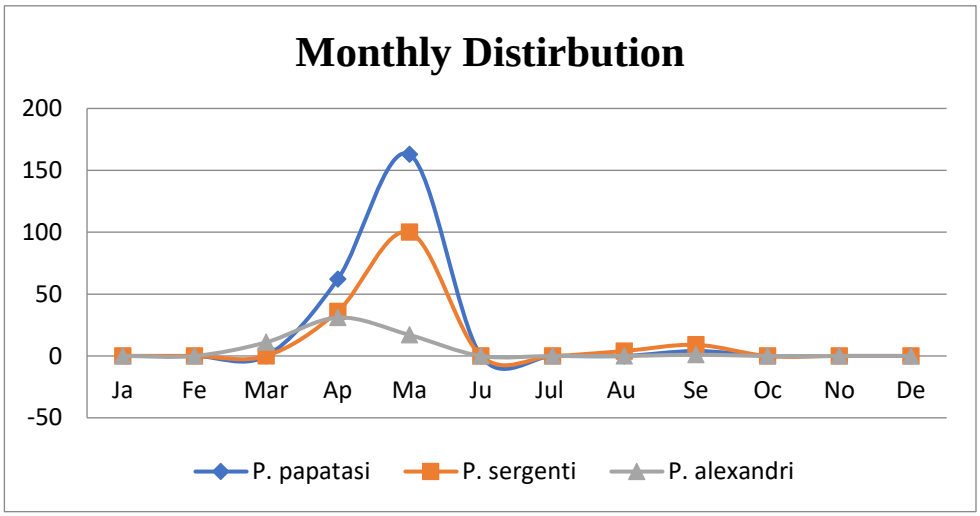


Figure 4.11. Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in Khabat region between January-December 2022

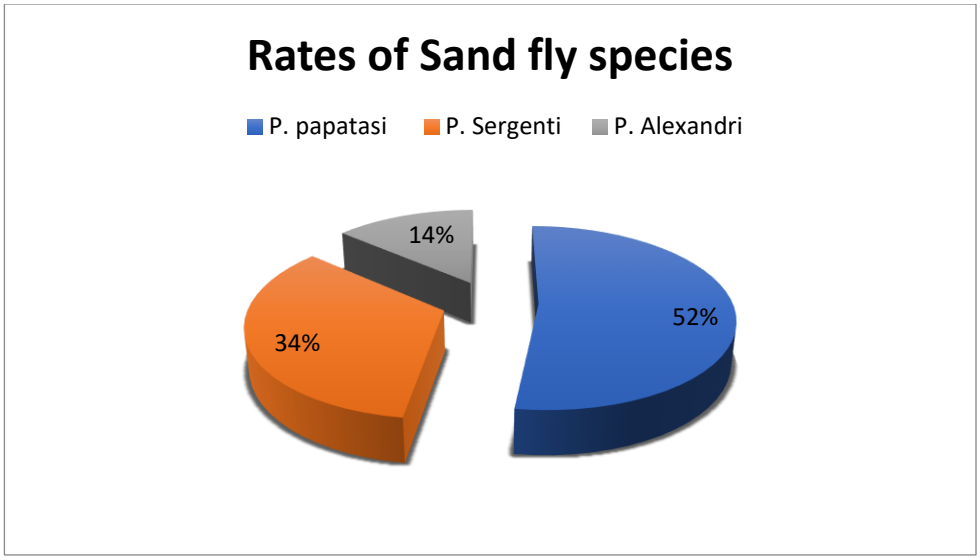


Figure 4.12. the rate of collected sand fly species in Khabat region between January-December 2022

Phlebotomus papatasi,

The most prevalent species in the Khabat region is P. papatasi. The (L.T) captured a total of 198 individuals 118 male and 80 female in this area. Out of the 17 which came to the oil paper trap (ST), 7 were female and 10 were male. One male and thirteen female

were present among the 14 individual who were aspirator-collected (ASP) (Table 4.3). It demonstrates that while (L.T and ST) traps are more effective at catching males than females, (ASP) traps are better at catching females. According to the sandfly species gathered in the Khabat region, P. Papatasi makes up 52% of the species overall and 29% of the males and 23% of the females (Tables 4.3. and Figure 4. 11). P. papatasi was found to start appearing in the Khabat region in April, reach its peak in May, then disappear in June, July and August, before beginning to reappear in September. P. papatasi was finally missing in this location for the remainder of the year (Figure 4.12).

Sergent's phlebotomus,

With a total of 149 individuals, 87 males, and 68 females in the Khabat region, P. sergenti was the second most prevalent species. According to the traps, 89% of P. sergenti was found in the (L.T), 6% in the (ST), and 5% in the ASP. The traps revealed the gender distribution of P. sergenti to be 78 male and 56 female for the (L.T), 7 male and 2 female for the (ST), and 2 male and 4 female for the (ASP) (Table 4.3 & Figures 4.11,), According to the data it can be shown that (L.T and ST) traps are more effective at catching males than females, while (ASP) traps are more successful at catching females than males. According to research, P. psergenti first came in the Khabat region in April, peaked in May, disappeared in June and July, reappeared in September, and eventually vanished during the remaining months of the year in this location (Figure 4.11, Figure 4.12).

Phlebotomus alexandri,

The P. alexandri, which had a total of 60 individuals, 36 males and 24 females, ranked third in terms of species abundance in this region. The percentage of flies caught in traps was discovered to be 86.3% for (L.T), 13.7% for (ST), and 0% for (ASP). According to the traps, P. alexandri had a sex distribution of 33 males and 19 females for the (L.T) population, 3 males and 5 females for the (ST) population, and 0 males and 0 females for the (ASP) population (Table 4.3, Figure 4.11). According to research, p.

alexandri first came in the Khabat region in March, peaked in April, and then gradually vanished during the remaining months of the year (Figures 4.12).

4.2.2.3 Gwer Region,

Three species of sandflies from the genus Phlebotomus were discovered in the Gwer region, which is similar to the Makhmur and Khabat districts in terms of sandfly species discovery. The most prevalent species was P. papatasi 136 (40%) in total, followed by P. sergenti 121 (36%) individuals. In the research region between January and December 2022 in Gwer district, P. alexandri 82 (24%) individuals were the least frequent species, (Figure 4.13, Figure 4.14 and Table 4.4).

Table 4.4 Distributions of collected sandfly species in Gwer region between January-December 2022

Gwer	Light Trap		Sticky paper		Aspirator		Total		Total
	M	F	M	F	M	F	M	F	
p. papatasi	72	62	2	0	0	0	74	62	136
p. sergenti	48	44	13	13	0	3	61	60	121
p. alexandri	20	29	10	15	3	5	33	49	82
Total	275		51		11		168	171	339

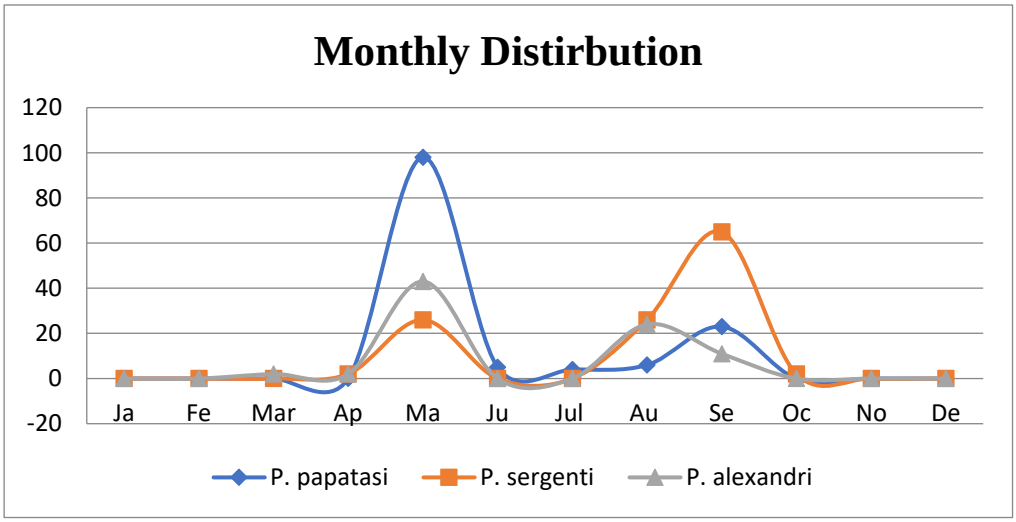


Figure 4.13 Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in Gwer region between January-December 2022

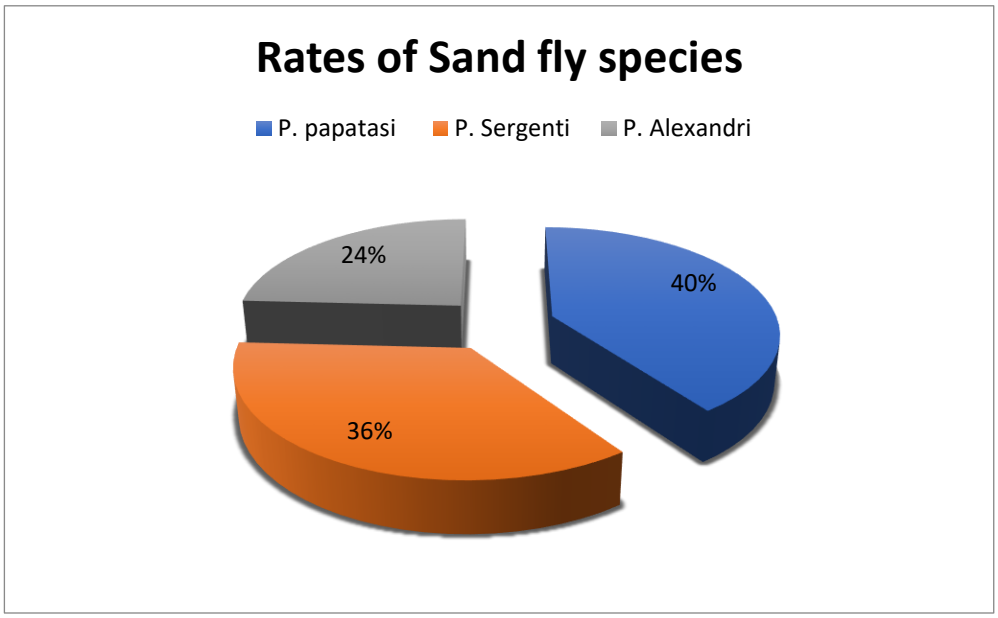


Figure 4.14 the rate of collected sand fly species in Gwer region between January-December 2022

Phlebotomus papatasi,

The most prevalent species in the Gwer area is P. papatasi. The (L.T) captured a total of 134 *P. papatasi* individuals 72 male and 62 female in this area. Out of the 2 which came from the oil paper trap (S.T), only 2 were male. Individual sand flies were not collected by using an aspirator (ASP) in this region. It demonstrates that (L.T and ST) traps are more successful in catching males than females. According to the sandfly species gathered in the Gwer region, P. Papatasi makes up 40% of all species and accounts for 21.8% of males and 18.2% of females (Table 4.4 and Figures 4.13). According to research, P. papatasi began to appear in the Khabat region in March, gradually increased until it reached its peak in May, then disappeared in June and July. It then started to reappear in August and reached its second peak in September before gradually declining in October and disappearing for the remainder of the year (Figure 4.14).

Sergent's phlebotomus,

P. sergenti, which totaled 121 individuals, 61 males, and 60 females, was the second most prevalent species in the Gwer region. According to the traps, P. sergenti was distributed as follows: 76.6% for (L.T), 21.4% for (ST), and 2% for (ASP). The traps revealed the gender distribution of P. sergenti to be 48 male and 44 female for the (L.T), 13 male and 13 female for the (ST), and 0 male and 3 female for the (ASP) (Table 4.4, and Figure 4.13). According to the data it can be shown that (L.T and ST) traps are more effective at catching males than females, while (ASP) traps are more successful at catching females than males. Based on the research, P. sergenti first appeared in the Gwer region in May, peaked in May as well, decreased in June and July, started to decline once more in August, reached its second peak in September, and finally vanished during the remaining months of the year in this area (Figure 4.14).

Phlebotomus alexandri,

P. alexandri, which had a total of 82 individuals, 33 males and 49 females, ranked third in terms of species abundance. The percentage of individualls caught using traps was determined to be 59.7% for (L.T), 30.4% for (ST), and 9.9% for (ASP). The traps revealed that there were 20 male and 29 female for the (L.T) population, 10 male and 15 female for the (ST) population, and 3 male and 5 female for the ASP population. It demonstrates that in this area, (L.T, ST and ASP) traps were more successful in catching females than males (Tables 4.4). (Table 4.4 and Figures 4.13). P. alexandri began to be observed in the Gwer region in March, peaked in May, then vanished in June and July. It then started to reappear in August and reached a second peak in August, declined in September, and finally vanished in the remaining months of the year in this region (Figure 4.14).

4.2.2.4. Koya Region

Two species of sandflies belonging to the genus Phlebotomus were discovered in the Koya region, which is different from the Makhmur, Khabat, and Gwer districts in terms of sandfly species. The most prevalent species was *P. papatasi* 95 (54.2%). The second most numerous species in the studied region between January and December 2022 in Koya district was P. sergenti, with 80 (47.8%) individuals (Figure 4.15 & table 4.5).

Table 4.5 Distirbutions of collected sandfly species in Koya region between January-December 2022

Koya	Light Trap		Sticky paper		Aspirator		Total		To
	M	F	M	F	M	F	M	F	
p. papatasi	45	36	7	5	0	2	52	43	95
p. sergenti	22	20	18	12	3	5	43	37	80
p. alexandri	0	0	0	0	0	0	0	0	0
Total	123		42		10		95	80	17

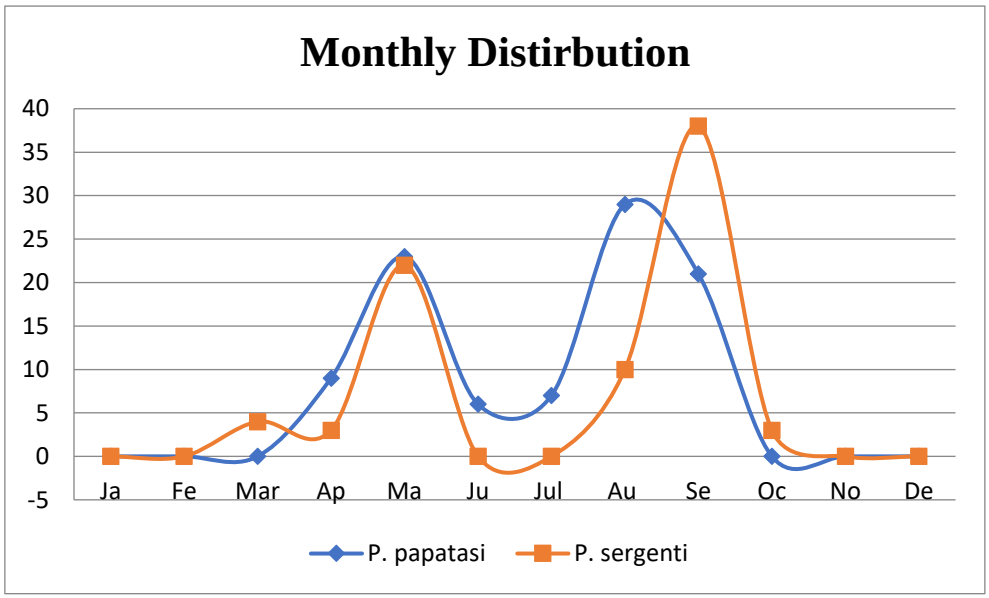


Figure 4.15. Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in Koya region between January-December 2022

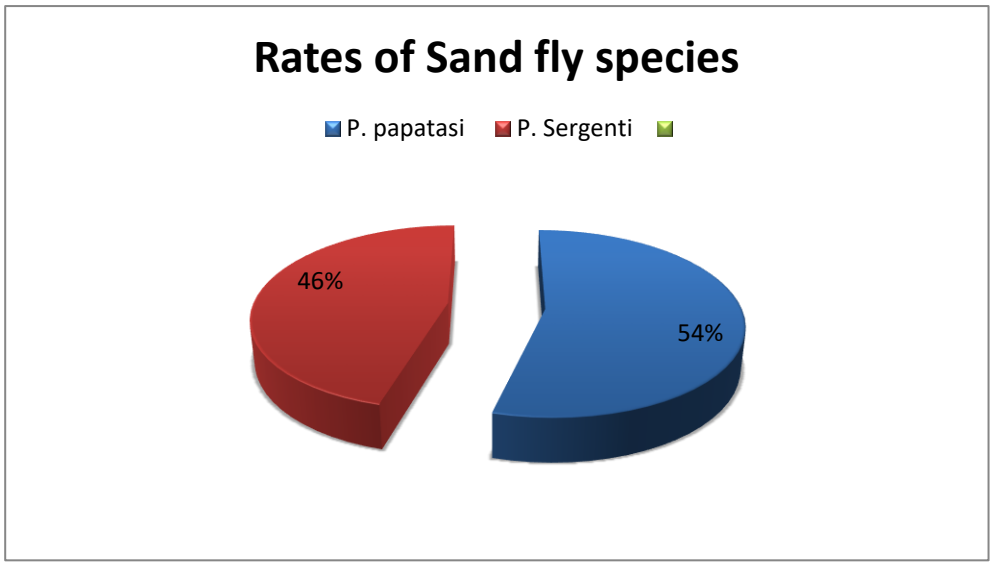


Figure 4.16 The rate of collected sand fly species in Koya region between January-December 2022

Phlebotomus papatasi,

With total of 95 individuals, *P. Papatasi* is the most prevalent species in the Koya area. The (L.T) caught a total of 81 individuals (45 males and 36 females) in this area. Only 7 male and 5 female, out of a total of 12, came from the oil paper trap (ST). Only 2 out of the 2 individualls that collected samples with an aspirator (ASP) were female. It demonstrates that while (L.T and ST) traps are more effective at catching males than females while (ASP) traps are better at catching females. The sex ratio of the sandfly species present in the Koya region was determined; P. Papatasi makes up 29.7% of the males, 24.8% of the females, and 54.5% of the species overall in this area (Table 4.5. and Figure 4.15). According to research, *P. papatasi* began to be seen in the Koya region in April, gradually increased until it reached its peak in May, then decreased in June and July before beginning to reappear in August and reaching a second peak in August before gradually declining in September and remaining absent in the remaining months of the year (Figure 4.15).

Phlebotomus sergenti,

With a total of 80 individuals, including 43 males and 37 females, P. sergenti was the least prevalent species in the Koya region. According to the traps, P. sergenti was distributed as follows: 52% for (L.T), 37% for (ST), and 11% for (ASP). According to the traps, P. sergenti had a sex distribution of 22 male and 20 female for the (L.T) population, 18 male and 12 female for the (ST) population, and 3 male and 5 female for the (ASP) population (Table 4.5 and Figure 4.15). It demonstrates that (L.T and ST) traps are more effective at catching males than females, whereas (ASP) traps are more successful at catching females. It has been determined that P. psergenti first appeared in the Koya region in March, peaked in May, disappeared in June and July, then reappeared in August, reached a second peak in September, declined in October, and finally vanished during the remaining months of the year in this area (Figure 4.16).

4.2.2.5. Soran Region

In terms of sandfly species, this region differs from the Makhmur, Khabat, Gwer, and Koya districts in that only one species of the genus Phlebotomus was discovered there. Throughout the study period in the Soran district between January and December 2022, *P. papatasi* 118 (100%) was the only species that was abundant (Figure 4.17 andTable 4.6).

Table 4.6 Distributions of collected sandfly species in Soran region between January-December 2022

Soran	Light Trap		Sticky paper		Aspirator		Total		Total
	M	F	M	F	M	F	M	F	
p. papatasi	45	30	18	25	0	0	63	55	118
p. sergenti	0	0	0	0	0	0	0	0	0
p. alexandri	0	0	0	0	0	0	0	0	0
Total	75		43		0		63	55	118

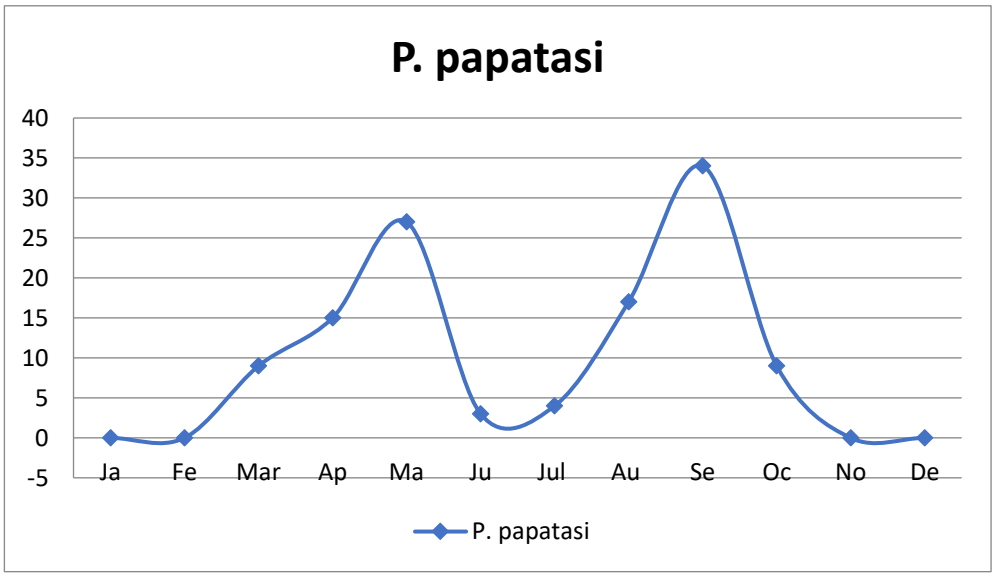


Figure 4.17. Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in Soran region between January-December 2022.

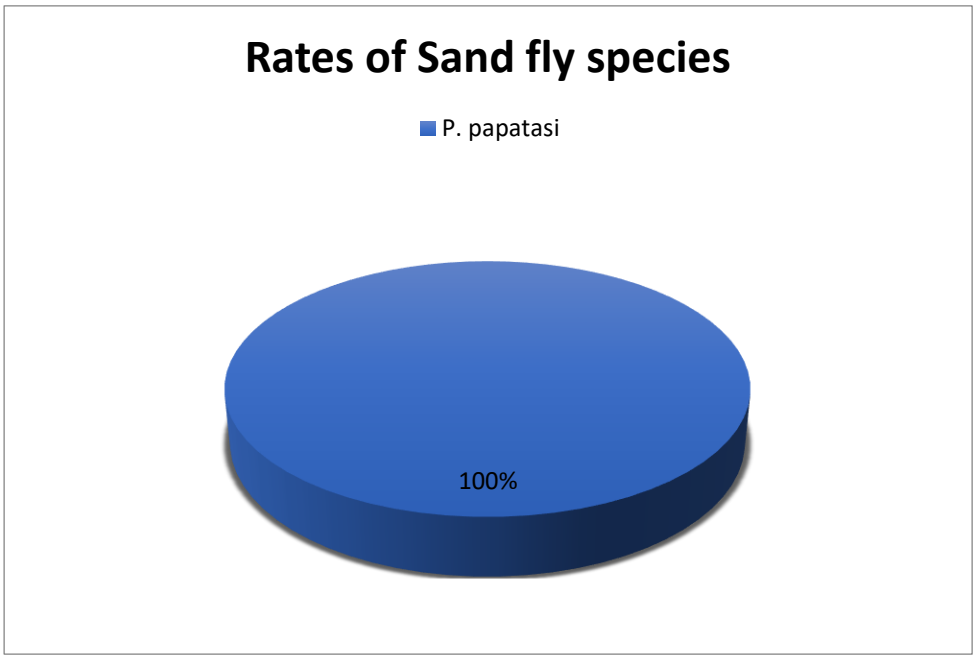


Figure 4.18 The rate of collected sand fly species in Soran region between January-December 2022

Phlebotomus papatasi,

The sole species found in the Soran region is *P. papatasi*. The light trap (L.T) captured a total of 75 (63% of the population), including 45 (38%) male and 30 (25%) female that were gathered in this area. Out of 43 (37%), there were only 18 (15%) men and 25 (21%) women which came from the oil sticky paper trap (ST) (Table 4.6). It demonstrates that (L.T) traps are more successful at catching males than females, but (ASP) traps were unsuccessful in this area in catching sand flies. According to the sandfly species collected in the area, P. Papatasi makes up 100% of the species overall and 54% of the males and 46% of the females (Table 4.16). It has been established that *P. papatasi* first appeared in the Soran region in March, gradually increased until reaching its peak in May, then decreased in June and July before beginning to decline once more in August and reaching its second peak in September before beginning to decline in October. In this region, it was, thankfully, absent for the remaining months of the year (Figure 4.17 & Table 6).

