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SAND-FLY *PHLEBOTOMUS PAPATASI* (PHLEBOTOMINAE): A GENERAL REVIEW WITH SPECIAL REFERENCE TO ZOONOTIC CUTANEOUS LEISHMANIASIS IN EGYPT

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Abstract

Leishmania are digenetic protozoa which inhabit two hosts, the sandfly where they grow as promastigotes in the gut, and the mammalian macrophage where they grow as amastigotes. Sandfly (or sand fly) is a colloquial name for any species or genus of flying, biting, blood-sucking Dipteran encountered in sandy areas. In the United States, sandfly may refer to certain horse flies that are also known as "greenheads" (family Tabanidae), or to members of the family Ceratopogonidae, also known in Florida and elsewhere as a sand gnat, sandflea, no-see-um (no-see-em, noseeum), granny nipper, chitra, punkie, or punky. Outside the United States, *sandfly* may refer to members of the subfamily Phlebotominae within the Psychodidae. Biting midges (Ceratopogonidae) are sometimes called sand flies or no-see-ums (no-see-em, noseeum). New Zealand sandflies are in the *Austrosimulium* genus, a type of black fly. Of 500 known phlebotomine species, only some 30 of them have been positively identified as vectors of the disease. Cutaneous leishmaniasis (ZCL) is a protozoan disease well documented not only in Egypt, but in nearly all the East Mediterranean Countries. It is prevalent in the Egyptian Sinai Peninsula with at least three identified foci.

Key words: Egypt, Zoonotic cutaneous leishmaniasis, *Phlebotomus papatasi* (Scopoli)

Review and Discussion

Only the female sandfly transmits the protozoa, infecting itself with the *Leishmania* parasites contained in the blood it sucks from its human or mammalian host in order to obtain the protein necessary to develop its eggs (Kvifte, 2014).

Some sandfly genera of the Phlebotominae subfamily are the primary vectors of leishmaniasis and pappataci fever; both diseases are confusingly referred to as sandfly fever. In the New World, leishmaniasis is spread by sand flies of the genus *Lutzomyia*; in the Old World, the disease is spread by sandflies of the genus *Phlebotomus*. Belize and Honduras are notorious in the Caribbean for their sandfly populations and travel pages frequently warn tourists to bring bug spray containing high concentrations of DEET. In the various sorts of sandfly only the female is responsible for biting and sucking the blood of mammals, reptiles and birds. She requires its protein in the blood to make her eggs (Aoun and Bouratbine, 2014).

According to the WHO, there are 2 million new cases each year and 1/10 of the world's population is at risk of infection (Gravino, 2004). In Egypt, *Phlebotomus* (*P.*) *langeroni* is the vector of *Leishmania* (*L.*) *infantum* (Youssef *et al*, 1989, Shehata *et al*, 1990; Doha and Shehata, 1992). *P. papatasi* is the vector of *L. major* (Wahba *et al*, 1990; Hanafi *et al*, 1998) from rodents to human (Morsy *et al*, 1987; Mansour *et al*, 1987; Morsy, 1996; El Hossary *et al*, 2000).

Phlebotomus papatasi is common in Egypt (El Sawaf *et al*, 1984; Lane, 1986; Soliman *et al*, 2001; Kamal *et al*, 2003; El-Naggar *et al*, 2006), where *L. major* was isolated and identified (Mansour *et al*, 1987; Wahba *et al*, 1990; Fryauff *et al*, 1993; Hanafi *et al*, 1999, El Hossary *et al*, 2000; El-Naggar *et al*, 2006). Shehata *et al.* (2009) isolated *L. tropica* from human cases in a Northern Sinai community bordering Gaza Strip. *P. papatasi* were found harbor-

ing *L. major*, however only non-infected individuals of *P. sergenti*, a vector for *L. tropica*, were caught. *Leishmania tropica* is viscerotropic (Jacobson, 2003).

P. papatasi and *P. sergenti* were experimentally infected by *L. major* promastigotes mixed with blood via chick skin membrane. *P. papatasi* was more susceptible to *L. major* than *P. sergenti*. The present study confirmed the fact that *P. papatasi* is the main vector of *L. major* in Egypt (Mansour *et al*, 1987; Wahba *et al*, 1990) and abroad (Killick-Kendrick, 1990; Abou El Ela *et al*, 1995). Vector-parasite correlations were found in sandfly-*Leishmania* domain, namely *Lu. whitmani-L. braziliensis* (Vexenat *et al*, 1986), *P. perniciosus-L. infantum* (Molina, 1991), *P. argentipes* and *P. papatasi-L. donovani* (Mukhopadhyay and Mishra, 1991), and *Lu. intermedia-L. braziliensis* (Rangel *et al*, 1992), *Lu. trapidoi* and *Lu. gomezi-L. braziliensis* and *L. panamensis* (Jaramillo *et al*, 1994), *P. langeroni-L. infantum* and *L. major* (El Sattar *et al*, 1991; Daba *et al,* 1997), *Lu. longipalpis -L. mexicana* (Ismaeel *et al*, 1998), *P. argentipes-L. donovani* (Kumar *et al*, 2001), *P. halepensis*, *P. duboscqi*, *P. sergenti*, and *Lu. longipalpis-L. major* and *L. tropica* (Sadlova *et al,* 2003) were reported.

Leishmania species are transmitted by sandflies. They are ingested with blood meals, multiply, and migrate forward in the gut to be regurgitated with bite to a vertebrate host for a blood meal and injected the parasite within its saliva (Killick-Kendrick, 1979; Melby, 2002; De Almeida *et al*, 2003; Molina *et al*, 2003; Yaghoobi-Ershadi *et al*, 2005).

Leishmania tropica was confused and grouped with *L. major*; *P. sergenti* being its potential vector (Jacobson, 2003). Vector competence of *P. papatasi* from Suez Governorate was carried out under laboratory conditions by feeding on lesion of a *L. major* of infected hamster or membrane feeding technique (El-Naggar *et al*, 2006). Data indicated that a total of 204 (51%) females engorged with infected blood meal through hamster lesion. Of females fed on hamster blood, 7.4% (15/204) harbored *L. major* parasites as compared to 64.3% (132/205) of those fed on membrane, a slightly high than in the present study.

Vaccination: Novel vaccination approaches are needed to prevent leishmaniasis. Live attenuated vaccines are the gold standard for protection against intracellular pathogens such as *Leishmania* and there would be new developments in this field. Zahedifard *et al.* (2014) reported that the nonpathogenic to humans' lizard protozoan parasite, *Leishmania* (L) *tarentolae*, has been used effectively as a vaccine platform against visceral leishmaniasis in experimental animal models. Correspondingly, pre-exposure to sand fly saliva or immunezation with a salivary protein has been shown to protect mice against cutaneous leishmaniasis. They concluded that a combination of recombinant *L. tarentolae* with a sand fly salivary antigen (PpSP15) and represents a novel promising vaccination approach against leishmaniasis. Abi Abdallah *et al*. (2014) in USA reported that mice were best protected against an intradermal needle challenge with *Leishmania major* and sand fly saliva when vaccinated intravenously. However, this protection was short-lived. Importantly, groups of vaccinated mice were protected long term when challenged with infected sand flies. Protection correlated with smaller lesion size, fewer scars, and better parasite control between 2 and 6 weeks postchallenge compared to the control group of mice vaccinated with the parent *L. monocytogenes* strain not expressing LJM11. Moreover, protection correlated with high numbers of CD4(+), gamma interferon-positive (IFN-γ(+)), tumor nepcrosis factor alpha-positive/negative (TNF-α (+/-)), interleukin-10-negative (IL-10(-)) cells and low numbers of CD4(+) IFN-γ(+/-) TNF-α(-) IL-10(+) T cells at 2 weeks postchallenge. Overall, our data indicate that delivery of LJM11 by *Listeria* is a promising vaccination strategy against cutaneous form

inducing long-term protection against ulcer formation following a natural challenge with infected sand flies.

Ramalho-Ortigão *et al.* (2015) reported that analysis of PpSP15 expression from field populations revealed significant intra- and interpopulation variation.. In spite of the variability detected for P. papatasi populations, common epitopes for MHC class II binding are still present and may potentially be used to boost the response against Le. major infections. They concluded that conserved epitopes of PpSP15 could potentially be used in the development of a salivary gland antigen-based vaccine.

In Egypt, Morsy *et al*. (1987) in North Sinai by isoenzyme characterization and biochemical typing identified *Gerbillus pyramidium* the animal reservoir host for *L. major*. Morsy *et al*. (1988) in Suez Governorate reported the huge abundance of *P. papatasi*

El Okbi *et al*. (1989) in Al-Agamy, west of Alexandria studied the seasonal and daily hours of activities of both *P. papatasi* and *P. langeroni* and the blood preference over two successive years. They found a bi-model or two peaks per year. The sandflies started to appear in the last of March or beginning of April and ended in last of November or beginning of December. The indoor activity started earlier and ended later than the outdoors one. The blood of *P. papatasi* was mainly human blood, while that of *P. langeroni* was mainly non-human blood.

Morsy *et al*. (1990) reported four species of *Phlebotomus* were reported; *P. papatasi, P. sergenti, P. langeroni* and *P. bergeroti.* While *P. papatasi* is a known vector of zoonotic cutaneous leishmaniasis, *P. langeroni* was an incriminated vector for infantile visceral leishmaniasis, *P. papatasi* is the predominant species in the Nile Delta, but *P. langeroni* is found in West Alexandria and as a very rare species at the Libyan Egyptian borders.

Morsy *et al.* (1992) experimentally evaluated the capability of three species of *Phlebotomus* for development of 4 species of *Leishmania* by feeding on infective blood via a membrane feeding technique and infected vertebrate hosts. The *Phlebotomus* were *P. papatasi* (Egyptian and Indian strains), *P. dubosqi* (Sinegal strain), and *P. perniciosus* (French strain). The *Leishmania* used were *L. tropica* (Afganistanian strain) in blood and *L. major* (Egyptian strain) in blood, in volunteer patient and in hamster, *L. major* (Morocco strain) in *Meriones shawi*, *L. infantum* (French strain) in dog and *L. enrietti* (Brazilian strain) in Guinea-pig. They found that Egyptian *P. papatasi* was the most suitable species for Egyptian *L. major* in blood followed by *P. dubosqui*. But, *P. dubosqui* was the most suitable species for *L. tropica* in blood followed by Indian *P. papatasi*. The Egyptian *P. papatasi* acquired *L. major* from hamster (38.2%) and *L. enrietti* from Guinea pig (1.9%), but not *L. major* from the patient. Indian *P. papatasi* acquired *L. major* from *M. shawi* (5.6%), *L. infantum* from dog (1.09%) but not *L. enrietti* from Guinea pig. The *L. major* was more engulfed by *P. papatasi* (2 strains) from artificial source (blood) than from the vertebrate hosts.

