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The role of mosquito in the transfer of blood Protozoa in Baghdad, Iraq

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Abstract
The current study revealed the presence of *Plasmodium* sp. in Anopheline female mosquitoes which were collected indoor from different areas in Baghdad city, this result proved that malaria is still in Baghdad in two area: Al Ameria (in the west) and Al Geraiat (in the north); While other nine areas were negative. In our study, nearly most of the phases of life cycle were appeared in gut and salivary of mosquitoes as well as Sporozoites, Gametocyte and Sporogony; these phases findings revealed to the parasites which dormant in mosquitoes (hypnozoites) that are the only hidden parasites obstacle to malaria eradication.
The current finding revealed to predictions of retransmission of malaria in Baghdad is probability at any time. This data can update the information system surveillance and strategies into the national program of malaria elimination.

Keywords: Mosquito, *plasmodium vivax*, anopheline, protozoa

1. Introduction

Mosquito as entomologic investigation of blood parasites that guide to the prevalence of the disease in certain area [1]. Anopheline mosquitoes are the criminal vector for Malaria disease [2]. The tropics and subtropics countries still have major public health problem Malaria, over 40% of the world's population are at risk, and it is endemic in 91 countries, mostly developing [3]. Eradication of malaria is difficult because of hidden parasites in vectors and host [4].

Three species of Anopheline mosquitoes were recorded in Iraq which criminal to cause malaria disease that *A. superpictus*, *A. sacarovi* and *A. pulcherrimus* by Macon [5], then Manoucheri [6] recorded them in southern, center and north regions of Iraq. However, Al Ghoury [7] revealed to high density of both *Anopheles stephensi* and *A. pulcherrimus* in Babylon province. Recently, Hantosh [8] recorded four species of *Anopheles*: (*An. pulcherrimus*, *An. stephensi*, *An. superpictus,* and *An. sacharovi*) and one species of *Culex* (*Cx. pipiens*) in a survey for all Iraq provinces accept Baghdad province.

The prevalence of malaria infection in Iraq from 1970-1975 was about 47395 cases [9]. Then from 1977- 1984 was about 20191 cases, [10]. However, Al-Muktar, [11] recorded the infected rate 7.44% in patient of Rabiea region in the north of Iraq.

According to the report of WHO [12]: "During 5 years ago, nearly one million people were killed by malaria globally each year, most of them were children. Iraq has participated and stands proud with its achievements today. The last 2 indigenous malaria cases were reported in Iraq in 2008. In 2014, two imported cases of malaria were diagnosed in Iraq among non-national individuals. In 2015, another 2 imported cases were reported; one was national and one non-national. There is risk of re-introduction of malaria from visitors, including those coming for religious tourism and employment, as well as mass population movement, either by refugees or internally displaced populations. Despite the critical situation in Iraq, great progress has been made in the field of malaria. Currently, Iraq is in the process of developing national strategy for malaria for 2016–2020. The main priorities for keeping the country malaria-free include."

According to the recent report of travel health prevention at 10 July 2018 [13]: "There is a very low risk of malaria in the rural northern area of Iraq below 1,500m, from May to November: awareness of risk and bite avoidance recommended. There is no risk in the rest of Iraq". That mean Baghdad city inside the risk area. For this reason, Baghdad city was selected as a study area.

Aim of the present study are:

1. Xenomonitoring of the vector mosquitoes (Diptera: Culicidae) to diagnose blood protozoa in different areas of Baghdad.
2. Determining areas of malaria disease prevalence in Baghdad.
3. Improve that hypnozoites occur in such mosquito-transmitted.

2. Materials and Methods

2.1. Sites of mosquito collection

A total of 433 females mosquito were trapped indoor from bedrooms and bathrooms, from five areas of Baghdad city the capital of Iraq, that were north area include Al-Shaab, Hi Al Benok and Al Geraiat; south area include Al- Doraa and Al Nahrwan; west area include Al- Ameria and Hi Al Jehad; east area include Al-Mashtal and Al Amin; center area include Bab Al-Muadum and Bab Al Karada; west area include Al America and Hi Al Jehad, during the period from March of 2017 to October of 2018.

All mosquito females were identified under dissecting microscope (Leica Microsystems GmbH, Wetzlar, Germany) and classified by Becker [14] key in Iraq Natural History Research Center and Museum.

All mosquitoes were dissected and stained with Giemsa, the gut and gland examined for oocysts and sporozoites [15].

3. Results and Discussion

The current study revealed to *Plasmodium* sp. still the first blood protozoa that transmitted by Anopheline mosquito in Baghdad city, Iraq.

3.1 Mosquito abundance in studied areas

A total of 433 mosquitoes that 42 (9.69%) were positive for *Plasmodium* sp.; From eleven studied areas in Baghdad city, two areas recorded positive for infection with *Plasmodium* sp.; These are Al Geraiat (19/47.5%) in north of Baghdad and Al America (23/65.71%) in west of Baghdad Table 1.

