The Journal of Medical Entomology and Parasitology is one of the series issued quarterly by the Egyptian Academic Journal of Biological Sciences. It is an important specialist journal covering the latest advances in that subject.

It publishes original research and review papers on all aspects of basic and applied medical entomology, parasitology and host-parasite relationships, including the latest discoveries in parasite biochemistry, molecular biology, genetics, ecology and epidemiology in the content of the biological, medical entomology and veterinary sciences.

In addition to that, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 11 (1):1 – 17 (2019)

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

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

Molecular Identification of Mammalian Blood Meals in Mosquito Vectors in Nile Delta, Egypt

Abdelbaset B. Zayed[1], Randa I. Eltaly[1]and Emtithal M. Abdel Samie[2].
1-Zoology Dept., Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt.
2- Entomology Dept., Faculty of Science, Cairo University, Cairo, Egypt
E-mail: randaebrahim2015@gmail.com

ARTICLE INFO
Article History
Received:1/1/2019
Accepted:3/2/2019

Key words:
Blood-feeding pattern, Mosquitoes, *Culex spp.*, host identification and host preference

ABSTRACT

The degree of contact of the vector and the vertebrate host is an important variable in determining the vectorial capacity of mosquito species for the arthropod-borne disease. This study conducted in Monufia Governorate, Egypt, to describe the mosquito community composition and species-specific host-feeding patterns. Mosquitoes were surveyed over a 2-years period and their host-feeding patterns were determined in relation to species relative abundance by polymerase chain reaction (PCR).This diagnostic technique was used to identify mammalian blood meals from female mosquitoes by sized DNA fragments following agarose gel electrophoresis. One universal reverse primer and five animal-specific forward primers included Human, Pig, Cow, Dog and Goat were used. Multiple blood meals from distinctive mammalian hosts were identified from single mosquito abdomens. Ninety-nine mosquito blood meals from four mosquito species were identified, 67.7% (67) were mixed blood. Both *Cx. pipiens* and *Cx. antennatus* fed on human and animals but feeding strategies differed from outdoors to indoors. Inside houses engorged female *Cx. pipiens* accounted for (94) 74% of collections and out of this, 53.8% fed on humans as single blood and 40% as mixed blood. However, outdoor, collected *Ochlerotatus caspius* constituted 7.1% of the collected females. Results suggested that, *Cx. pipiens* an important bridge of disease vector to humans in Egypt.

INTRODUCTION

Mosquitoes feed on a wide range of different vertebrate hosts such as human, monkeys, horses, camels, dogs, pigs, other ruminants, birds, etc. However, some species have developed a characteristic host preference, feeding on humans (anthropophagic), animals (zoophagic) or birds (ornithophagic) and others are facultative (Rao, 1984). Blood meal source affects feeding rates and reproduction as avian blood contains nucleated blood cells which have been hypothesized to influence fecundity as it contains more nutrition and due to different rates of digestion, while mammalian blood contains a nucleated cells (Dowen and Archer, 1975). Biting activity is one of the important aspects of the biology of vectors to which disease transmission is depended. The intensity of transmission of filarial infection

depends upon the high biting density, anthropophily and high survival rates of the vector (Wattal, 1976 and Agrawal, 2015). The host preference pattern of vector mosquitoes influences greatly the dynamics of transmission of infection in the community. However, in many mosquito species, there is a genetically determined host preference, this preference influenced by environmental conditions (Takken, 2013) as well as the ecology and the behavior of both host and vector (Gillies, 1988). The collection of blood-fed female mosquitoes and identification of blood meals gives a valuable data on the host feeding patterns of mosquitoes in nature (Service, 1993) and (Raweewan *et al*., 2014). Identifying the blood meal source is an important component to study the transmission dynamics (vector-host-pathogen interaction dynamics) and determining the role of different mosquito species that causing a biting nuisance to humans and animals (Mukabana *et al*., 2002). This identification has already been performed with the precipitin test, latex agglutination test, and enzyme-linked immunosorbent assay (ELISA) based on serological techniques (Delatte *et al.,* 2010). The PCR technique is an additional method using mitochondrial DNA, which identifies a mosquito's blood-meal origin (Ngo and Kramer, 2003; Kent and Norris, 2005 and Townzen *et al.*, 2008).

In Egypt, *Culex* mosquitoes have been implicated in the transmission of filariasis and various viral diseases, including Rift Valley fever and West Nile Virus (Darwish and Hoogstraal, 1981 and Gad *et al*., 1999). All of these diseases occur in the Nile Delta and in El-Monufia governorate, where this study was conducted. The impact of an arthropod species as a vector can be determined after detailed epidemiological studies into the relationships between the vector- host and the role of the arthropod species in the disease transmission cycle. Mosquito host feeding in Egypt has been studied in several areas representing different ecological conditions (Kenawy *et al*. 1987; Beier *et al*. 1987 and Gad *et al*. 1999). Host-feeding patterns of mosquitoes, such as those revealed in this study, add much to the knowledge of these relationships. As far as we know the first study on mosquito feeding patterns in Egypt was conducted in 1956 during an epidemiological survey of West Nile virus infection in the Barada- Sindbis-Quaranfil area of the Nile Delta Hurlbut and Weitz (1956); Washino and Tempelis (1983). Genetic and environmental factors include temperature, photoperiod and changes in host densities; produce mosquito feeding variables (Edman, 1974). The aim of this study was to identify the vertebrate blood meal hosts and study community composition of mosquitoes at El- Monufia governorate using Multiplex PCR assay.

MATERIALS AND METHODS

Collection of Blood-Fed Mosquitoes and Study Area:

Blood-fed female mosquitoes were collected throughout five villages follow Berket El-Sabie district (east of Nile Delta); El-Ganzor (30° 40.490'Nand 31° 1.667'E), El-Roda (30° 39.763'Nand 31°4.418'E), El-Shaheed Fekry (30° 40.897'N and 31° 4.246'E), Mit Om-Saleh (30° 38.863'N and 31° 2.527'E) and Shintina Al-Hagar (30°38.661''N and 31° 3.173'E) villages. The distance between each village and the other ranged from about 2 to 4 kilometer. The study was conducted during two years from Spring 2016 to Winter 2018 seasonally (4 visits annually) during April, July, October and January. Blood-fed female mosquitoes were surveyed using CDC light traps from indoors and outdoors. Rested mosquitoes were also collected seasonally outdoors and indoors using window traps.

Preservation Method:

Immediately after collection, mosquitoes from the light-trap and window trap were knocked down by freezing, only blood-fed females were identified morphologically to species using Harbach (1985) key. Engorged specimens were separated, placed by species and collection set in the Eppendorf tube and kept at-20 °C

until blood meals identification could be processed (WHO, 1975).

DNA Isolation from Blood-fed and Unfed Mosquitoes:

DNA was isolated from the abdominal contents of blood-fed mosquitoes individually by using the QIAamp DNA Mini Kit, (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The DNA was extracted from unfed female mosquito to be considered as negative control using QIAamp DNA Mini Kit, (Qiagen, Valencia, CA, USA) **Fig. (1)**.