Merdan *et al.* (1992) in North Sinai Governorate studied the seasonal abundance and sex ratio of *P. papatasi* and to search for natural infected in wild caught females. CDC miniature light traps were used for adult collections, dissection was used for demonstration of the promastigotes and biochemical typing was used for identification of isolated strain. They found that (a) the seasonal activities started in April and ended by the end of November, with a well-marked bimodal distribution in one year, (b) the sex ratio (male to female) 1:2.9 (c) 0.14% of 4208 females were naturally infected with promastigotes and typing proved to be *L. major*, zymodeme London 70.

Aboul Ela *et al.* (1993) established a base line susceptibility level for Egyptian *P. papatasi* to five insecticides. These insecticides were: BHC and DDT (chlorinated hydrocarbon), permethrin (synthetic pyrethroides),

malathion (Organophosphorus) and propoxur (carbamate). They found that laboratory bred *P. papatasi* were more susceptible to the insecticides than the wild caught ones. Insecticidal efficiency based on LC_{50} in the following descending order was propoxur, permethrin, BHC, DDT and malathion for laboratory bred flies and propoxur, permethrin, BHC, malathion then DDT for wild caught flies. Least LC_{50} was by using propoxur for both the wild caught flies (0.0014%) and laboratory bred ones (0.00043%). The least LT50 was when using propoxur for both the wild caught flies (4.8 seconds) and the laboratory bred flies (2.2 seconds).

Morsy *et al*. (1993a) used modified Counter immunoelectrophoresis for the identification of the blood meals of wild P. *papatasi* collected from different sites in Qualyobia Governorate. They used eight antisera; human, cat, dog, rat, sheep, goat, bovine and avian. Modification was to secure save of time and least amount of materials. They found that 99.52% of 832 female *P. papatasi* contained human blood, 0.12% (one female) contained only avian blood and 0.36% or three females each contained mixed blood of human together with dog blood (One), rat blood (one) or avian blood (one). *P. papatasi* is an anthropophilic insect, but in rare cases, it is zoophilic.

Morsy *et al*. (1993b) in Greater Cairo studied the seasonal abundance, nocturnal activity and breeding sites as well as other relevant behavior **of** *P. papatasi.* They found that: (a) the seasonal activity started in April and ended in November or beginning of December. (b) female outnumbered male indoors (7.4:1) and v.v. outdoors (0.14:1). (c) blood fed females were 97.7% indoors and 29.4% outdoors. (d) nocturnal activity ranged between 6 p.m. to 6 a.m. indoors and 8 p.m. to 6 a.m. outdoors. (e) immature stages of *Phlebotomus* were successfully recovered from rodent burrows and poultry sheds.

Morsy *et al.* (1993c) evaluated the residual effect of four known insecticides prepared in olive oil and sprayed on cement plastered walls was estimated against the Egyptian strain of *P. papatasi* (Scopoli). The results showed that propoxur reduced the number of sandflies to 16.5%, 24.1%, 46.4%, 53.4% & 76.7%, 75 days post treatment after an exposure time for 5, 10, 15, 20 & 30 minutes. Permethrin gave zero, 17.5%, 25.0%, 41.4% & 51.9%; malathion caused 4.3%, 10%, 26.7%, 35.9% & 49.1% while BHC caused zero, 10%, 30%, 36.3% & 48.1% for the same exposure time respectively. They concluded that propoxur had the highest residual effect and BHC had the lowest one.

El Sawaf *et al*. (1994) infected *P. papatasi* and *P. langeroni* with *L. major* and *L. infantum* by membrane feeding. Each sand fly ingested approximately 200 parasites per blood meal. Higher mortality in both sand fly species was seen with mixed infections than with a single parasite species. There was no significant difference between infections with either *L. major* or *L. infantum* in their natural vectors or experimental hosts. Infection significantly depressed the mean number of eggs laid per female.

Morsy (1996) gave a review of c.L.in Egypt. He mentioned that Leishmania is primarily characterized by existing in two stages in its life cycle, each occurs in a distinct host. The amastigote stage was found in the cytoplasm of the reticulo- endothelial cells, monocytes and other phagocytic cells of the vertebrate host. The promastigote stage was found in the gut of its insect vector. Leishmaniasis comprised several diseases of wide diversity of manifestations caused by different species of the genus Leishmania. Because of the virtual morphological identity of the organisms throughout the genus, they are classified according to the clinical conditions which they produce in man, under three main headings: (1) Cutaneous leishma-niasis (CL.), (2) Mucocutaneous leishmaniasis (MCL.), (3) Visceral leishmaniasis (VL). Generally speaking, leishmaniasis is an example of a zoonosis that reaches man through an insect vector. The great majority of the *Leishmania* species are

maintained by mammalian reservoir hosts in natural foci of infection. Rodents, dogs, wild cats, jackals, foxes, sloths, hyraxes and other carnivores are the animal reservoirs which maintain the infection in nature. The insect vectors are over 50 species of the genus *Phlebotomus* in the Old World and genus *Lutzomyia* in the New World.

Hanafi *et al*. (1998) The ability of three populations of *P. papatasi* collected from different areas of Egypt (Sinai, Aswan and Delta regions) to acquire successfully and transmit *L. major* (Sinai sandfly isolate IPAP/EG/89/SI-177) was laboratory evaluated. They found that Aswan population had significantly lower feeding rate (16.2%) than the Sinai (51.2%) and Delta (69.7%) populations ($P<0.0001$). The infection rate for the Sinai population was significantly higher (65.9%) than the rate for the Delta (52.3%; $P < 0.05$) and slightly higher than that for the Aswan (62.5%). No differences were in the intensity of *L. major* infection in the midguts of the sandflies examined from any of the three populations. When flies from each population were fed naturally on BALB/c mice infected with *L. major*, the feeding rates of the three populations showed a similar pattern to that in the membrane feeds, with the Aswan population having the lowest rate. In each of two separate trials for each population, a group of artificially infected flies was refed on uninfected BALB/c mice. Thirty-six days following exposure to the infected sandflies in the Sinai population, a leishmanial lesion was on the corner of one animal's mouth. They concluded that *P. papatasi* is a vector of L. major in Egypt.

Wahba *et al.* (1999) treated adult and immature stages of *P. papatasi* by the bacterial insecticide, *Bacillus thurnigiensis* var. *israelensis* with different concentrations mixed with fructose and glucose and assayed against the adults, while the immature stages were treated by offering larval diet contaminated by *B.t.i.* with different concentrations diluted by distilled water. *B.t.i.* induced mortality to half of the larval and pupal population at 0.26 x 10(-5) g/L. The median lethal doses of adults which were fed on contaminated surgery diet with serial dilutions of *B.t.i.* were 1.3 x 10(-2) g/L with fructose, and 3 x 10(-2) g/L with glucose after 48 and 72 hrs. Longevity period of larvae and pupae fed on contaminated larval diet showed negative correlation with bacterial concentrations except for highly concentrations. They concluded that bacterial control of adult *P. papatasi* particularly adults

Hanafi *et al*. (1999) studied the biological activities of two populations of *P. papatasi* collected from Sinai and Aswan and the effect of *L. major* on such activities were investigated under insectary conditions. They found a significant reduction in the number of eggs laid by the infected females when compared to the non-infected ones of the two populations. The immatures of the Aswan strain required a longer period to complete larval development. No significant difference was observed for the effect of L. major on the survivorship of tested populations. The mean generation time for the non-infected and infected females of Sinai and Aswan were 47.5, 49.8, 50.8, 49.5 days, respectively. Mean productivity (the number of females produced by one female) of the non-infected females of Sinai strain (18.1 female female/female) was significantly higher than Aswan strain (12.3 female female/female) while the productivity for infected females for both Sinai and Aswan strains (10.8 and 7.3 female female/female) was significantly reduced. They concluded that *L. major* affected the fertility and productivity of both populations.

Wahba (2000) studied the impact of contaminated larval food with *B. sphaericus* on some biological aspects of *P. papatasi* and also the latent effect on the fecundity of survived females from previously treated larvae. He found that fecundity of females survived from previously treated larvae, was sharply decreased. Many variations were noticed in the tissues of mid-gut, fat tissues, and Malpighian tubules, although no bacter-

aemia was detected.

Kassem *et al*. (2001) studied the potential of avermectins as environmentally safe agents for the control of the sandfly. Female *P. papatasi* and *P. langeroni* were fed either blood-meals containing laboratory-grade ivermectin or sugar-meals containing a commercial-product based on abamectin. The feeding of females of both species with generally sublethal doses (LC_{30}) of ivermectin in blood led to marked reduced survival and fecundity (i.e. No. of eggs laid/ ovipositing female). However, addition of ivermectin to the bloodmeal (or of abamectin to the sugarmeal) of the females had no statistically significant effect on the proportion of their eggs that hatched. They concluded that very small amounts of avermectin in their blood- or sugar-meals could control *P. papatasi* and *P. langeroni*, and that ivermectin reduced the fecundity of the survivors.

Elnaiem *et al*. (2005) investigated possible antigenic variation in this protein and examined genetic polymorphism of SP-15 in 100 *P. papatasi* in a natural population from Sudan & four laboratory colonies from Egypt, Jordan, Israel and Saudi Arabia. They found that many variants of SP-15 in nature, differrences among them with minimal (M+/-SD pairwise differences was 1.69+/-0.83% for forty nucleotide sequences & 3.06+/-1.13% for thirty amino acid sequence variants). They reported that a vaccine based on SP-15 protein gave a uniform immune response.

Wahba *et al*. (2005) investigated the salivary glands of *P. papatasi* and *P. langeroni* for their immunomodulatory properties on vertebrate hosts. To determine the influence of saliva homogenate (SGH) were performed ELISA using sera from injected hamsters by SGH. Frequent feeding of *P. papatasi* on hamsters was carried out to relate the anti-body titre with the biting rate. C*x. pipiens* was allowed to feed on pre-exposed hamsters to *P. papatasi* bites. Antibodies correlated with the saliva of both species showed low titre and not related either to the time (after feeding and injection) or to the number of fed flies. Saliva of *P. langeroni* (non-vector) recorded higher antibody titre than *P. papatasi.* The preliminary experiment of *Cx. pipiens* on pre-exposed hamsters on its saliva showed cross reactions between biting of mosquitoes and sand flies. They concluded that more studies were needed to use the fly salivary proteins as vaccines for leishmaniasis.

