EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
E
MEDICAL ENTOMOLOGY & PARASITOLOGY

ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)

Egypt. Acad. J. Biolog. Sci., 12 (2):19 – 25 (2020)

Egyptian Academic Journal of Biological Sciences
E. Medical Entom. & Parasitology

ISSN: 2090 – 0783
www.eajbse.journals.ekb.eg

The Midgut Ultrastructure of the Sand Fly Vector, *Phlebotomus Langeroni* (Diptera: Psychodidae)

Sanaa A. El Sattar[1*] and Bahira M. El Sawaf[2]

1. Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt.
2. Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt.

E-mail: badersanaa@yahoo.com

ARTICLE INFO

Article History
Received:20/5/2020
Accepted:12/8/2020

Keywords:
Sand fly
Phlebotomus langeroni
Midgut ultrastructure
Transmission electron microscope

ABSTRACT

The midgut ultrastructure of *Phlebotomus langeroni*, the vector of visceral leismaniasis was studied by the transmission electron microscope. The midgut region warrants a special investigation since it is directly relevant to the development of *Leishmania* promastigotes. Observations performed on females at time-intervals following a bloodmeal were analyzed and compared. The epithelium of the midgut consisted of a single layer of columner cells surrounded by the basal lamina. Under which there is a musculature consisted of circular and longitudinal fibers. The cells were characterized by large nuclei, densely packed microvilli, and intricate basal labyrinth. The cytoplasm was filled with numerous mitochondria. Morphological changes occurred in the cellular structures during the blood digestion process. The peritrophic matrix was present up to 3 days after the bloodmeal and completely disintegrated at day 4 after bloodmeal. At day 6 after bloodmeal, lipid inclusions were few within the cell cytoplasm and vacuoles were depicted. At day 8, the area of the microvilli was greatly reduced probably due to reduced functional activity along with the end of the digestive cycle. The changes depicted in the midgut of *P. langeroni* during the process of blood digestion were comparable to those described for other hematophagous dipterans.

INTRODUCTION

Phlebotomus langeroni is a member of subgenus *Larroussius*. The subgenus *Larroussius* harbours important sand fly vectors or potential vectors of *Leishmania infantum* in the Mediterranean basin (northern Africa, southern Europe, and southern Iran. The principal reservoir host is the dog, while the role of cats (Asgari *et al*., 2020) and rabbits (Diaz Sáez *et al*., 2018) as well as various wild canids is also expected.

Phlebotomus (*Larroussius*) *langeroni* Nitzulescu, 1930 is the proven vector of *Leishmania infantum* in the northwestern coast of Egypt (El Sawaf *et al*., 2012). *P. langeroni* meets the five criteria set up by Killick-Kendrick (1990) for the incrimination of a sand fly species as a vector. Essentially, it is an anthropophilic sand fly that also feeds on dogs; its spatial distribution is associated with human cases of visceral leishmaniasis; it was naturally infected by *L. infantum* and transmits the disease by bite (Kassem *et al*., 2017). The vector species acquires its highest epidemiological relevance in El-Agamy, Alexandria, Egypt, where noticeable abundance was found.

More recently the spread of this important sand fly vector was demonstrated in southern Spain associated with wild rabbits. *Leishmania infantum* DNA was found in both *P. langeroni* and the wild rabbit *Oryctolagus cuniculus* (Diaz Sáez *et al*., 2018), which may increase visceral leishmaniasis incidence. Several modelled scenarios based on anticipated changes of climate variables in Europe suggest a possible spread of other *Larroussius* species into non-endemic regions (Fischer *et al*., 2011and Haeberlein *et al*., 2013). Hence, more studies on the vector-parasite interphase will be of epidemiological significance.