4.2.2.6. Shaqlawa Region

Only one species of sandfly from the genus Phlebotomus was discovered in the Shaqlawa region, which is similar to the Soran district in terms of sandfly species discovery. P. papatasi was the sole abundant species in the research region in the Shaqlawa district from January to December 2022. In this area, out of 81 (100%) individuals, 45 (53%) were men and 36 (47%) were women (Figure 4.19, Figure 4.20 & table 4. 7).

Table 4.7. Distirbutions of collected sandfly species in Shaqlawa region between January-December 2022

Shaqlawa	Light Trap		Sticky paper		Aspirator		Total		Total
	M	F	M	F	M	F	M	F	
p. papatasi	40	32	5	4	0	0	45	36	81
p. sergenti	0	0	0	0	0	0	0	0	0
p. alexandri	0	0	0	0	0	0	0	0	0
Total	72		9		0		45	36	81

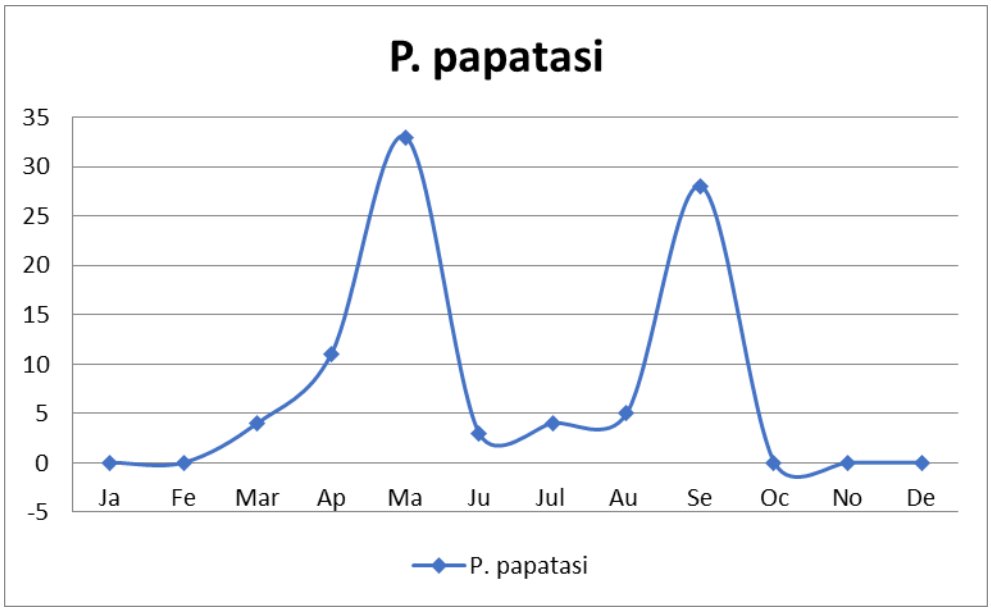


Figure 4.19. Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in Shaqlawa region between January-December 2022

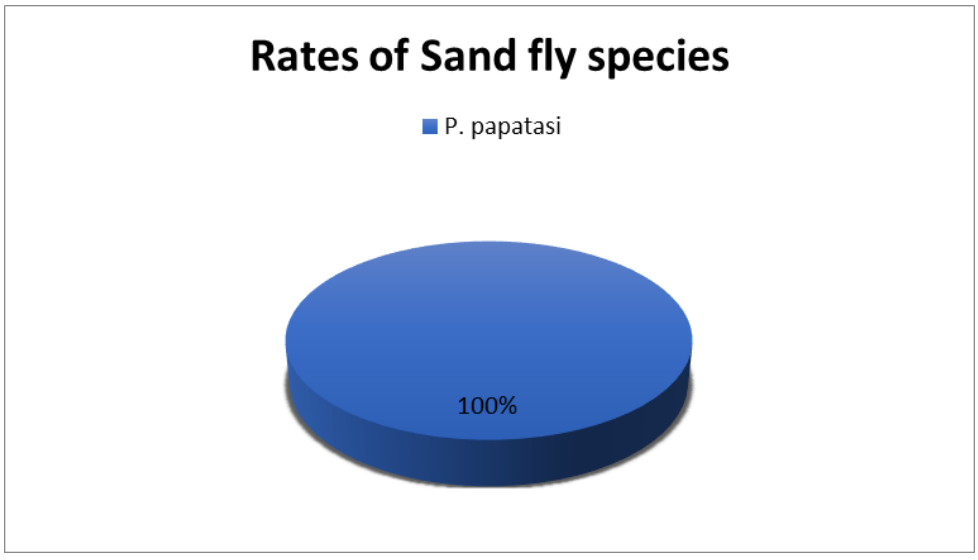


Figure 4.20 The rate of collected sand fly species in Shaqlawa region between January-December 2022

Phlebotomus papatasi,

The sole species found in the Shaqlawa region is *P. papatasi.* The light trap (L.T) captured a total of 72 (88.2%) individuals, including 32 (39.3%) female and 40 (49.7%)

male that were gathered in this area. Only 5 (6%) men and 4 (5.8%) women out of a total of 9 (11.8%) flies came from oil paper trap (ST) (Table 4.7). It demonstrates that (LT and ST) traps are more successful at catching males than females, but (ASP) traps were unsuccessful in this area in catching sand flies. According to the sandfly species gathered in the area, *P. Papatasi* makes up 100% of the species overall and 57% of the males, 43% of the females (Figure 4.19). It has been established that *P. papatasi* began to appear in the Shaqlawa region in March, progressively increased until it reached its peak in May, was dabsent in June and July, then started to grow again in August, reaching its second peak in September. In this region, it was absent for the remaining months of the year (Figure 4.19, & Table 4. 7).

4.2.2.7. City Center Region,

Two species of sandflies from the genus Phlebotomus were discovered in the City Center region. The majority of the population 194 (89%) belonged to *P. papatasi*. *P. sergenti* was the second most prevalent species in the research area between January and December 2022 in the City center region, with 23 (11%) individuals (Figure 4.21, 4.22 & table 4.8).

Table 4.8 Distributions of collected sandfly species in City center between January-December 2022

City center	Light Trap		Stickypaper		Aspirator		Total		Total
	M	F	M	F	M	F	M	F	
p. papatasi	95	60	15	13	3	8	113	81	194
p. sergenti	0	13	0	6	0	4	0	23	23
p. alexandri	0	0	0	0	0	0	0	0	0
Total	0		0		0		113	104	217

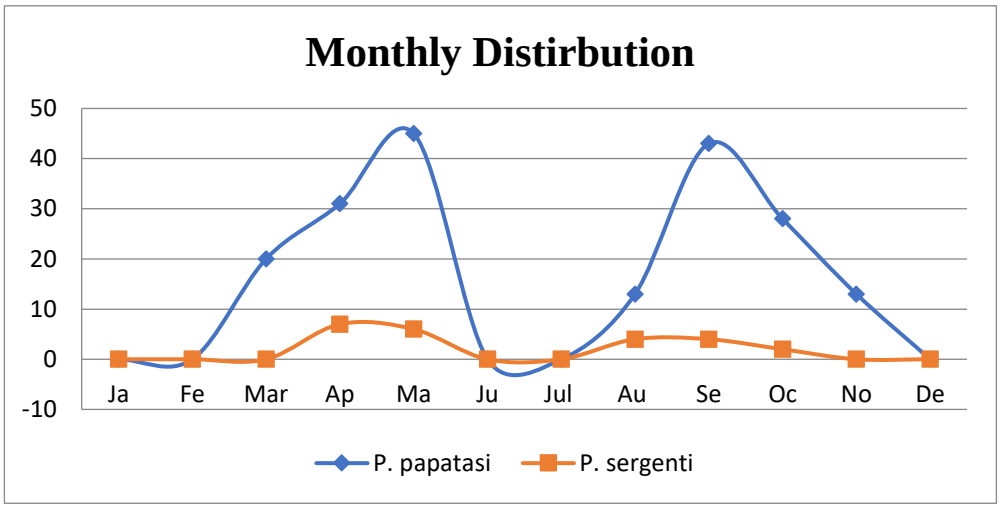


Figure 4.21. Monthly populations of sand fly species collected with light trap, oily sticky paper and aspirator in City center between January-December 2022

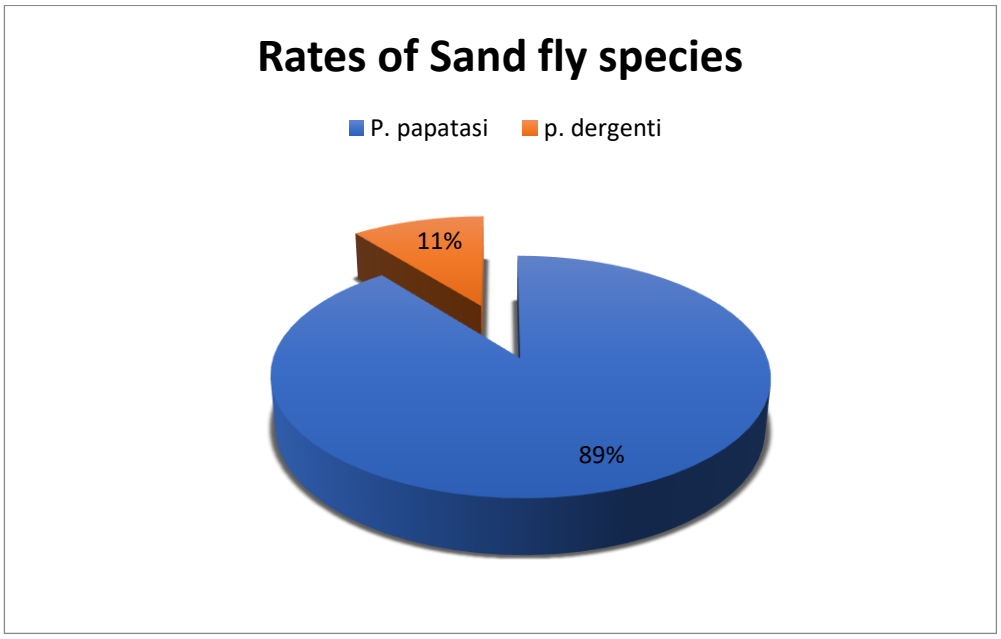


Figure 4.22. The rate of collected sand fly species in City center region between January-December 2022

Phlebotomus papatasi,

The most prevalent species in the Center region is *P. papatasi.* The light trap (L.T) captured 155 (%79) individualls in total, of which 60 (31%) were female and 95 (48%)

were male. Only 15 (7.5%) men and 13 (6.5%) women out of a total of 28 individualls capturedby the oil paper trap (ST). Out of 11 (7%), 3 (2.7%) men and 8 (4.3%) women were collected using an aspirator (ASP) (Table 4.8). It demonstrates that men were easier to catch using (L.T and ST,) traps than females. The sandfly species gathered in the Center region had a 52 percent male to 37 percent female sex ratio, making up 89% of the species there overall (Tables 4.8). It has been determined that *P. papatasi* began to appear in the Center region in April, gradually increased until it reached the maximum level in May, then disappeared in June and July. It then started to reappear in August, reached a second peak in September, and then began to decline gradually in October and November, respectively. In this location, it was completely missing in the final month of the year, December (Figure 4.21, 4.22 and Table 4.8).

Phlebotomus sergenti,

With a total of 23, 13 male and 10 female, *P. sergenti* was the least prevalent species in this area. According to the traps, P. sergenti was distributed as follows: 56% for (L.T), 26% for (ST), and 18% for (ASP). According to the traps, there were 13 males for (L.T), 6 female for (ST), and 4 female for (ASP) in the *P. sergenti* sex distribution (Table 4.8). It demonstrates that (L.T) traps were only successful in catching males, while (ST and ASP) traps were only successful in catching females (Tables 4.8). According to research, P. psergenti first appeared in the City Center region in March, peaked in April, and then withdrew in June and July. It then started to reappear in August, reached a second peak in September, declined in October, and finally vanished in the remaining months of the year (Figure 4.21 & Table 4.8).

4.2.3. Sandfly Distribution in Relation to Climate Variables During the Study's Months

This study found a negative correlation between sandfly individuals and high temperature, whereas sandflies are active and numerous at moderate temperatures and significantly decline in number during the hot months ($P<0.05$). Sandflies are affected by rainfall; however they are more active and abundant in moderate precipitation, and their numbers significantly decline in months with no or high precipitation ($P<0.05$). Additionally, this study showed that humidity may have an impact on the presence and activity of sandflies ($P<0.05$), sandflies being more active in moderate humidity (Figure 4.23 & Table 4.9).

Table 4.9. Shows the seasonal variation of sand fly population in the study's months in Erbil province between January-December 2022

Month	Mean temperature (C°)	Mean Rain amount(mm)	Mean humidity (%)	Sandflies	
				No.	(%)
January	9.21	133.9	57.18	0	0
February	10.99	97.7	55.42	79	3.8
March	14.93	94.7	54.34	177	8.6
April	20.69	91.5	46.46	422	20.5
May	27.99	45.4	31.07	867	42.2
June	34.77	2.63	17.56	14	0.7
July	38.35	0.37	14.21	15	0.7
August	37.75	0.19	14.64	139	6.8
September	32.68	3	18.03	284	13.8
October	9.21	133.7	57.18	44	2.1
November	10.99	97.7	55.42	13	0.6
December	14.93	144.7	54.34	0	0
Total / mean±SD	44.33±11.052	23.61±6.511	33.41±11.514		
P-value*	>0.05	>0.05	>0.05		

*Logistic regression test; **NS=non-significant; mm=millimeter; m/s=meter/second; No. =number; %=percentage; SD=standard deviation

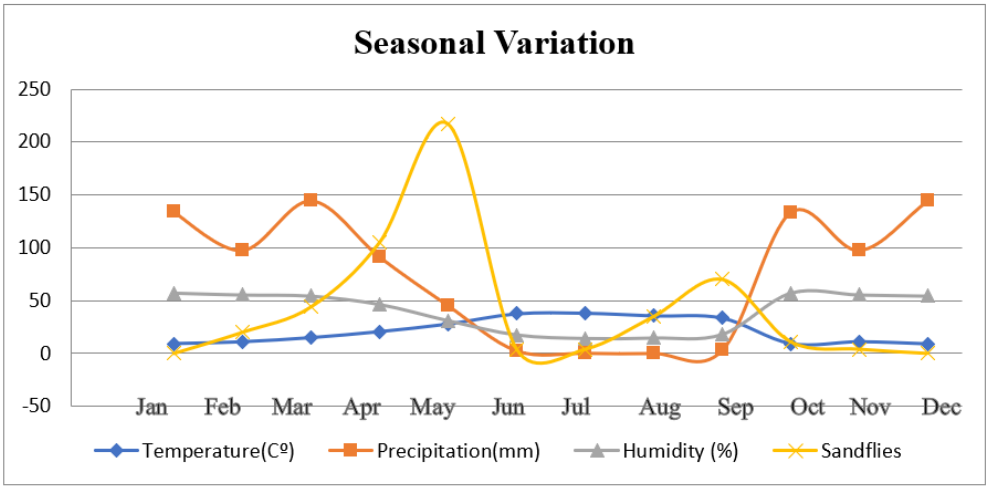


Figure 4.23. shows the seasonal variation of sand fly population in the study's months in Erbil province between January-December2022

May, April, and September were the months with the most sandflies observed. where there was a moderate quantity of precepitation, temperature, and humidity. Sandflies can grow and function normally in these circumstances (Khan, 2012; Karimi *et al*., 2018).

This investigation aligns with a previous study carried out by (Alten et al., 2016). However, in June, July, and August, no sandflies were collected for the study. This is due to the high temperatures, dry weather, low humidity, and strong winds, all of which have an impact on the existence, development, and reproduction of sandflies.

These percentages bear strong resemblance to other research conducted in Iraq, which demonstrated that moderate levels of humidity, temperature, and wind speed provide an ideal habitat for the emergence of sandflies. In hot months of the year, when the high temperatures prevent these insects from spreading, the population density of these insects falls (Abdulwahab, 2013; Ali, 2018).

The study’s findings were compiled based on a study by (Coleman et al., 2007), which found that the number of sandflies was low in April, high in May, at its peak from mid-September, and rapidly declined in late October. According to studies on sandflies'

nocturnal activity, during the cooler months, activity was generally high in the evening, while during the hotter months, activity was higher at night. The findings were in line with the Tikrit study conducted by (AL-Obiadi, 2000), but not with the findings published in Iran (Talari et al., 2006) and Afghanistan (Faulde et al., 2009).

The activity of the sandflies may be the cause of this monthly peak variation. The maturity of female insects and their blood supplementation throughout their life cycle for the growth and development of eggs in the spring may be contributing factors to these variations in leishmaniasis distribution (Khalaf et al., 2016). (Coleman et al., 2007) discovered that *P. papatasi* were primarily prevalent during the hot season (August and September).

According to a study conducted in the Diyala governorate, between 2012 and 2016, the rates of CL peaked in the winter (68.3%), followed by the spring (19.3%), summer (7.3%), and autumn (5.1%). This may have to do with how many sandflies there are and how active they are during the cooler months as opposed to the warmer ones. According to (Pereira et al., 2017), the peak activity period coincided with rising humidity and moderate temperature, suggesting that these two factors are important for the sandflies' survival as part of the ecosystem's environmental dynamics. Some researchers have confirmed the positive link between temperature and humidity and the population abundance of sand flies (Gramiccia and Gradoni, 20017).

In Saudi Arabia, a study by (Al-Ajmi et al., 2015) also identified sand flies into five species; three of them belong to genus Phlebotomus (*P. papatasi, P. bergeroti, P. sergenti*), and two belong to genus Sergentomyia (*S. antennata* and *S. clydei*). In Sudan, (Adam et al., 2017) recorded 10 species of sandflies were three Phlebotomus species and seven Sergentomyia species of these sandflies, *P. rodhaini* and *P. orientalis*). “In Egypt, (Ali et al., 2016) collected 143 of *P. papatasi* the highest prevalence was 44.8% in Al Hawareya, while Marakya was free of sand flies, with male to female sex ratio 1:1.6 and two peaks of abundance in both July and September. while the lowest monthly abundance

was in November. In Palestin, (Sawalha et al., 2017) collected sand flies from different districts (Phlebotomus and Sergentomyia) the genera Phlebotomus and Sergentomyia are represented by 13 and nine species and subspecies, respectively.

4.2.4. Distributions of Sandflies According to Sex

Throughout the study, in total, 2054 sand flies were gathered from 7 zones and distributed to 1137 (55.4%) males and 917 (44.6%) females in Erbil province between January-December 2022 (Table 4.10).

Table 4.10 Distribution of sandflies according to sex in the study area in Erbil province January-December 2022

Sex	**Sandflies**	
	No.	**(%)**
Male	1137	55
Female	917	45
Total	2054	100
P-value*	=0.2	

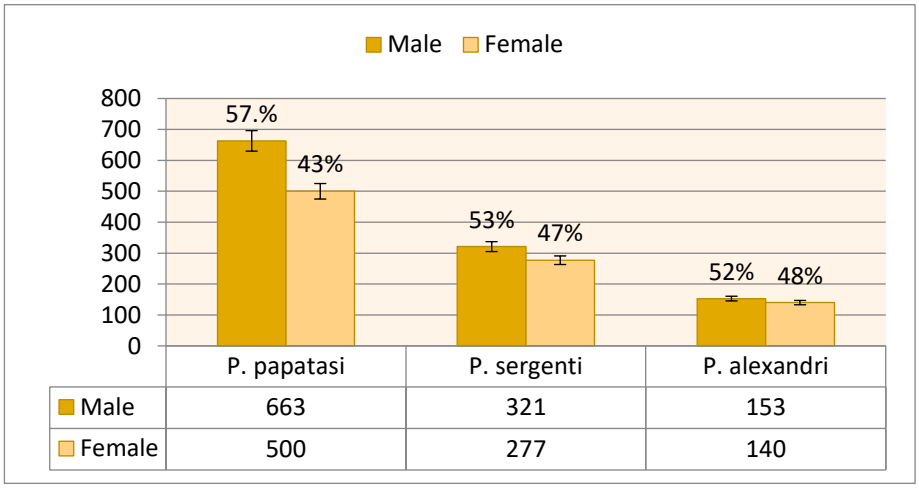


Figure 4. 24. Distribution of sandflies according to sex in the study area in Erbil province January-December 2022

Throughout the study, in total, 2054 sand flies were gathered from 7 zones and distributed to 1137 (55. %) males and 917 (45. %) females (Table 4.10). All specimens were identified morphologically in accordance with the Morphological Classification of Sand Flies key for the Psychodidae family in Iraq. Three species of the genus Phlebotomus, including *P. papatasi, P. sergenti,* and *P. alexandri,* were identified. *P. papatasi* was recorded as the most prevalent sand fly species in the study area, with 1163 (56.6%) individuals, including 663 (58%) males and 500 (43%) females. while, *P. sergenti* a constituted 598 (29.1%) of all sand flies collected, including 321 (53.7%) males, 277 (46.3%) females and *p. alexandri* a constituted 293 (14.3%) of all sand flies collected, including 153 (52.2%) males, 140 (47.8%) females (Figure 4.24). here is statistically no significant association between gender and sand fly species at ($P<0.2$).

This study is in agreement with a study which conducted by (Al-Abbas et al., 2018), between January and December 2016, which identified 1376 male and 1102 female sandflies were gathered from five distinct collection regions. Furthermore, this study is in greement with a study that carried out by (Toprak and Ozer, 2005). The distribution of the other minor species discovered in this study in Turkey Using morphological and molecular methods, this study was carried out to determine the fauna and yearly activity patterns of sandflies, were %58 males and % 42female.

However, this study is disgreement with a study that carried out by (Al-Awadi, 2019) carried out a second study in the province of Thi-Qar to look into the species of sand flies that are present there and act as the disease's vector. A total of 6527 sand flies were collected using aspirators, oil traps (sticky papers), and light traps. Of these, 3064 females and 3463 males were distributed. The two species, *P. papaatasi* and *P. sergenti*, belonged to the same genus, Phlepotomus.

4.2.5. Distribution of Sandflies According to the Capture Rates

Considering the capture rates of the species found in the study area, *P. papatasi* is the most common species with a 1163 (56.6)%. Of the 1163 *P. papatasi* caught in the whole study area, 992 (85.2%) were caught by (L.T), 153 (13.1%) by oil paper trap (ST) and 18 (1.5%) by aspirator (ASP). Followed by *P.sergenti,* of the 598 (29.1%). *P.sergenti* caught in the whole study area, 482 (80.6%) were caught by (L.T), 99 (16.5%) by oil paper trap (ST) and 17 (2.9%) by aspirator. However, *P. Alexandri* is the least common species with a 229 . Of the 293 (14.3%). *P. Alexandri* caught in the whole study area, 229 (78.1%) were caught by (L.T), 52 (17.7%) by oil paper trap (ST) and 3 (1.02%) by aspirator (ASP) (Table 4.11 & Figure 4.25).