DNA Extraction of A Collected Blood Sample from Animals (positive control):

The blood of each sample was aliquoted in a microcentrifuge tube (1 ml. per each) prior to DNA extraction. These tubes were centrifuged at 4000 r. p. m. for 15 min. The plasma and buffy coat were discarded. The pellets of blood corpuscles (RBCs) were washed in phosphate buffer saline (PBS) pH 7.4 by centrifugation at 14000 r. p. m. for 15 min. and the supernatant was discarded **(Calder, 1991)**. The DNA was extracted after this by using QIAamp DNA Mini Kit, (Qiagen, Valencia, CA, USA) **Fig. (1)**.

Primer Design and PCR:

Mitochondrial cytochrome *b* gene was amplified using diagnostic primers **(Table1).** One universal reverse primer and five animal-specific forward primers were selected from a multiple alignments of cytochrome *b* sequences (Kent and Norris, 2005). PCR reactions were performed with the 5x Green Go Taq Buffer Plus Master Mix Kit (Qiagen, Valencia, CA, USA) according the following protocol: Cycling conditions were 95°C for 5 min, followed by 35 cycles at 95°C for 1 min., 58°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 7 min. Each 50-µL PCR consisted of 10 µL of 5x Go Taq Buffer, 6µL of 25mM $MgCl_2$, 2µL of 2.5mMdNTPs,3.4 of each primer (Pig573F, Human741F, Goat894F, Dog368F, Cow121F, and UNREV1025 primers), 6.1µL of PCR water, 0.5 µL of Go Taq flex DNA polymerase and5 µL of extracted DNA from engorged mosquito. The advantage of this PCR diagnostic test is that mammalian blood hosts can be identified directly by size specific fragments following agarose gel electrophoresis. This diagnostic test was designed for use in differentiating between blood meals of mosquitoes caught in villages where the potential hosts are primarily humans and domestic animals.

DNA amplifications were completed and visualized after electrophoresis on Ethidium bromide–stained agarose gel. Electrophoresis was conducted with Gene Ruler 100-basepair (bp) molecular mass marker (Jena, Bioxinea).

Blood Meal Identification:

Positive identification and host species detection were made when exact or nearly exact PCR product sizes were obtained **(Table1**). Sequences that did not meet the product size were assumed as unknown.

Table 1. Diagnostic primer sequences and sizes for the identification of mammalian blood meals in female mosquitoes. (Kent and Norris, 2005).

Primer	Name Species	Primer Sequence (5' – 3')	Size (bp)
PIG573F	Pig	CCTCGCAGCCGTACATCTC	453
HUM741F	Human	GGCTTACTTCTCTTCATTCTCTCCT	334
GOAT894F	Goat	CCTAATCTTAGTACTTGTACCCTTCCTC	132
DOG368F	Dog	GGAATTGTACTATTATTCGCAACCAT	680
COW121F	Cow	CATCGGCACAAATTTAGTCG	561
Unrev1025	Universal Reverse	GGTTGTCCTCCAATTCATGTTA	–

RESULTS

During the study, a total of 292 blood-fed mosquitoes representing four different species; *Cx. pipiens*, *Cx. antennatus*, and *Cx. perexiguus* were collected from all 5 villages where blood fed while, *Ochl caspius* was collected from one village only either outdoor and indoor **(Table 2)**.

The results indicated that the prevalence of blood-fed mosquitoes varied among the 5study villages as a large number of blood-fed females were collected from El- Ganzor village 31.8% (93) followed by El-Shaheed village 19.5%(57) then El-Roda and Mit Om Saleh with 19%(56). The smallest number was collected from Shintina 10.2% (30).The most frequently blood fed collected species was *Cx. pipiens* represented roughly 71.2 % (208) of the blooded mosquitoes collected. However, *Cx. antennatus* and *Cx. perexiguus* represented 13% (38) and 13.6% (40) respectively. The four mosquito species showed marked differences in blood fed relative abundance as 14.3% (42) of blood-fed mosquitoes were collected from outdoor and 43.4% (127) from indoors. The remaining was collected resting indoor 41.1% (120) and 1% (3) resting outdoor **(Table2)**. In the study, the host species was identified in 48% *Cx. pipiens*, 30% *Cx. perexiguus* 27%*Cx. antennatus* and only 6% *Ochl. caspius* **(Table2).**

Table 2. Mosquito species blood fed composition that collected by CDC and by window traps both indoor and outdoor from April 2016 to January 2018 at 5 villages, El-Monufia Governorate.

Species	No. of blood fed mosquitoes collected (% of total) [a]				
	Indoor	Outdoor	Rested indoor	Rested outdoor	Total no. for each species
Cx. pipiens	94(74)	37(88)	74(61)	3 (100)	208
Cx. antennatus	10(7)	2(4.7)	26(21)	0-	38
Cx. perexiguus	20(15)	0-	20(16)	0-	40
Ochlerotatuscaspius	3(2.4)	3(7.1)	0-	0-	6
Total (%) [a]	127 (43)	42(14)	120(41)	3(1)	292

[a]Percentage of each blood-fed female collected from each collection set (village).

Blood-fed *Cx. pipiens* females collected indoor accounted for 74% of over 127 total mosquitoes that collected indoor. However, *Ochl. caspius* constituted 2.4% of indoor collections; thus, the other *Culex* species accounted for over 23.6% of all indoor-collected mosquitoes. From outdoor resting sites, only blood fed collected species was *Cx. pipiens* and collected in lower number **(Table2).** During the study, a total of 99 blood-fed mosquitoes of four species were successfully tested for blood meal identification and the four mosquito species showed marked differences in feeding behavior and relative abundance **(Table 3).** In addition, most engorged *Cx. pipiens* female fed on mixed blood 43.9% over all tested blood from *Cx. pipiens* female (41)and fed on single blood 34.2% (14) (Table 3). Humans were a primary blood source (53.8%) and as mixed blood (40%) for *Cx. pipiens* collected indoor and for that rested indoor (13.3%). However, cow blood was the primary blood for engorged females collected outdoor (50%) as mixed blood and 20% as single blood followed by dog blood (50%) as mixed and 10% as single **(Table3).** The study found that, *Cx. antennatus* was the 2nd collected species fed mainly on human (10%) and human mixed blood (100%) from

indoor collections, as well as cow blood (66.6%) in mixed blood. However, *Cx. perexiguus* that collected from indoor fed commonly on cow blood (100%) as mixed blood then dog blood as (90%)**.** Although, pigs are not common in Monufia governorate, pig blood was detected in engorged female *Cx. pipiens* and *Cx. perexiguus* that collected from Shintina village **(Table3)**. From all tested blood-fed mosquitoes 31.3% of the tested were contained unidentified blood mixed with other identified blood meal source and 16.1% of the tested mosquitoes were contain unidentified single blood meal. Mixed meals were recognized in the 4 mosquito species from the 5 villages (Table 3). Of 67.7% mixed blood meal females, 55.2% (37) were double feeds and 11.9% (8) were triple feeds.