In the gut of the sand fly vector, *Leishmania* parasites develop as extracellular promastigotes that are attached to the alimentary canal lining (Killick-Kendrick, 1979). Most of the developmental stages of suprapylarian *Leishmania* are confined to the midgut. In the midgut, promastigotes attach by inserting their flagella between microvilli, whereas in the foregut parasites attach to the cuticular surface by forming flagellar hemidesmosomes (Walters *et al*., 1989 and Warburg *et al*., 1989). Thus, the structure of the midgut is directly relevant to the development of *Leishmania* promastigotes within it. Some studies have been performed related to the morphology of the midgut of different female sand flies and the formation of the peritrophic matrix (Davis, 1967; Gemetchu, 1974; Rudin and Hecker, 1982; Jobling, 1987; Blackburn *et al*., 1988; Billingsley, 1990; Andrade-Coêlho *et al*., 2001 and Warburg, 2008).

In this paper, we described for the first time the ultrastructure of the midgut epithelium of *P. langeroni* after bloodmeal ingestion. The study of the midgut epithelium of *P. langeroni* is a prerequisite for future investigations on *L. infantum/ P. langeroni* association.

MATERIALS AND METHODS

Sand fly Species:

Laboratory reared *P. langeroni* was originally collected from El-Agamy, Alexandria, Egypt. Sand fly was reared following the procedure described by Schmidt (1964).

Study Samples:

Females (five-day-old) were allowed to feed on anesthetized hamsters. Engorged females were taken and placed in cardboard cups provided with a piece of cotton wetted with a 30% sucrose solution and maintained at room temperature (24-26 ^{o}C) and 60-70% RH.

Transmission Electron Microscopy:

Females sampled at 3,4,6 and 8 days after a bloodmeal were anesthetized and kept on ice and transferred individually to glass slides with a drop of saline solution and dissected. Suitable guts attached to the head were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4 ^{o}C overnight. Then washed in the same buffer for 20 h. Post fixation was in 1% osmium tetroxide in cacodylate buffer at 4 ^{o}C for 1.5 h. Guts were rinsed in distilled water and stained with 0.5% uranyl acetate at room temperature (22 ^{o}C) for 30 min. The fixed guts were dehydrated in an ethanol/propylene oxide series, followed by embedment in fresh TAAB resin overnight at room temperature. Blocks were polymerized at 60 ^{o}C for 12-24 h. Ultrathin sections stained with 0.1% lead citrate were examined and photographed with JEOL C×100 transmission electron microscope.

The transmission electron microscopy (TEM) was performed at the electron microscope unit. London School of Tropical Medicine and Hygiene, UK.

RESULTS

The ultrastructural features of the midgut epithelium of females sampled at 3,4,6 and 8 days after bloodmeal were described.

The epithelium of the midgut consisted of a single continuous layer of columner cells, with evident oval nuclei and densely packed apical microvilli. Underneath the columner cell layer, demarcating the epithelium was the basal lamina, made up of a complex web of invaginations, formed of

space demarcated by their membrane, and commonly known as basal labyrinths. Cells were connected together with a belt of intercellular junctions (*Zonula continua*). A musculature consisted of circular and longitudinal muscle fibers was present (Fig.'s 1C and D).

The sections examined 3 days after bloodmeal showed that the ingested blood was confined to the abdominal midgut and separated from the epithelium by the peritrophic matrix (PM). The PM remained mostly intact or was slightly disintegrated. The blood not completely digested. Microvilli were well defined, filled with secretions and products of blood digestion. Mitochondria were spread throughout the cytoplasm under the microvilli. Vacuoles were observed in the cytoplasm especially around the nucleus (Fig. 1A).

At day 4 after bloodmeal, the epithelial cell displayed clear vacuoles and lipid-inclusions. The PM no longer appeared and the gut-lumen was clear of any blood remnants. Mitochondria were spread throughout the cytoplasm (Fig. 1B). The epithelial cells were coated with long dense microvilli that filled almost the whole luminal space.

The sections examined at day 6 after the bloodmeal showed an undulated basal lamina and the basal labyrinths were clearly visible. The cell nuclei were large and filled with clumped chromatin granules. Mitochondria were spread throughout the cytoplasm. The microvilli were dense and arranged in parallel. Clear vacuoles and few lipid inclusions were seen in the cells (Fig.'s 1C, D, and E). At day 8 after bloodmeal, the nuclei were oval-shaped. Vacuoles were abundant within the cytoplasm. Mitochondria were few. The microvilli area was greatly reduced or even detached, suggesting the end of the digestive cycle (Fig. 1F).