Wahba and Riera (2006) compared protein components in salivary glands of sandflies from different species and origins. The salivary gland homogenate (SGH) of laboratory reared Egyptian *P. papatasi, P. sergenti* and *P. langeroni* Sinai and El-Agamy strains respectively were compared with Spanish *P. perniciosus* and *P. ariasi* collected 20 km southwest of Barcelona. They found that 8-14 prominent proteins bands with molecular masses ranging from 8-70 kDa were visualized by Commassie blue gel code staining in each SGH and that saliva composition varied between species and sub-species, and increased between different sub-genera and geographical areas.

El-Naggar *et al.* (2006) in Suez G studied sand fly and reservoirs, and found that *P. papatasi* reached its highest density in September. The successfully colonized P. papatasi facilitated its biology and competence study. An autogenous trait was proven within *P. papatasi* population indicated its ability to survive and breed during adverse conditions. Hamsters and BALB-c mice inoculated with *L. major* developed ZCL lesions.

Kassem and Osman (2007) tested the intraspecific transmission of *Wolbachia* in crosses between infected females and uninfected males, or those between uninfected females and infected males, a PCR based on *Wolbachia* -specific wsp primers was used and, subsequently, 50 individual flies from the F(3) generation. All the individual flies tested from F(1) progeny of the crosses between infected males and uninfected females were found to be uninfected. In the crosses involving infected females and uninfected males, however, *Wolbachia* were found in

the progeny of five matings out of 23 that produced viable eggs. In the F(3), *Wolbachia* were not detected in any individuals resulted from the cross between uninfected females and infected males but they were detected in 52% (26) of 50 tested individuals resulting from cross between infected females and uninfected males. No evidence of cytoplasmic incompatibility (CI) was in any of the crosses. They concluded that absence of CI expression and relatively low frequencies of maternal transmission could hamper the potential use of *Wolbachia* in a transgenic strategy for the control of leishmaniases.

Hanafi *et al.* (2007) conducted a longitudinal survey for sandflies from 1989 to 1991 at a focus of ZCL in Northeast Sinai, within the border region monitored by multinational peace-keepers. They found that *P. papatasi* was the only anthropophagic species, which comprised more than 94% of the sandfly population, with two population peaks (May, July) in both survey years. Density of *P. papatasi* in underground bunkers was higher than outside but inflated by a greater proportion of male flies. During 1990, the proportion of gravid P. papatasi increased progressively during the 5 months period from May to September and averaged 29.5% and 29.7% for interior and exterior collections, respectively. Density of *P. papatasi* was greater during 1991, but proportions of gravid flies were significantly lower in each survey month and averaged 14.9% & 12.3% for interior and exterior collections, respectively. Seasonal rates of *Leishmania*-infected *P. papatasi* averaged 0.8% and 0.9% in 1989 and 1990, but fell to zero in 1991, suggested an unstable focus of *L. major* transmission. Proportions of gravid flies were a valid indicator of the physiological age and epidemiologic importance of the vector sandfly population. The strong correlation of sticky trap indices to human-landing/biting rates shows that this is an accurate, inexpensive, and no-risk alternative to human bait collections.

Abdel-Hamid (2007) studied the effect of blood of human, Guinea pig and hamster on the different biological aspects of *P. papatasi* under laboratory conditions of 28 +/- 2 degrees C, 75 +/- 5% RH & a 14:10 hr. (L:D) photoperiod regime was evaluated. He found that man was the most preferable host followed by hamster, and then Guinea pig. Human blood gave the highest yield of eggs (mean: 65.15 eggs/female), highest hatchability (mean: 96.23%), shortest egg incubation period (mean: 7.37 days), shortest larval duration (mean: 22.59 days), shortest adult emergence period (mean: 39.73 days) and highest productivity of the adult progeny (mean; 81.75%).

Hamadto *et al.* (2007) studied the status of ZCL in North Sinai Governorate, the reservoir host(s) and insect vector(s) in Sinai. They found six species of 50rodents trapped from areas or nearby areas where human ZCL cases were *Mus musculus* (10), *Rattus r. alexandrinus* (18), *R. norvegicus* (2), *Gerbillus gerbillus* (4), *G. pyramidum* (12) and *Jaculus jaculus* (4). One *G. pyramidumn* had natural infection with *L. major* as indicated by smears and culture. The spot light surveys carried out by the sticky paper traps and the CDC light traps in four centers; Al Hassanah, Nakhil, Al Arish, and Bir Al-Abd yielded 1320 sandflies. They were *P. papatasi* (1150) and *P. sergenti* (170) in a ratio of 7:1. A total of three isolates of zymodeme London 70 undistinguished from the formerly obtained human and rodent isolates were enzymatically identified in *P. papatasi.*

Hoel *et al.* (2007) used modified CDC light traps with light-emitting diodes (LED) and compared against a control trap (incandescent light) to determine the effectiveness of blue, green, and red lights against standard incandescent light routinely used for sand fly surveillance. Light traps were baited with dry ice and rotated through a 4 x 4 Latin square design during May, June, and July, 2006. Trapping over 12 trap nights yielded a total of 2,298 sand flies in the village of Bahrif, 6 km north of Aswan on the east bank of the Nile River. *P. papatasi*

comprised 94.4% of trap collections with five other species collected in small numbers. Over half (55.13%) of all sand flies were collected from red light traps and significantly more sand flies ($P < 0.05$) were collected from red light traps than from blue, green, or incandescent light traps. Red light traps collected more than twice as many sand flies as control (incandescent) traps and > 4 x more than blue and green light traps. Results indicate that LED red light is a more effective substitute for standard incandescent light when surveying in areas where *P. papatasi* was predominant species. Each LED used about 15% of energy that a standard CDC lamp consumes, extending battery life and effective operating time of traps.

Hogsette *et al*. (2008) in the village of Bahrif in Aswan studied diurnal resting sites of adult phlebotomine sand flies by aspiration from low (30-45 cm high) irregular piles of mud bricks found under high date palm canopies between the village and the Nile River. There were 5 males and 7 females of *P. papatasi* and 3 males of *Sergentomyia schwetzi*. Six of the 7 females were engorged with blood. A total of 78 sand flies were captured on 3 glue boards placed overnight on the ground next to the mud bricks. Attempts to aspirate sand flies from adjacent walls and plants were unsuccessful. The identification of diurnal resting sites in less structured habitats might ultimately lead to more effective adult sand fly control.

Shehata *et al*. (2009) reported the first isolation of *Leishmania tropica* from human cases of CL in a Northern Sinai community bordering Palestine. Parasite culturing, real-time PCR, gene sequencing, and restriction fragment length polymerphism (RFLP) analyses indicate CL cases in this community were caused by either *L. major* or *L. tropica* (three cases each). Two wild-caught rodents (*Gerbillus pyramidum floweri*) were infected with *L. tropica. P. papatasi* were harbored *L. major*, however only non-infected individuals of *P. sergenti*, a vector for *L. tropica*, were caught. *L. tropica* patients did not travel from the region in over a year, might suggest these cases to be autochthonous. This scenario is consistent with an incursion of *L. tropica* from bordering countries and raises concerns about expansion of this parasite further into Egypt.

Kassem *et al*. (2009) studied sandflies in two villages; Kafr-Tahla (Qalyubiya) and el Quantara el Beida (Kafr el-Sheikh) from September 2003 to August 2005. A total of 9529 were all *P. papatasi*. Sand fly activity started from April to December with a bimodal annual pattern, with significantly male biased sex. Their densities were strongly correlated to tempe-rature but not to relative humidity or wind velocity. Variation in the densities of *P. papatasi* in both villages did not show a significant effect due to lunar phases. However, sand fly activity was highly positively correlated to fraction illumination.

Darwish *et al*. (2011) studied the vectorial competence of *P. papatasi* for two old world species, *L. major* and *L. tropica*. *P. papatasi* collected from Suez Governorate, were membrane fed on homogenized hamster's lesion infected with *L. major*, MHOM/EG/06/RTC-63, and *L. tropica,* MGER/EG/06/RTC-74 identified from patients with suspected CL in Northern Sinai, Egypt. They concluded that transmission by bites in case of *P. papatasi/L. tropica* failed. A characteristic *L. major* lesion was developed on the foot pads region 120 days post infective bites on healthy hamster. They concluded that *P. papatasi* is a much more effective vector for *L. major* than for *L. tropica.*

Obenauer *et al*. (2011) described a simple, economic, and effective method for constructing sticky bottle traps that can be used to capture adult sand flies from rodent burrows. Although sand fly surveillance activities often employ light- or CO2-baited traps, sticky papers secured to a post or placed on the ground can also be used. However, in arid environments, sand and other debris often collect on the sticky surface, reducing

trap effectiveness, capacity, and a means for rapid discrimination and enumeration of adult specimens.

Hoel *et al.* (2011) in Egypt baited CDC light traps were with carbon dioxide (CO2) produced from three different sources to compare the efficacy of each in collecting phlebotomine sand flies in Bahrif village, Aswan Governorate, Egypt. Treatments consisted of compressed CO2 gas released at a rate of 250 ml/min, 1.5 kg of dry ice (replaced daily) sublimating from an insulated plastic container, CO2 gas produced from a prototype FASTGAS (FG) CO2 generator system (APTIV Inc., Portland, OR), and a CDC light trap without a CO2 source. Carbon dioxide was released above each treatment trap's catch opening. Traps were placed in a 4 x 4 Latin square designed study with three replications completed after four consecutive nights in August 2007. During the study, 1,842 phlebotomine sand flies were collected from two genera and five species. Traps collected 1,739 (94.4%) *Phlebotomus papatasi* (Scopoli), 19 (1.0%) *P. sergenti*, 64 (3.5%) *Sergentomyia schwatzi,* 16 (0.9%) *Sergentomyia palestinensis*, and four (0.2%) *S. tiberiadis*. Overall treatment results were dry ice (541) > FG (504) > compressed gas (454) > no CO2 (343). Total catches of *P. papatasi* were not significantly different between treatments, although CO2-baited traps collected 23-34% more sand flies than the unbaited (control) trap. They found that the traps baited with a prototype CO2 generator were as attractive as traps supplied with CO2 sources traditionally used in sand fly surveillance efforts. The field-deployable CO2 generators were particularly advantageous in remote areas where dry ice or compressed gas was difficult to obtain.