Table 3. Identification of blood meals in adult female mosquitoes collected indoor and outdoor using light and window traps from April 2016 to January 2018 from the 5 studied villages, Monufia Governorate.

Species	Habitat	No. tested	Human (%a)	Goat (%a)	cow (%[a])	Dog (%[a])	Mixed[b]							Unknown (%[a])	-Ve DNA extracted
							Total	Cow (%[a])	Goat (%[a])	Dog (%[a])	Pig (%[a])	Human (%[a])	Unknown (%[a])		
Cx. pipiens	I	15	7 (53.8)	-		-	5 (38.5)	4 (80)	-	1 (20)	-	2 (40)	4 (80)	1 (7.7)	2 (13.3)
	O	15	-	1 (10)	2(20)	1 (10)	4 (40)	2 (50)	-	2 (50)	1 (25)	1 (25)	2 (50)	2 (20)	5 (33.3)
	R.I	15	2 (13.3)	-		-	8 (53.3)	3 (37.5)	-	2 (25)	-	7 (87.5)	5 (62.5)	5 (33.3)	-
	R.O	3	-	-		1 (33.3)	1 (33.3)	1 (100)	-			1 (100)	-	1 (33.3)	-
Cx. antennatus	I	10	1 (10)	-		-	9 (90)	6 (66.6)	-	-	-	9 (100)	3 (33.3)	-	-
	O	2	-	-		-	2 (100)	2 (50)	-	2 (50)	-	-	-	-	-
	R.I	15	-	-		3 (20)	11 (73.3)	5 (45.5)	-	2 (18.1)	-	9 (81.8)	7 (63.6)	1 (7.7)	-
Cx. perexiguus	I	15	1 (6.6)	1 (6.6)		1 (6.6)	10 (66.6)	10 (100)	-	9 (90)	2 (20)	-	2 (20)	2 (13.3)	-
	R.I	15	-	-		-	13 (86)	8 (61.5)	-	-	3 (23.1)	13 (100)	5 (38.4)	2 (13.3)	-
Ochl. caspius	I	3	-	-		-	2 (66.7)	1 (50)			-	1 (50)	2 (100)	1 (33.3)	-
	O	3	-	-		-	2 (66.7)		1 (50)	1 (50)	-	1 (50)	1 (50)	1 (33.3)	-

1 Indoor.**O.** Outdoor **RI.** Rested indoor **RO.** Rested outdoor
[a]Percentage of each blood meal from the total number of mixed blood. [b]Mixed blood meals

Cow blood was detected mixed with a human in large number from females collected indoor and outdoor from Ganzor village than other villages. However, goat blood was detected from female *Cx. pipiens* and *Cx. perexiguus* that collected from indoor from El Shaheed village. The study found that, dog blood was detected from all females collected from five villages. An alternative primer combination using primers Pig 573F, Human741F, Goat 894F, Dog 368F, Cow 121F, and UNREV1025 primers, primers gave an array of diagnostic fragments distinguishable by size by agarose electrophoresis **(Fig. 1, 2, 3, 4, 5).** During the studied fragment that did not meet the product size was assumed as unknown

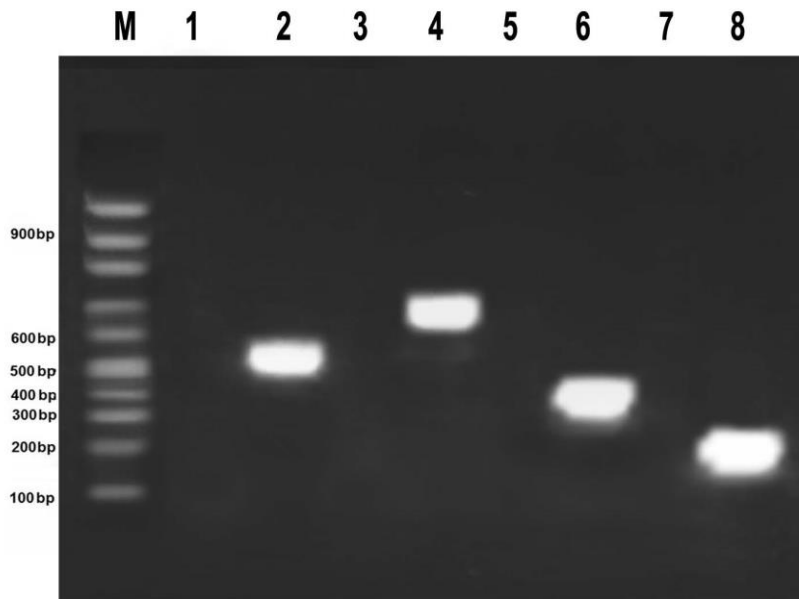

Fig. 1. Ethidium bromide–stained agarose gel showing positive controls amplified from animal whole blood extractions and negative control. Lane1,3,5,7,9 negative control; lane 2 cow; lane4 dog blood; lane 6 human; lanes 8 goat. M. DNA ladders. bp = basepairs.

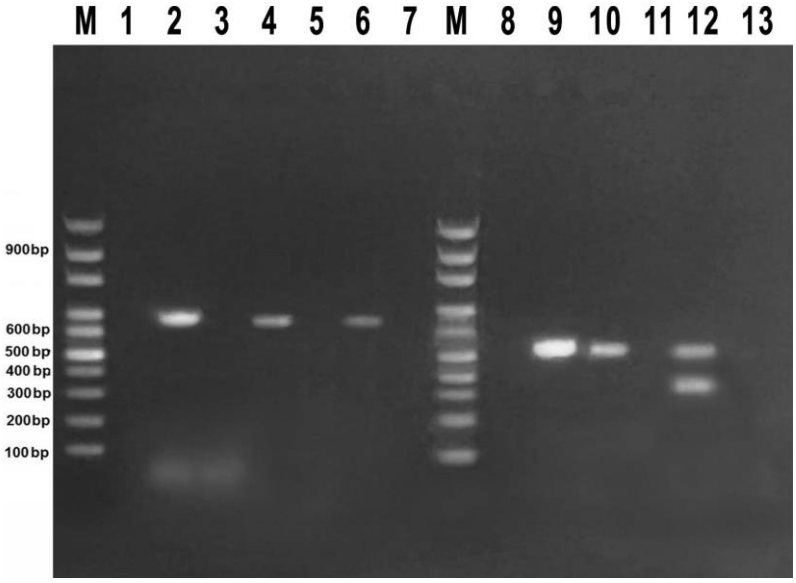

Fig. 2. Ethidium bromide–stained agarose gel showing host specific cytochrome *b* polymerase chain reaction products amplified from whole blood DNA extractions. Lane1,8 negative control; lane 2 dog (positive control); lane 3 indoor resting *Cx.pipiens* fed on unknown; lane5 indoor resting *Cx. antennatuus* fed on unknown; lane4 outdoor resting *Cx.pipiens*fed on dog blood; lane6 indoor*Cx. perexiguus* fed on dog blood, lane7 outdoor resting *Cx. pipiens* fed on unknown blood. lane9 cow blood (positive control); lanes 10 outdoor *Cx. pipiens* fed on cow blood; lane11 indoor resting *Cx. pipiens*fed on unknown; lane12 outdoor *Ochl. caspius* fed on cow blood and unknown; lane 13 indoor *Cx.pipiens* fed on unknown blood;. M. DNA ladders. bp = basepairs.