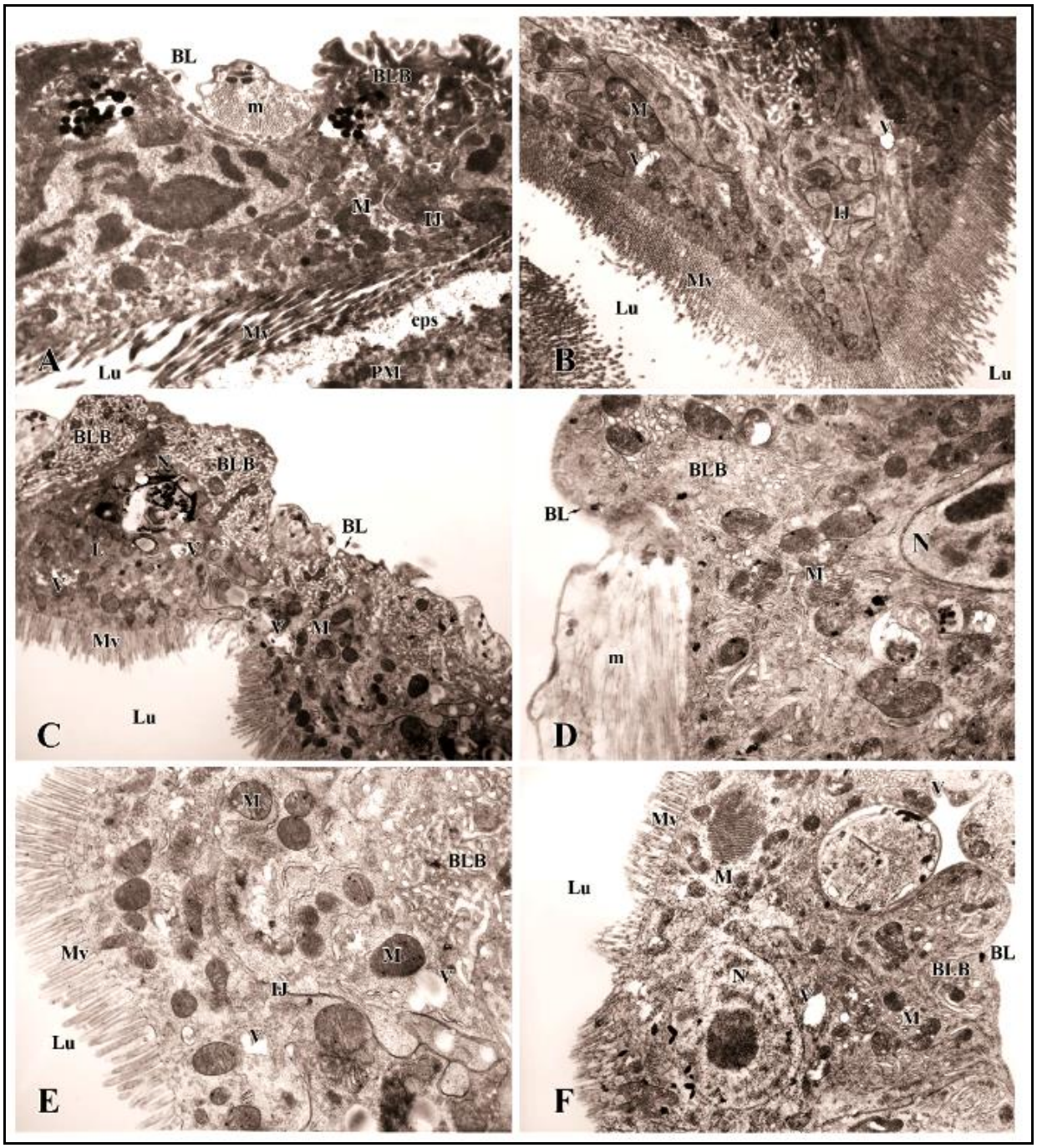

Fig. 1. Electron micrographs of cross sections of the abdominal midgut of *P. langeroni* females, showing the process of blood digestion.