Hanafi *et al*. (2011) injected hamsters subcutaneously with two standard IVM treatments (200 and 400µg/kg body weight) and allowed cohorts of *L. major*-infected *P. papatasi* to blood-feed on these animals at various post-treatment time points (4 h, 1, 2, 6, & 10 days). Infected and uninfected sand flies were treated and untreated hamsters served as controls. Serum levels of IVM in low- and high-dose-treated hamsters were determined at the five time points. Sand fly mortality following blood feeding was recorded at 24-h intervals and, in relation to IVM treatment, was time and dose dependent. Mortality was most rapid and greatest among infected flies that fed nearest to time of dosing. Mean survival of infected sand flies after feeding on untreated hamsters was 11.5 days, whereas that of infected sand flies that fed 4 h, 1 day, or 2 days post-treatment on high-dose-treated hamsters (400µg/kg) was 1.6, 2.1, and 2.7 days, respectively. Infected and uninfected sand flies that blood fed 6 days following low-dose IVM treatment (200µg/kg) still experienced significantly greater mortality ($p < 0.02$) than controls. Promastigotes dissected out of surviving flies that fed on IVM-treated hamsters showed typical motility and survival. Moreover, 21.7% of IVM-treated hamsters developed lesions after being fed on infected sand flies. *L. major* promastigotes appeared to be tolerant to ng/mL blood levels of IVM that caused significant mortality for up to 10 days post-treatment in blood-feeding *P. papatasi.*Abdel-Hamid (2012) maintained *P. papatasi* females on different diets (30% sucrose solution, Guinea pig blood and sucrose followed by blood) under laboratory conditions for offspring emergence to examine the survival period expressed as the median emergence time (E50) and female fecundity (females/female). Life table was constructed including the mean life expectancy at emergence (e0) as a measure of longevity and the mortality rate per day (qx). He found that the females fed on sucrose-blood had the highest fecundity and the shortest E50 compared to those fed on other diets. The mean life expectancy at emergence (e0) differed significantly with the highest value being for females fed on sucrose. He concluded that calculated expectancies for female life beyond the infective

age (8 days) indicated that more flies would survive to become infective when fed on sucrose-blood meals than those offered blood alone which increases its capability for *Leishmania* transmission.

Shoukry and Morsy (20110 in a preliminary survey in Toshka District reported *Psammomyes obesus* Cretzchmar, 1828 the main reservoir of ZCL and *Phlebotomus papatasii* (Scopoli), but did not found human cases of ZCL. El-Shazly *et al*. (2012) found that the monthly density of the sand fly, *P. papatasi* was monitored during 2009 at Burg El-Arab, a rural district located close to the Mediterranean coast of Egypt. The annual generations and the efficacy of microbial control by the entomopathogenic fungus, *Metrahizium anisopliae* were determined in the laboratory under atmospheric conditions, simulating those of the animal shelters in the study area. They used two collecting techniques; CDC light traps and oiled paper traps, to quantify sand fly density inside houses and in the open field. Adult flies exhibited a seasonal range from April to December. The seasonal pattern was bimodal, with one peak in July and the second one in October and that a significant role of temperature and relative humidity in the monthly abundance of the sand flies in the study area. *P. papatasi* colony completed seven annual generations under semifield conditions, but the mean developmental time of each immature stage and the mean total duration of development from egg to adult for each generation varied according to the prevailing temperature. The longest generation time was observed in winter (M ± SD was 118±11.70 d), and the shortest one occurred at the highest temperatures in summer (M±SD was 25.21±2.04 d). In microbial control studies, the entomopatho-genic fungus, *M. anisopliae*, was used at 15×10(8) spores/g food as a standard dose against the second-instar larvae of *P. papatasi* at the different seasons during 2009. Mortality reached 100% in winter and decreased to 56.0% as prevailing temperature increased during the summer season.

Kassem *et al*. (2012) reported that Southern Sinai was characterized by a diverse sand fly fauna (eight *Phlebotomus* species), probably attributable to highly variable landscape and environmental factors. *Phlebotomus alexandri, P. kazeruni* and *P. sergenti* were widespread and abundant, *P. papatasi* and *P. bergeroti* were less frequent, and *P. arabicus, P. major* and *P. orientalis* had highly restricted distributions. Logistic regression models indicated that elevation and climatic conditions were limiting determinants for the distributions of sand flies in southern Sinai. Based on the predicted distribution of *P. papatasi*, a recognized vector of L. major, about one-quarter of southern Sinai may be at high risk of ZCL. Risk areas for the suspected ZCL vector *P. bergeroti* had a more patchy distribution. Results suggest that future studies should include other factors related to vector abundance, vector competence, human population, and parasite and reservoir host(s) to produce more comprehensive ZCL transmission risk maps, thus helping in planning effective prevention and control strategies.

Zayed *et al.* (2013a) evaluated susceptibility of *P. papatasi* Scopoli (Diptera: Psychodidae) larvae to the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Ma79) (Hypocreales: Clavicipitaceae) at two different temperatures. They found that fungus reduced the adult emergence at 26 +/- 1 degrees C when applied to larval diet. Six spore concentrations were used in the bioassays ranging from 1 x 10(6) to 5 x 10(8) spores/ ml. Mortality decreased significantly at temperature of 31 +/- 1 degrees C at all tested concentrations. Fungus-treated vials were assayed against sand fly larvae at different time lapses without additional reapplication of the fungus in the media to determine whether the level of inocula persisting in the media was sufficient to re-infect healthy sand flies. Twenty weeks post-application, there were still enough infectious propagules of Ma79 to infect 40%

of *P. papatasi* larvae. A comparison between the infectivity of 10 subsequent in vitro cultures and the host-passed inocula of the fungus against sand fly larvae was conducted. Mortalities of *P. papatasi* larvae changed significantly when exposed to inocula passed through different insects. They concluded that *M. anisopliae* as an effective control agent against the main cutaneous leishmaniasis old-world vector *P. papatasi*. Zayed *et al.* (2013b) evaluated the toxicity and duration of three residual insecticides against the Old World sand fly, *P. papatasi*, on 2 types of tent material used by the US military in Afghanistan and the Middle East. Vinyl and cotton duck tent surfaces were treated at maximum labeled rates of lambda-cyhalothrin, bifenthrin and permethrin (Insect Repellent, Clothing Application, 40%), then subsequently stored in indoor, shaded spaces at room temperature (60%-70% relative humidity (RH), 22°C-25°C), and under sunlight and ambient air temperatures outdoors (20%-30% RH, 29°C-44°C). They found that Lambda-cyhalothrin treated cotton duck tent material stored indoors killed *P. papatasi* for 8 months, while the complementary sun-exposed cotton duck material killed adult flies for 1 month before the efficacy dropped to less than 80%. Sand fly mortality on permethrin- and bifenthrin-treated cotton duck decreased below 80% after 2 weeks exposure to sunlight. Shade-stored permethrin and bifenthrin cotton duck material killed more than 80% of test flies through 5 months before mortality rates decreased substantially. They concluded that Vinyl tent material provided limited control (less than 50% mortality) for less than one month with all treatment and storage regimes. Lambda-cyhalothrin-treated cotton duck tent material provided the longest control and produced the highest overall mortalities (100%) for 8 months (shaded), more than (90%) for 1 month (sunlight-exposed) of both cotton duck and vinyl tents.

Hanafi *et al.* (2013) mentioned that *Pachyuromys duprasi* is a common burrowing rodent found across the northern Sahara Desert from Morocco to Egypt, with overlap in its geographical distribution and ecological habitats, several Old World *Leishmania* species, and numerous sand fly vectors. They experimentally found that Captive-born *P. duprasi* inoculated subcutaneously (s.c.) in the tail with promastigotes or amastigotes of *L. major* Egyptian strain and monitored for signs of infection. They found that tissue density of amastigotes in the gerbil's tail lesions after inoculating with either stage of *L. major* was significantly lower than that produced in the footpads of BALB/c mice by the same parasite and incubation period. *L. major* to *P. duprasi* by sand fly bite was demonstrated and acquisition of *L. major*, by bite, from tail lesions of infected *P. duprasi* to laboratory-reared *P. papatasi* was also achieved with 10% of biting flies developing promastigote infections. They concluded that the susceptibility of *P. duprasi* to *L. major* delivered at low densities by sand fly bites indicate that fat-tailed gerbils could serve as a natural host and reservoir of *L. major*.

Morsy (2013) stated that only the cutaneous form leishmaniasis is a self-curing, which might develop a certain degree of immunity against the parasite, resulting in healing of the lesion(s). However, the parasites probably never disappear completely, since in situations where immune system is compromised, as in AIDS, or suppressed by cancer chemotherapy or in organ transplantation, *Leishmania* spp. may suddenly reappear. The cell-mediated immunity is responsible for skin lesion healing but humeral response played a protective role against the disease. He added that cutaneous leishmaniasis especially ZCL in the hot dry areas pave the way to the mutation and the development of skin cancer.

Samy *et al.* (2014) identified six sandfly species collected from different districts in North Sinai: *P. papatasi, P. kazeruni, P. sergenti, P. alexandri, Sergentomyia antennata* and *S. clydei. Leishmania* (-)-like flagellates

were identified in 15 *P. papatasi* individuals (0.5% of 3,008 dissected females). Rodent populations were sampled were: *Rattus norvegicus* (n=39), *R. r. frugivorous* (n=13), *R. r. alexandrinus* (n=4), *Gerbillus pyramidum floweri* (n=38), *G. andersoni* (n=28), *Mus musculus* (n=5), *Meriones sacramenti* (n=22) and *M. crassus* (n=10). Thirty-two rodents were found positive for *Leishmania* infection (20.12% of 159 examined rodents). Only *L. major* was isolated and identified in 100% of the parasite samples. The diversity of both the vector and rodent populations was examined using diversity indices and clustering approaches.

Samy *et al*. (2014a) in North Sinai carried out a comprehensive study over seven years (January 2005-December 2011) to track ZCL transmission with respect to vectors and animal reservoirs by using diversity indices and clustering approaches. They identified *P. papatasi, P. kazeruni, P. sergenti, P. alexandri, Sergentomyia antennata* and *S. clydei. Leishmania* (-)-like flagellates were identified in 15 *P. papatasi* individuals (0.5% of 3,008 dissected females). Rodent populations were sampled in the same districts where sandflies were collected and eight species were identified: *Rattus norvegicus*, *Rattus r. frugivorous, Rattus r. alexandrinus, Gerbillus pyramidum floweri, G. andersoni, Mus musculus, Meriones sacramenti* and *M. crassus*. Thirty-two rodents were positive for *Leishmania* infection. Only *L. major* was isolated and identified in 100% of the parasite samples.