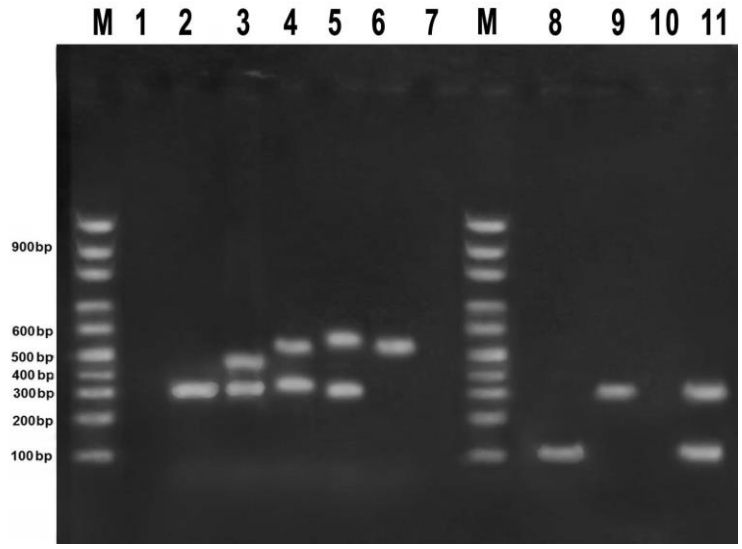

Fig. 3. Ethidium bromide–stained agarose gel showing host specific cytochrome b polymerase chain reaction products amplified from whole blood DNA extractions. Lane1 negative control; lane 2 human positive control; lane 3 indoor resting *Cx. pipiens* fed on human and unknown; lane4 indoor *Cx. antennatus* fed on unknown and cow; lane5 indoor resting *Cx. pipiens* fed on human and unknown, lane 6 outdoor *Cx. pipiens* fed on cow blood. Lane7 indoor *Cx. perexiguus* fed on unknown blood; Lane 8 goat positive control (positive control); Lane9 indoor *Cx. pipiens* fed on human; lane10 indoor *Cx. perexiguus* fed on a human blood; lane 11 indoor *Ochl. caspius* fed on human blood and goat. M. DNA ladders. bp = basepairs.

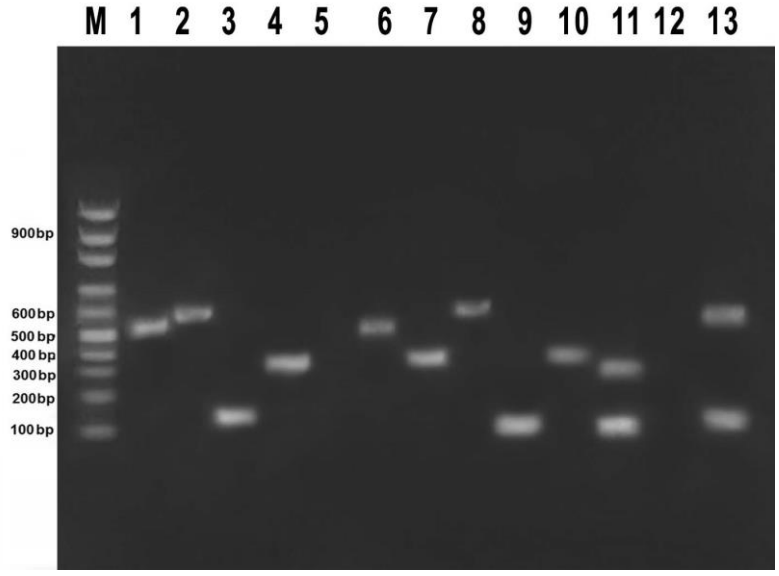

Fig. 4. Ethidium bromide–stained agarose gel showing host specific cytochrome b polymerase chain reaction products amplified from whole blood DNA extractions. Lane 1, 2, 3, 4 positive control (human, cow, dog and goat blood); lane5 negative control; lane6 outdoor *Cx. pipiens* fed on cow blood; lane 7 indoor *Cx. pipiens* fed on human blood; lane8 outdoor *Cx. pipiens* fed on dog blood; lane 9,10 indoor *Cx. perexiguus* and indoor *Cx. pipiens* fed on goat and human blood respectively. Lane11 indoor *Cx. pipiens* fed on human and goat blood; lane12 outdoor *Ochl. caspius* fed on unknown blood; lane13 indoor *Ochl. caspius* fed on a dog and unknown. M. DNA ladders. bp = basepairs

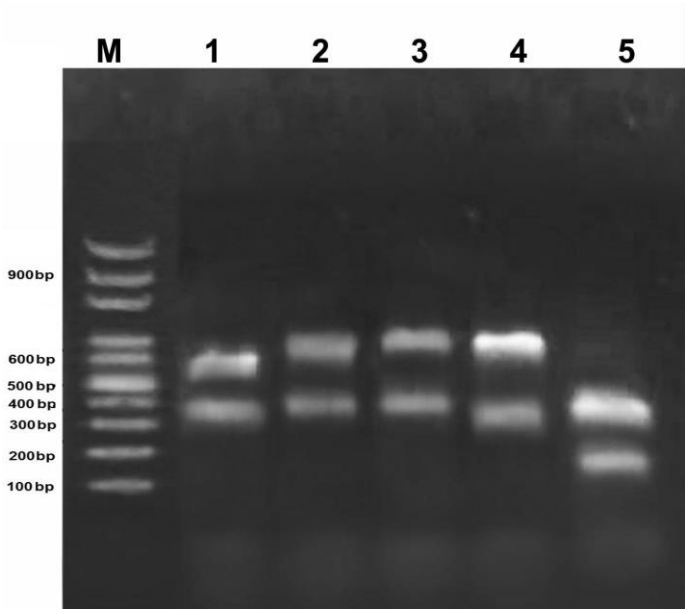

Fig. 5. Ethidium bromide–stained agarose gel showing blood meal identifications of engorged females. Lane1,outdoor resting *Cx. pipiens* fed on mixed blood (cow and human); lane2,3, 4 outdoor *Cx. pipiens* fed on mixed blood (pig and unknown blood meal) and (human and unknown blood) respectively. Lane 5 outdoor *Ochl .caspius* fed on mixed blood (human and unknown). M. DNA ladders. bp = basepairs.