Table 4.11. According to the traps and general capture rates of the species found in the study area

Species	Traps %			Total (%)
	Light trap(L.T)	Sticky papar(ST)	Aspirator(ASP)	
P. papatasi	992 (57.5)	153 (52.7)	18 (50)	1163 (56.6)
p. sergenti	482 (28.6)	99 (31.7)	17 (31)	598 (29.1)
p. alexandri	229 (13.9)	52 (15.7)	3 (19)	293 (14.3)
Total %	1712 (83.3)	300 (14.6)	42 (2)	2054

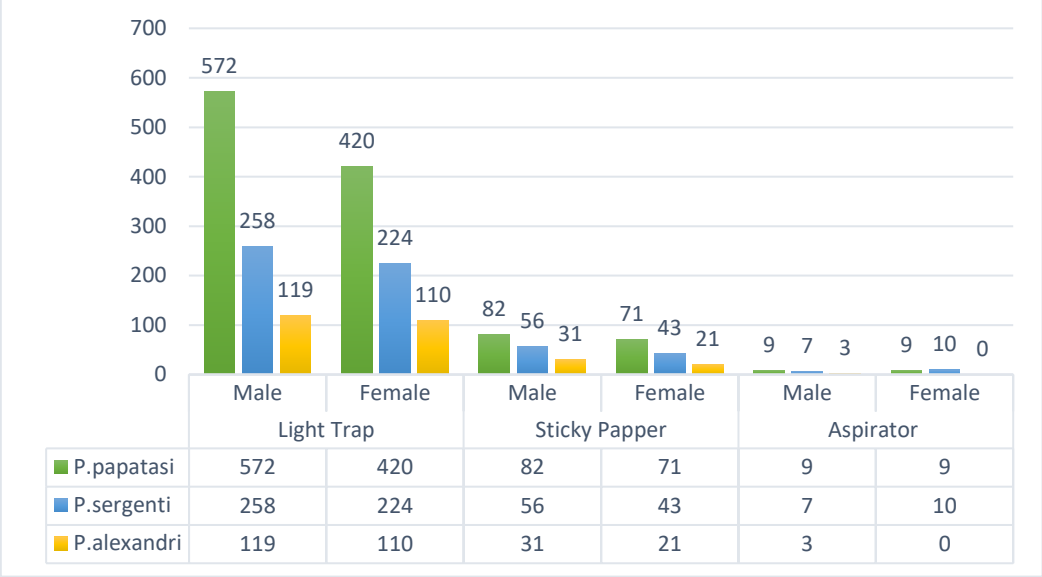


Figure 4. 25. Trap efficiency to sample for the sandflies species in the study area, Erbil province-Iraq January-December 2022

The light trap was most effective for collecting sandfly individuals and all sandfly species which were collected 1712 (83.3%) out of 2054 by aid of (L.T). The sticky paper trap came second in order of efficiency which were collected 300 (14.6%) out of 2054 collected sandflies and the aspirator trap showed the least efficiency in all collection sites, which were collected 42 (2%) out of 2054 collected sandflies, as seen in (Table 11 and Figure 4.25). According to statistical analyses (Pearson Chi-square test), a significant difference was seen among trap preference of the species at (p=0.00). According to the efficacy of traps in districts, the study reveals that light trap was the most effective trap which captured sandflies in 6 districts, while aspirator trap was the least effective, capturing sandflies only in 3 districts, staticall analysis shows significant associassion between effeciency of traps in districts at (p=0.00). Out of all sandflies identified, light trap was captured (33.2%) of male sandflies and (33.5%) of female sandflies, but sticky paper captured (15%) male sandflies and (14.2% female sandflies. While aspirator was captured (1.8%) male and (2.3%) female sandflies, there were no significant differences between trap efficiency and the gender at (p=0.2).

This study is in agreement with a study which conducted by (Al-Abbas and et al., 2018), between January and December 2016, which identified 1376 male and 1102 female sandflies were gathered from five distinct collection regions by using traps, the most effective trap was light trap, followed by oil trap. Also, this study is lined with a study that carried out by (Al-Awadi, 2019) carried out a second study in the province of Thi-Qar to look into the species of sand flies that are present there and act as the disease's vector. A total of 6527 sand flies were collected using aspirators, oil traps (sticky papers), and light traps. Of these, 3064 females and 3463 males were distributed. This study declared that the %51 captured sand flies were by using light trap.

Furthermore, this study is matched with a study that carried out by (Toprak and Ozer, 2005). The distribution of the other minor species discovered in this study in Turkey, using traps, light trap, aspirator and oil trap, the most effective trap was light trap for capturing sand fly samples.

4.2.6. Monthly Distribution of Species Found in the Study Area

Population distirbutions of sandfly species in Erbil province shows some differences from species to species. *P. papatasi* species, which is found all over the study area, starts to appear in February, gradually increased its population density in March April and, reached the high level in May, then started to decrese in June and July, while it started to increase in August and got second peak in September, and shows a rapid decline in October. In some parts of the study area, collection of *P. papatasi* was also available in November (Figure 4. 26).

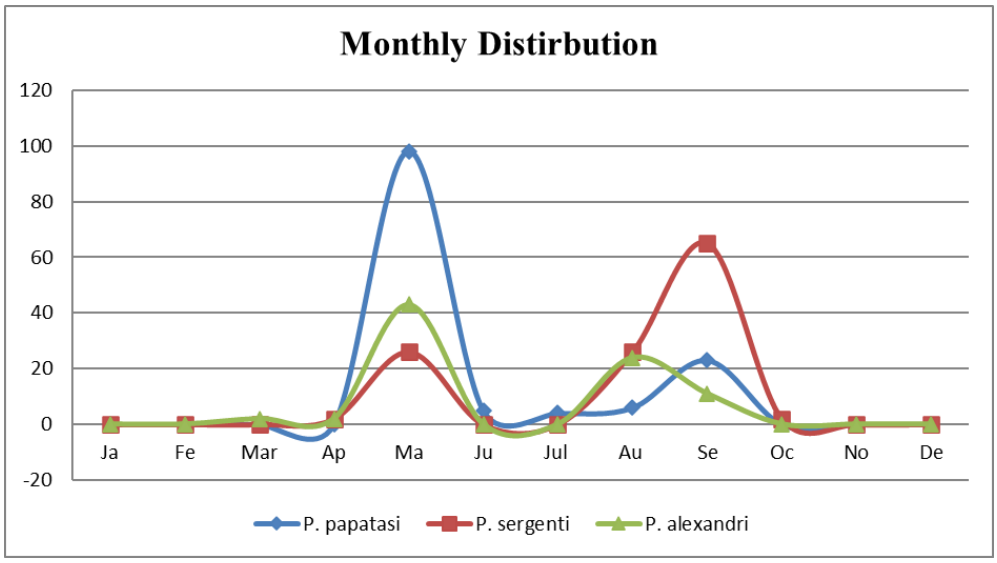


Figure 4.26. The monthly distribution of three species of sand flies, *P. papatasi, P. sergenti, and p. alexandri* in Erbil province January-December 2022

The monthly distribution of three species of sand flies, *P. papatasi, P. sergenti,* and *p. alexandri* in Erbil province, is shown in (figure 4.26). *P. papatasi* is observed to begin in February, March, and April to be very rare in June and July, and again observed to increase in August and with high dentistry in May and September, but disappearing in January and December. While, *P. sergenti* appeared in March, and april it again reappeared in August and October, with high density in May and September, then disappeared in June, July, November, December, January and February. Lastly, *P. alexandri* began to appear in March, and April, and it again reappeared in August with high density in May and September, while it disappeared in January, February, June, July, October, November and December. Generally, there were two peaks of existing sandflies along the year, first peak was in May and the second peak was in September. A statistical analysis has shown that there is a significant association between sand fly species of the periods at ($p<0.00$).

This study is inagreement with a study which conducted by (Al-Abbas et al., 2017) between January and December 2016, which identified 1376 male and 1102 female sandflies were gathered from five distinct collection regions. The findings indicate that the number of sandflies declined throughout the winter's cold months (December, January,

and February) and reached 0%. In contrast, the insect became more active during the warmer months, particularly in August and September, when its percentages were 16.10 and 13.95 percent, respectively.

Also, this study is ingreement with a study that carried out by (Al-Awadi, 2019) carried out a second study in the province of Thi-Qar to look into the species of sand flies that are present there and act as the disease's vector. A total of 6527 sand flies were collected using aspirators, oil traps (sticky papers), and light traps. Of these, 3064 females and 3463 males were distributed. The two species, *P. papaatasi* and *P. sergenti*, belonged to the same genus, Phlepotomus. The density of sand flies has two peaks: the first one occurred in May, and the second one occurred in September.

However, this study is disgreement with a study that carried out by an inquiry was done in Paveh County, Kermanshah Province, west of Iran, to ascertain the biodiversity and seasonal activity of sand flies. Sand flies were caught in five locations in Paveh County between May and October of 2015 using sticky traps. Sand fly activity peaked in early October and ended in late April. 2110 phlebotominae in total (64.6%) were collected outside and 35.4% were collected indoors, with 71.1% of the males and 28.9% of the females. In the area, *Phlebotomus alexandri* accounted for 50% of all sand flies collected, while *Ph. brevis* made up just 0.04% of specimens (Mawloudi and associates, 2018).

Phlebotomus papatasi,

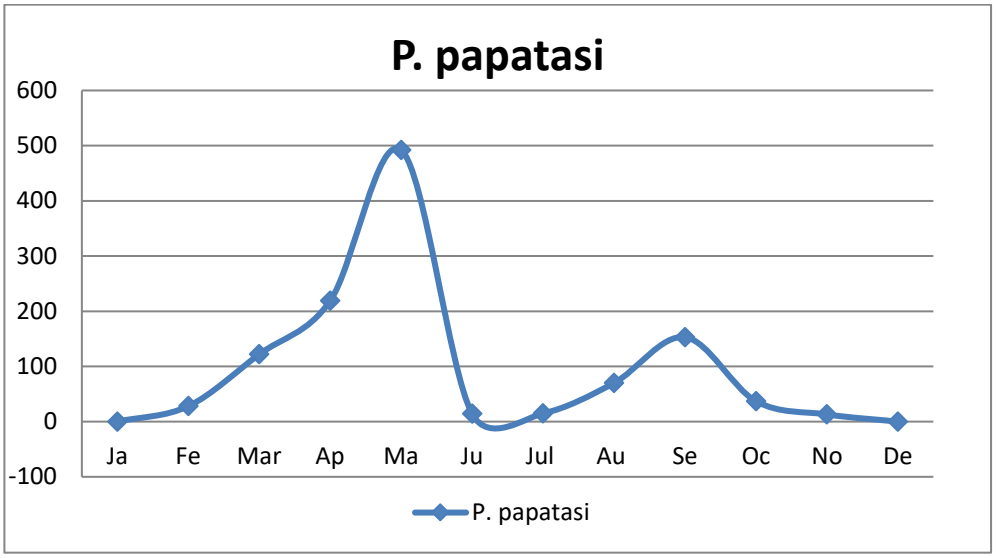


Figure 4.27. Monthly distirbution of P. papatasi caught with light trap, sticky paper trap and aspirator between January-December 2022 in Erbil province

P. papatasi, which has a 1163 (56.6%) capture rate in the study area, was found in all sampling areas. The population density was in high level in Makhmur, While the population density decreased in the Central and Koya regions, it increased significantly in the Makhmur, Khabat and Gwer regions. Considering the level of capture by months, May is the month in which the most samples were caught. Population density of P. papatasi decreased significantly in June and July, while it started to appear in August again and got the second peak in September. Rapidly decreased in October and November, the population disappears only in January and December generally in Erbil province during the study (Figure 4.27).

Phlebotomus sergenti,

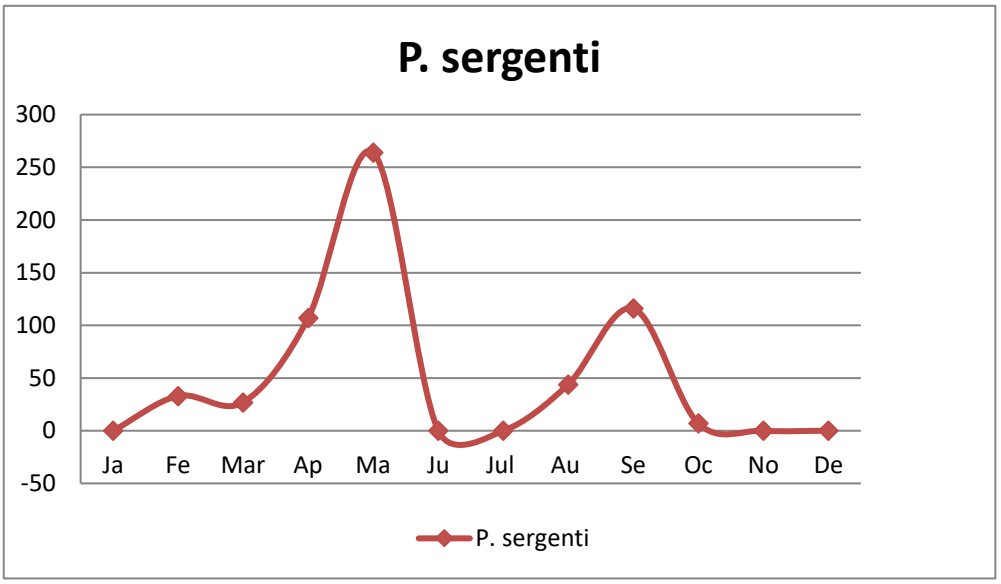


Figure 4.28 Monthly distirbution of P. sergenti caught with light trap, sticky paper trap and aspirator between January-December 2022 in Erbil province

Although P. sergenti was the second most abundant species with a catch rate of 598 (29.1%) in the study area, it was found in five sampling areas. The population density was in high level in Makhmur, While the population density decreased in the Central and Koya regions, it increased significantly in the Khabat and Gwer regions. While the population density was at the highest level in May, Population density of P. Sergenti decreased significantly in June and July, while it started to appear in August then it got second peak in September. It was absent again in October and November, it disappeared only in January and December generally in Erbil province during the study (Figure 4.28).

Phlebotomus alexandri,

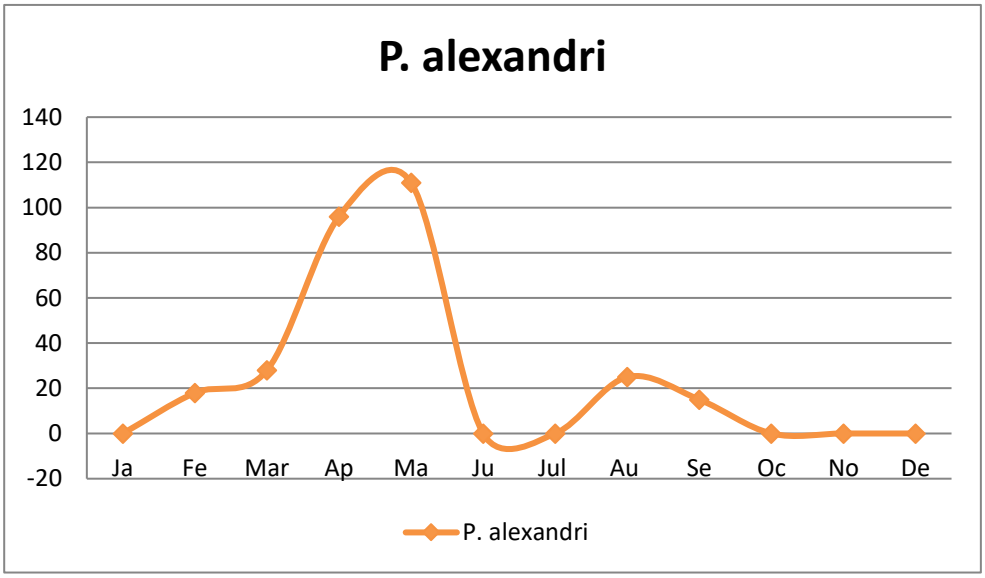


Figure 4.29. Monthly distirbution of P. alexandri caught with light trap, sticky paper trap and aspirator between January-December 2022 in Erbil province

Throughout the study area, P. alexandri is the third most abundant species with a catch rate of 293 (14.3%) and was collected in 3 sampling regions. While population density decreased in Gwer and Khabat regions. P. alexandri population is seen for the first time in february, reaches the highest level in May and disappears towards the end of June and July, then again appeared in august and got the second peak in August, significantly decreased in September, finelly dissappered in the rest months of the year (Figure 4.29).

4.3. Molecular Identification of Sand Flies

Compared to the traditional morphology-based total classification, the genotyping technique has been demonstrated to be more accurate and user-friendly for the identification of sand fly species. It also requires less expertise and poses less risk of misunderstandings. except from the fact that sample damage, which frequently affects morphologic classification, no longer affects the genotyping analysis (Terayama et al.,

2008; Al-Ajmi et al., 2015). In the current study, PCR-direct sequencing is utilized to detect and identify *P. papatasi, P. sergenti, and P. alexandri*, which are endemic CL from the Erbil area. We also used the nucleotide sequence to estimate their phylogenetic chronology with other known lines.

This study used a DNA extraction kit made by Thermo Scientific called the Isolation Kit Gen JET Genomic DNA Purification Kit to molecularly identify sand flies for the first time in the province of Erbil by mitochondrial mt DNA COI and molecular average length 600 base pair of *P. papatasi, P. sergenti, and P. alexandri.*

4.3.1. DNA Extractıon

In order to extract DNA from the sand fly samples, individual body ethanol-fixed specimens were homogenized and lysed using a DNA extraction kit made by Thermo Scientific called the Isolation Kit Gene JET Genomic DNA Purification Kit. The PCR gene amplification process was then applied to 8µl portions of the DNA genomic samples. 1) Added 200 µl of PBS to a 1.5 ml microcentrifuge tube. Transfered the body sandfly samples into PBS solution, left the specimen at room temperature for 3 hours.

2) At the end of the holding period, added 20 µl of proteinase K to a new tube. Transfer the sandfly samples to this new tube. Add 200 µl of lysis buffer (buffer AL) to it. The samples were Crushed well in this solution.

3)Centrifuged the tubes at 3000 rpm for 5 minutes.

4)Spin the tubes for 15 seconds. Then placed the tubes in heat block at 56°C overnight.

5)Added 200 µl of ethanol (96–100%) the next day. Mixed thoroughly by vortexing.

6)Pipetted the mixture onto the (filtre tube) QIAamp Mini spin column (in a 2 ml collection tube) and centrifuge at 6000 x g (8000 rpm) for 1 minute. Discarded the collection tube.

7)Placed the (filtre tube) QIAamp Mini spin column in a new 2 ml collection tube and added 500 µl of Buffer AW1 (wash buffer 1). Centrifuged for 1 minute at 6000 x g (8000 rpm). Discarded the collection tube.

8)Placed the (filtre tube) QIAamp Mini spin column in a new 2 ml collection tube and added 500 µl of Buffer AW2 (wash buffer 2). Centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 minutes. Discarded the collection tube.

9)Placed the (filtre tube) QIAamp Mini spin column in a new 1.5 ml microcentrifuge tube, added 200 µl of Buffer AE (elution buffer) and wait 1 minute at room temperature. Centrifuge for 1 minute at 6000 x g (8000 rpm) to separate the DNA.

10)Finally the DNA will be ready to put in to the Electrophoresis equipment and then read the bands wheather was the DNA extracted or not, the bands will appear or not?

4.3.2. Loading and Running DNA in the Agarose Gel

2µl DNA was mixed with 3µl bromophenol blue (loading dye) and loaded into wells of the 2% agarose gel. The gel was run at 100 V for 30 minutes, and then DNA extracred were examined and visualized by using ultraviolet trans-illuminator (ex: under a UV trans-illuminator) (Figure 4. 30).

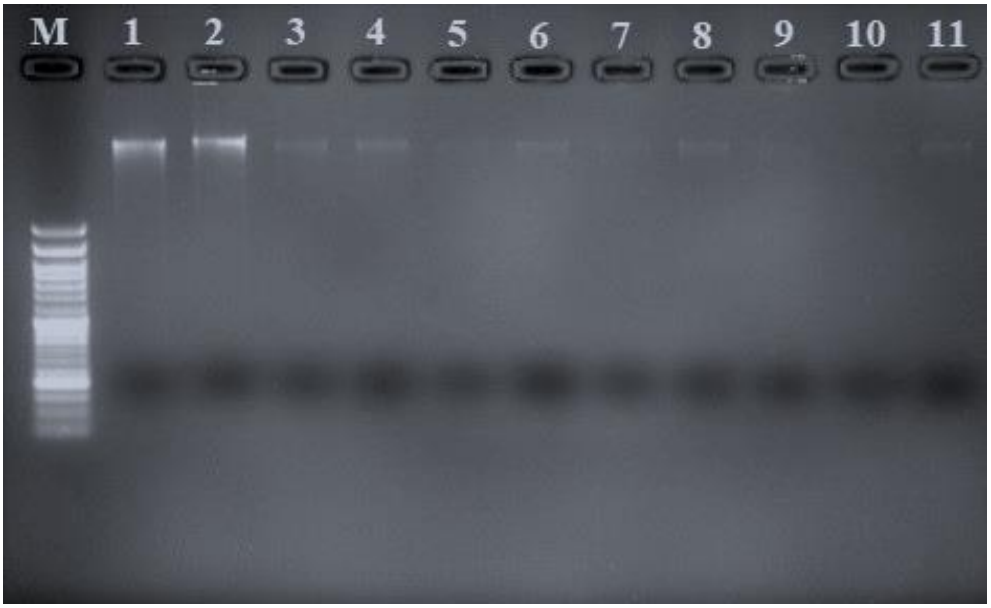


Figure 4.30. Agarose gel electrophoresis image that showed the DNA extracted analysis of mtDNA COI gene in 2% agarose gel at 100 V/cm^2 for 30 min. DNA was visualized under U.V. light after staining with Gel Stain-GREEN. Where M: DNA ladder, lane (1, 2, 3, 4, 5, 6, 7,8, 9,10 and 11) positive for Phlebotomus species

4.3.3. PCR Amplification of mt DNA COI Genes

The universal primers LCO1490 (Forward), LCO1490 (Reverse) and chemicals for detection Sandfly spp., (Table 3.11) were used to amplify the genomic DNA and generate an average 600 bp sequence. After adjusting the Polymerase Chain Reaction settings, the 600 bp area of the mt DNA COI was amplified under PCR conditions (Table 4.15). In order to see the bands formed by the oxidized LCO1490 areas as a result of the PCR, a 2% agarose gel was prepared. PCR technique was performed for detection sand flies: *Phlebotomus papatasi, Phlebotomus sergenti* and *Phlebotomus alexandri* were designed in this study.

4.3.3.1. PCR Master Mix Preparation

PCR master mix was prepared by using Maxime PCR Pre Mix and done according to company instructions as following (Table 4.14).

Table 4.14. Standard PCR reaction for each specimen

PCR master mix	Volume
Genomic DNA	8µL
Buffer	2.5µL
$MgCl_2$	2µL
Taq DNA polymerase	0.1µL
dNTP	0.5µL
reverse primers (10pmol)	1µL
forward primers (10pmol)	1µL
D. water	9.9 µL
Total	25µL

4.3.3.2. External Thermocycler Reaction Conditions

PCR Thermocycler conditions was done by using (Optimase protocol writer) online application and based on methods described in company instructions (Table 4.15).

Table 4.15. The Conditions of PCR Thermocycler

PCR cycle	Repeat	Temp.	Time
Initial denaturation	1	95C	3min
Denaturation	40	95C	30sec.
Annealing		48C	30sec
Extension		72C	45sec
Final extension	1	72C	5min

Note: All sand flies species were done at same PCR Thermocycler conditions.

The PCR master mix reaction components were then added to standard PCR tubes containing the PCR PreMix along with the other materials including the components listed in table (4.14) required for the PCR reaction. The tube was then inserted into a vortex. It was then put into a PCR thermocycler.

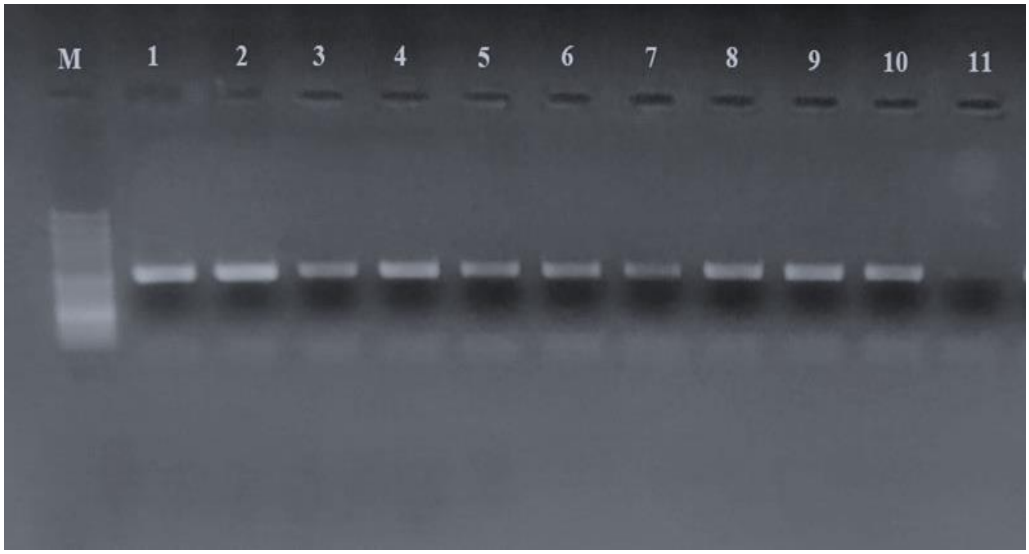


Figure 4.31. Is an image of an agarose gel electrophoresis showing the examination of PCR products from the mt DNA COI gene in the gel at 100 V/cm2 for 30 minutes

The PCR product was stained with Gel-Stain-GREEN and then observed under ultraviolet lighting. Where M: DNA ladder; lanes (1, 2, 3, 4,) positive for Phlebotomus

papatasi at (600bp); (5, 6, 7) lanes positive for Phlebotomud sergenti at (600bp), (8, 9, 10, and 11) lanes positive for Phlebotomus alexandri at (600bp).