Samy *et al.* (2014b) evaluated coarse-resolution aspects of the ecology of leishmaniasis transmission, by collecting records for sandflies and *Leishmania* species were obtained from diverse sources and characterized environmental variation across the country, they used multitemporal Land Surface Temperature (LST) and Normalized Difference Vegetation Index (NDVI) data from the Moderate Resolution Imaging Spectroradiometer for 2005-2011. They found niche similarity only between one vector species and its corresponding parasite species (*P. papatasi* with *L. major*), suggesting that geographic ranges of ZCL and its potential vector may overlap, but under distinct environmental associations. Other associations (*P. sergenti* with *L. major*) were not supported. Mapping suitable areas for each species suggested that northeastern Egypt is particularly at risk because both parasites have potential to circulate. They concluded that the ecological niche modeling approaches could constraints on the geography of transmission patterns of leishmaniasis.

Kaldas *et al.* (2014) investigated the effect of *Ricinus communis* (Euphorbiaceae) and *Bougainvillea glabra* (Nyctaginaceae), on transmission of leishmaniasis using them as diets for *P. papatasi* to monitor their effect on life-history traits. *P. papatasi* were allowed to feed separately on both plants then offered a blood-meal. Fed-females were observed daily for egg-laying and subsequent developmental stages. They found that feeding on these plants not only decreased sand fly survival rates but incurred negative effects on fecundity. Findings indicate that planting high densities of *R. communis* and *B. glabra* in sand flies-endemic areas would reduce population sizes and reduce the risk of *L. major* infections.

Palacios *et al.* (2014) profiled the immunoreactivities of plasma antibodies to sand fly salivary gland sonicates (SGSs) from 229 human blood donors residing in different regions of sand fly endemicity throughout Jordan and Egypt as well as 69 US military personnel, who were differentially exposed to *P. papatasi* bites and *L. major* infections in Iraq. Compared with plasma from control region donors, antibodies were significantly immunoreactive to five salivary proteins (12, 26, 30, 38, & 44 kDa) among Jordanian and Egyptian donors, with immunoglobulin G4 being the dominant anti-SGS isotype. US personnel were significantly immunoreactive to only two salivary proteins (38 & 14 kDa). Using k-means clustering, donors were segregated into four clusters distin-

guished by unique immunoreactivity profiles to varying combinations of the significantly immunogenic salivary proteins. They found that SGS-induced cellular proliferation was diminished among donors residing in sand fly-endemic regions.

Geraci *et al*. (2014) profiled the immunoreactivities of plasma antibodies to sand fly salivary gland sonicates (SGSs) from 229 human blood donors residing in different sand fly endemic regions throughout Jordan and Egypt as well as 69 US military personnel, who were differentially exposed to *P. papatasi* bites and *L. major* infections in Iraq. They compared with plasma from control region donors, antibodies were significantly immunoreactive to five salivary proteins (12, 26, 30, 38, &44 kDa) among Jordanian and Egyptian blood donors, with IgG4 being the dominant anti-SGS isotype. US personnel were significantly immunoreactive to only two salivary proteins (38 & 14 kDa). Using k-means clustering, donors were segregated into four clusters distinguished by unique immunoreactivity profiles to varying combinations of the significantly immunogenic salivary proteins. SGS-induced cellular proliferation was diminished among donors residing in sand fly-endemic regions. They concluded that these data gave a clearer picture of human immune responses to sand fly vector salivary constituents.

On the other hand, Aoun and Bouratbine (2014) mentioned that in North African countries, cutaneous leishmaniasis transmission has been increasing since the 1980s, with a significant increase in the incidence of cases and a spread of the geographical distribution and currently represents a major public health problem with a productivity gap and an impediment for development, with dramatic socioeconomic and psycho-sanitary impacts. They added that in Egypt, only a few dozen cases per year are reported, mainly in the Sinai Peninsula. Three *Leishmania* species, associated with distinct eco-epidemiological and clinical patterns, are involved, namely *L. infantum*, *L. major*, and *L. tropica*. However, *L. major* was commonest in Algeria, Libya, and Tunisia, with more than 90% of the registered cases. It is mainly encountered in rural areas under semi-arid, arid and Saharan climates. *L. tropica* is more prevalent in Morocco, reaching 30-40% of isolates in some districts. They concluded that much data was still missing concerning the risk factors of the infection and the lesion development, as well as vector and reservoir ecology and behavior.

Sandflies in Egypt nearby countries:

Abdel-Dayem *et al.* (2012) In Libya stated that cases of cutaneous leishmaniasis vectored by *P. papatasi* increased. Besides, Obenauer *et al.* (2012) in the coastal towns of northwestern Libya where ZCL was endemic; found *P. papatasi* and P. *longicuspis*.

Khalil *et al*. (2012) in Sudan stated that ZCL and *P. papatasi* were common. They isolated *L. donovani* parasites from CL lesions of some patients who contracted the disease in Khartoum State. Also, Hassan *et al.* (2012) reported that *P. papatasi* vector of ZCL were most widely spread in Sudan. They added that malathion and propoxur resistance in the sand fly population probably resulted from anti-malarial control activities carried out during the past 50 years.

Morsy and el Ajlouny (1984) in Jordan reported *P. papatasi* and CL. Also, Saliba *et al.* (1997) reported the endemicity ACL caused by *L. tropica* in a focus of the disease around Eira and Yarqa in Salt District, Jordan.

Sawalha *et al*. (2003) in the Palestinian West Bank reported *P. papasati* and ZCL. Also, Hamarsheh and Amro (2011) in Jerusalem, Palestine reported great number of *P. papatasi.* Faiman *et al.* (2013) for the first reported implicating *Microtus guentheri* and *Meriones tristrami* as reservoirs of ZCL. They added that the widespread co-distribution of *M. guentheri* and *P. papatasi,* suggested a significant threat from the spread of ZCL in the Middle East, central Asia and southern Europe.

Maroli *et al*. (2009) in Syria identified three foci of anthroponotic cutaneous leishmaniasis (ACL) in Aleppo Governorate. The common sandflies were *P. papatasi* (68%); *P. sergenti* (25.4%); *Sergentomyia minuta* (6.4%); *P. tobbi* (0.1%), and *P. mascittii canaaniticus* (0.1%). They added that *P. sergenti* were caught indoors (246 specimens) and outdoors (222), whereas *P. papatasi* was significantly more abundant indoors (1096 specimens) than outdoors (156). They tested the blood preference and found that *P. sergenti* was an opportunistic feeder, imbibing human, ovine, avian, bovine and feline blood, although more blood-meals were taken from humans and cattle than expected in relation to the relative proportions of potential hosts present.

Morsy and, Shoura (1976) identified *P. papatai* and foci of ZCL in Riyadh district. Sebai and Morsy (1976) reported P. papatasi abundant in an endemic ZCL in Bisha To wn, south-western Saudi Arabia. Killick-Kendrick *et al*. (1985) identified a focus of ZCL in the Western Saudi Arabia and incriminated *P. papatasi* as the main vector. Morsy (1988) in Saudi Arabia identified about 22 species of *Phlebotomus* and *Sergentomyia* and added that all clinical forms of leishmaniasis were encountered. El-Beshbishy *et al*. (2013) stated that CL, which is caused by various species of the genus *Leishmania* was considered a major health problem in different areas of Saudi Arabia including Al-Madinah Al-Munawarah Province. They added that *P. papatasi* and *P. sergenti* and other species were abundant. By the molecular characterization they concluded that the semi-nested PCR method against kDNA and the ITS1-PCR-RFLP analysis are useful tools for molecular identification of both *L. major* and *L. tropica.*

Prevention and Control of sand-fly:

Most adult sand fly control techniques are the following: 1- Residual sprays: residual spraying of houses and animal-shelters is probably the most useful and the most utilized. The application of long-lasting insecticides as DDT and malathion exhibited less sand fly-specific toxicity than did the pyrethroid insecticides as resmethrin and cyfluthrin to surfaces, often interior walls, to either kill or repel biting insects (Orshan *et al*, 2006), 2- Space sprays: the dispersal of an insecticide in droplets or smoke to kill insects in the treated space, leaving little residual effect, 3- Barriers and treated netting/ clothing: Preventing sand flies from entering a cantonment inside the treated area, 4-Use topical repellents, and 5-Applications of insecticides and rodenticides in reservoir burrows.

The CDC (2013) mentioned that there were neither vaccines nor specific drugs to prevent infection. The best way for travelers to prevent infection is to protect themselves from sand fly bites. CDC added that to decrease the risk of being bitten, follow these preventive measures: Avoid outdoor activities, especially from dusk to dawn, when sand flies generally are the most active.

I- When outdoors (or in unprotected quarters): 1-Minimize the amount of exposed (uncovered) skin. To the extent that is tolerable in the climate, wear long-sleeved shirts, long pants, and socks; and tuck your shirt into your pants. As the Bed nets, repellents, and insecticides should be purchased before traveling and can be found in hardware, camping, and military surplus stores. Bed nets and clothing that already have been treated with a pyrethroid-containing insecticide also are commercially available. 2- Apply insect repellent to exposed skin and under the ends of sleeves and pant legs. Follow the instructions on the label of the repellent. The most effective repellents generally are those that contain the chemical DEET (N,N-diethylmetatoluamide). II- When indoors: 1- Stay in well-screened or air-conditioned areas. 2- Keep in mind that sand flies are much smaller than mosquitoes and therefore can get through smaller holes. 3- Spray living/ sleeping areas with an insecticide to kill insects. 3- If you are not sleeping in a well-screened or air-conditioned area, use a bed

net and tuck it under your mattress. If possible, use a bed net that has been soaked in or sprayed with a pyrethroid-containing insecticide. The same treatment can be applied to screens, curtains, sheets, and clothing (clothing should be retreated after five washings). González *et al*. (2015) concluded that using insecticides to reduce sandfly numbers was effective at reducing CL incidence in some settings. However, there was insufficient evidence to know whether it was better to spray the internal walls of houses or to use insecticide impregnated bed-nets, curtains, bed-sheets or clothing. No evidence that these measures being effective or not in reducing incidence of VL. Policy decisions must consider local sand-fly epidemiology and behavior, and diversity of transmission scenarios (including vector and animal or human reservoirs) when designing and applying leishmaniasis control programs.