A (x6,000) 3 days after bloodmeal. Midgut epithelium separated from the blood by the peritrophic matrix (PM) and ectoperitrophic space (eps), small food particles penetrating among the microvilli (Mv). Basal labyrinths (BLB), basal lamina (BL), muscle (m), mitochondria (M) and intercellular junction (IJ).

B (x5,000) 4 days after bloodmeal. Clear lumen (Lu), mitochondria (M), vacuoles (V) and intense microvilli (Mv).

C (x3,300), **D** (x6,600) and **E** (x8,300) 6 days after bloodmeal. Clear vacuoles (V), few lipid inclusions (L), clear undulated basal lamina (BL) and basal labyrinths (BLB).

F (x5,000) 8 days after bloodmeal. Large nuclei (N) filled with chromatin, abundant vacuoles (V) and reduced microvilli (Mv).

DISCUSSION

The description of the midgut epithelium of *P. langeroni* based on TEM was performed for the first time. The sections examined showed that the apical end of the columner cells contained microvilli providing a wide surface area for absorbing material from the lumen. Underneath the columner cell layer, demarcating the epithelium, was the basal lamina, made up of invaginations called basal labyrinths, which, like the microvilli, provide a large surface across which materials can be transported. Mitochondria were found in some areas of the cytoplasm associated with the basal labyrinths. The basal lamina was granular. These structures were also described by Romoser (1996) for hematophagous insects. The Golgi apparatus was not detected in our study, probably due to the limited samples examined. However, the Golgi apparatus has already been demonstrated in the cells of other dipterans by many researchers (for *e,g.* Gemetchu, 1974; Rudin and Hecker, 1982 and Billingsley, 1990). We observed all the structures cited by Andrade-Coêlho *et al.* (2001) for the sand fly *Lutzomyia intermedia* except the rough endoplasmic reticulum, probably because it is not an easy structure to find or because that after the bloodmeal (as was the condition of the present study) this structure spreads around the cell cytoplasm and underwent clear morphological changes, as in *Aedes aegypti* females (Bertram and Bird, 1961). The rough endoplasmic reticulum might be responsible for all the secretory and absorptive functions ascribed to the midgut (Bertram and Bird, 1961). Gemetchu (1974) analyzed the fine structure of the midgut in *P. longipes* females and only found the whorled rough endoplasmic reticulum beginning four days after emergence.

In the present work, the PM remained intact or slightly disintegrated, in the females 3 days after a bloodmeal. The PM was present surrounding the ingested blood and performing its function of protecting the epithelium. The PM (previously known as the peritrophic membrane) is present in most insects and is classified into type I and type II. Usually, PM II occurs in the larval stage and some adult Diptera. In general, the name PM is commonly used as a reference for type I in hematophagous Diptera (Secundino *et al.*, 2005). In nematoceran Diptera including sand flies, females produce a type I PM, which is secreted by the entire midgut in direct response to the distention of the midgut caused by the blood-feeding (Jacobs-Lorena and Oo, 1996).

The blood-fed females examined at day 4 after bloodmeal indicated the absence of PM completely and showed a clear lumen. Andrade-Coêlho *et al.* (2001) found that PM was not seen 72 h after the bloodmeal in *Lu. intermedia*. We believe that the time of degradation of PM is species-specific. This agrees with the view of Gemetchu (1974) that the kinetics of PM formation and degradation is totally related to the ingestion and the time of digestion of the bloodmeal, which varies according to distinct insect species.

At day 6 after bloodmeal, which appears to represent the end of the digestive cycle. Clear vacuoles around the nucleus were evident. Lipid inclusions in the apical region of the epithelium were scarce or disappeared. These observations in *P.langeroni* are in agreement with those observed in *Lu. intermedia* (Andrade-Coêlho *et al.*, 2001) and *Lu. longipalpis* (Rudin and Hecker, 1982). Indicating that after blood digestion the cell apparatus was reduced and lipid inclusions and glycogen deposits disappeared from the abdominal region.