*After PCR, the marker was used on an agarose gel to measure the length of the products the marker produced. The correct region was found to be oxidized as the target region is 600 bp long.

4.3.4. Sequencing And Phylogenetic Analyses of the *mtDNA COI* Gene

4.3.4.1. DNA Sequence Results

According to a phylogenetic tree analysis using the Phlebotomus species standard NCBI BLAST program, local *Phlebotomus papatasi, Phlebotomus sergenti, and Phlebotomus alexandri* isolates from various places in the Erbil province were subjected to DNA sequencing. Phlebotomus *papatasi*, Phlebotomus *sergenti*, and Phlebotomus *alexandri* isolates from the local area were used to analyze the mtDNA COI gene sequence using M'EGA 6.0, a multiple alignment analysis tool, and the NCBI-GenBank Phlebotomus species based Clustal Walignment analysis. As shown in figure (4.32), the multiple alignment analysis revealed similarities (*) and differences in the nucleotide sequences of the mt DNA COI gene.

Phlebotomus papatasi, Phlebotomus sergenti, and Phlebotomus alexandri isolates from the local area were employed for phylogenetic tree analysis based on the partial sequence of the mt DNA COI gene for genetic relationship analysis and confirmative genetic detection. The local *P. alexandri* isolates (Query_367435, Query_367436) showed (99%) similarity with the NCBI blast *P. alexandri*, while the local *P. papatasi* isolates (Query_367437, Query_367438) showed (100%). While the local *P. sergenti* isolates (Query_367439) showed (98%) similarity with the NCBI blast *P. sergenti*, similarity with the NCBI blast *P. papatasi.* As shown in (Table 4.16), there is homology in the sequences

of the local isolates of *Phlebotomus Papatasi, Sergenti, and Alexandri* and the Phlebotomus species in the NCBI GenBank.

The Neighbor Joining (NJ), maximum parsimony, and maximum likelihood approaches were used to infer phylogenetic analyses. The evolutionary distances were calculated using the MEGA 6.0 version of the phylogenetic UPGMA tree type. Phylogenetic trees (Figure: 4.32.) revealed moderate degrees of interspecific variability between species of the same genus. The full sequencing of the mt DNA COI genes of the local *P. papatasi* isolates (Query_367437, Query_367438) demonstrated that they were genetically close to the local *P. papatasi* strains according to NCBI-Blast. The local P. *sergenti* isolates (Query_367439) revealed tight genetic relationships to *local P. sergenti* using NCBI-Blast. The local *P. alexandri* isolates (Query_367435, Query_367436) revealed tight genetic relationships to the local *P. alexandri* in NCBI-Blast. The current work used PCR-direct sequencing to find and *identify P. papatasi P. sergenti and P. alexandri* originated from Erbil provice endemic CL.

Figure 4.32. Using M'EGA 6.0, a tool for multiple alignment analysis, multiple sequence alignment was performed on the partial mt DNA COI gene sequence from local isolates of *Phlebotomus papatasi, Phlebotomus sergenti, and Phlebotomus alexandri*. In the mtDNA COI gene nucleotide sequence, the multiple alignment analysis revealed similarities (*) and differences

4.3.4.2. Phylogenetic tree analysis

Furthermore, as shown in (Figures 4.33 & Table 4.16) of the current study, the phylongenetic tree of several Phlebotomine species based on the Neighbor-Joining (NJ) and Maximum Likelihood methods indicates that each species is closely linked to the species listed as reference species in the GenBank.*P. papatasi, P. sergenti, and P. alexandri* were detected and identified in the current investigation using PCR-direct sequencing, which was used to identify endemic CL from the Erbil province.

Different techniques can be employed in the study of mtDNA findings to establish the genetic separation between species, and Neighbor Joining (NJ) joining diagrams created in accordance with these techniques. To graphically represent genetic distances, the neighbor joining tree is constructed.

Phylogenetic tree:

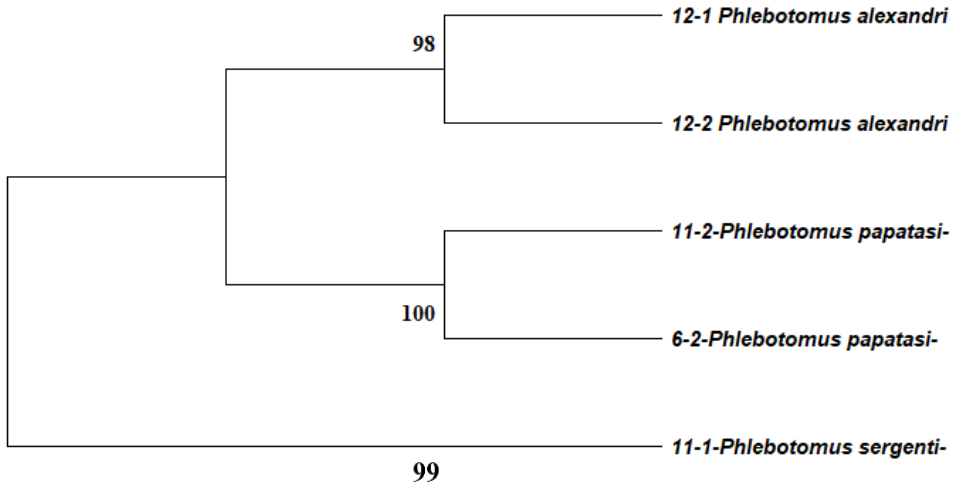


Figure 4.33. Neighbor-joining tree of the mt DNA COI gene, *P. papatasi, P. sergenti, and P. alexandri.* The investigation yielded new sequence ancestors for the sandfly species. For each sequence, the sandfly species and GenBank accession numbers are provided

Table 4.16. Homology from NCBI-BLAST Sequence similarities between local isolates of the *Phlebotomus papatasi, Phlebotomus sergenti, and Phlebotomus alexandrini* species and those in the NCBI-Genbank

Morphological isolate (local isolate)	Genbank submission accession number	NCBI-BLAST Homology Sequence identity		
	code	NCBI species name	Accesion number	Identify (100%)
P. papatasi	11-2	*Phlebotomus papatasi*	Query_367437	100
P. papatasi	6-2	*Phlebotomus papatasi*	Query_367438	100
P. alexandri	12-2	*Phlebotomus alexandri*	Query_367436	98
P. alexandri	12-1	*Phlebotomus alexandri*	Query_367435	98
P. sergenti	11-1	*Phlebotomus sergenti*	Query_367439	99

4.3.4.3. Bioedit Image

The Chromas-Pro computer application was used to convert these data into base sequences. The Bioedit computer tool was used to compare this sequence to the reference sequence downloaded from the Gene bank. (Figure 4.34).

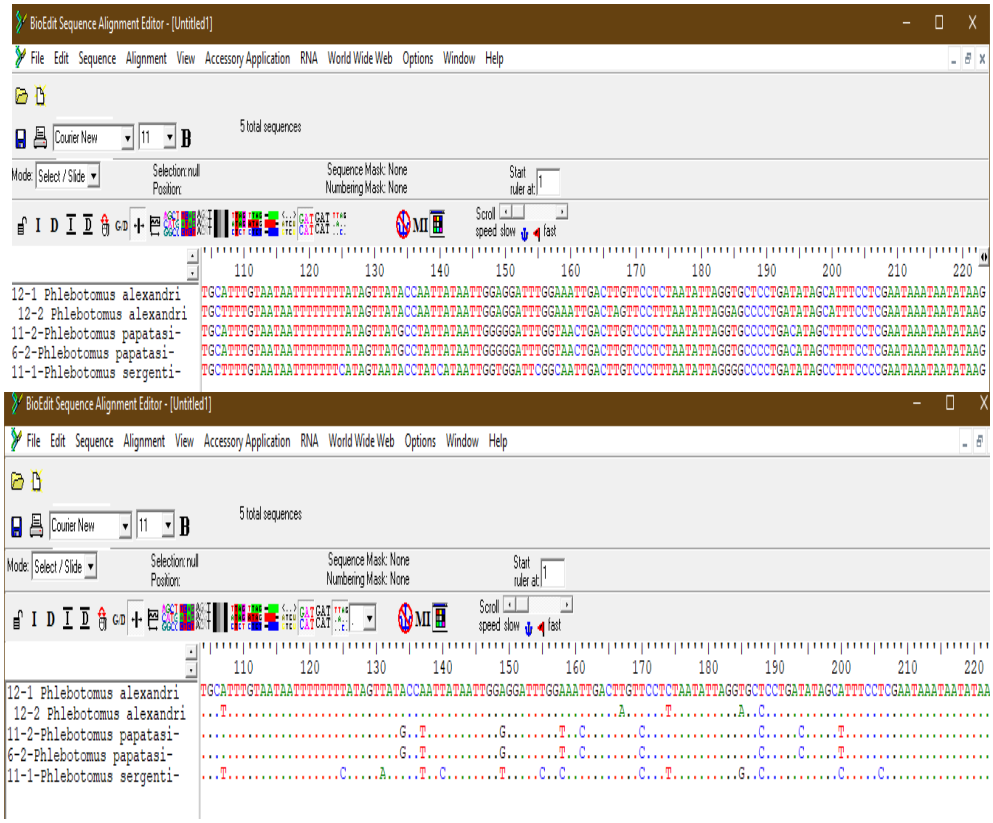


Figure 4.34. Bioedit image of a portion of the mtDNA COI region of *P. papalati, P. sergenti and P. alexandri individuals*

* The result of gene sequence analysis of *P. paparasi, P. sergenti and P. alexandri* individuals can be seen in the Chromas-Pro computer program. By means of this program, the peaks of the base sequences were carefully examined. **(Appendix 4).**

4.3.4.4. mt DNA COI sequences for *Phlebotomus papatasi, P. sergenti and P. alexandri* individuals

mtDNA COI gene sequences for *Phlebotomus papatasi,*

>11-2-Phlebotomus papatasi-

TCTTAATTCGAGCAGAACTTGGCCATCCTGGAGCTTTAATTGGC
GATGATCAAATTTATAATGTAATTGTAACAGCTCATGCATTTGT
AATAATTTTTTTTATAGTTATGCCTATTATAATTGGGGGATTTGG
TAACTGACTTGTCCCTCTAATATTAGGTGCCCCTGACATAGCTTT
TCCTCGAATAAATAATATAAGTTTTTGACTATTACCCCCTTCATT
AACTCTATTATTAACAAGAAGAATAGTTGAAACTGGGGCAGGA

>6-2-Phlebotomus papatasi-

TCTTAATTCGAGCAGAACTTGGCCATCCTGGAGCTTTAATTGGC
GATGATCAAATTTATAATGTAATTGTAACAGCTCATGCATTTGTA
ATAATTTTTTTTATAGTTATGCCTATTATAATTGGGGGATTTGGT
AACTGACTTGTCCCTCTAATATTAGGTGCCCCTGACATAGCTTTT
CCTCGAATAAATAATATAAGTTTTTGACTATTACCCCCTTCATTA
ACTCTATTATTAACAAGAAGAATAGTTGAAACTGGGGCAGGAAC

mtDNA COI gene sequences for *Phlebotomus sergenti* individuals.

>11-1-Phlebotomus sergenti-

CTTCCCTAAGAATTTTAATTCGAGCTGAACTGGGCCATCCTGGA
GCTTTAATTGGTGATGACCAAATTTATAATGTAATTGTTACAGCA
CATGCTTTTGTAATAATTTTTTTCATAGTAATACCTATCATAATT
GGTGGATTCGGCAATTGACTTGTCCCTTTAATATTAGGGGCCCCT
GATATAGCCTTTCCCCGAATAAATAATATAAGTTTTTGATTACTC
CCCCCTTCCTTAACCCTTTTACTAACCAGTAGAATAGTTGAAACT

>12-1_Phlebotomus alexandri

GAATAGTAGGAACTTCTTTAAGAATTCTTATTCGAGCTGAACTT
GGACATCCCGGAGCTTTAATTGGAGATGACCAAATTTATAATGT
AATTGTTACTGCTCATGCATTTGTAATAATTTTTTTTATAGTTATA
CCAATTATAATTGGAGGATTTGGAAATTGACTTGTTCCTCTAATA
TTAGGTGCTCCTGATATAGCATTTCCTCGAATAAATAATATAAG
ATTTTGATTATTACCACCTTCCTTAACTCTTCTATTAACAAGTAG
AATAGTTGAAACTGGGGCAGGAACTGGATGAACTGTTTATCCCC

>_12-2 Phlebotomus alexandri

GGTACTTCCTTAAGAATTTTAATTCGAGCTGAATTAGGTCATCCT
GGAGCTTTAATTGGAGATGATCAAATTTATAATGTAATTGTTACT
GCCCATGCTTTTGTAATAATTTTTTTTATAGTTATACCAATTATAA
TTGGAGGATTTGGAAATTGACTAGTTCCTTTAATATTAGGAGCCC
CTGATATAGCATTTCCTCGAATAAATAATATAAGATTTTGATTAC
TACCCCCTTCCTTAACTCTATTATTAACAAGTAGAATAGTTGAAA

This study used sequencing analysis of the mtDNA COI gene region to verify the results of morphological identification of sandfly species in Erbil province, Iraq, a location with leishmaniasis sickness. In the genetic examination of populations, nucleotide diversity is a sensitive technique (Nei and Li, 1979). The foundation of biological study is the identification of sand fly species. The external morphology of adults and the features of the cibarium, pharynx, and spermatheca are commonly used to categorize sandflies. However, it can be difficult to identify a species from its physical traits (Zhang et al., 2012; Zhang et al., 2013).

According to (Xiong et al., 2016), female sandflies can be easily identified since they are larger than males and have distinctive spermatheca. This means that it is more

likely to be successful to identify a species through dissection of females. In our investigation, a greater percentage of the samples were male sandflies. Three species from one genus were discovered after we dissected all of the sandfly samples we had gathered for morphological identification. Following morphological identification, 70 sand fly samples were directly sequenced, and the species of each were designated based on the sequence traits. But it is not easy to identify them accurately by morphological characters.

Previous studies about molecular identification of sand flies in Iraq are very rare, except previous studies in Iraq, in Al-Qadisiya province by (Al-Hassani, 2016) who used method of polymerase chain reaction (PCR) for molecular identification of sand flies for the first time in Iraq through the detection of gene Mitochondrial cytochrome b (Cytb) DNA gene and an output along the molecular 575bp for *P. papatasi* and the length of molecular 600bp for P. sergenti.

This study agreement with the following studies: in Bangladesh by (Alam et al. 2012) that employed the RFLP-PCR analysis, local Phlebotomus spp., *P. argentipes, P. papatasi, and Sergentomyia species*. Agarose gel electrophoresis picture. Where M is the marker (1500-100bp), lanes 1, 4, 5, 7, 8, 9, and 10 are positive for the Phlebotomus papatasi PCR product (801bp), and lanes 2, 3, and 6 are positive for the Phlebotomus sergenti PCR product (916bp). The species of 64.4% of the 1055 female sand flies that were successfully analyzed for individual species identification were categorized as Sergentomyia species, 35.6% as *P. argentipes, and P. papatasi* was not discovered.

Ye et al. (2015), examined the first mitochondrial genomes from Phlebotomus (P. chinensis and P. papatasi). Both genomes contain an A + T-rich area, 22 transfer RNA genes, two ribosomal RNA genes, and 13 protein-coding genes. The mitochondrial genomes of Phlebotomus have the same gene order as the ancestors of insects.

The partial sequence of the mitochondrial cytochrome oxidase gene subunit I (COI) was now employed in Colombia by (Gutiérrez et al., 2014) to distinguish species in many animal taxa, including insects. This partial sequence is referred to as a barcoding sequence. This study looked into the usefulness of using a DNA barcode to identify phlebotomine sand flies.

Al-Ajmi, et al. (2015), conducted research in Saudi Arabia by setting up a polymerase chain reaction (PCR) and direct partial sequence of the 18S ribosomal RNA (rRNA) gene using specially designed primers. The 18S subunits of the rRNA gene from several direct PCR-amplified sequences of each species revealed a moderate degree of interspecific heterogeneity between species of the same genus and species of other genera.

According to study that conducted by (Ramazani et al., 2018), Iran. (The cytochrome b (Cytb) – mt DNA fragment PCR result. P. kabulensis' male and female were recently discovered Iranian representatives of the subgenus Adlerius. preliminary DNA study revealed how unique this species is. According to the findings, the *P. kabulensis* female may be distinguished from other Adlerius female groups by comparing morphometric traits and DNA sequencing. *Phlebotomus papatasi, P. similis, P. killicki, Sergentomyia minuta, and S. dentata* are among the five species of sand flies that can be found in the Middle East. Dokianakis et al., (2018) employed DNA barcoding to identify individuals of these flies.

A study in India by (Tiwary et al., 2012) employed PCR-RFLP (Restriction Fragment Length Polymorphism) as a quick and precise method to distinguish between closely related organisms. Different species of morphologically similar sand fly exist in the areas of India where (VL) is endemic, but only female Phlebotomus argentipes is the vector for VL that was developed to target the 18S rRNA encoding gene that exhibited amplification in all the major sand fly species observed.

The first mitochondrial genomes of Phlebotomus (P. chinensis and P. papatasi) are provided by (Ye et al., 2015). Both genomes have an A + T-rich area, 22 transfer RNA genes, two ribosomal RNA genes, and 13 protein-coding genes. The mitochondrial genomes of Phlebotomus share the same ancestral gene order as insects. Tanyderidae and Psychodidae are sister taxa, according to phylogenetic analyses.

A study by (Al-Ajmi et al., 2015) in Saudi Arabia revealed the phylogenetic relationships of taxa utilizing multiple direct PCR-amplified sequences of each species that were evaluated using Maximum Composite Likelihood (MCL), Neighbor-Joining (NJ), and MEGA 5 methods. The 18S rRNA gene data that were acquired revealed a moderate degree of interspecific heterogeneity both within and across species of the same genus.

A study by (Al-Huchaimi et al., 2018) in Najaf Provice used sequencing and phylogenetic inference analysis to confirm that local *P. papatasi* isolates were shown to be closely related to the NCBI, P. papatasi reference sequence (AF161214.1), local *P. sergenti* isolates showed high similarity with the NCBI, *P. sergenti* sequence (AF161216.1), and local S. sintoni isolates showed high homology with the NCBI, S.

It is possible to identify species using only DNA sequences. The PCR approach has proven to be a reliable method for distinguishing one sand fly species from another, according to earlier investigations. This species' uniqueness has been demonstrated by preliminary DNA research. In order to effectively control this species, additional molecular comparisons, entomological studies, and research into its distribution and abundance are required (Ramazani et al., 2018).

As compared to the traditional morphology-based categorization, the genotyping method has been found to be more precise, user-friendly, and accurate for identifying

sand fly species. It also carries a lower risk of incorrect interpretation. Aside from that, sample damage, which frequently impacts morphologic classification, has no impact on the genotyping analyses. (Terayama et al., 2008; Al-Ajmi et al.,2015).

4.4 Morphological Detection of Leishmania Parasite in Patients

4.4.1. Direct Leishmania microscopic examination

The (CDC, 2011), states that a tiny amount of material from the margin of the skin lesion that is easily stained with leishman stain is required for the direct staining smears, which are thought to be an excellent initial test for CL. The microscopic investigation was carried out using oil immersion light microscopy (100X) subsequent to the staining of smear samples, 82 (65.6%) with CL. out of 135 obtained from patients with human cases. (Figure 4.36, A and Figure 4.36, B) display smears stained with Leishman stain. From 135 suspected cases of CL, 82 (65.6%) of the human samples were positive (Table 4.17).

Table 4.17. shows the rate of infection based on human cases of CL cases

Sample	Sample collected	Positive	Negative
	No, (%)		
Suspected cases	135	82(65.6)	53(34.4)

Without requiring a thorough examination of the sample, staining facilitates sample identification by changing the color of the smear, making it easier to see the sample's morphology, size, and form. Leishman stain smears can yield negative results when the parasite is either undetectable or has vanished. This has been ascribed to numerous factors, including the patients' course of therapy, timing errors in staining, smear thickness, and the potential for cell wall distortion, (Mustafa et al., 2017; Younis et al., 2018).

In many Middle Eastern and Mediterranean nations (CL), a parasite disease conveyed by vectors, poses a major threat to public health. There is a large prevalence of

it in Iraq. In practically every region in the nation (Markle and Makhoul, 2004; WHO, 2020).

The results of this study are in line with other studies carried out in several Iraqi regions, including (Abdulsadah, 2011) in Baghdad, where in direct microscopy was used to determine the diagnosis in 60% of the cases and 85 out of 100 patients received a positive result. Additionally, it agrees with (Hassan, 2017) regarding the possibility of hygienic contamination leading to contaminated lesions, which can postpone treatment or create an environment conducive to subsequent infection contamination.

CL had a significant frequency and was the most frequent endemic disease in the Makhmur district. In this investigation, 82 patients (65.6%) were shown to have CL infection in a lab setting.

4.4.2. Leishmania cultivation in modified NNN media

Using a modified Novy-MacNeal-Nicolle medium, 135 human CL. samples (68) were cultivated.

4.4.2.1. Growth of Leishmania on Human Blood Modified N.N.N.

Fifty-seven out of sixty-eight (83.8%) suspected human CL samples were negative, eleven (16.2%) CL cases were Contaminated, while zero (0%) CL cases were positive, as seen in (Figure 4. 35 and Table 4.18).

Table 4.18. Leishmania cultivation on human blood-modified N.N.N

Host	No. of samples cultivated with N.N.N + human blodd	(%)	No. of positive samples	No. of negative samples	No. of contaminated samples
Human	68	50.3	0	57 (83.8%)	11 (16.2%)

Fifty-seven out of sixty-eight (83.8%) suspected human CL samples were negative, eleven (16.2%) CL cases were Contaminated, while zero (0%) CL cases were positive, figure 4.35. The samples became (negative) due to uncontrollable factors including temperature fluctuations and power outages inside the incubator throughout the growing phase.

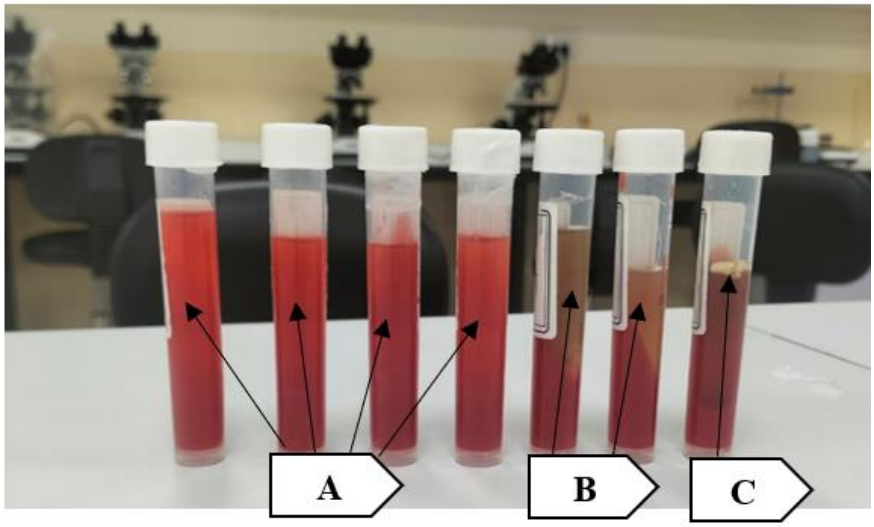


Figure 4.35. inoculated CL blood sample on NNN media reveals negative and contaminated results: A) Negative results, B) contaminated with yeast fungi, C) contaminated with mold fungi

The results of the study are in line with the following studies: According to a study conducted by (Abdulwahab, 2013) found that 75% of samples cultivated on N.N.N. were negative and 25% of samples were positive. A further study carried out by (Younis, 2018) in Baghdad, out of 75 skin lesion samples, 12 grow exclusively on (NNN) medium, 38 are contaminated, and the remaining 25 samples do not grow at all.