On the other hand, Warburg (1991) experimentally determined susceptibility of different *P. papatasi* strains to a cytoplasmic polyhedrosis virus (CPV) by feeding polyhedra to larvae. He found that Indian *P. papatasi*, 15.6% were infected, but Egyptian ones were refractory. Infect- ion rates were not augmented in the Jordanian Valley flies, 23.8% of which were naturally infected with CPV. With *Serratia marcescens* and *Beauvaria bassiana* infectivity to *P. papatasi*, a suspension of *B. bassiana* spores or *S. marcescens* bacteria, ingested by *P. papatasi* in sucrose solution, gave no significant augment mortality rates or reduce eggs oviposited number. *B. bassiana* spores smeared on a filter paper constituting 1 or 5% of surface area induced 100% mortality of *P. papatasi* on 5^{th} & 4^{th} days respectively. All *L. longipalpis* died on 4^{th} day, with markedly lower mortality rates in control groups and more eggs were produced by *P. papatasi*: Colombian *Lutzomyia* spp., a nonfluorescent pseudomonas, an *Entomophthorales* fungus, and a Trypanosomatid (*Leptomonas*?) were isolated in culture media. *Ascogregarina saraviae*, *Tylenchida* and *Spirurida* were recorded. Opportunistic aggregates of bacteria, yeast, and fungi on the tarsi of colonized *L. longipalpis* and *P. papatasi* hindered their mobility and reduced colony vigor. *Aspergillus flavus, B. bassiana,* and *S. marcescens* were isolated from laboratory-bred *P. papatasi* adults.

Hurwitz *et al*. (2011) in India demonstrated the paratransgenic manipulation of the sand fly vector, *P. argentipes*, under laboratory conditions. They added that the use of an environmental commensal bacterium for delivery of foreign genes to developmental stages of the sand fly served as a platform to consider paratransgenic approaches in field conditions as a tool to control vectorial transmission of *L. donovani*. They found that the anti-microbial peptide, mellitin, exerted potent activity against promastigote forms of *L. donovani* at micro-molar concentrations and that *L. donovani* underwent transformation from amastigotes to dividing and infectious promastigotes at the midgut. The targeted delivery of leishmaniacidal molecules by a commensal bacterium within this region of the adult sand fly disrupted this developmental transition, thereby generating paratransgenic sand flies refractory to *L. donovani* infection. They concluded that this approach proved highly advantageous in decreasing the visceral leishmaniasis burden.

Telleria *et al.* (2013) reported that *L. longipalpis* modulates defensin expression upon bacterial and *Leishmania* infection, with patterns of expression that are distinct among bacterial species and routes of infection. Also, Heerman *et al*. (2015) stated the midgut microbial community in insect vectors of disease proved crucial for an effective immune response against infection with various human and animal pathogens. They added that that during development, the sand flies harbor a wide variety of Gram-negative and Gram-positive bacteria, acquired as larval stages from the soil, and the abundance and distribution of these microorganisms varied depending on the sand fly species or

the breeding site. They assessed the distribution of two bacteria commonly found within its gut, *Pantoea agglomerans* and *Bacillus subtilis*, which were able to differentially infect the larval digestive tract, and regulate the immune response in sand fly larvae. Besides, bacterial distribution, and likely the ability to colonize the gut, is driven, at least in part, by a gradient of pH present in gut.

Conclusion

All the reviewed data given here within proved that the *Phlebotomus papatasi* is the main vector of zoonotic cutaneous leishmaniasis caused by *Leishmania major* not only in Egypt but also in all the nearby countries.

References

Abdel-Dayem, MS, Annajar, BB, Hanafi, HA, Obenauer, PJ, 2012: The potential distribution of *Phlebotomus papatasi* (Diptera: Psychodidae) in Libya based on ecological niche model. J. Med. Entomol. 49, 3:739-45.

Abdel-Hamid, RM, 2007: The effect of the host blood on the biology of the sand fly, *Phlebotomus papatasi* (Diptera: Psychodidae) under laboratory conditions. J. Egypt. Soc, Parasitol. 37, 3:1091-100.

Abdel-Hamid, YM, 2012: Life table characteristics of the female sandfly, *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae) under three food regimes. J. Vector Borne Dis. 49, 4: 226-9.

Abi Abdallah, DS, Pavinski Bitar, A, Oliveira, F, Meneses, C, Park, J, *et al*, 2014: A *Listeria monocytogenes*-based vaccine that secretes sand fly salivary protein LJM11 confers long-term protection against vector-transmitted *Leishmania major*. Infect. Immun. 82, 7:2736-45.

Aboul Ela, RG, Morsy, TA, el-Gozamy, BM, Ragheb, DA, 1993: The susceptibility of the Egyptian *Phlebotomus papatasi* to five insecticides. J. Egypt. Soc. Parasitol. 23, 1:69-94.

Abou El Ela, RGh, Morsy, TA, Rifaat, MMA, Al Dakhil, MA, 1995: Morphological studies on *Phlebotomus papatasi* (Scopoli) in Riyadh City, Saudi Arabia. J. Egypt. Soc. Parasitol. 25, 3: 631-48.

Aoun, K, Bouratbine, A, 2014: Cutaneous Leishmaniasis in North Africa: a review. Parasite 21:14. doi: 10.1051/parasite/2014014

CDC, 2013: Leishmaniasis: Prevention and Control. www.cdc.gov/parasites/leishmaniasis/prevent. html

Daba, S, Mansour, NS, Youssef, FG, Shanbaky, NM, Shehata, MG, El Sawaf, BM, 1997: Vector-host-parasite relationships in leishmaniasis. III. Impact of blood meal from natural vertebrate hosts on the survival and development of *L. infantum* and *L. major* in *P. langeroni* (Diptera: Psychodidae). J. Egypt. Soc. Parasitol. 27, 3:781-94.

Darwish, AB, Tewfick, MK, Doha, SA, Abo-Ghalia, AH, Soliman, BA, 2011: Vectorial competence of *Phlebotomus papatasi* (Diptera: Psychodidae) to transmit two old world *Leishmania* species: *Leishmania major* and *L. tropica*. J. Egypt. Soc. Parasitol. 41, 3:565-72.

De Almeida, MC, Vilhena, V, Barral, A, Barral-Netto, M, 2003: Leishmanial infection: analysis of its first steps: A review. Mem. Inst. Oswaldo Cruz 98, 7:861-70.

Doha, S, Shehata, MG, 1992: *Leishmania infantum* MON-98 isolated from naturally infected *P. lan-geroni* (Diptera: Psychodidae) in El Agamy. Egypt. J. Med. Entomol. 1:417-25.

El-Beshbishy, HA, Al-Ali, KH, El-Badry, AA, 2013: Molecular characterization of *Leishmania* infection in sand flies from Al-madinah Al-munawarah province, western Saudi Arabia. Exp. Parasitol. 134, 2:211-5.

El Hossary, SS, Shehata, MG, Helmy, N, Lane, RP, El Sawaf, BM, 2000: Studies on the phle-botomine sand flies (Diptera: Psychodidae) and rodents in cutaneous leishmaniasis focus in Nekhel, North Sinai, Egypt. J. Union Arab. Biol. Zool. 13, A:53-64.

El Okbi, LM, Morsy, TA, Khalid, MLM, Salama, MMI, Bebars, M.A, *et al*, 1989: Some aspects of sandflies of the genus *Phlebotomus* in El Agamy, Alexandria, Egypt. J. Egypt. Soc. Parasitol. 19, 2: 437-46.

El Sattar, S, Shehata, M, El Sawaf, B, 1991: The development of *L. infantum* in *P. langeroni nitzulescu* (Diptera: Psychodidae). Parassitologia 33:199-204.

El Sawaf, BM, Beier, JC, Hussein, SM, Kassem, HA, Sattar, SA, 1984: *Phlebotomus langeroni*: a potential vector of Kala Azar in the Arab Republic of Egypt. Trans. R. Soc. Trop. Med. Hyg. 78:421-9.

El Sawaf, BM, el Sattar, SA, Shehata, MG, Lane, RP, Morsy, TA, 1994: Reduced long-

evity and fecundity in *Leishmania*-infected sand flies. Am. J. Trop. Med. Hyg. 51, 6:767-70.

El-Naggar, MH, Shoukry, NM, Soliman, BA, Darwish, AB, El-Sawaf, BM, 2006: Ecology, biology and susceptibility of *P. papatasi* to *Leishmania* experimental infection in Suez Governorate. J. Egypt. Soc. Parasitol. 36, 1:127-38.

Elnaiem, DE, Meneses, C, Slotman, M, Lanzaro, GC, 2005: Genetic variation in the sand fly Salivary protein, SP-15, a potential vaccine candidate against *Leishmania major*. Insect Mol. Biol. 14, 2: 145-50.

El-Shazly, MM, Soliman, MM, Zayed, A, 2012: Seasonal abundance, number of annual genera- tions, and effect of an entomopathogenic fungus on *Phlebotomus papatasi* (Diptera: Psychodidae). En-viron. Entomol. 41, 1:11-9.

Faiman, R, Abbasi, I, Jaffe, C, Motro, Y, Nasereddin, A, *et al*, 2013: A. A newly emerged cutaneous leishmaniasis focus in northern **Israel** and two new reservoir hosts of *Leishmania major*. PLoS. Negl. Trop. Dis. 7, 2:e2058.

Fryauff, DJ, Modi, GB, Mansour, NS, Kreutzer, RD, Soliman, S, Yous-sef, FG, 1993: Epidemiology of cutaneous leishmaniasis at a focus monitored by the multi-national force and observers in the Northeastern Sinai desert of Egypt. Am. J. Trop. Med. Hyg. 49, 5:598-607.

Geraci, NS, Mukbel, RM, Kemp, MT, Wadsworth, MN, Lesho, E, *et al*, 2014: Profiling of human acquired immunity against the salivary proteins of Phlebotomus papatasi reveals clusters of differential immunoreactivity. Am. J. Trop. Med. Hyg. 90, 5:923-38

González, U, Pinart, M, Sinclair, D, Firooz, A, Enk, C, *et al*, 2015: Vector and reservoir control for preventing leishmaniasis. Cochrane Database Syst. Rev. 2015 Aug 5;8:CD008736.