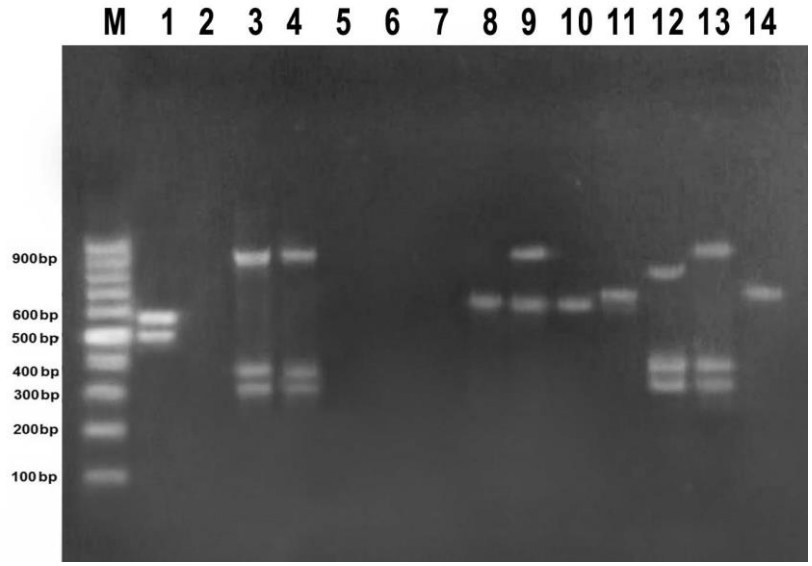

Fig. 6. Ethidium bromide- stained agarose gel showing PCR products obtained from different mosquito species resting indoor, lane 1*Cx. pipiens* fed on cow and unknown, lane3, 4*Cx. pipiens* and *Cx. antennatus* fed on mixed blood (human and unknown blood), lane 6,7 *Cx. pipiens* fed on unknown blood; lane8*Cx.antennatus* fed on mixed blood (dog and unknown), lane 8, 10, 9 *Cx. antennatus* fed on dog blood and (dog and unknown); lane11,12 *Cx. pipiens* fed on unknown and mixed blood (human and unknown blood); lane 13 *Cx. perexiguus* fed on (unknown blood and human) lane 14 *Cx. perexiguus* unknown. Control unfed female mosquitoes are shown in lane 5. Outside lanes are 100-basepair DNA ladders. bp = basepairs.

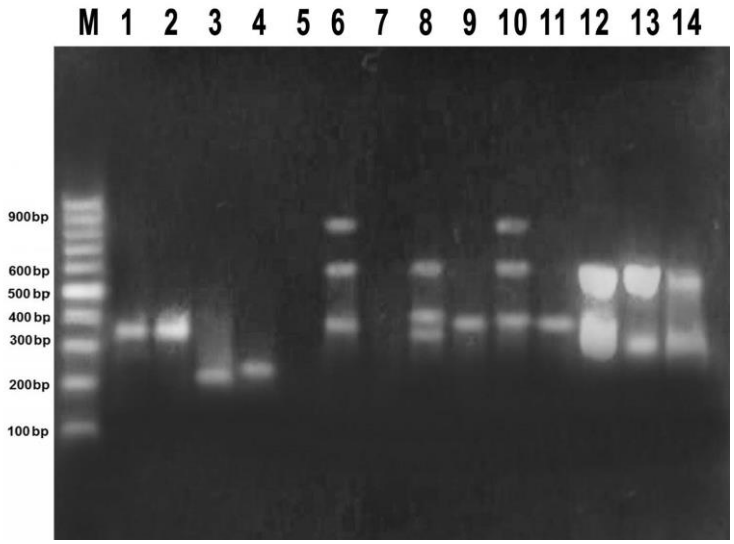

Fig. 7. Ethidium bromide-stained agarose gel showing multiple blood meals were also detected in most indoor and outdoor samples. Lane1, 2 indoor *Cx.pipiens* fed on (human); lane 3, 4 outdoor *Cx. Pipiens* fed on unknown blood; lane 6 *Cx. pipiens* indoor fed on mixed blood (dog and unknown). Blood meal identification from mosquito resting indoor showed that, lane 7, 8 *Cx. pipiens* fed on unkown and mixed blood (dog and unknown), respectivily. lane 9 indoor *Cx. antennatus* fed on a human; lane 10 *Cx. pipiens* fed on mixed bood (dog, humanand unknown), lane 11 *Cx. pipiens* fed on (human blood), lane12 indoor *Cx. antennatus*and lane 13,14 *Cx. pipiens* fed on a mixed blood (cow and unknown). Unfed mosquitoes are shown in lanes 5 (negative control). Outside lanes are 100-basepair DNA ladders. bp = basepairs.

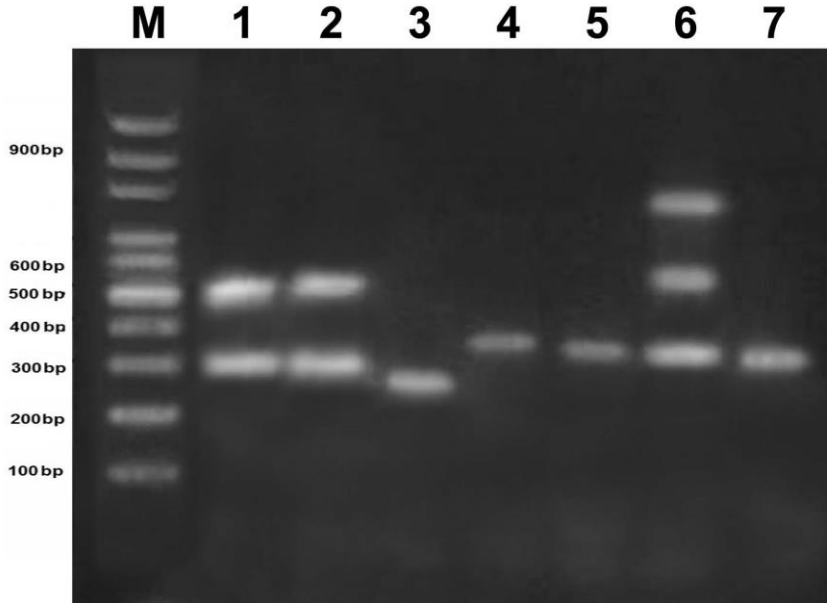

Fig. 8. Ethidium bromide-stained agarose gel showing PCR products obtained from mosquitoes collected indoor fed on different ratios of mixed blood. Lane1,2,4 *Cx. pipiens* fed on mixed blood (cow and unknown) and human blood, respectively; lane3,5*Cx. perexiguus* fed on mixed blood (unknown) and (human), respectively; lane6 outdoor female *Ochl. caspius* fed on (human, cow and unknown); lane7 indoor resting *Cx. pipiens* fed on (human) blood