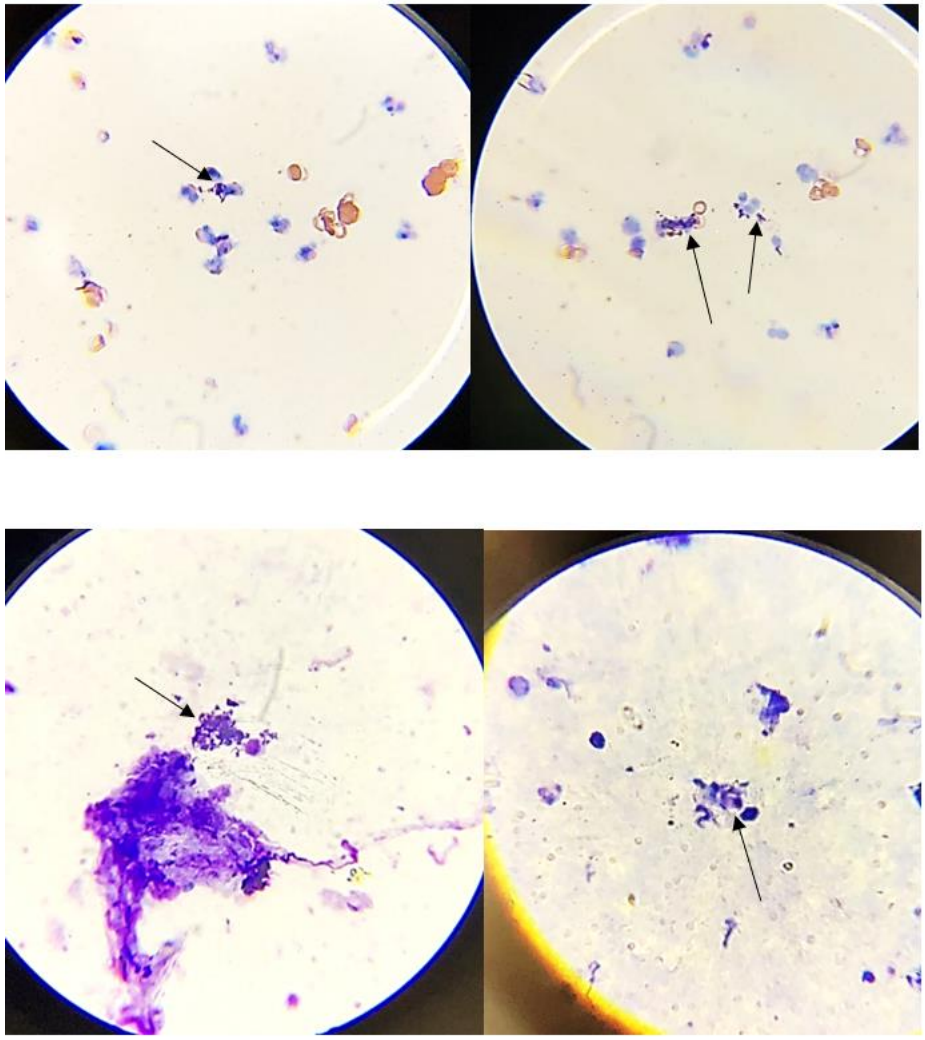


Figure 4.36 (A). Smears of Leishman stain from lesions reveal amastigotes in and outside of macrophages submerged in oil (100X)

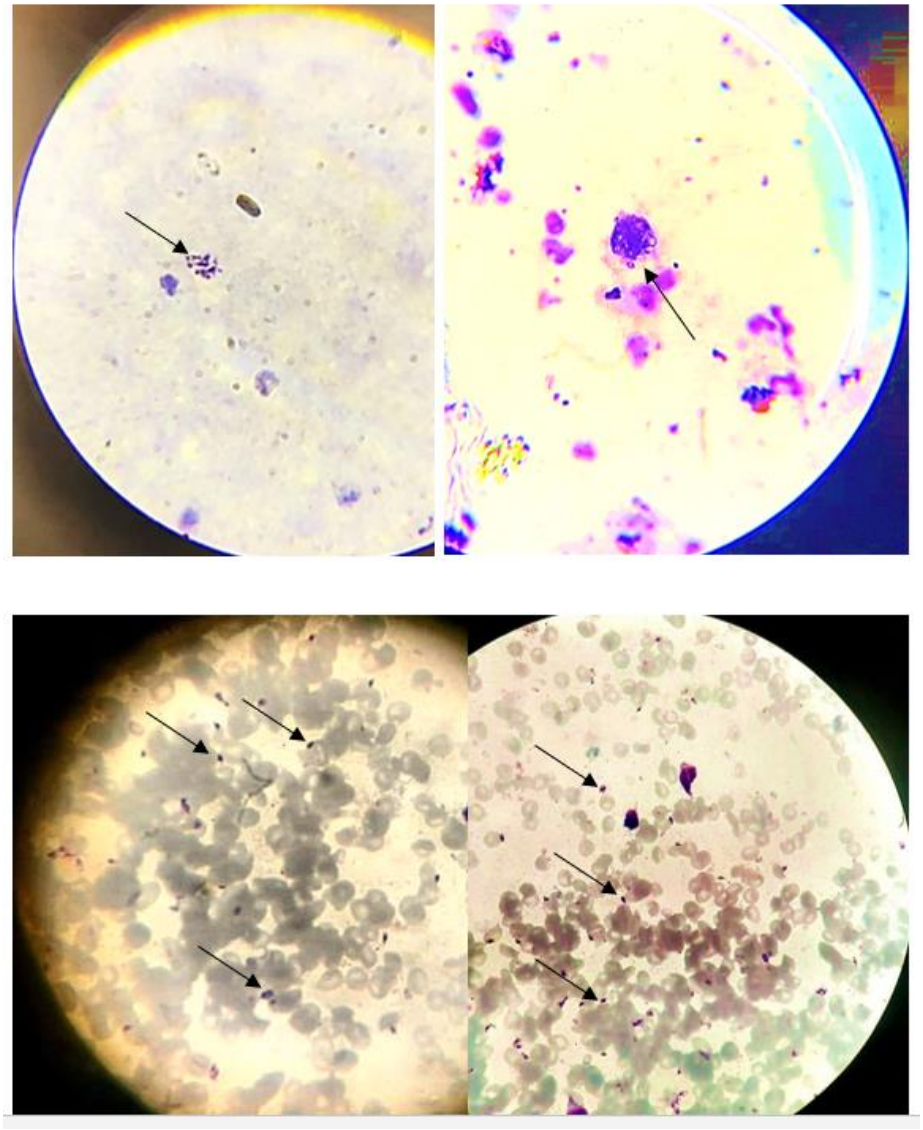


Figure 4.36 (B). Smears of Leishman stain from lesions reveal amastigotes in and outside of macrophages submerged in oil (100X)

4.4.3. The consequences of the human population

4.4.3.1. Infection rate according to patient age

Two of the patients were younger than a year old. Ages varied from one year to over sixty-five. Patients from 1 year to 25 years old had the highest rates of CL infection 63 (46.6%), followed by those from 26 to 50 years old 44 (32.5%), 51 to 65 years old 19

(14.7%), and the group older than 65 years old 9 (6.6%). As a result of stastical analysis, with some significant variations (P<0.05), the rate of CL infection was higher in younger age groups, and gradually decreased in older age groups (Table 4.19) and (figure 4.37, A).

Table 4.19. Infection rate according to patient age

Age group (years)	CL. Cases		Patients with positive CL.	
	No.	(%)	No.	(%)
1-25	63	46.6	45	54.9
26-50	44	32.5	18	22
51-65	19	14.07	14	17.1
65+	9	6.6	5	6.1
Total	135	100	82	100
*Two patients younger than a year; maximum age: above 65, P<0.05				

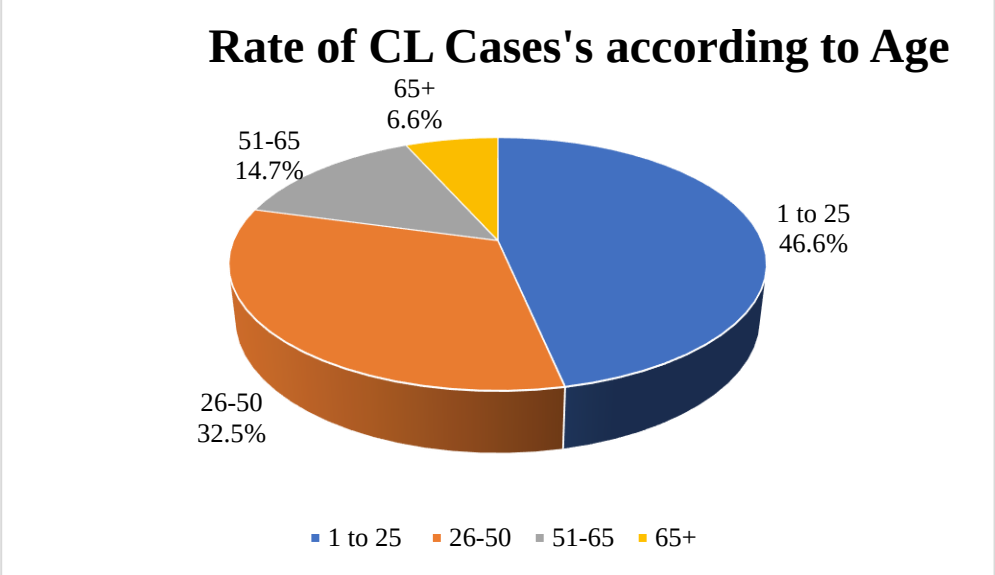


Figure 4.37 (A). Infection rate according to patient age

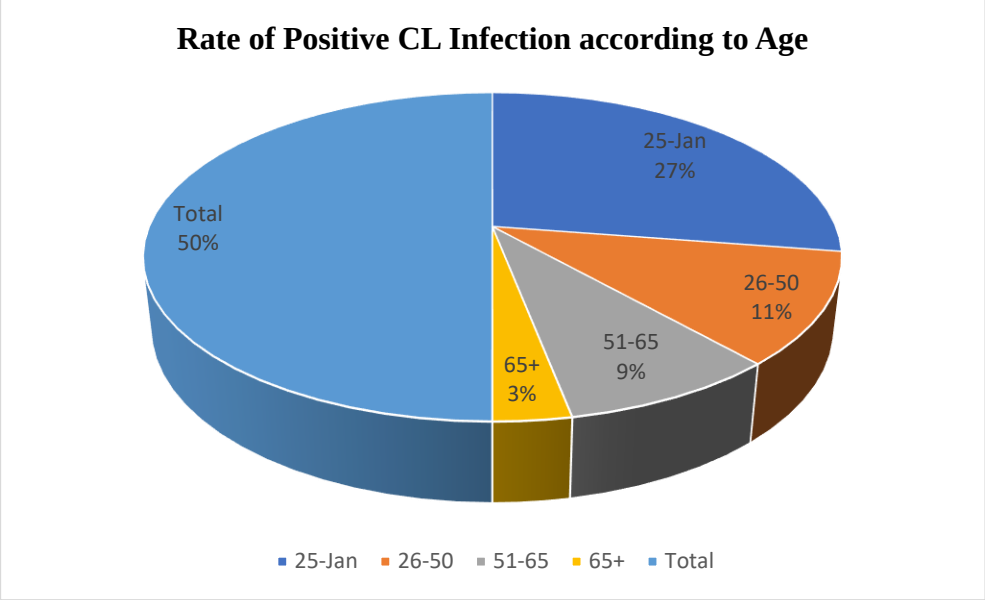


Figure 4.37 (B). Infection rate according to patient age

A CL infection is more likely to affect younger age groups. The investigation discovered two examples of severe and numerous CL lesions in children younger than a year old. At 63 cases (46.6%), CL was most common in the 1 to 25-year-old age range. Furthermore, it is explained by the fact that children and teenagers in this age group enjoy playing outside for extended periods of time, which increases their exposure to sandfly bites and renders them more vulnerable to the flies' preference to pierce their skin during feeding (Al-Jubori et al., 2019).

These results are consistent with those of a different study conducted in the Iraqi city of Kirkuk, which found that 43% of cases affected people between the ages of one day and 25 (Obaid and Shareef, 2018). A recent study conducted in Al-Najaf city by (Ghezzai et al., 2020) found that 6.2% of CL patients were younger than 10 years old. According to our research, people over 60 were less likely to have CL infection, with those between the ages of one year and 25 being the most vulnerable. This is because children in these age groups have extremely thin skin in comparison to other age groups (Al-Jubori et al., 2019).

4.4.3.2 Infection rate according to patient gender

There was significant difference between the genders (P<0.05) in the CL rate, which was higher in males than females 52 (62.2%) versus 30 (37.8%) (Table 4.20 and Figure 4.38).

Table 4.20. shows the infection rate according to patient gender

Sex	CL. Cases		Patients with positive CL.	
	No.	(%)	No.	(%)
Male	84	62.2	52	63
Female	51	37.8	30	37
Total	135	100	82	100
P<0.05				

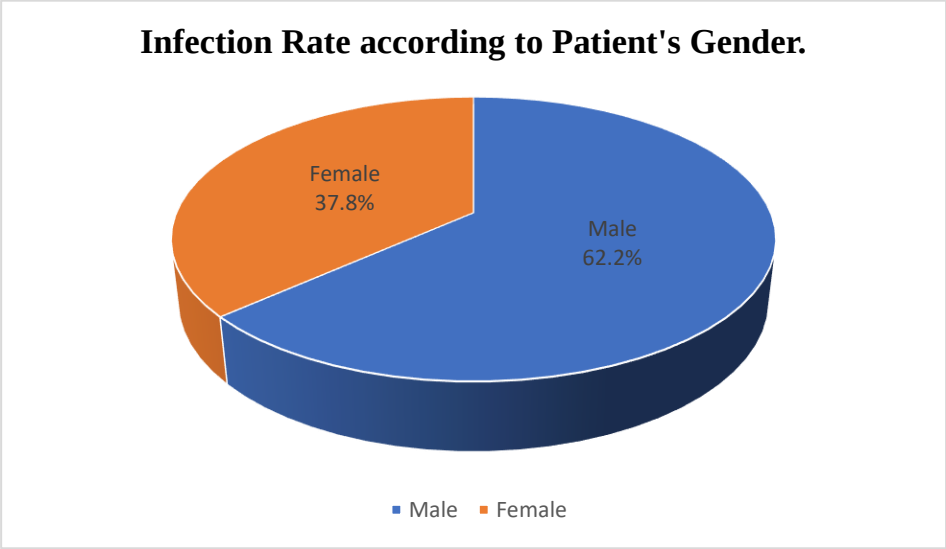


Figure 4.38. shows the infection rate according to patient gender

There were appreciable variations in the exposure to CL infection between the sexes. The impact of sex on society, traditions, and rituals may help to explain this. Because men walk around and labor in the fields and farms, they are more likely to become infected than women, who typically stay at home and wear long clothing, especially in Islamic

nations. Women have fewer exposed body parts than men do as a result. Men are more likely to get bitten by sandflies for all of the previos reasons. Additional research conducted in Iraq corroborated these conclusions (Aldifaei, 2013). (Aksoy et al., 2017) reported similar outcomes in Turkey.

Our findings, however, are not consistent with those of (Saki et al., 2010)'s study conducted in Iran, which discovered that females had a higher prevalence of CL (54.68%) than males (45.31%). According to (Hassan's, 2017) epidemiological study, females in Al-Tuz had a higher prevalence of CL infection than males.

4.4.3.3 Infection rate of patients according to Erbil districts

Makhmur district (73 cases, 54.07%) had the greatest infection incidence, followed by Khabat (34, 25.18%), Koye (13, 9.6%), and Gwer (12, 8.88%), %). Erbil Center (3, 2.2%) had the lowest prevalence, (table 4.21 and figure 4.39& 40).

Table 4.21. Infection rate by patient's residential area

No.	District	CL. Cases		Patients with positive CL.	
		No.	(%)	No.	(%)
1.	Makhmur	73	54.07	56	68.3
2.	Khabat	34	25.18	9	11
3.	Gwer	12	8.88	6	7.3
4.	Koya	13	9.6	8	9.8
5.	Erbil Center	3	2.2	3	3.7
	Total	135	100	82	100
P<0.05					

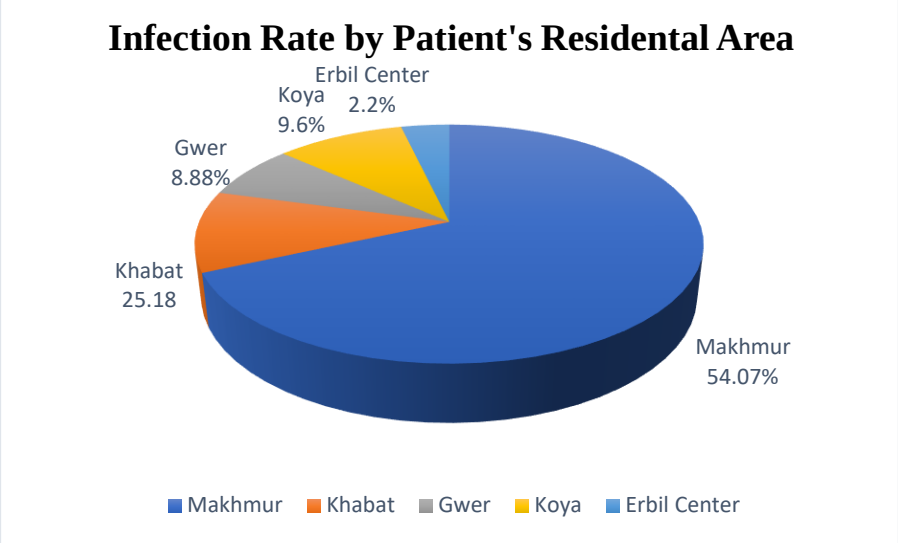


Figure 4.39. Infection rate by patient's residential area

Makhmur City was classified as a rural area in this study due to the climate, the environment, the distribution of marshes around the town. In all of Erbil, this is the city where CL is most common. Sandflies have been spreading more widely recently as a result of the local government's reduced or nonexistent insecticide spraying efforts. Furthermore, no control measures exist for stray animals, such as dogs and rodents, which serve as the parasite's reservoir hosts in addition to domestic canines.

This study's results agree with the results of study that Ali (2018) found, the study found that over the years 2012 to 2016, the rate of CL in rural areas was the greatest at 87.9%, while the rate in urban areas was the lowest at 12.1% in the province of Diyala.

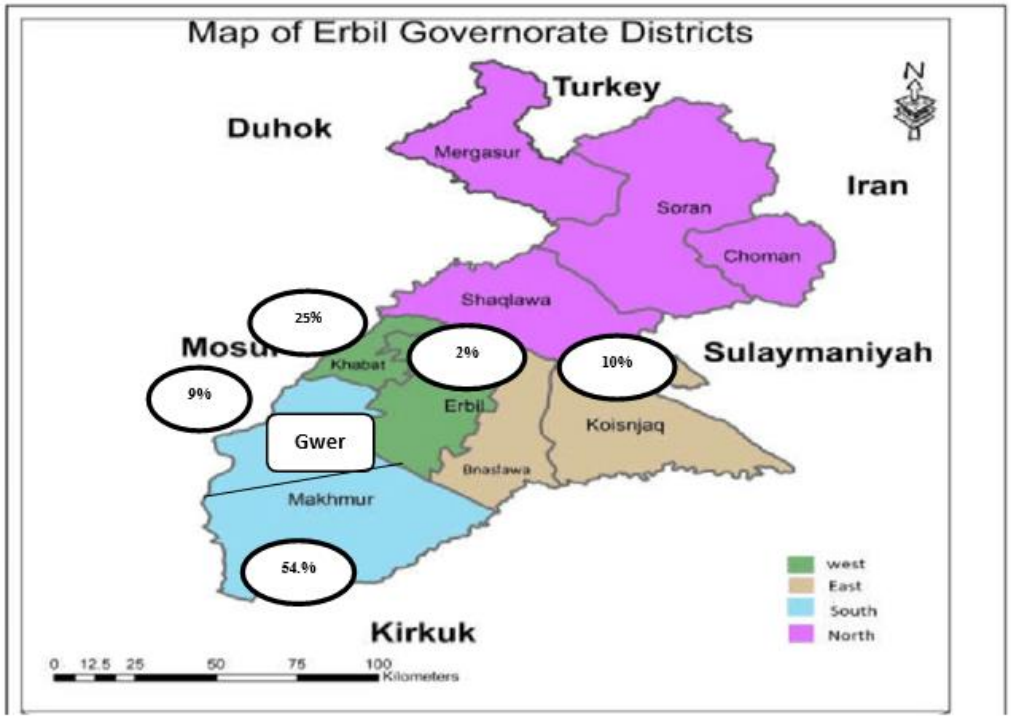


Figure 4.40. Map of CL distribution in the province of Erbil based on sample sites used from January to December 2022

The present findings reveal significant differences between all districts at $p< 0.05$. This could be attributed to the high infection rates in the district of Makhmur which are marked by the spread of reservoirs like dogs and rodents. These areas are home to many breeding animals that offer an ideal environment for the presence of the vector insect, and organic materials serve as an ideal growing medium for sand fly larvae.

4.4.3.4. Patients with Cutaneous Leishmaniasis distributed by type of habitation

Patients with CL are distributed according to the type of habitation. Infections with CL are more common in rural regions (93 cases, or 68.8% of the total), whereas they are less common in urban areas (42 cases, or 31.2% of the total). According to the present findings, there are significant differences between the types of occupancy at $P< 0.05$. (Table 4.22).

Table 4.22. Distribution of Patients with Cutaneous Leishmaniasis by Type of Habitation

Habitation	**CL. Cases**		**Patients with positive CL.**	
	No.	(%)	No.	(%)
Rural	93	68.8	65	79.3
Urban	42	31.2	17	20.7
Total	135	100	82	100
P<0.05				

There is similarity between the study and a study which conducted by (AL-Difaie, 2014) reported on another study conducted in the province of Al-Diwaniya, which found that the rate of infections was higher in rural regions (87.28%) compared to Urban ones (12.72%). The latest conclusion was in line with a 2014 study by Atshan, which discovered that the prevalence of CL infections was higher in rural than in urban regions, reaching 64.80% and 38.10%, respectively. Furthermore, the current study's findings conflict with those of a prior investigation conducted in the same province by (Al-Obaidi et al., 2016), who discovered that infections in urban areas (45 cases) were greater at 52.3% than in rural areas (41 cases), at 47.7%. According to (Abul-Doanej's, 2014) report, the percentage of CL infections in Maisan province was greater in urban areas—62.5%—than in rural regions (39.52%).

The villages, a rural area surrounded by agricultural land and horticulture, are the subject of the current study's focus on the districts and countryside. There are communities and clans that do agricultural and animal husbandry where the majority of its population resides. So, the spread of sandflies to provide breeding grounds and living conditions in moist agricultural areas, along with the presence of animals for the parasite, particularly dogs and rodents, and the high humidity brought on by agriculture, are the reasons for the high number of infections in rural areas.

4.4.3.5. CL infection rate and patient's accommodations

(Table 4.23) displays the CL infection rates according to the patients' accommodations. A substantial difference (P<0.05) was found between the proportion of infected patients who lived in block-building houses (106/135, 78.5%), those who lived in mud-brick buildings (21/135, 15.5%) and the least number of patients (8/135, 5.9%) lived in tents.

In reference to the characteristics in close proximity to the residences, we observed that the prevalence of infection was elevated in areas adjacent to marshes and streams in 92/135 (68.1%) and 23/135 (17.03%), respectively. With a significant difference (P<0.05), the lowest infection rate was 7/135 (5.1%) in areas adjacent with river.