Gravino, AE, 2004: Interpretation of laboratory data during cryptic leishmaniasis in dog. Parasitologia 46, 1/2: 227-9.

Hamadto, HA, Al-Fakahany, AF, Farrag, AB, Abdel-Maksoud, MK, Morsy, TA, 2007: Zoonotic cutaneous leishmaniasis: reservoir host and insect vector in north Sinai, Egypt. J. Egypt. Soc. Parasitol. 37, 3:843-50.

Hamarsheh, O, Amro, A, 2011: Characterization of simple sequence repeats (SSRs) from *Phlebo- tomus papatasi* (Diptera: Psychodidae) expressed sequence tags (ESTs). Parasit Vectors. Sep. 29; 4:189. doi: 10.1186/1756-3305-4-189.

Hanafi, HA, El Sawaf, BM, Fryauff, DJ, Beavers, GM, Tetreault, GE, 1998: Susceptibility to *L. major* of different populations of *P. papatasi* (Diptera: Psychodidae) from endem- ic and nonendemic regions of Egypt. Ann. Trop. Med. Parasitol. 92, 1:57-64.

Hanafi, HA, El-Sawaf, BM, Beavers, GM, 1999: The effect of *L. major* on some biological parameters of *P. papatasi* (Diptera: Psychodidae) from endemic and non-endemic areas in Egypt. J. Egypt. Soc. Parasitol. 29, 2: 293-305.

Hanafi, HA, el-Sawaf, BM, Beavers, GM, 1999: The effect of *Leishmania major* on some biological parameters of *Phlebotomus papatasi* (Diptera: Psychodidae) from endemic and non-endemic areas in Egypt. J. Egypt. Soc. Parasitol. 29, 2:293-305.

Hanafi, HA, Fryauff, DJ, Kittell, CE, 2013: Evaluation of the fat-tailed gerbil, *Pachyuromys duprasi* (Rodentia: Gerbillidae), as a new animal model for studies of *Leishmania major* infection and transmission. Vector Borne Zoonotic Dis. 13, 9:650-6.

Hanafi, HA, Fryauff, DJ, Modi, GB, Ibrahim, MO, Main, AJ, 2007: Bionomics of phlebotomine sandflies at a peacekeeping duty site in the north of Sinai, Egypt. Acta Trop. 101, 2:106-14.

Hanafi, HA, Szumlas, DE, Fryauff, DJ, El-Hossary, SS, Singer, GA, *et al*, 2011: Effects of ivermectin on blood-feeding *Phlebotomus papatasi,* and promastigote stage of *Leishmania major*. Vector Borne Zoonotic Dis. 11, 1: 43-52.

Hassan, MM, Widaa, SO, Osman, OM, Numiary, MS, Ibrahim, MA, *et al*, 2012: Insecticide resistance in the sand fly, *Phlebotomus papatasi* from Khartoum State, Sudan. Parasit Vectors Mar 7; 5:46. doi: 10.1186/1756-3305-5-46.

Heerman, M, Weng, JL, Hurwitz, I, Durvasula, R, Ramalho-Ortigao, M, 2015: Bacterial infection and immune responses in *Lutzomyia longipalpis* sand fly larvae midgut. PLoS Negl. Trop. Dis. Jul 8;9(7):e0003923. doi:10.1371/journal.pntd.

Hoel, DF, Butler, JF, Fawaz, EY, Watany, N, El-Hossary, SS, *et al*, 2007: Response of phlebotomine sand flies to light-emitting diode-modified light traps in southern Egypt. J. Vector Ecol. 32, 2:302-8.

Hoel, DF, Zollner, GE, El-Hossary, SS, Fawaz, EY, Watany, N, Hanafi, HA, *et al*, 2011: Comparison of three carbon dioxide sources on phlebotomine sand fly capture in Egypt. J. Med. Entomol. 48, 5:1057-61.

Hogsette, JA, Hanafi, HA, Bernier, UR, Kline, DL, Fawaz, EY, *et al*, 2008: Discovery of

diurnal resting sites of phlebotomine sand flies in a village in southern Egypt. J. Am. Mosq. Control Assoc. 24, 4:601-3.

Hurwitz, I, Hillesland, H, Fieck, A, Das, P, Durvasula, R, 2011: The paratransgenic sand fly: a platform for control of *Leishmania* transmission. Parasit Vectors May 19;4:82. doi 10.1186 /1756-3305-4-82.

Ismaeel, AY, Garmson, JC, Molyneux, DH, Bates, PA, 1998: Transformation, development and transmission of axenically cultured amastigotes of *L. mexicana* in vitro & in *Lu. longipalpis*. Am. J. Trop. Med. Hyg. 59, 3: 421-5.

Jacobson, RL, 2003: *Leishmania tropica* (Kinetoplastida: Trypanosomatidae)-a perplexing parasite. Folia Parasitol. (Praha) 50, 4:241-50.

Jaramillo, C, Travi, BL, Montoya, J, 1994: Vector competence of some neotropical sandflies for the *L.* (*viannia*) *braziliensis* complex. Med. Vet. Ento- mol. 8, 1:1-7.

Kaldas, RM, El Shafey, AS, Shehata, MG, Samy, AM, Villinski, JT, 2014: Experimental effect of feeding on *Ricinus communis* and *Bougainvillea glabra* on the development of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae) from Egypt. J. Egypt. Soc. Parasitol. 44, 1:1-12.

Kamal, HA, Doha, SA, El Hosary, S, Shehata, MG, El Sawaf, BM, 2003: Human zoonotic cutaneous leishmaniasis and associated sand flies (Diptera: Psychodidae) in Sheikh Atiya village, Southern Sinai, Egypt. J. Egypt. Soc. Parasitol. 33:795-803.

Khalid, NM, Aboud, MA, Alrabba, FM, Elnaiem, DE, Tripet, F, 2012: Evidence for genetic differentiation at the microgeographic scale in *Phlebotomus papatasi* populations from Sudan. Parasit. Vectors 5:249-54.

Kassem, HA, El-Sayed, YA, Baz, MM, Kenawy, MA, El Sawaf, BM, 2009: Climatic factors influencing the abundance of *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae) in the Nile Delta. J. Egypt. Soc. Parasitol. 39, 1:305-16.

Kassem, HA, Osman, G, 2007: Maternal transmission of *Wolbachia* in *Phlebotomus papatasi* (Scopoli). Ann. Trop. Med. Parasitol. 101, 5: 435-40.

Kassem, HA, Siri, J, Kamal, HA, Wilson, M L, 2012: Environmental factors underlying spatial patterns of sand flies (Diptera: Psychodidae) associated with leishmaniasis in southern Sinai, Egypt. Acta Trop. 123, 1:8-15.

Kassem, HA, Tewfick, MK, El Sawaf, BM, 2001: Evaluation of avermectins as sandfly control agents. Ann. Trop. Med. Parasitol. 95, 4: 405-11.

Killick-Kendrick, R, 1979: The biology of *Leishmania* in Phlebotomine sandflies. In: Biology of the Kinetoplastida, V. II, By W.H.R. Lumsden & D.A. Evans. Academic Press, London.

Killick-Kendrick, R, 1990: Phlebotomine vectors of the leishmaniases: a review. Med. Vet. Entomol. 4, 1:1-24.

Killick-Kendrick, R, Leaney, AJ, Peters, W, Rioux, JA, Bray, RS, 1985: Zoonotic cutaneous leishmaniasis in Saudi Arabia: the incrimination of *Phlebotomus papatasi* as the vector in the Al-Hassa oasis. Trans. R. Soc. Trop. Med. Hyg. 79, 2:252-5.

Kumar, V, Kishore, K, Palit, A, Keshari, S, Sharma, M, *et al*, 2001: Vectorial efficacy of *P. argentipes* in Kala-azar endemic foci of Bihar (India) under natural and artificial condtions. J. Commun. Dis. 33, 2:102-9.

Kvifte, GM, 2014: Nomenclature and taxonomy of Telmatoscopus Eaton and Seoda Enderlein; with a discussion of parameral evolution in *Paramormiini* and *Pericomaini* (Diptera: Psychodidae, Psychodinae). Zootaxa 3878, 4:390-400.

Lane, RP, 1986: The sand flies of Egypt (Diptera: Phlebotominae). Bull. Br. Mus. Nat. Hist. (Entom.) 52:1-35.

Mansour, NS, Youssef, FG, Mohareb, EW, Dees, WH, Karuru, E, 1987: Cutaneous leishma-niasis in North Sinai. Trans. R. Soc. Med. Hyg. 81:747-52.

Maroli, M, Jalouk, L, Al Ahmed, M, Bianchi, R, Bongiorno, G, *et al*, 2009: Aspects of the bionomics of *Phlebotomus sergenti* sandflies from an endemic area of anthroponotic cutaneous leishmaniasis in Aleppo Governorate, Syria. Med. Vet. Entomol. 23, 2:148-54.

Melby, PC, 2002: Vaccination again-st cutaneous leishmaniasis: current status. Am. J. Clin. Dermatol. 3, 8:557-70.

Merdan, AI, Morsy, TA, Shoukry, A, Wahba, MM, 1992: Two successive years studies on *Phlebotomus papatasi* in North Sinai Governorate, Egypt. J. Egypt. Soc. Parasitol. 22, 1:91-100.

Molina, R, 1991: Experimental infections of a *P. perniciosus* colony using different procedures. Parasitologia 33:425-9.

Molina, R, Gradoni, L, Alvar, J, 2003: HIV and the transmission of *Leishmania*. Ann. Trop. Med. Parasitol. 1:29-45.

Morsy, TA, 1996: Cutaneous leishmaniasis in Egypt: Review and comment. J. Egypt. Soc. Parasitol. 26, 1:105-30.

Morsy, TA**, 1997:** Visceral leishmaniasis with special reference to Egypt (review and comment). J. Egypt. Soc. Parasitol. 27, 2:373-96.

Morsy, TA, 2013: Cutaneous leishmaniasis predisposing to human skin cancer: forty years local and regional studies. J. Egypt. Soc. Parasitol. 43, 3:629-48.

Morsy, TA, Shoura, MI, 1976: Some aspects of cutaneous leishmaniasis in Riyadh. Saudi Arabia. J. Trop. Med Hyg. 79, 6:137-9.

Morsy, TA, el Ajlouny, AK, 1984: Cutaneous leishmaniasis in Jordan. J. Egypt. Soc. Parasitol. 14, 1: 257-64

Morsy, TA, Shoukry, A, Schnur, LF, Sulitzeanu, A, 1987: *Gerbillus pyramidum* is a host of *Leishmania major* in the Sinai Peninsula. Ann. Trop. Med. Parasitol. 81, 6:741-2.