DISCUSSION

A blood meal is a source of protein which is essential for female ovarian and eggs development (Clements, 1992). Study the blood-feeding behavior of *Culex* mosquitoes provides information about their relative roles as enzootic and epidemic vectors of arboviral diseases in this region. In this study blood, feeding analysis was conducted by using PCR-based method (Townzen *et al.*, 2008). The using of especially cytochrome *b* gene in this study is due to it has been used over earlier analyses and showed specificity to identify the blood-meal source to the species level. Species-specific cytochrome *b* fragments of the predicted size were increased from a cow, human, pig, goat, and dog blood employing a novel, multiplexed, vertebrate-specific primer set developed by Kent and Norris (2005). Effective amplification was gotten utilizing template DNA from entirety blood and blood-fed field mosquitoes (Figures 1). For field specimens where blood meal identification failed, host DNA concentrations may be deficiently for detection due to blood feast volume, the forms of absorption may have denatured DNA, or the mosquito may have nourished on an animal not included within the demonstrative measure. In field specimens from which blood meals fizzled to increase initially, reamplification of those clearly negative PCR products resulted in unmistakable products, suggesting that host DNA was at a low concentration within the source extraction. An important advantage of PCR diagnostic assay was developed by Kent and Norris (2005). A final important advantage of this assay is that, it identifies the mammalian host directly by size-differentiated DNA fragments on agarose gels in a single step and it successfully detecting blood within 48 hours (Raweewan *et al*., 2014). During the study collection of engorged females was conducted after 12 hours from placing the traps and preserved in -20 °C as DNA could be detectable in frozen mosquito abdomens from 24 to 30 hours of post-feeding (Kent and Norris, 2005). However, the rate of blood-meal digestion depends on the species because of different digestive mechanisms (Siriyasatien*et al.*, 2010). As female *Ae. Aegypti* completely digested a blood meal within 36 hours at 27 ^{O}C (Briegel and Lea, 1975). Beier *et al.* (1988) demonstrated that, blood in *Anopheles* has been detected at up to 32 hrs for dried mosquitoes, and 23 hours for fresh ones post-feeding. The avian blood meals were detected in *Cx pipiens pipiens* L. up to 72 hours' post-feeding (Ngo and Kramer, 2003) and DNA from human blood was detected in mosquitoes until 48 hrs post-feeding by PCR (Raweewan*et al.*, 2014). Also, preservation method and detection technique affect the length of time that blood can be detected, for this reason engorged females were preserved at 20 ^{O}C to prevent the degradation of host DNA by the digestion of the blood meal occurs in the insect gut and maintain all the blood meal

sources (Oshaghi *et al.*, 2006). The obtained results indicated that, the prevalence of blood fed adult mosquitoes varied among the 5 study villages as a large number of mosquito species collected from Ganzor village, this is might due to the presence of suitable breeding sites water bodies thus creating a big pool of potential vector risks for filariasis and RVF in Nile Delta. The study showed that, *Cx. pipiens* is the most abundant species (71.2%) as compared with the other collected species, this indicated by other studies in other areas throughout Egypt (Hoogstraal *et al*., 1979 Zayed 1989 and Zayed *et al.*, 2015). *Cx antennatus* was the 2nd collected mosquito species fed mainly on human 100% from indoor as mixed blood and 10% as single blood meal, as well as cow blood 66.6% because of that this species act as a secondary vector of filariasis and in arbovirus transmission (Darwish and Hoogstraal, 1981, Zayed 1989 and Zayed et al., 2015).

The results may be agree with Mattingly (1969), who stated that, no species of mosquito feeds exclusively on man. Nor are vector species necessarily predominantly anthropophilic. *Culex* mosquito transmitting various forms of encephalitis seems to owe their efficiency as vectors mainly to their unusual plasticity with regard to avian and mammalian hosts. *Cx. pipiens* for all its close association with man, feed extensively on birds, including domestic poultry even under urban conditions.

Other study conducted by Chandler *et al.*(1975) using a CDC light trap indoors, demonstrated that, the *Cx. antennatus* host range is wider as 54% of the *Cx. antennatus* meals were from human, 20% from cattle and 18% from birds.

In the present, study *Ochl. caspius* were collected from only two villages (6%) this is might due to the presence of suitable breeding sites in these two villages as water of these breeding places were stagnant or slow running, turbid with high level of solid substances specially floating plants and garbage which indicated that, this species preferred breeding in these places. This geographic variation in host-feeding by mosquito species in Egypt has been attributed to relative host abundance, which reflects the ecological conditions and human customs (Zimmerman *et al*. 1985, Beier *et al*. 1987, Zayed 1989, and Wassim *et al.* 2013). The study demonstrated that, the 4 dominant mosquito species feed on a wide variety of hosts, this feeding behavior, confirming by earlier reports (Kenawy *et al.*1987 and Gad *et al.* 1995). This is important because these mosquitoes may have served as a bridge vector between humans and domestic animals. The study also found that for collected species more blood-fed was collected during Spring this might be indicated that, female flight activities (e.g. host searching) increased during favorable temperature conditions (Lebl *et al*., 2013). However, the lower number of blood-fed females during Winter season reaffirms that, blood feeding does not likely occur

among diapausing females during the winter and early spring in the hibernacula (Mitchell, 1983).

During the study there was marked variation in feeding behavior between each collection set as *Cx. pipiens, Cx. antennatus, Cx. perexiguus* and *Ochl. caspius* predominated in outdoor trap collections, half fed and fed mosquito comprised a lower portion of outdoor collections. However, the majority were collected as unfed and gravid which indicated the endophagic behavior of *Culex* species (Kaul and Wattal, 1968). The collection of engorged *Ochl. caspius* during the study from indoor indicated that, this species known to bite indoor before nightfall (Gillet, 1972). The study found that, the higher collection of blood-fed mosquito species were from indoor 43% (127) than outdoor 14% (42), which indicates the endophilic nature of the *Culex* species and tendency of this species to rest in the site of feeding after feeding thus may account for most human infections. Similar results were reported by Gowda and Vijayan (1992) as a large proportion rest indoors before completion of their gonotrophic cycle. The relatively high proportions of blood-fed mosquitoes collected in light traps indicated that, these traps may indeed be used successfully to collect engorged mosquitoes after they have fed and are seeking a resting place (Pappa *et al.*, 2011).

During the study most of the blood-fed field *Culex* species contained mixed blood this indicated the gonotrophic discordance, or taking multiple blood meals during the gonotrophic cycle which reported in different mosquito species in other studies (Boreham *et al*., 1979and Scott *et al.*, 2000) and if the blood meal is interrupted, mosquito is able to bite several hosts (Enguehard *et al.*, 2018). The study found that, collected *Culex* species inside houses, fed predominantly on humans and other large mammals this indicate the endophagic nature of this species. In addition, the results indicated that, blood fed *Cx. pipiens* rested indoor 41% (120) than resting outdoor 1% (3) so it is endophilic species and may account for most human infections. The study indicated that, *Cx. pipiens* and *Cx. perexiguus* fed on goat blood indifferent range from El Shaheed village this is have been important in virus dissemination during RVF epidemics due to sheep is an important reservoir host of RVF virus (Kenawy *et al*., 1987). Previous studies in Egypt indicated that *Cx. pipiens* females were mostly fed on man (anthropophilic species) and the vertebrate mammals represented the most host (Zayed, 1989). Although pig is not common in El Monufia its blood was detected with human blood from outdoor and outdoor resting *Cx. pipiens* and indoor *Cx. perexiguus* which induce transmission of Japanese encephalitis virus where pigs and humans are hosts (Eldridge *et al*., 2000). The study also found that, most blood-fed field *Culex* species from indoor contain mixed blood from both human and animal sources, this is due to houses generally were associated with animal sheds where domestic