Additionally, look into the kind of house and water supplies where the patients are staying. According to research conducted in Diyala by (Ali, 2018), the characteristics of the walls and other parts of the house affect how quickly sandflies spread, which could have an impact on the rate of CL infection. Because they create a humid atmosphere ideal for insect growth, ponds, swamps, river branches, and marshes are all strongly associated with the presence of sandflies, whether or not a garden is located next to the house.

Table 4.23. CL infection rate and patient accommodations

Variable		No. of patients	(%)
Accommodation types	Mud bricks	21	15.5
	Block building	106	78.5
	Tent	8	5.9
	Total	135	100
P<0.05			
Nearby water source	Marshes	92	68.1
	Mini-branches of river	13	9.6
	River	7	5.1
	Streams	23	17.03
	Total	135	100
P<0.05			

Unsanitary and unsuitable living conditions, improper waste disposal, or exposed, standing water can all contribute to the elevation of sandfly resting spots and human entry points. Sandflies appear to be congested in dwellings because they provide a good source of blood meals (Ali, 2018). Asmaa et al. (2017) discovered that the location, which was close to year-round freshwater holes and a stream, was associated with the highest percentage of CL cases in Shara'b District, Yemen. These features give sandflies an ideal habitat for completing their life cycle and increasing reproduction.

4.4.3.6. Rates of CL infection in relation to climate factors

February 2022, January 2022, March 2022, December 2022 and November 2022 all had higher infection rates (31.9%, 20%, 9.6%, 6.7% and 5.9% respectively). Following that, there was a significant decline in the rates. According to the findings, the rate of infection was higher in the months of February, January, and March at moderate mean temperatures (C^o). In contrast, the rate decreased in the hotter months of April, May, June, July, August, and September (infection rates: 4.4%, 6.7%, 3.7%, 3.7%, 3.7%, and 3.7%, respectively), but it was zero in October. Significant variations in the rate of CL were seen in these data ($P<0.05$).

A significant rate of CL infection was observed in Makhmur district as a result of the high mean humidity (68%). With notable exceptions, high humidity was linked to increased infection rates in February 2022, January 2022, March 2022, December 2022 and November 2022 all had higher infection rates (31.9%, 20%, 9.6%, 6.7% and 5.9% respectively). Moderate humidity and fairly high infection rates were observed in September and October, respectively, with significant differences ($P<0.05$). As shown in (Table 4.25 and Figure 4.41& 4.42).

Regarding precipitation, the rates of CL infection were highest in the wet months of January, February, March, November, and December, and lowest in the other months, with

the exception of April and May, where the rates were 4.4% and 6.7%, respectively with significant differences (P<0.05).

The months with the most rainfall had the greatest documented infection rate of CL, with moderate temperatures and high humidity. The growth and activity of the vector host—sandflies—are compatible with these conditions. In the summer, when there was little humidity, high temperatures, and no rain, the study found that there were less CL infection.

Table 4.25. CL infection rates in connection to climate variable

Month	Mean temperature (C°)	Mean Rain amount(mm)	Mean humidity (%)	CL. Cases	
				No.	(%)
January	9.21	133.9	57.18	27	20
February	10.99	97.7	55.42	43	31.9
March	14.93	94.7	54.34	13	9.6
April	20.69	91.5	46.46	6	4.4
May	27.99	45.4	31.07	9	6.7
June	34.77	2.63	17.56	5	3.7
July	38.35	0.37	14.21	5	3.7
August	37.75	0.19	14.64	5	3.7
September	32.68	3	18.03	5	3.7
October	9.21	133.7	57.18	0	0
November	10.99	97.7	55.42	8	5.9
December	14.93	144.7	54.34	9	6.7
P-value*	<0.05	<0.05	<0.05	135	100

*Logistic regression test; mm=millimeter; No. =number; %=percentage

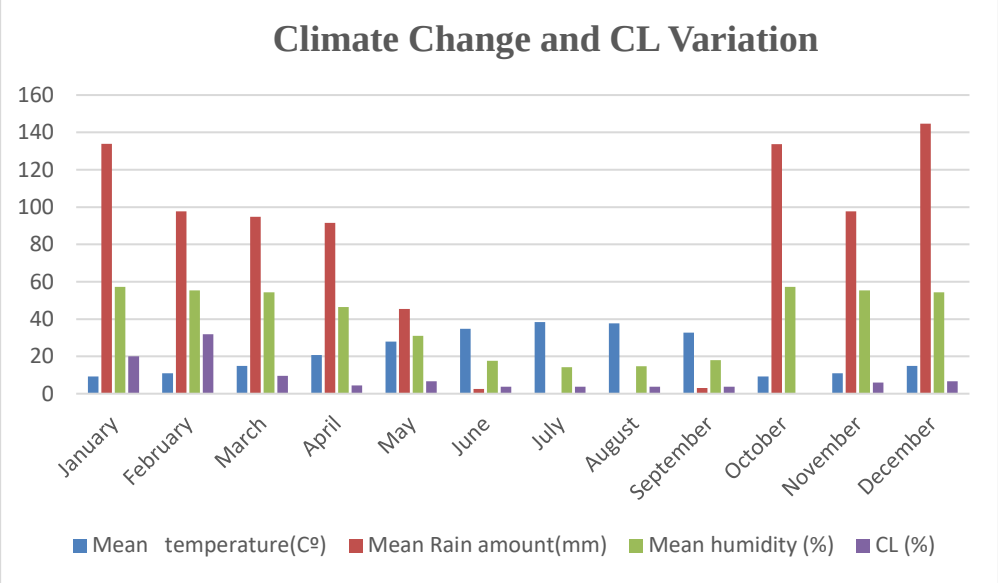


Figure 4.41. The relationship between the rate of CL infection and mean temperature (Cº), precipitation (mm), and mean humidity (%)

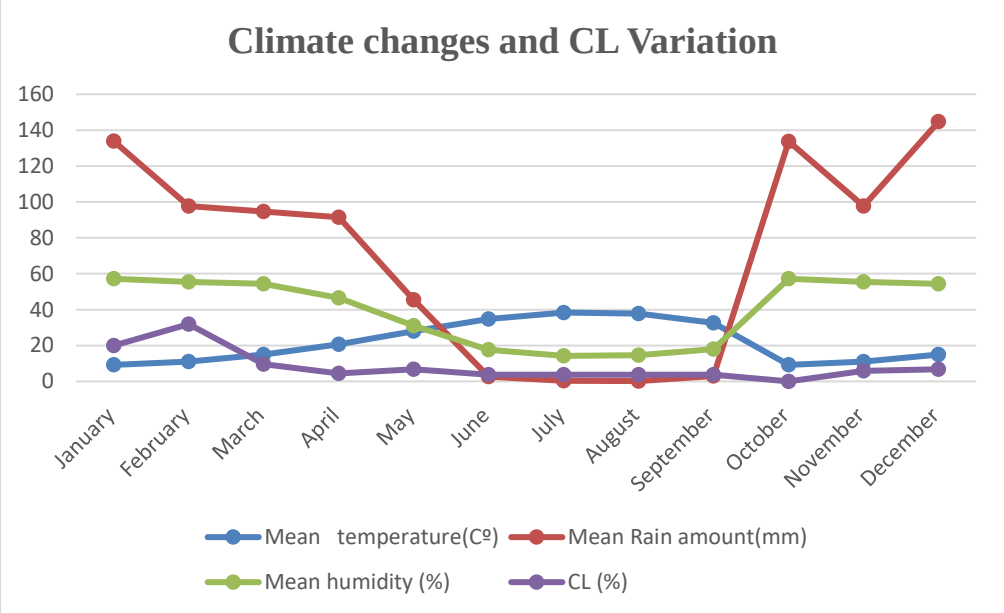


Figure 4.42. The relationship between the rate of CL infection and mean temperature (Cº), precipitation (mm), and mean humidity (%).

These findings corroborated those of earlier research carried out in Iraq, which discovered that the greatest frequency of CL was observed in February (36.66%), followed by January (23.33%), and May (1.66%), with the lowest rate (Abdulwahab, 2013). These

conditions affect the presence, growth, and reproduction of the sandfly and thus the rate of infection.

These results are in line with a study conducted by (Habeeb) from 2012 to 2016 on the seasonal distribution of CL, which found that the highest rate of leishmaniasis was recorded in the winter (68.3%), followed by the spring (19.3%), the summer (7.3%), and the autumn (5.1%). However, the study's findings do not line up with those from Iran (Talari et al., 2006). Sandflies require a suitable environment with moderate temperatures and humidity for them to emerge. During the warmer months of the year, when the high temperatures prevent these insects from appearing, their population density will subsequently decrease (Habeeb, 2005).

The monthly peak variation may be attributed to the activity level of sandflies or the total population during the wet season. Alternatively, it could be linked to the development of female sandflies and their requirement for blood during the life cycle for egg maturation and production, particularly during the spring months (Khan, 2012).

4.4.4 The Clinical Outcomes of Individuals with CL

4.4.4.1. CL Infection Rate in Relation to Lesion Sites

In terms of clinical outcomes, the most frequently affected areas by CL were the upper limbs in 62/135 patients (45.9%), the lower limbs in 39/135 patients (28.9%), and the face (including the ears and nose) in 28/135 patients (20.7%). Additionally, 6/135 patients (4.4%) had several organs in their bodies where CL lesions were found. $P<0.05$ indicates that these results are statistically significant as illustrated in (table 4.26) and as seen in (Figures 4.43, 4. 44. 4.45. 4.46. 4.47, 4.48, 4. 49, 4.50 and 4.51).

Table 4.26. Rate of CL infection according to lesion sites

Site of Infection	CL. Cases	
	No.	(%)
Upper limbs	62	45.9
Lower limbs	39	28.9
Face	28	20.7
Multiple organs	6	4.4
Total	135	100
P< 0.05		

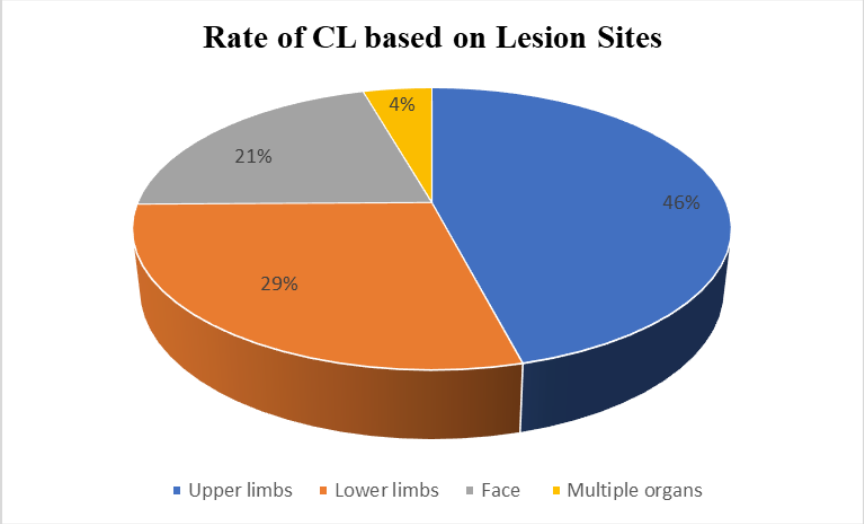


Figure 4.43. Rate of CL infection according to lesion sites

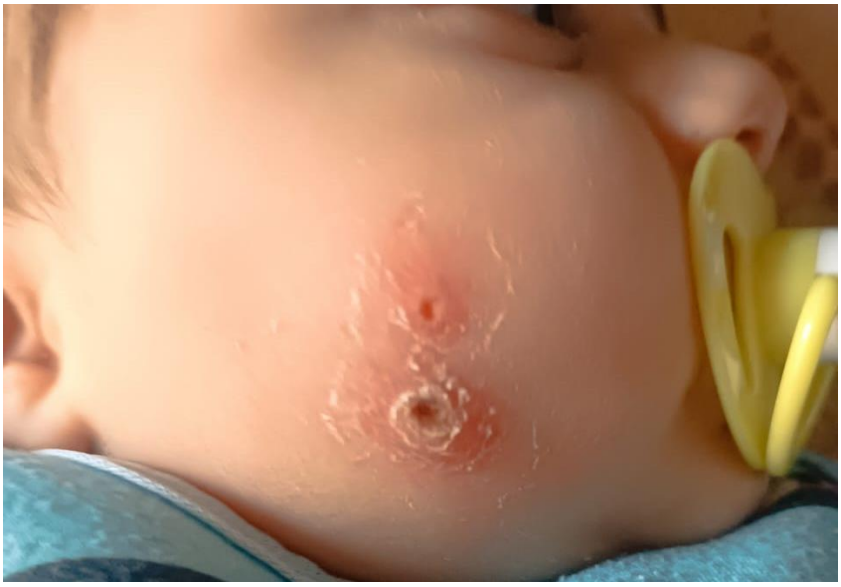


Figure 4.44. Two cresting CL lesions on the face of one year-old infant (check)

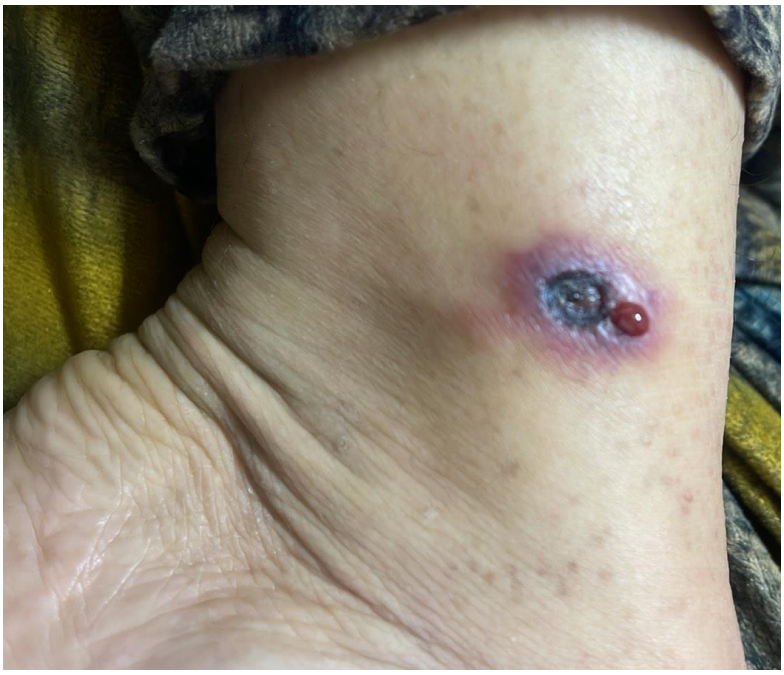


Figure 4.45. A big ulcerative lesion on the left leg of a 47-year-old lady

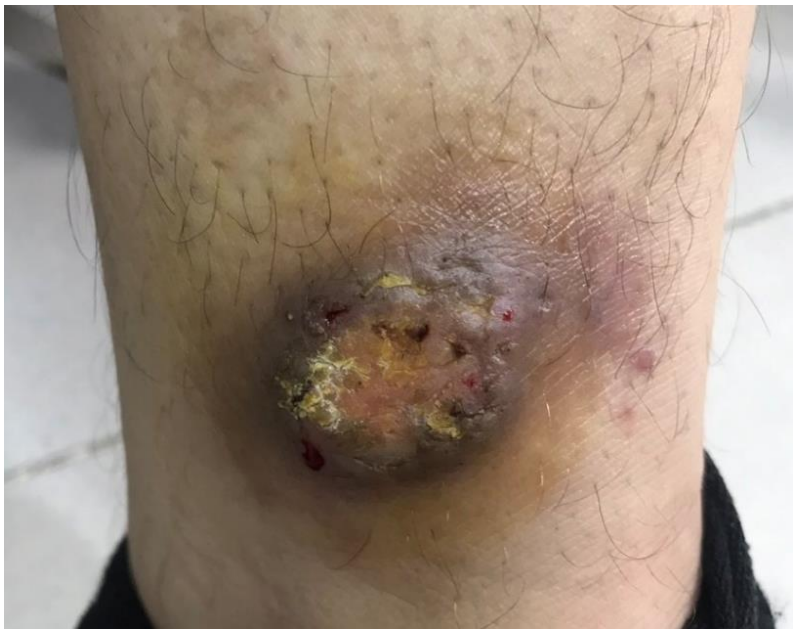


Figure 4.46. A woman in her fifties, whose right leg had a single, big ulcerative lesion

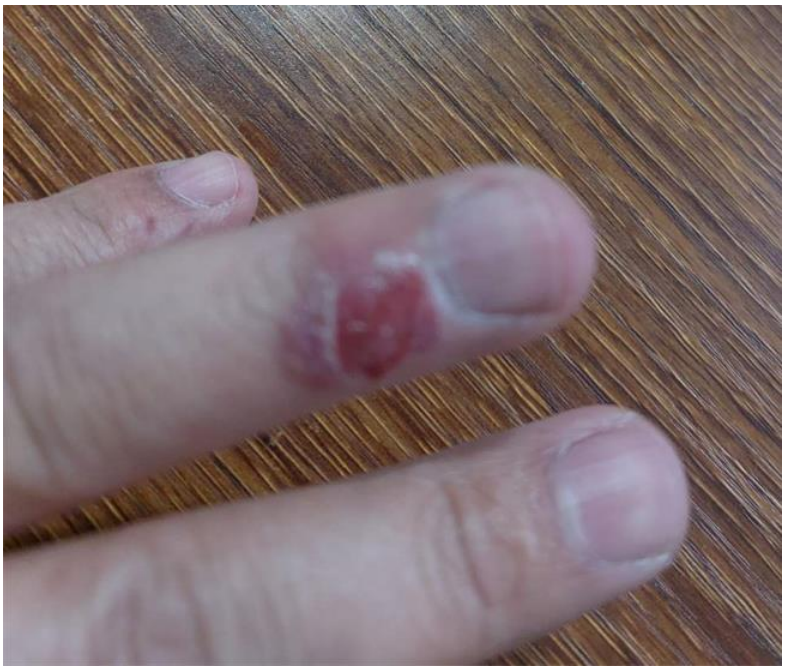


Figure 4.47. shows a 45-year-old man with a single, sizable CL lesion on his right hand

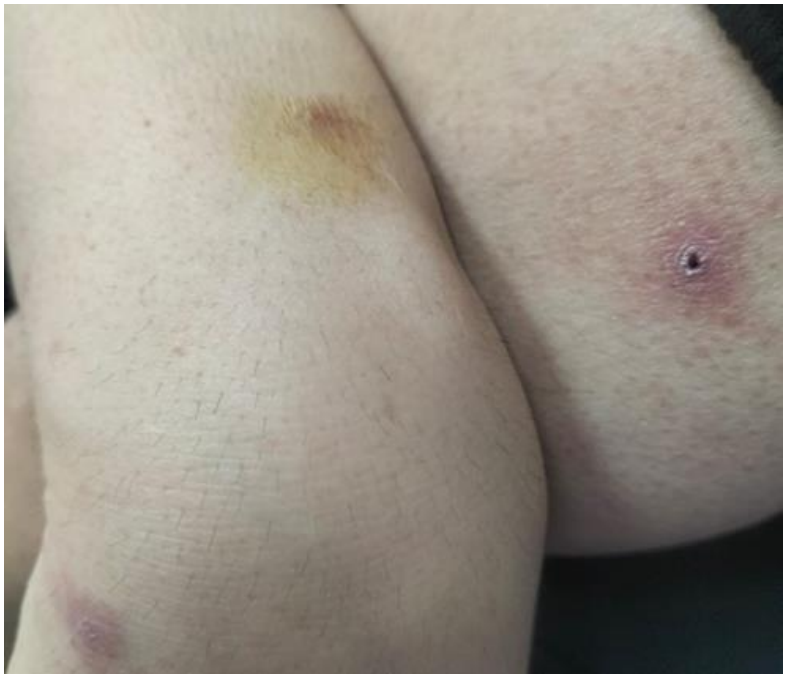


Figure 4.48. A 24-year-old man with single CL lesions on his right hand

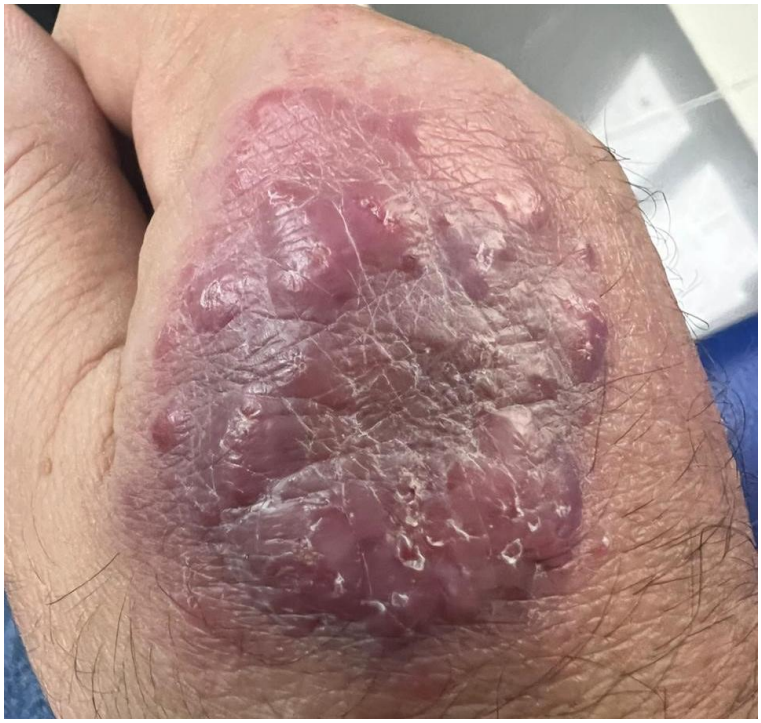


Figure 4.49. A 24-year-old female right hand has three CL lesions after taking medications

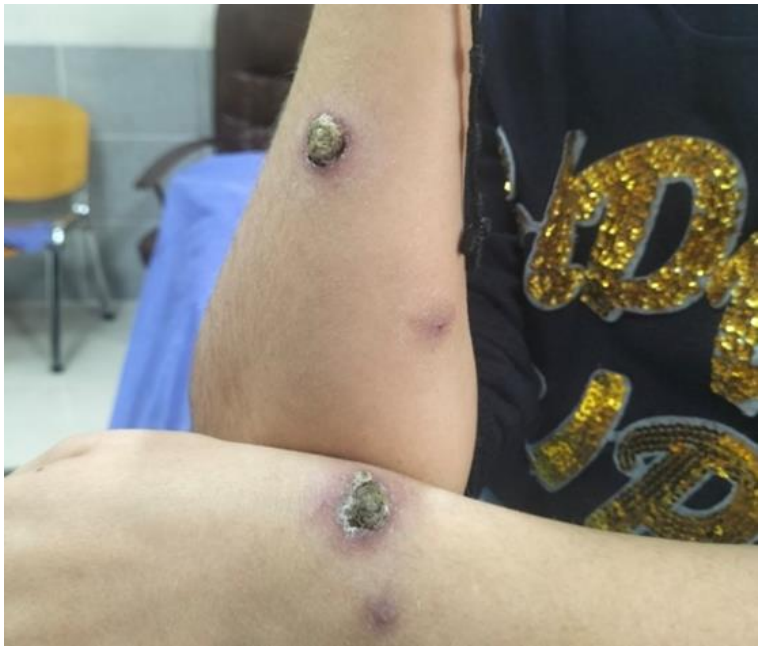


Figure 4.50. A thirteen-year-old girl with several CL lesions on several organs

Figure 4.51. A thirteen-year-old girl with several CL lesions on several organs

The most frequently affected areas by CL were the upper limbs in 62/135 patients (45.9%), the lower limbs in 39/135 patients (28.9%), and the face (including the ears and nose) in 28/135 patients (20.7%), Wearing long sleeves, long pants, and socks will help reduce the amount of unprotected skin exposed to sandfly biting to a manageable level depending on the weather, according to recommendations from the CDC (CDC, 2004).

The research supports a study conducted in the province of Maisan by (Abul-Doanej, 2014), which indicated that the common infection (face, trunk, and lower limbs) was 22.58% and that the highest percentage was discovered in the upper limbs (59.67%). The current study's findings conflict with those of a study conducted in the same province by (Atshan, 2014), who discovered that the head had the highest percentage of infections (44 cases) at 38.30%, followed by the lower limbs (25 cases) at 27.40%" 128 and the trunk had the lowest percentage of infections (1 case) at 1.09%.