Morsy, TA, Bassili, WR, Fayad, ME, El Okby, LM, Saleh, MS, 1987: Rodents in relation to cutaneous leishmaniasis in North Sinai Governorate, Egypt. J. Egypt. Soc. Parasitol. 17, 2: 427-37.

Morsy, TA, el-Missiry, AG, Kamel, AM, Fayad, ME, el-Sharkawy, IM, 1990: Distribution of *Phlebotomus* species in the Nile Delta, Egypt. **J.** Egypt. **Soc. Parasitol.** 20, 2:589-97

Morsy, TA, El Okbi, LMA, Fawzy, AFA, Arafa, MAS, Shouma, MIM, 1988: Studies on blood sucking insects in Suez City, Egypt. J. Egypt. Soc. Parasitol. 18, 1:81-6.

Morsy, TA, Merdan, AI, Shoukry, A, el Said, S, Wahba, MM, 1992: Experimental efficiency of *Phlebotomus papatasi* in maintaining development of four species of *Leishmania*. J. Egypt. Soc. Parasitol. 22, 1:241-51.

Morsy, TA, Aboul Ela, RG, Abdelmawla, MM, el Gozamy, BM, 1993a: Counter immunoelectrophoresis, a modified technique for the identification of blood meals of sandflies collected from Qualyobia Governorate, Egypt. J. Egypt. Soc. Parasitol. 23, 1:109-32.

Morsy, TA, Aboul Ela, RG, Sarwat, MA, Arafa, MA, el Gozamy, BM, 1993b: Some aspects of *Phlebotomus papatasi* (Scopoli) in greater Cairo, Egypt. J. Egypt. Soc. Parasitol. 2: 399-416.

Morsy, TA, Aboul Ela, RG, el Gozamy, BM, Salama, MM, Ragheb DA, 1993c: Residual effect of four insecticides applied for indoor control of *Phlebotomus papatasi* (Scopoli). J. Egypt. Soc. Parasitol. 23, 2:485-92.

Mukhopadhyay, AK, Mishra, RN, 1991: Development of *L. donovani* in *P. argentipes* and *P. papatasi* fed on Kala-azar patients in Bihar. Indian J. Med. Res. 93:152-4.

Obenauer, PJ, Villinski, JT, Zayed, A, Watany, N, El-Hossary, SS, 2011: Sticky bottle traps: A simple and effective method for collecting adult phlebotomine sand flies from rodent burrows in a *Leishmania*-endemic region of Egypt. J. Am. Mosq. Control Assoc. 27, 3:333-5.

Obenauer, PJ, Annajar, BB, Hanafi, HA, Abdel-Dayem, MS, El-Hossary, SS, *et al*, 2012: Efficacy of light and nonlighted carbon dioxide-baited traps for adult sand fly (Diptera: Psychodidae) surveillance in three counties of Mesrata, Libya. J. Am. Mosq. Control Assoc. 28, 3:179-83

Orshan, L, Szekely, D, Schnur, H, Wilamowski, A, Galer, Y, *et al*, 2006: Attempts to control sand flies by insecticide-sprayed strips along periphery of a village. J. Vector Ecol. 31:113-7

Palacios, G, Tesh, RB, Savji, N, Travassos da Rosa, AP, Guzman, H, *et al*, 2014: Characterization of the Sandfly fever Naples species complex and description of a new Karimabad species complex (genus Phlebovirus, family Bunyaviridae). J. Gen. Virol. 95, Pt 2:292-300.

Ramalho-Ortigão, M, Coutinho-Abreu, IV, Balbino, VQ, Figueiredo, CA, Jr, Mukbel, R, *et al,* 2015: *Phlebotomus papatasi* SP15: mRNA expression variability and amino acid sequence polymorhisms of field populations. Parasit Vectors 2015 May 29;8:298. doi: 10. 1186/s13071-015-0914-2.

Rangel, EF, Barbosa, AF, Andrade, CA, Sousa, NA, Wermelinger, ED, 1992: Development of *L.* (*viannia*) *braziliensis vianna*, 1911 in *Lu. intermedia* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) under experimental conditions. Mem. Inst. Oswaldo Cruz. 87, 2: 235-8.

Rasheid, KhAS, Morsy, TA, 1998: Efficacy of ivermectin on the infectivity of *Leishmania major* promastigotes. J. Egypt. Soc. Parasitol. 28, 1: 207-12.

Sádlová, J, Hajova, M, Volf, P, 2003: *Phlebotomus* (*Adlerius*) *halepensis* vector competence for *L. major* and *L. tropica.* Med. Vet. Entomol. 17, 3:244-50

Saliba, EK, Saleh, N, Oumeish, OY, Khoury, S, Bisharat, Z, *et al*. 1997: The endemicity of *Leishmania tropica* (zymodeme MON-137) in the Eira-Yarqa area of Salt District, Jordan. Ann. Trop. Med. Parasitol. 91, 5:453-9.

Samy, AM, Doha, SA, Kenawy, MA, 2014a: Ecology of cutaneous leishmaniasis in Sinai: linking parasites, vectors and hosts. Mem. Inst. Oswaldo Cruz 109, 3:299-306.

Samy, AM, Campbell, LP, Peterson, AT, 2014b: Leishmaniasis transmission**:** Distribution and coa-rse-resolution ecology of two vectors and two parasites in Egypt. Rev. Soc. Bras. Med. Trop. 47, 1:57-62.

Sawalha, SS, Shtayeh, MS, Khanfar, HM, Warburg, A, Abdeen, ZA, 2003: Phlebotomine sand flies (Diptera: Psychodidae) of the Palestinian West Bank: potential vectors of leishmaniasis. J. Med. Entomol. 40:321-8.

Sebai, ZA, Morsy, TA, 1976: Cutaneous leishmaniasis in Bisha Town, Saudi Arabia. J. Trop. Med. Hyg. 79, 4:89-91.

Shehata, M, El Sawaf, B, El Said, S, Doha, S, El Hossary, S, *et al*, 1990: *Leishmania infantum* (MON-98) isolated from El Agamy, Egypt. Trans. R. Soc. Trop. Med. Hyg. 84:227-8.

Shehata, MG, Samy, AM, Doha, SA, Fahmy, AR, Kaldas, RM, *et al*, 2009: First report of *L. tropica* from a classical focus of *L. major* in North-Sinai, Egypt. Am. J. Trop. Med. Hyg. 81, 2: 213-8.

Shoukry, NM, Morsy, TA, 23011: Arthropod borne diseases at Toshka, Upper Egypt. World J. Zool. 6, 2:126-33.

Soliman, BA, Hassan, MM, El Houssary, SS, 2001: Species composition of Phlebotomine sandfly (Diptera: Psychodidae) in the south eastern transect of Upper Egypt. Proceed. Efflatoun's 1st Conf. Entomol. Cairo, Egypt.

Telleria, EL, Sant'Anna, MR, Alkurbi, MO, Pitaluga, AN, Dillon, RJ, *et al*, 2013: Bacterial feeding, *Leishmania* infection and distinct infection routes induce differential defensin expression in *Lutzomyia longipalpis*. Parasit Vectors Jan 11;6:12.doi: 10.1186/1756-3305-6-12.

Vexenat, JA, Barretto, AC, Rosa Ade, C, 1986: Experimental infection of *Lu. whitmani* in dogs infected with *L. braziliensis*. Mem. Inst. Oswaldo Cruz. 81, 1: 125-6.

Wahba, MM, 2000: The influence of *Bacillus sphaericus* on the biology and histology of *Phlebotomus papatasi*. J. Egypt. Soc. Parasitol. 30, 1:315-23.

Wahba, M, Riera, C, 2006: Salivary gland composition of some Old World vector sand fly. J. Egypt. Soc. Parasitol. 36, 1:289-96.

Wahba, M.M, Schnur, LF, Morsy, TA, Merdan, A, 1990: Characterization of *L. major* from *P. papatasi* caught in Northern Sinai, Egypt. Trans. Roy. Soc. Trop. Med. Hyg. 84:785-6.

Wahba, MM, Labib, IM, el Hamshary, EM, 1999: *Bacillus thuringiensis* var. *israelensis* as a microbial control agent against adult and immature stages of the sandfly, *P. papatasi* under laboratory conditions. J. Egypt. Soc. Parasitol. 29, 2:587-97

Wahba, M, Riera, C, Abdel-Hamid, YM, Kamal, H, 2005: Immunomodulatory properties of sand fly saliva and its role on vertebrate host. J. Egypt. Soc. Parasitol. 35, 3:1135-47.

Yaghoobi-Ershadi, MR, Akhavan, AA, Zahraei, AR, Jalali, AR, Piazak, N, 2005: Bionomics of *P. papatasi* (Diptera: Psychodidae) in an endemic focus of zoonotic cutaneous leishmaniasis in central Iran. J. Vector Ecol. 30, 1:115-8.

Youssef, M, Shehata, MG, El Sawaf, BM, Boulos, L, Pratlong, F, *et al*, 1989: *Leishmania infantum* MON-98, a new zymodem isolated from human visceral leishmaniasis in Egypt (El Agamy). Ann. Parasitol. Hum. Comp. 64, 2:152-63.

Zahedifard, F, Gholami, E, Taheri, T, Taslimi, Y, Doustdari, F, *et al*, 2014: Enhanced protective efficacy of nonpathogenic recombinant *Leishmania tarentolae* expressing cysteine proteinases combined with a sand fly salivary antigen. PLoS. Negl. Trop. Dis. 2014 Mar 27; 8(3):e2751. doi: 10.1371 / journal.pntd.0002751.

Zayed, A, Soliman, MM, El-Shazly, MM, 2013a: Infectivity of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) to *Phlebotomus papatasi* (Diptera: Psychodidae) under laboratory conditions. J. Med. Entomol. 50, 4:796-803.

Zayed, AB, Hoel, DF, Tageldin, RA, Fawaz, E Y, Furman, BD, *et al*, 2013b: Efficacy and duration of three residual insecticides on cotton duck and vinyl tent surfaces for control of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae). US Army Med. Dep. J. 2013 Apr-Jun: 66-72.

Warburg, A, 1991: Entomopathogens of phlebotomine sand flies: laboratory experiments and natural infections. J. Invertebr. Pathol. 58, 2:189 -202