At day 8 after bloodmeal, it was observed that the area occupied by the microvilli was greatly reduced, and the microvilli were in a state of degeneration, probably due to reduced or no functional activity by the end of digestive cycle.

In *P. langeroni* the blood digestion process lasted over 4 days and may reach 6 days. This duration was longer than that observed for *Lu. longipalpis* (Rudin and

Hecker, 1982) and *Lu. intermedia* (Andrade-Coêlho *et al.,* 2001). The bloodmeal digestion process varies from one species to another depending on the type of blood and digestive enzymes activities (Dillon and Lane, 1993) and many other factors. However, the size of the bloodmeal ingested cannot be excluded. It is known that while feeding on the vertebrate, sand fly females take a bloodmeal that is directed to the abdominal midgut and surrounded by PM in the so-called peritrophic sac or endoperitrophic space (Dvorak *et al.,* 2018). The amount of bloodmeal varies from 0.6 µl in *P. argentipes* or *P. orientalis* to 0.9 µl in *P. papatasi* (Pruzinova *et al.,* 2015) and 0.85 µl in *P. langeroni* a closely related species to *P. orientalis* (Daba *et al.,* 2004).

Finally, our study has revealed, the possible roles of the different internal structures of *P. langeroni* midgut. This may help in understanding the parasite infection and adaptation in this sand fly vector.

ACKNOWLEDGEMENT

We thank Engineer Khaled Abu Almajd (freelance graphic designer), for the photo design of the electron micrographs.

REFERENCES

Andrade-Coêlho, C.A.; Santos-Mallet, J.; Souza, N.A.; Lins, U.; Meirelles, M.N.L and Rangel, E.F. (2001). Ultrastructural features of the midgut epithelium of females *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae). *Memórias do Instituto Oswaldo Cruz*, 96: 1141-1151.

Asgari, Q.; Mohammadpour, I.; Bozorg-Ghalati, F.; Motazedian, M.H.; Kalantari, M. and Hosseini, S. (2020). Alarming: high prevalence of *Leishmania infantum* infection in cats from southern Iran based on molecular and serological methods. *Annals of Parasitology*, 66: 143-156.

Bertram, S.D. and Bird, G.D. (1961). Studies on mosquito-borne viruses in their vectors. The normal fine structure of the midgut epithelium of the adult female *Aedes aegypti* (L.) and the functional significance of its modification following a bloodmeal. *American Journal of Tropical Medicine and Hygiene*, 55: 404-411.

Billingsley, P.F. (1990). The midgut ultrastructure of hematophagous insects. *Annual Review of Entomology,* 35: 219-248.

Blackburn, K.; Wallbanks, K.R.; Molyneux, D.H.; Havin D.R. and Winstanley, S.L. (1988). The peritrophic membrane of the female sand fly *Phlebotomus papatasi. Annals of Tropical Medicine and Parasitology,* 82:613-619.

Daba, S.; Daba, A.; Shehata, M.G and El Sawaf, B.M. (2004). A simple micro-assay method for estimating bloodmeal size of the sand fly, *Phlebotomus langeroni* (Diptera: Psychodidae). *Journal of Egyptian Society of Parasitology,* 34: 173-182.

Davis, N.T. (1967). Leishmaniasis in the Sudan Republic. 28. Anatomical studies on *Phlebotomus orientalis* Parrot and *P. papatasi* (Scopoli) (Diptera: Psychodidae). *Journal of Medical Entomology*, 4: 50-65.

Diaz Sáez, V.; Marillas-Marquez, F.; Merino-Espinosa, G.; Corpas-López, V.; Morales-Yuste, M.; Pesson, B.; Barón-López, S.; Lucientes-Curdi, J. and Martin-Sánchez, J. (2018). *Phlebotomus langeroni* Nitzulescu (Diptera, Psychodidae) a new vector for *Leishmania infantum* in Europe. *Parasitology Research*, 117: 1105-1113.

Dillon, R.J. and Lane, R.P. (1993). Bloodmeal digestion in the midgut of *Phlebotomus papatasi* and *Phlebotomus langeroni. Medical and Veterinary Entomology*, 7: 225-232.