The results of the study are in line with those of (Younis, 2018) in Baghdad, who found that, in comparison to other infection sites, the upper limbs had the highest percentage (48%) followed by the legs (26.66%), feet (16%), face (9.3%), and ears (2.9%).

Our findings were in line with those reported by (Sharquie et al., 2002), who discovered that lesions in Saudi patients mostly affected the upper limbs and less often the face. Our results, however, conflict with a study by (Al-Obaidi et al., 2016) that discovered the face and feet to be the areas most frequently impacted by lesions. Similar findings were observed by several studies conducted around the globe, including Turkey (Akcali et al., 2007) and Iran (Talari et al., 2006).

The findings are consistent with those of (Al-Obaidi et al., 2016) in Thi-Qar province, where the trunk had the lowest rate of infection at 1.2%, while the upper limbs had the highest rate of infection at 40 cases, followed by lower limbs at 22 cases, 25.6%, and the face at 10 cases. The results of the current study, on the other hand, matched those of other previous studies conducted in other provinces in Iraq, by (Moker, 2006) in Basra province, which exposed parts of the body that were involved with a predominance of upper and lower limbs (18 cases) 35.3% each, followed by and facial infection 19.6%.

Due to the fact that exposed body parts that are not covered by clothing are more vulnerable to sandfly attacks, the upper limbs were the most often bitten areas; these findings are consistent with those of other studies. According to some writers, the face (25%) and lower limbs and feet (21.5%) were the most common areas of the body to contract CL, with the lower limbs and hands (38.5%) having the lowest prevalence of infection (15%) compared to other body parts (CDC, 2014).

4.4.4.2. CL Infection Rates According to the Number of Lesions

At the time of evaluation, 117 out of 135 patients (86.66%) had fewer than 4 lesions, whereas 18 out of 135 (13.4%) patients had more than 4 lesions; this difference was significant ($P<0.05$) as shown in (table 4.27 and figures 4.52 and 4.53).

Table 4.27. CL infection rates according to the number of lesions

Number of lesions	CL. Cases	
	No.	(%)
<4	117	86.6
>4	18	13.4
Total	135	100
P<0.05		

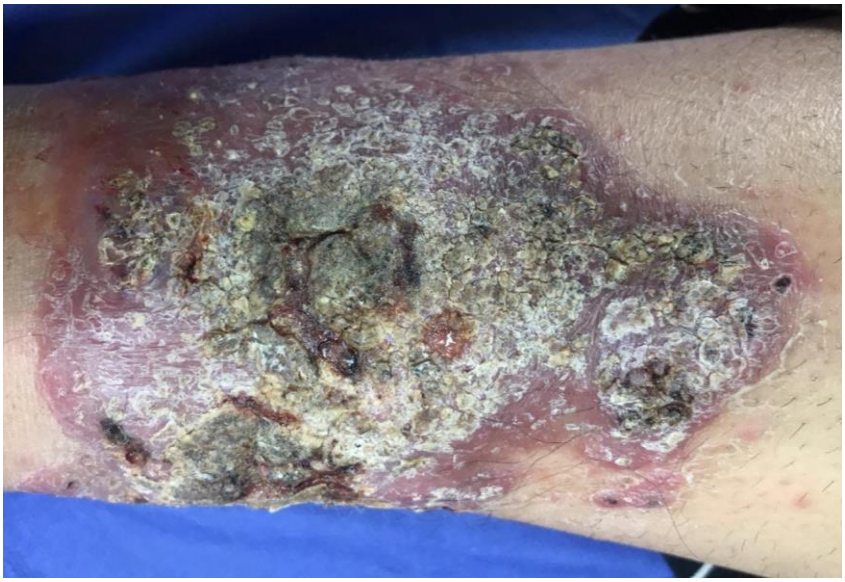


Figure 4.52. One huge, severe CL infection on the right leg of a 23-year-old lady

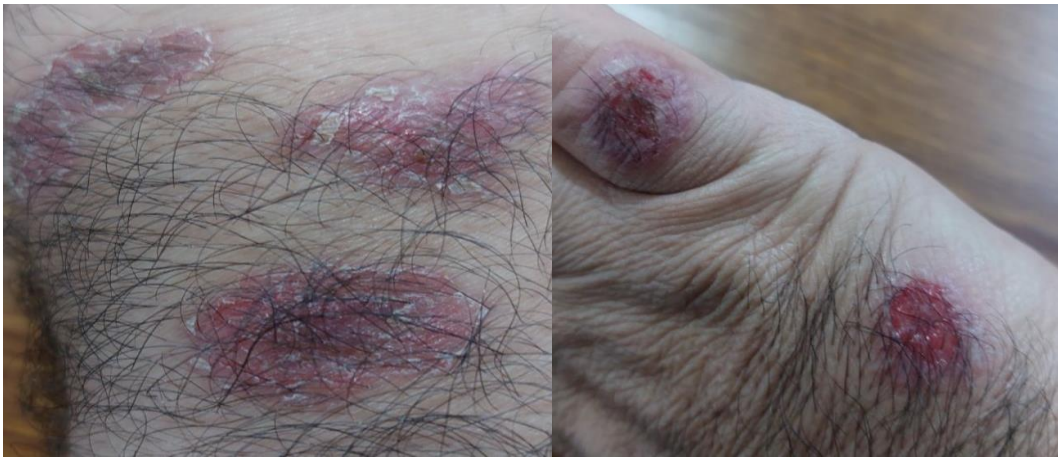


Figure 4.53. A male 25-year-old with five CL lesions on his arm and left hand

13.4 percent of patients had more than four lesions, while 86.66 percent of patients had fewer than four lesions. This matched the findings of (Abdulwahab, 2013), who found that 20% of individuals had more than ten skin lesions and that 79.9% of cases had one to nine lesions. Here, CL patients' prevalence of multiple lesions matched that of another Iraqi study (Al-Samaria and Al-Obaidi, 2009).

In 49 (65.33%) of the patients, (Younis, 2018) found one ulcerated lesion; in 24 (32%) of the patients, several lesions (2–9) were found. The high population density of sandflies in Iraq and extended periods of contact to them may be the cause of this (Al-Samaria and Al-Obaidi, 2009).

4.4.4.3. The Correlation Between the Severity of CL Lesions and the Infection Rate.

89/135 patients (65.9%) had intermediate types of CL lesions, which were the most prevalent presentation. Severe cases (32/135, 23.7%) and mild cases (14/135, 10.3%) were the next most common presentations, with a significant difference ($P<0.05$) (Table 4.28 and figure 4.54).

Table 4.28 The correlation between the severity of CL lesions and the infection rate

Severity of lesions	CL. Cases	
	No.	(%)
Severe	32	23.7
Mild	14	10.3
Moderate	89	65.9
Total	135	100
P<0.05		

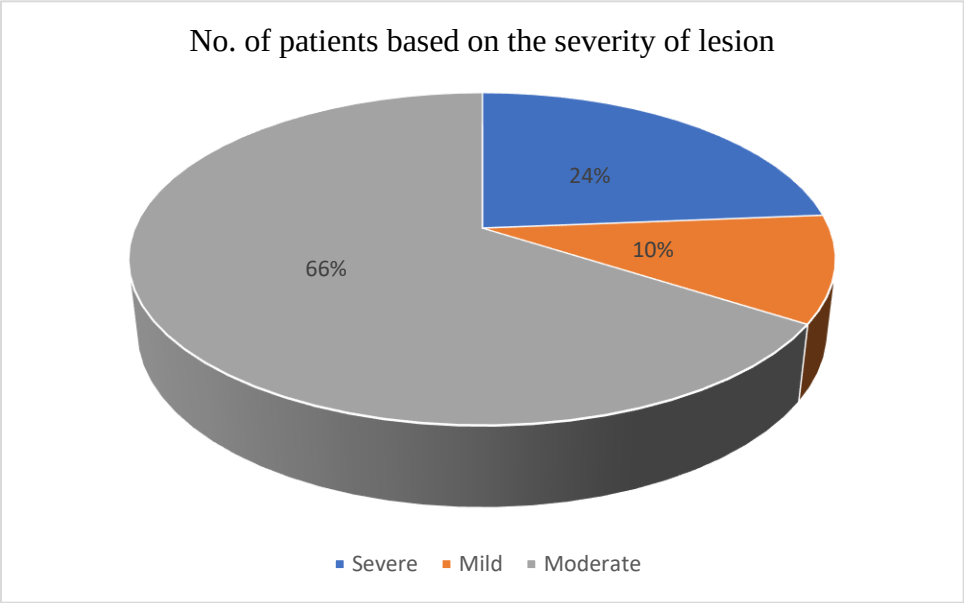


Figure 4.54. The correlation between the severity of CL lesions and the infection rate

4.4.4.4. The Correlation Between the Number of CL Cases and The Seasonal Distribution of Sandflies

Population distributions of sandfly species in Erbil province shows some differences from species to species. The monthly distribution of three species of sand flies, *P. papatasi, P. sergenti, and p. alexandri* in Erbil province, is shown in (table 4.29 and figure 4.55 & 4.56). P. papatasi is observed to begin in February, March, and April to be very rare in June and July, and again observed to increase in August and with high dentistry in May and September, but disappearing in January and December. While, P. sergenti appeared in March, and april it again reappeared in August and October, with high density in May and September, then disappeared in June, July, November, December, January and February, P. alexandri began to appear in March, and April, and it again reappeared in August with high density in May and September, while it disappeared in January, February, June, July, October, November and December. Generally, there were two peaks of existing sandflies along the year, first peak was in May and the second peak was in September. In contrast, CL's case was reported in

January, February, March, November, and December. The winter months had the highest number of CL. cases, whereas the summer months had the lowest number, which vanished in October, there were two peaks of existing CL. along the year, first peak was in February and the second peak was in December. When comparing the emergence of Sandflies and CL cases, regarding to the Leishmania parasite incubation period, the statistical analysis reveals significant differences at ($P<0.05$). there was a strong correlation between emerging sand fly spp. and CL disease, regarding to the incubation period of leishmania parasite. as illustrated in (table 4.29).

Table 4.29. The correlation between the number of instances of cutaneous leishmaniasis and the seasonal distribution of sandflies

Month	Sandflies Collected		CL. Cases	
	No.	(%)	No.	(%)
January	0	0	27	20
February	79	3.8	43	31.9
March	177	8.6	13	9.6
April	422	20.5	6	4.4
May	867	42.2	9	6.7
June	14	0.7	5	3.7
July	15	0.7	5	3.7
August	139	6.8	5	3.7
September	284	13.8	5	3.7
October	44	2.1	0	0
November	13	0.6	8	5.9
December	0	0	9	6.7
Total	2054	100	135	100
P<0.05				

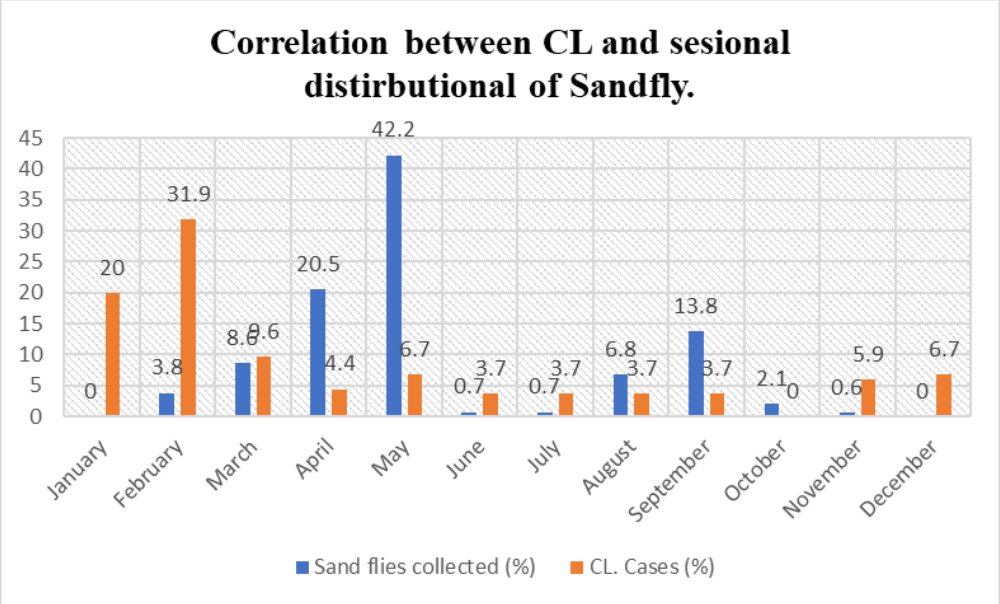


Figure 4.55. The correlation between the number of instances of cutaneous leishmaniasis and the seasonal distribution of sandflies

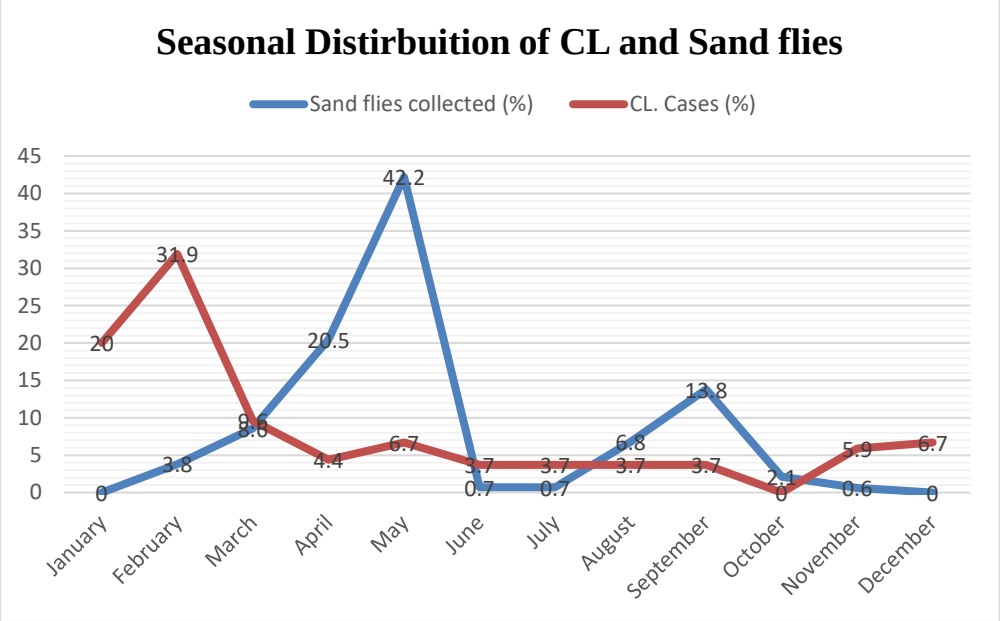


Figure 4.56. The correlation between the number of instances of cutaneous leishmaniasis and the seasonal distribution of sandflies

The two most prevalent species of leishmaniases that cause CL are L. tropica and L. major. The incubation periods for these parasites are 4–8 months for L. tropica and 2-4 months for L. major, with the disease being transmitted from sand flies to humans between September and May. A variety of sand fly species serve as vectors for the zoonotic illness (CL), which affects humans as vertebrate hosts. The risk of infection spreading is greatest between dusk and dawn because this is when sand flies are typically most active. The length of the infection also depends on factors like therapy, immunity levels, lesion count, and location of infection.

The results of the current study were consistent with findings made by (Atshan, 2014) in the Thi-Qar province regarding the relationship between the prevalence of seasonal sand flies and CL. It has been noted that sand flies emerge in the spring and summer, peak in May and October, and then disappear in the winter months of December, January, and February.

The current study's findings were consistent with earlier research by Abul-Hab and (Al-Hassani, 2016), which established that the infection happened in May and that the disease's four to eight-month incubation period for L. tropica and two to four months for L. major corresponds with the time when the disease is transmitted to humans by vectors in the months of September and May. Within the province of Maisan, (Abul-Doanej, 2014) found that the incidence in urban areas was 62.5%, while the incidence in rural regions was 39.52%. The largest number of infections, 42.85%, was seen in February.

The findings of our investigation aligned with earlier research conducted in other nations. (Bakdash et al., 2012) noted that P. papatasi was detected in Syria in a firm across all locations. In the Homs governorate, there were two density peaks for Phlebotomus activity: one in May and one in September. According to (Azizi et al. 2016), natural infection to L. major was found in P. papatasi (25 out of 130 sand flies, or 19.2% of the population), and the species' monthly activity in Iran spanned from April to the end of

November. The species' density curve had two peaks, one in June and the other in September.

4.4.4.5. Infection Rate Compared to The Kind of CL Lesions

A significant difference (P<0.05) was seen between the percentage of patients with CL lesions (97/135, 71.8%) that were almost moist and the remaining patients (38/135, 28.2%) that had dry lesions (Table 4.30 and figure 4.57).

Table 4.30. Infection rate compared to the kind of CL lesions

Nature	CL. Cases	
	No.	(%)
Wet	97	71.8
Dry	38	28.2
Total	135	100
P<0.05		

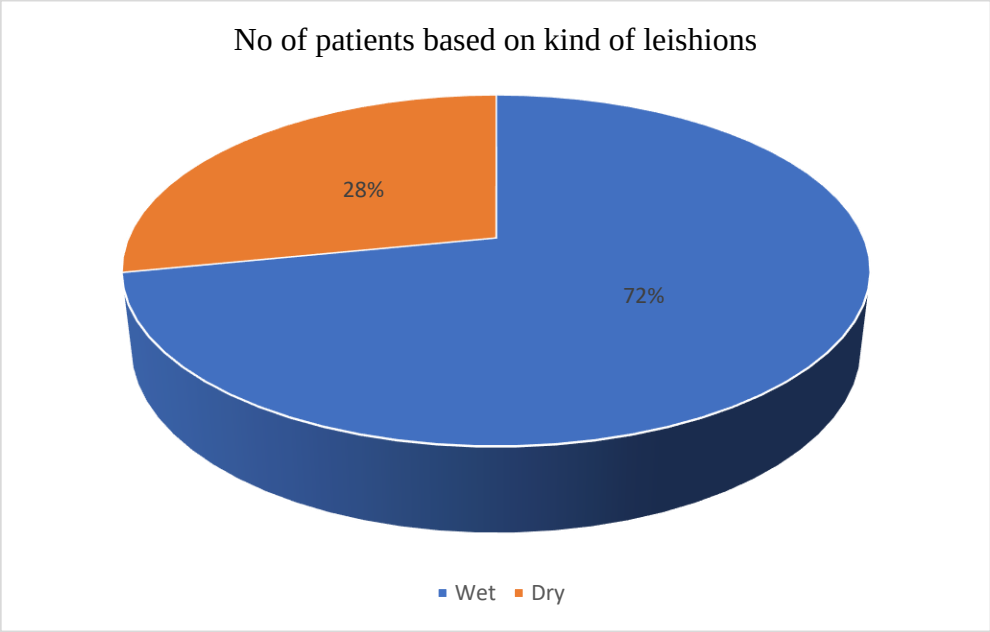


Figure 4.57. Infection rate compared to the kind of CL lesions

In this study, the majority of lesions evaluated were wet lesions, with a lower percentage of dry lesions found. These results were in line with a study conducted in Iraq (AlSamaria and AlObaidi, 2009), as well as those conducted in Pakistan (ul Bari and Rahman, 2006) and Iran (Talari et al., 2006). However, they were at odds with a study conducted in India by (Singh and Sivakumar, 2003). The high proportion of wet forms could be attributed to the abundance of reservoir hosts, particularly dogs and rats, in some areas (Al-Samaria and Al-Obaidi, 2009). Wet CL lesions accounted for 71.8 percent of all lesions seen in this investigation. In terms of CL severity, the most common pattern was moderate, with severe and mild disease being less common, (Bamorovat et al., 2015).

Factors that may influence this include patients' immunity, the length of time before seeking dermatological advice, the type and quantity of lesions, and the effectiveness of treatment. Increased human-caused risk factors are generally to blame for the rise in leishmaniasis prevalence. Immunosuppression, environmental variables, population, mass migration, deforestation, and urbanization are the main ones. Changes in the environment and population migration may have affected the quantity, range, and density of reservoirs and vectors. CL have an impact on the less fortunate people, and outbreaks happen around harvesting season. Additional circumstances include sleeping on the ground or outside, having damp earthen floors, and residing in a house made of cracked mud. Because they provide diurnal resting places, breeding grounds, and moisture, these factors can help sandflies survive and increase vector numbers (Oryan et al., 2013).

4.4.4.6. CL Infection Rate in Correlation with the Length of Lesions

The time interval between being bitten by a sandfly and needing to visit the hospital varied from one to more than fourteen weeks. Most of the patients, 85/135 (62.9%) came to the Dermatological Hospital after ten to fourteen weeks, while 33/135 (24.4%) came after six to nine weeks, 12/135 (8.8%) after four to six weeks, and 5/135 (3.7%) after more than fourteen weeks. The severity of CL was significantly impacted by these varying durations, and this association was very significant ($P<0.05$), (Table 4.31 and figure 4.58).

Table 4.31. shows the relationship between the length of lesions and the rate of CL infection

Duration (weeks)	CL. Cases	
	No.	(%)
4-6	12	8.8
6-9	33	24.4
10-14	85	62.9
>14	5	3.7
Total	135	100
P< 0.05		

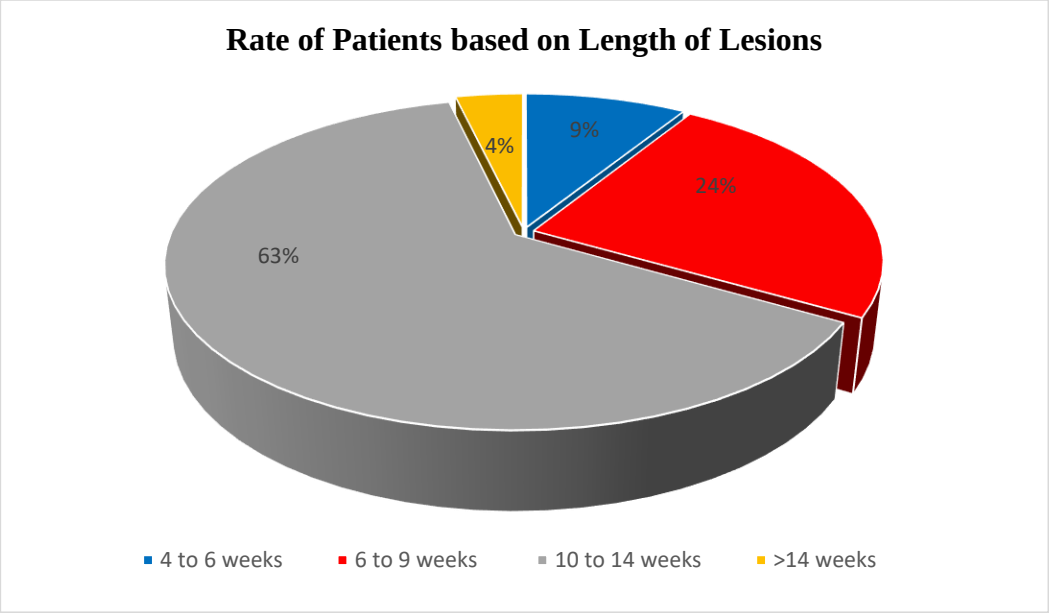


Figure 4.58. shows the relationship between the length of lesions and the rate of CL infection.

There were four distinct times from the start of the symptoms to the dermatologist's visit. The most common time frame was between 10 and 14 weeks, which was followed by 6 and 9 weeks. Better management, shorter treatment times and costs, and earlier diagnosis are all associated with earlier therapy. The Iraqi Journal of Veterinary Medicine released research by (Kadir and El-Gorban, 2006) that said that lesions could take anywhere from two to sixteen weeks to be diagnosed with CL.

4.4.4.7. Monthly Distribution of Cases with Cutaneous Leishmaniasis

February 2022, January 2022, and March 2022 all had higher infection rates (31.9%, 20%, and 9.6%, respectively). According to the findings, the rate of infection was higher in the months of February, January, and March. In contrast, the rate decreased in the hotter months of April, May, June, July, August, and September (infection rates: 4.4%, 6.7%, 3.7%, 3.7%, 3.7%, and 3.7%, respectively), but it was zero in October as seen in (figure 4.59). The winter months of January, February, and December had the highest percentage, while the summer and fall had the lowest. The details are explained in (table 4.32). The results of statistical analysis indicate significant variations between the months at ($P<0.05$).