animals (horses, donkeys, cattle, buffalo, goats, and sheep) were kept at night this acting as the primary link between animals in those villages. The highest number of contained mixed blood meals was indicated by other studies (Zinser *et al.*, 2004). This host-feeding behavior can influence pathogen transmission through increased frequency of vector-human contact, or possibly reduce vector-human contact if some blood meals are taken from alternative mammalian hosts. However, mixed-source blood meals have been reported for a number of *Culex* species by using different methods for blood-meal identification (Magnarelli *et al*., 1977 and Apperson *et al*., 2002). This mention by other studies as Molaei *et al*. (2008)who demonstrated that, host-feeding patterns and preferences vary according to environmental factors, host availability and abundance, flight and feeding behavior of mosquitoes. This study and most studies have shown that, *Cx. pipiens* is the most abundant species in other areas throughout Egypt (Hoogstraal *et al*., 1979 and Zayed *et al.*, 2015).

The study investigated host sources of blood meals from four mosquitoes. However, some unidentified blood meals may be attributed to blood meals acquired from hosts not considered in this study, including cats and donkeys or even avian blood which usually found associated with human and indoor. This is true also in other studies that conducted in Nile Delta, (Zimmerman *et al*., 1985) other study conducted in Sudan also detected 42% of blood meal as unknown (Gad *et al.*, 1999).

Overall Conclusions

Our study assessed to understand the distribution, composition and feeding behavior of the mosquito population in El-Monufia governorate. We found that *Cx. pipiens* was the dominant mosquito species in outdoor and indoor thus creating a big pool of potential vectors for viruses like Rift Valley Fever (RVF), West Nile Virus (WNV), and other arboviruses. The other species found in our study sites, *Cx. antennatus, Cx. perexiguus* and *Ochl caspius.* Although pig is not common in El Monufia, but its blood was detected with human blood from outdoor and outdoor resting *Cx. pipiens* and indoor *Cx. perexiguus* which induce transmission of some virus where pigs and humans are hosts.

REFERENCE

Agrawal, S. K. (2015): Host Selection and Multiple blood feeding behavior of *Culex quinquefasciatus* in Raipur city, Chhattisgarh state, India. International J. of Applied Research. 1, 4: 30-33, 2015.

Apperson, C.S.; Harrison, B.A.;Unnasch, T.R.; Hassan, H.K.; Irby, W.S. and Savage, H.M.(2002): Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of Culex mosquitoes. J.Med.Entomol. 39:777–785, 2002.

Bahgat, I. M. (2000): Host-feeding patterns of *Culex pipiens* (Diptera- Culicidae) in El-Abtal village, Ismailia Governorate, Egypt. Egyptian J. Biol, Vol. 2, 139-143.

Beier, J. C.; Perkins, P.V.; Wirtz, R. A.; Koros, J.; Diggs, D.; Gargan, T.P. and Koech, D.K. (1988):Blood meal identification by direct enzyme- linked immunosorbent assay (ELISA), tested on Anopheles (Diptera: Culicidae) in Kenya. J. Med. Entomol. 25, 1:9-16, 1988.

Beier, J. C.; Zimmerman, J. H.; Kenawy, M. A.; El Said, S. and Abbassy, M. M. (1987): Host feeding patterns of the mosquito community (Diptera: Culicidae) in two Faiyum Governorate villages. J. Med. Entomol.24:28-34, 1987.

Boreham, P.F.; Jenahan J.K.; Boulzaguet, R.; Storey, J.; Ashkar, T.S. and Nambian, M. T.(1979): Studies on multiple feeding by *Anopheles gambiae*s.l. in a Sudan savanna area of northern Nigeria. Trans. R. Soc. Trop. Med. Hyg. 73: 418–423, 1979.

Briegel, H. and Lea, A.O. (1975): Relationship between protein and proteolyptic activity in the midgut of mosquitoes. J. Insect Physiol. 21:1597-604, 1975.

Chandler, J.A.; Highton, R.B. and Hill, M.N. (1975): Mosquitoes of the Kano plain, Kenya I. Results of indoor collections in irrigated and non-irrigated areas using human bait and light traps. J. Med. Entomol.12: 504-510, 1975.

Clement, A.N. (1992): The Biology of Mosquitoes Development, Nutrition and Reproduction. Chapman and Hall, London. 1, 1:509, 1992.

Darwish, M. A. and Hoogstraal, T. (1981): Arboviruses infecting humans and lower animals in Egypt: a review of thirty years of research. J. Egypt. Pub. Hlth. Assoc. 56: 111-112, 1981.

Dowen, A. E. R. and Archer, J. A. (1975): The effects of different blood meal sources on digestion and egg production in *Culex tarsalis* Coq. (Diptera: Culicidae). J. Med. Entomol. 12: 431-437, 1975.

Delatte, H.; Desvars, A.; Bouétard, A.; Bord, S.; Gimonneau, G.; Vourc'h, G. and Fontenille, D. (2010):Blood-feeding behavior of *Aedes albopictu*, a vector of Chikungunya on La Réunion. J. Vector Borne Zoonotic Disease. 10, 3:249–258, 2010.

Edman, J. (1974): Host-feeding patterns of Florida mosquitoes, three *Culex (Culex)* and *Culex (Neoculex)*. J. Med. Entomol. 8, 6: 687–695, 1974.

Eldridge, B.F.; Scott, T.W.; Day, J.F. and Tabachnick,T.J.(2000): Arbovirus Diseases. In: Eldridge, B.F. and Edman, J.D. editors. Medical Entomology: A Textbook on Public Health and Veterinary Problems Caused by Arthropods. Dordrecht, the Netherlands: Kluwer Academic Publishers.415–461, 2000.

Enguehard, M. (2018): Interaction between Chikungunya and dengue viruses during co-infection in *Aedes* mosquito cells and in *Aedes aegypti* mosquito. Virology. Université de Lyon.Lifescience.2, 1: 35, 2018.

Gad, A.M.; Farid, H.A.; Ramzy, R.R.; Riad, M.B.; Presley, S.M.; Cope, S.E.; Hassan, M.M. and Hassan, A.N. (1999): Host feeding of mosquitoes (Diptera: Culicidae) associated with the recurrence of Rift Valley fever in Egypt. J. Med. Entomol. 36, 6:709–714, 1999.

Gillet, J.D. (1972): Common African Mosquitoes and their Medical Importance. William Heinemann Medical Books Ltd, London. 106,1972.

Gillies, M.T. (1988): Anopheline mosquitos: vector behavior and bionomics. In wernsdorfer, W.H. and McGregor, I. eds. Malaria, principles and practices of malariology. Churchillliving stone, Edingburgh.769-779, 1988.

Gowda, N.M. and Vijayan, V.A. (1992): Indoor resting density, survival rate and host preference of *Culex quinquefasciatus* say (Diptera: Culicidae) in Mysore city. J. Comm. Dis. 24, 1: 20-28, 1992.