Dvorak, V.; Shaw, J. and Volf, P. (2018). Parasite Biology: The Vectors. The leishmaniasis: Old Neglected Tropical Diseases. F. Bruschi, L. and Gradoni L. (eds). *Springer International Publishing AG 2018.*

El Sawaf, B.M.; Doha, S.A. and Imam, M.I. (2012). Transmission of *Leishmania*

infantum MON-98 to hamsters by the bite of *Phlebotomus langeroni* Nitzulescu (Diptera: Psychodidae). *Acta Tropica,* 121: 44-46.

Fischer, D.; Moeller, P.; Thomas, S.; Naucke, T. and Beierkuhnlein, C. (2011). Combining climatic projections and dispersal ability: a method for estimating the responses of sand fly vector species to climate change. *PloS Neglected Tropical Diseases,* 5: e1407.

Gemetchu, T. (1974). The morphology and fine structure of the midgut and peritrophic membrane of the adult female, *Phlebotomus longipes* Parrot and Martin (Diptera: Psychodidae). *Annals of Tropical Medicine and Parasitology,* 68: 111-124.

Haeberlein, S.; Fischer, D.; Thomas, S.M.; Schleicher, U.; Beierkuhnlein, C. and Bogdan, C. (2013). First assessment for the presence of phlebotomine vectors in Bavaria, Southern Germany, by combined distribution modeling and field surveys. *PloS ONE,* e 81088.

Jacobs-Lorena, M. and Oo, M.M. (1996). The peritrophic matrix of insects. In: J. Beaty and W.C. Marquardt (eds). The biology of disease vectors. *University Press of Colorado, Boulder,* pp 318-332.

Jobling, B. (1987). Anatomical Drawings of Biting Flies. *British Museum (Natural History), London.*

Kassem, H.A.; Beier, J.G. and El Sawaf, B.M. (2017). Historical overview of infantile visceral leishmaniasis in El Agamy, Alexandria, Egypt. *Acta Tropica,* 176:335-339.

Killick-Kendrick, R. (1979). Biology of *Leishmania* in phlebotomine sand flies, In W.H.R Lumsden and D.A. Evans [eds], Biology of the kinetoplastida Vol. 2. Academic, New York, pp 395-460.

Killick-Kendrick, R. (1990). Phlebotomine vectors of the leishmaniasis: a review. *Medical and Veterinary Entomology,* 4: 1-24.

Pruzinova, K.; Sadlova, J.; Seblova, V.; Hamola, M.; Votypka, J. and Volf, P. (2015). Comparison of bloodmeal digestion and the peritrophic matrix in four sand fly species differing in suspectibility to *Leishmania donovani. PLoS ONE* (DOI: 10.1371/journal.pone.0128203)

Romoser, W.S. (1996*).* The biology of disease vectors. *University press of Colorado,* 606 pp.

Rudin, W. and Hecker, H. (1982). Functional morphology of the midgut of a sand fly as compared to other hematophagous nematocera. *Tissue and cell*, 14:751-758.

Schmidt, M.L. (1964). A laboratory culture of two *Phlebotomus* species, *P. papatasi* and *P. orientalis. Bulletin of the World Health Organization*, 31: 577-578.

Secundino, N.F.; Eger-Magrich, I.; Braga, E.M.; Santoro, M.M. and Pimenta, P.F. (2005). *Lutzomyia longipalis* peritrophic matrix: formation, structure and chemical composition. *Journal of Medical Entomology,* 42: 928-938.

Walters, L.L.; Modi, G.B.; Chaaplin, G.L. and Tesh, R.B. (1989). Ultrastructural development of *Leishmania chagasi* in its vector *Lutzomyia longipalis* (Diptera: Psychodidae). *American Journal of Tropical Medicine and Hygiene,* 41: 295-317.

Warburg, A. (2008). The structure of the female sand fly *(Phlebotomus papatasi)* alimentary canal. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 102: 161-166.

Warburg, A.; Tesh, R.B. and McMahon-Pratt, D. (1989). Studies on the attachment of the *Leishmania* flagellate to sand fly midgut epithelium. *Journal of Parasitology,* 36: 613-617.