Table 4.32. Monthly Distribution of Patients with Cutaneous Leishmaniasis

Month	CL. Cases	
	No.	(%)
January	27	20
February	43	31.9
March	13	9.6
April	6	4.4
May	9	6.7
June	5	3.7
July	5	3.7
August	5	3.7
September	5	3.7
October	0	0
November	8	5.9
December	9	6.7
Total	135	100
P<0.05		

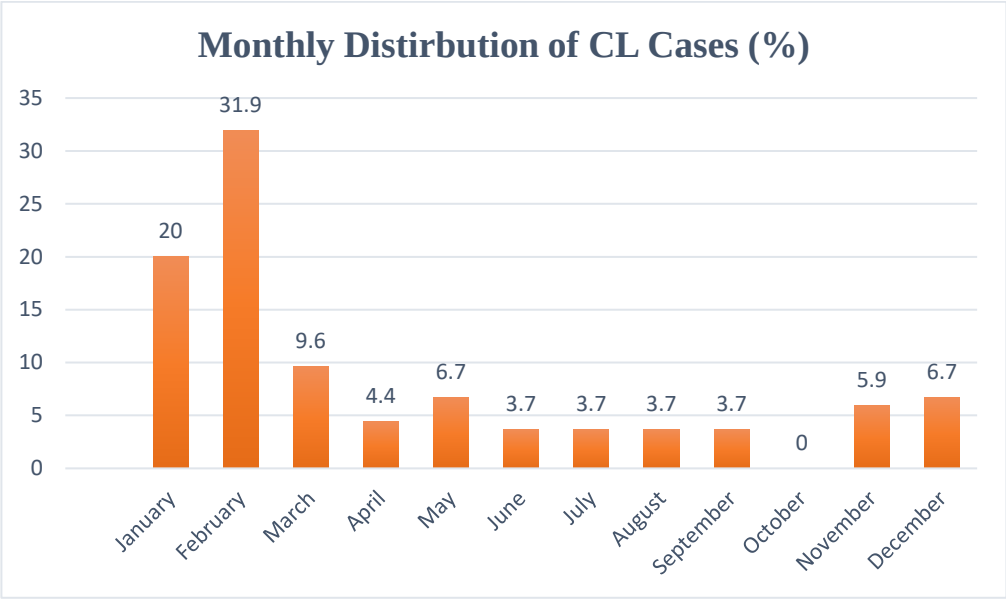


Figure 4.59. Monthly Distribution of Patients with Cutaneous Leishmaniasis

According to (AL-Yazachi 1974), the incubation period for species L. major is 2-4 months, whereas that of species L. tropicala is 6-8 months. The peak of the infections of the Baghdad boil in Iraq occurs in January and disappears in July. Additionally, according to (Abul-Hab and Baghdadi 1972 b), the infection happened in September.

Comparing the study's cases to the typical type, we discovered that the predicted infection happens in September and manifests itself in January following a four-month incubation period, or the length of incubation for L. major. (It is also in line with the World Health Organization's (WHO, 1998) report, which stated that the incidence of CL infections in Iraq peaks in the winter and spring, climbs in January and March, and then declines as summer approaches. (This might also be related to the peak of the sand fly epidemic in Iraq and the 4–6-month life cycle of the parasite, which has the highest sand fly populations in May and September and the lowest infection rates in January and February.) This could also explain the high density of vectors in the time leading up to January and February. The CL infection was present in the spring and early winter but was not present in the summer (Al-Taee et al., 2011).

The current study's findings were compared to those of other earlier investigations conducted in a few southern Iraqi areas. According to (Jafar et al., 2014), the province of Karbala "recorded the highest percentage (14 and 13 cases) in the months of November and December, 19.1% and 17.8% respectively."According to (Abul-Doanej, 2014), the province of Maisan had no infections in the months of July 2013, August, September, and November, while the greatest ratio of infections CL in February 2014 was 42.85%.

(Al-Hassani, 2016) discovered that in the province of Missan, the month of January had the largest percentage of CL infections (497 cases) at 34.4%. After that, the infections progressively declined, with the lowest percentage (6 cases) at 0.4% in the month of August. According to (Abul-Hab, 1982), the bite of a sand fly near the end of summer, along with the activity of the sand fly carrier and the intensity and duration of parasite incubation, by type, heat, food, and response to the host, are the primary causes of infections and their spread to temperature.

5. CONCLUSIONS and RECOMMENDATIONS

5.1. Conclusion

In the light of the findings of this study, the percentages of *P. papatasi* were much higher than *P. sergenti* and *P. alexandri* in all districts in Erbil province, it was found in all districts, which illustrated that *P. papatasi* more dangerous than other species in transmitting CL diseases in the study area. The light trap had the highest success rate (56.6%) at catching sand flies. The first peak of the seasonally abundant CL vectors occurred in May, while the second peak occurred in early September. There was a considerable statistical difference between the zones (rural and urban sides) and the distribution of sand fly species at ($p<0.00$), with the number of species in rural areas being 1556 (75.8%) and in urban areas being 498 (24.2%).

From 135 CL cases, 82, 65.6 % of the human samples were positive. Two of the patients were younger than a year old. Ages varied from one year to over sixty-five. Patients from 1 year to 25 years old had the highest rates of CL infection (63, 46.6%), followed by those from 26 to 50 years old (44, 32.5%), 51 to 65 years old (19, 14.7%), and the group older than 65 years old (9, 6.6%). As a result, with some significant variations ($P<0.05$), the rate of infection was higher in younger age groups and gradually decreased in older age groups. The winter months had the highest number of CL. cases, whereas the summer months had the lowest number, which vanished in October, there were two peaks of existing CL. along the year, first peak was in February and the second peak was in December. When comparing the emergence of Sandflies and CL cases, regarding to the Leishmania parasite incubation period, the statistical analysis reveals significant differences at ($P<0.05$). there was a strong correlation between emerging sand fly spp. and CL disease, regarding to the incubation period of leishmania parasite.

The most frequently affected areas by CL were the upper limbs in 62/135 patients (45.9%), the lower limbs in 39/135 patients (28.9%), and the face (including the ears and

nose) in 28/135 patients (20.7%). There was significant difference between the genders (P>0.05) in the CL rate, which was higher in males than females (84(62.2%) versus 51 (37.8%).

There were appreciable variations in the exposure to CL infection between the sexes. The impact of sex on society, traditions, and rituals may help to explain this. Because they walk around and labor in the fields and farms, men are more likely to become infected than women, who typically stay at home and wear long clothing, especially in Islamic nations. Women have fewer exposed body parts than men do as a result. Men are more likely to get bitten by sandflies for all of these reasons. Additional research conducted in Iraq corroborated these conclusions (Aldifaei, 2013). Patients with CL are distributed according to the type of habitation. Infections with CL are more common in rural regions (93 cases, or 68.8% of the total), whereas they are less common in urban areas (42 cases, or 31.2% of the total). According to the present findings, there are significant differences between the types of occupancy at $P < 0.05$.

Makhmur City was classified as a rural area in this study due to the climate, the environment, the distribution of marshes around the town, in all of Erbil, this is the city where CL is common. According to the findings, the rate of infection was higher in the months January, February, March, November, and Decembe at moderate mean temperatures (C^{o}). In contrast, the rate decreased in the hotter months of April, May, June, July, August, and September (infection rates: 4.4%, 6.7%, 3.7%, 3.7%, 3.7%, and 3.7%, respectively), but it was zero in October. Significant variations in the rate of CL were seen in these data ($P<0.05$).

A significant rate of CL infection was observed in Makhmur district as a result of the high mean humidity (%). With notable exceptions, high humidity was linked to increased infection rates in November, December, January, and February. Moderate humidity and fairly high infection rates were observed in November, December, January, and February respectively, with significant differences ($P<0.05$).

Regarding precipitation, the rates of CL infection were highest in the wet months of January, February, March, November, and December, and lowest in the other months, with the exception of April and May, where the rates were 4.4% and 6.7%, respectively with significant differences ($P<0.05$).

The months with the most rainfall had the greatest documented infection rate of CL, with moderate temperatures and high humidity. The growth and activity of the vector host—sandflies—are compatible with these conditions. In the summer, when there was little humidity, high temperatures, and no rain, the study found that there were less CL infection. In this study, the majority of lesions evaluated were wet lesions, with a lower percentage of dry lesions found.

Outcomes of These results can serve as the foundation for the implementation of vector control measures, which may aid in reducing vector density and, as a result, managing CL in the research region associated with controlling cutaneous leishmaniasis vectors. Public health officials should take advantage of this knowledge to develop optimum vector control tactics in Erbil province and the surrounding area since sand flies are strongly connected with temperature. To further lower the prevalence of CL in the tested region, effective educational initiatives centered on the disease's transmission and prevention strategies are required, as well as active monitoring to swiftly identify and treat cases.

5.2. Recommendation

1. Despite the fact that this study examined the physical traits and molecular identification of sandfly samples taken from the Erbil province of Iraq, certain conclusions require more evidence for further confirmation because, it supposed that there are more sand fly species in the region.

2. More samples need to be collected for molecular studies in order to literally confirm the species of sandflies distributed in Erbil province and whether they are species complex or not in the future.

3. For the following researchers that want to identify leishmania parasite by culturing on NNN media, they need to consider the following points, not cut off an electricity or putting USB device while incubation the inoculated NNN media, do not let to contaminate the samples while taking samples from CL cases and do not culture the CL specimen after taking medicines. The previous factors prevent the growth of Leishmania parasite on NNN media.

4. This study recommends to Erbil governorate to take action to spread out specific repellent against the sand fly depending on the findings of this study when they are in sharps during the year, as sand fly a risky vector for many pathogens.

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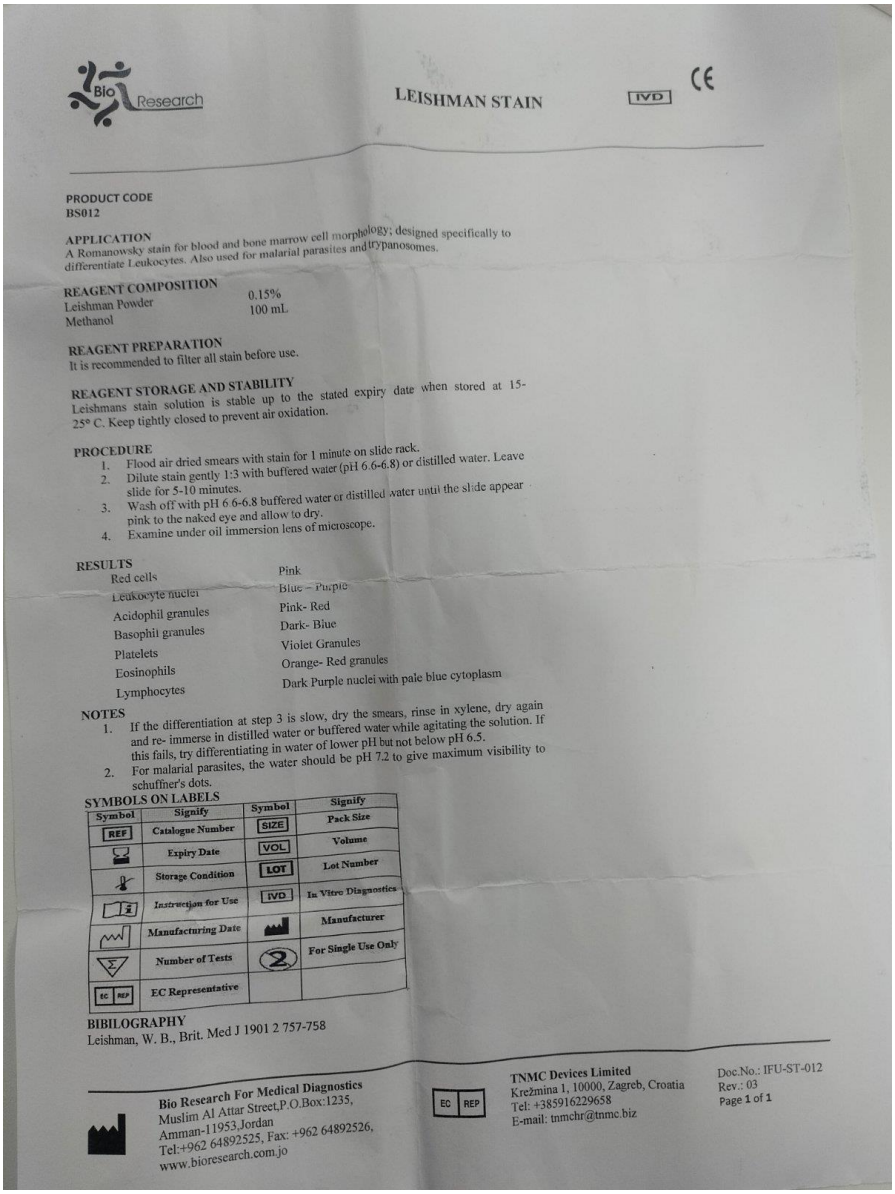
APPENDICES

Appendix (1): Questioner sheet for Cutaneous Leishmaniasis Patients

فۆرمی پشکنینی نەخۆشی لیشمانیا 2022

ناوی نەخۆش/	ڕەگەز/	تەمەن/	پیشە/
پارێزگا/	قەزا/	ناحیە/	
کەرت/	گەڕەك/	گوند/	ژ.مۆبایل/
ناونیشانی پێشتر/			
جۆری نەخۆشی لیشمانیا/	شوێنی بڕین لەجەستە/	ژمارەی بڕین/	
ئاژەڵدارن/	جۆری ئاژەڵ/		
سەگیان هەیە/	سەگی بەڕەڵا/	سەگی ماڵی/	
پێشوتر ئەندامێکی خێزانەکەی تووشی ئەم نەخۆشیە بووە:			
پێشوتر ئەم نەخۆشیە لە دەڤەرەکەی هەبووە:			
دەڤەرەکە ڕەش کرایە:			
جۆری خانوو(بلۆك،کەڵپووچ، قوڕ، خەیوەت):			
پاشماوەی ئاژەڵ لەنزیك ماڵەکەیان هەیە:			
لەگەڕەکە کە دووکەڵکردنی بۆکراوە:			
نەخۆشەکە سەردانی هیچ شوێنێکی کردوە پێش بە توشبوونی بە (3) مانگ: شوێنی سەردان:			
چارەسەری وەرگرتووە:			
ڕێکەوتی توشبوونی (2022/ /)		ڕێکەوتی پڕکردنەوەی فۆرم (2022/ /)	

Appendix. (2): Leishman stain preparation, storage and stability.



Bio Research

LEISHMAN STAIN

IVD CE

PRODUCT CODE
BS012

APPLICATION
A Romanowsky stain for blood and bone marrow cell morphology; designed specifically to differentiate Leukocytes. Also used for malarial parasites and trypanosomes.

REAGENT COMPOSITION

Leishman Powder	0.15%
Methanol	100 mL

REAGENT PREPARATION
It is recommended to filter all stain before use.

REAGENT STORAGE AND STABILITY
Leishmans stain solution is stable up to the stated expiry date when stored at 15-25° C. Keep tightly closed to prevent air oxidation.

PROCEDURE
1. Flood air dried smears with stain for 1 minute on slide rack.
2. Dilute stain gently 1:3 with buffered water (pH 6.6-6.8) or distilled water. Leave slide for 5-10 minutes.
3. Wash off with pH 6.6-6.8 buffered water or distilled water until the slide appear pink to the naked eye and allow to dry.
4. Examine under oil immersion lens of microscope.

RESULTS

Red cells	Pink
Leukocyte nuclei	Blue – Purple
Acidophil granules	Pink- Red
Basophil granules	Dark- Blue
Platelets	Violet Granules
Eosinophils	Orange- Red granules
Lymphocytes	Dark Purple nuclei with pale blue cytoplasm

NOTES
1. If the differentiation at step 3 is slow, dry the smears, rinse in xylene, dry again and re- immerse in distilled water or buffered water while agitating the solution. If this fails, try differentiating in water of lower pH but not below pH 6.5.
2. For malarial parasites, the water should be pH 7.2 to give maximum visibility to schuffner's dots.

SYMBOLS ON LABELS

Symbol	Signify	Symbol	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
Σ	Number of Tests	2	For Single Use Only
EC REP	EC Representative		

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E-mail: tnmchr@tnmc.biz

Doc.No.: IFU-ST-012
Rev.: 03
Page 1 of 1

Appendix. (3): Preparation of (NNN Medium, Novy, MacNeal-Nicolle)

Technical Data

NNN Modified Medium (Twin Pack) M681

NNN Modified Medium (Twin Pack) is used for cultivation of Leishmaniae and Trypanosomes.

Composition**

Ingredients	Gms / Litre
Part A	-
Meat extract	3.000
Peptone	5.000
Sodium chloride	8.000
Agar	15.000
Final pH (at 25°C)	7.3 ± 0.2
Part B	-
Sodium chloride	8.000
Potassium chloride	0.200
Calcium chloride	0.200
Monopotassium dihydrogen phosphate	0.300
Dextrose	2.500
Final pH (at 25°C)	7.0 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Part A: Suspend 31 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 10% of sterile defibrinated rabbit or human blood after inactivation at 56°C for 30mins. Mix well and dispense in 5 ml amounts in test tubes or 25 ml amounts in flasks. Allow tubed media to cool in slanted position.

Part B : Suspend 11.2 grams of Part B in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool and add approximately 2 ml in tubes or 10-15 ml in flasks over solidified Part A medium.

Principle And Interpretation

The protozoan family *Trypanosomatidae* includes members from the genera *Leishmania* and *Trypanosoma* , which are flagellates that inhabit the blood and tissues of humans.

NNN Medium was developed by Novy, McNeal (1) and modified by Nicolle (2). NNN Modified Medium is a modification of the original medium and consists of two phases, blood agar (Part A) and Lockes solution (Part B) (3). This modified medium is commonly used for diagnostic work (4, 5).

This medium consists of a blood agar base and an overlay medium. The blood agar base is a highly nutritious medium that supports the growth of fastidious organisms like *Leishmania* and *Trypanosoma* . The specimens are inoculated into the liquid phase of the diphasic medium and incubated. This favours the development of organisms in the insect vector. The amastigotes transform to promastigotes in about 24 hours (5).

Quality Control

Appearance

Part A : Cream to tan homogeneous free flowing powder Part B: White to cream homogeneous free flowing powder

Gelling

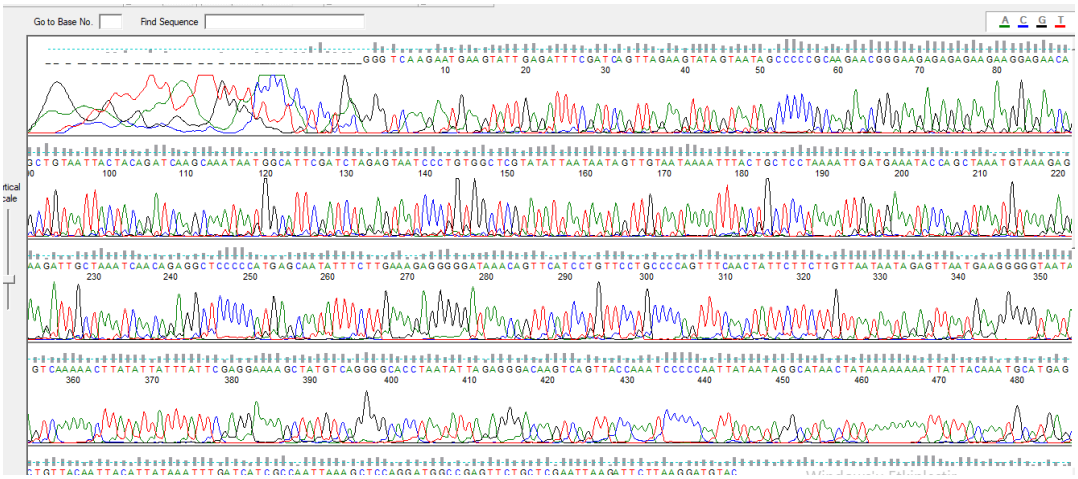
Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared medium

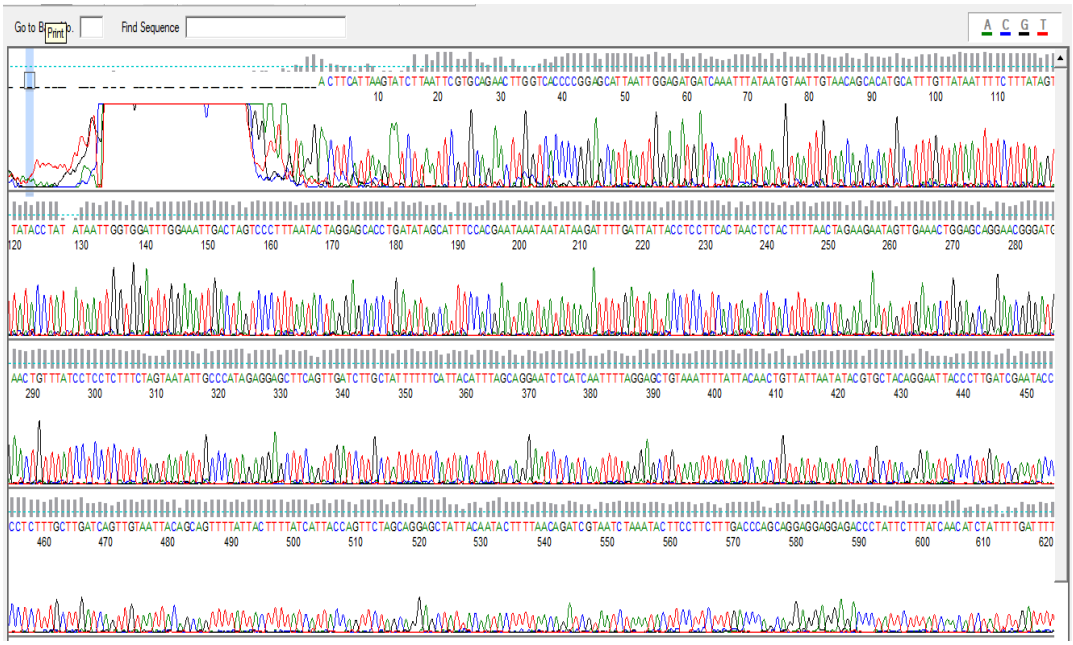
Basal medium :Light amber clear to slightly opalescent gel. After addition of sterile defibrinated rabbit or human blood : Red coloured opaque gel Part B : Colourless clear liquid

Appendix. (4): The result of gene sequence analysis of P. paparasi, P. sergenti and P. alexandri individuals can be seen in the Chromas-Pro computer program. By means of this program, the peaks of the base sequences were carefully examined.

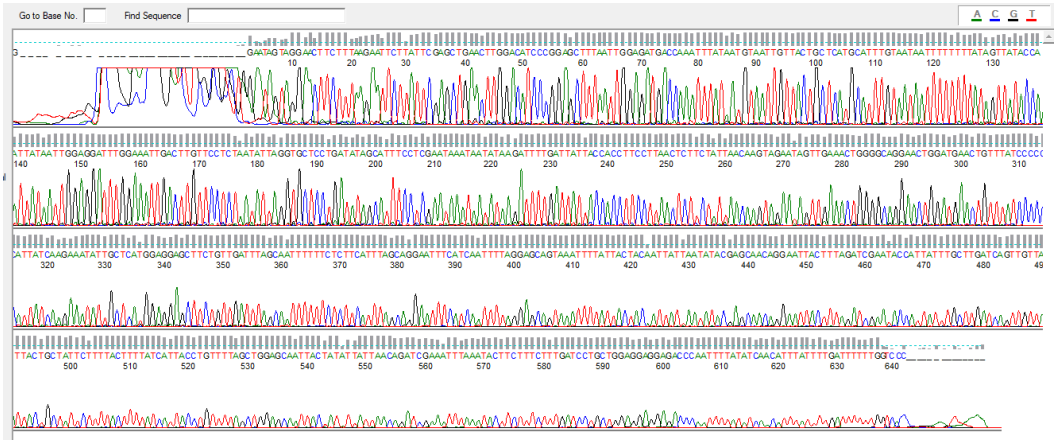
Finith-TV image of samples 11-2 (P. papatasi)



Finith-TV image of samples 11-1 (P. Sergenti)



Finith-TV image of samples 12-1 (P. alexandri)



Finith-TV image of samples 12-2 (P. alexandri)