Harbach, R.E. (1985): Pictorial keys to the genera of mosquitoes, subgenera of *Culex* and the species of *Culex* (*Culex*) occurring in southwestern Asia and Egypt, with a note on the subgeneric placement of *Culex deserticola*. Mosquito Systematics.17, 2: 83-107, 1985.

Hoogstraal, H.; Meegan, J.M.; Khalil, G.M. and Adham, F.K. (1979): The Rift Valley fever epizootic in Egypt 1977–1978. 2. Ecological and entomological studies. J. Trans. Royal Soc. Trop. Med. Hyg. 73: 624–629, 1979.

Hurlbut , H. S. and Weitz, B.(1956): Some Observations on the Bionomics of the Common Mosquitoes of the Nile Delta. J. Am. Soc. Trop. Med. Hyg. 5, 5:901– 908, 1956.

Kaul, H.N. and Wattal, B.L. (1968): Studies on the culicines mosquitoes. IV. Influence of climate on the feeding behavior of *Culex fatigans* females in village Artala, Near Delhi. Bull. of the Indian Society for Malaria and other Comm. Dis. 5:45-52, 1968.

Kenawy, M. A.; Beier, J. C.; Zimmerman, J. H.; El Said, S. and Abbassy, M. M. (1987): Host feeding patterns of the mosquito community (Diptera: Culicidae) in Aswan Governorate, Egypt. J. Med. Entomol. 24:35-39, 1987.

Kent, R.J. and Norris, D.E. (2005): Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. J. of Am. Trop. Med. Hyg. 73, 336–342.

Lebl, K.; Brugger, K. and Rubel, F. (2013): Predicting *Culex pipiens restuans* population dynamics by interval lagged

weather data. J. Parasites and Vectors. 6:129, 2013.

Magnarelli, L.A. (1977): Host feeding patterns of Connecticut mosquitoes (Diptera: Culicidae). J. Am J Trop Med Hyg. 26:547–52, 1977.

Mitchell, C.J. (1983): Differentiation of host-seeking behavior from blood-feeding behavior in overwintering Culex pipiens (Diptera: Culicidae) and observations on gonotrophic dissociation. J. Med. Entomol. 20: 157-163, 1983.

Molaei, G; Andreadis, T.G.; Armstrong, P. M. and Diuk-Wasser, M. (2008):Host-feeding patterns of potential mosquito vectors in Connecticut, USA: molecular analysis of blood meals from 23 species of *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Psorophora*, and *Uranotaenia*. J. Med. Entomol. 45:1143–1151, 2008.

Mukabana, W.R.; Takken, W.; Seda, P.; Killeen, G.F.; Hawley, W.A. and Knols, B.G.J. (2002): Extent of digestion affects the success of amplifying human DNA from blood meals of *Anopheles gambiae* (Diptera: Culicidae), J. Bull Entomol. Res. 92: 233–239, 2002.

Ngo, K.A. and Kramer, L.D. (2003): Identification of mosquito blood meals using polymerase chain reaction (PCR) with order-specific primers. J. Med Entomol. 40: 215–222, 2003

Oshaghi, M.A.; Chavshina, A.R.; Vatandoost, H; Yaaghoobi, F.; Mohtarami, F. and Noorjah, N. (2006): Effect of post- ingestion and physical conditions on PCR amplification of host blood meal DNA in mosquitoes. Exp. Parasitology. 112,4: 232-236, 2006.

Pappa, V, Reddy M. , Overgaard H. J., Abaga S. and Caccone A. (2011):Estimation of the Human Blood Index in Malaria Mosquito Vectors in Equatorial Guinea after Indoor Antivector Interventions, Am J Trop Med Hyg. 84, 2 :298–301, 2011.

Rao, T.R. (1984): The Anophelines of India. Second Edition. Malaria Research Centre. New Delhi: Indian Council of Medical Research.193-195, 1984.

Raweewan, S.; Sungsit, S.; Maxime, J.; Narumon, K.; Chamnarn, A.; Jean Pierre, D. and Sebastien, B. (2014): preserving blood-fed *Aedes albopictus* from field to laboratory for blood source determination. 3: 31:39, 2014.

Scott, T.W.;Amerasinghe, P.H.; Morrison, A.C.; Lorenz, L.H.; Clark, G.G.; Strickman, D.; Kittayapong, P. and Edman, J.D.(2000): Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. J Med.Entomol. 37: 89–101, 2000.

Service, M.W. (1993): Mosquito ecology, Field sampling methods. London, Elsevier Applied Science Publisher Ltd, Vol. 1, PP. 954.

Siriyasatien, P.; Pengsakul, T.; Kittichai, V.; Phumee, A.; Kaewsaitiam, S.; Thavara, U.;

Tawatsin, A. Asavadachanukorn, P. and Mulla, M.S. (2010): Identification of blood meal of field caught *Aedes aegypti* (L.) by multiplex PCR. Southeast Asian J. Tropical Medicine Public Health. 41:43–47.

Takken, W. and Verhulst, N.O. (2013): Host Preferences of Blood-Feeding Mosquitoes. Annuale Review Entomology.58:433–453.

Townzen, J.S.; Brower, A.V. and Judd, D.D. (2008): Identification of mosquito blood meals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. J. Med. and Vet.Entomol. 22: 386–393, 2008.

Washino, R.K. and Tempelis, C.H. (1983): Mosquito host blood meal identification.J.Annual review of Entomol. 28:179–201, 1983.

Wassim, N.M.; Soliman, B.A.; Yamani, D.F. Ismail, M. and Nabi, A. (2013): Rapid assay to differentiate the two forms of Egyptian *Aedes caspius* (Diptera: Culicidae) using acetylcholineestrase gene. The J. of basic and applied zoology. J. Basic and Applied for Zoology. 66:12-17, 2013.

Wattal, B.L. (1976): Entomological parameters and their relevance in filariasis control. J. Comm. Dis. 8:328.

WHO (1975): Manual on practical entomology in malaria. Part (I). Vector bionomics and organization of anti-malaria activities.WHO Division of Malaria and other Parasitic Diseases, WHO, Geneva.

Zayed A. B. (1989): Ecology of filaria bancrofti vectors in certain endemic and non-endemic areas in lower Egypt. M.Sc. thesis, Faculty of Science, Ain Shams University.

Zayed, A.B.; Britch, S.C.; Soliman, M.S. and Linthicum, K.J. (2015): Mosquitoes and the environment in Nile Delta villages with previous Rift Valley fever activity. J.Am.Mosq. Control Assoc. 31, 2:139-148, 2015.

Zimmerman, J.H.; Hanafi, H.A. and Abbassy, M.M. (1985): Host-feeding patterns of Culex mosquitoes (Diptera: Culicidae) on farms in Gharbiya Governorate, Egypt. J.Med. Entomol. 22:82–87, 1985.

Zinser, M.; Ramberg, F. and Willott, E. (2004): *Culex quinquefasciatus* (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: Blood meal analysis indicates feeding on both humans and birds. J. Insect Sci. 4:20, 2004.