Table 1: Distribution of infected mosquitoes with *plasmodium* sp. in studied areas in Baghdad city, Iraq.

	Region	Site	No. of mosquitoes	Result	No. of positive	%
1	Al Shaab	North	24	Negative	-	-
2	Hi Al Benok	North	25	Negative	-	-
3	Al Geraiat	North	40	Positive	19	47.5
4	Al Dora	South	45	Negative	-	-
5	Al Nahrawn	South	35	Negative	-	-
6	Al Mashtal	East	40	Negative	-	-
7	Al Amin	East	45	Negative	-	-
8	Bab Al Muadum	Center	50	Negative	-	-
9	Al Karada	Center	54	Negative	-	-
10	Al Ameria	West	35	Positive	23	65.71
11	Hi Al Jehad	west	40	Negative	-	-
			433		42	9.69

The current study revealed the presence of *Plasmodium* sp. in Anopheline female mosquitoes which collected indoor from different areas in Baghdad city, this result proved that malaria is still in Baghdad in two area (Al Ameria and Al Geraiat); While other areas were negative, it was because collected mosquitoes were not *Anophelse* sp. but comprises of *Culex*, *Aedes* or *Culicin*. This is similar to Abd Shar and Ahmed [16] who recorded *Culex quinquefasciatus* genetically in Nahrawan region south of Baghdad city. These finding is regarded as early warning of malaria epidemic.

528 species of *Anopheles* mosquitoes have been recorded, and approximately 80 of them transmit *Plasmodium* sp. and cause malaria, [17]. There is difficulty in precisely identifying sibling species that similar in morphology lead to misidentification of Anopleles, [18]. However, Choochote and Saeung [19] concluded that cytological markers (characteristics of metaphase chromosomes/ karyotypic forms), together with the data on comparative sequences of some specific genomic regions (rDNA and mtDNA) aid in diagnosis sibling species or subspecies members of Anopheles species complexes. According to Al-Ghoury *et al*. [7], *A. stephensi* is the major malaria vector in the central and southern regions of Iraq. So the density of *A. stephensi* indoor was as an indicator of epidemic risk.

Anopheles stephensi is a competent vector for both *Plasmodium falciparum* and *P. vivax*, the most virulent malaria-associated species [20]; While, Couto *et al*. [21] proved that *A. stephensi* transmit *Plasmodium berghei* by transcriptomics analysis. So *Anopheles stephensi* may be transmitting more than one species of *Plasmodium*.

3.2 Phases of life cycle

The current study summarized the life cycle in one drawing figure (figure 1) which is divided into two parts, Left: A fresh blood meal from the humans by mosquitoes. Right: Mosquito phase of malarial life cycle.

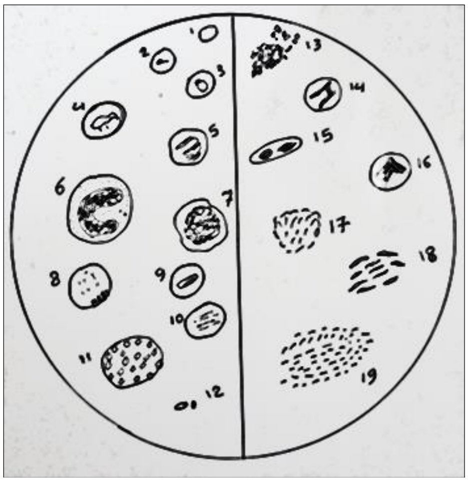

Fig 1: Left- 1. Normal red blood cell (comparative with the increased size of those containing parasite), 2. Very young trophozoite with a few Schüfuer's dots already in evidence, 3. Ring stage, 4. Trophozoite exhibiting characteristic amoeboid form, 5. Trophozoite, (probably a young gametocyte), 6. Polymorphnuclear leucocyte, 7. Larg monocyte, 8. A trophozoite simulating the marginal or "appligue" forms, 9. Trophozoite illustrating the typical band form, 10. A schizont with the chromatin is already divided more than once, 11. An erythrocyte exhibiting basophilic stippling, 12. Ring forms of *Plasmodium* lying free in blood. Right- 13. Group of merozoites with mass of pigment, 14. Trophozoite that may have started to divide precociously, 15. Ookinete, 16. A microgametocyte with a typical large marginal chromatin mass, 17. Daisy shape merozoites, 18. Sporozoites in salivary gland of mosquito, 19.Swarm of sporozoites.

A normal RBCs., trophozoite in fresh RBC, and very young trophozoite with a few Schüfuer's dots were shown in figure 2. Ring forms of *Plasmodium* lying free outside the RBCs was shown in figure 3.

Figure 4 showed five phases of parasites: Trophozoite in fresh RBCs of mammals, ring stage marginal, trophozoite illustrating the typical band form, a microgametocyte with a typical large marginal chromatin mass, and erythrocyte with three trophozoites. Trophozoite exhibiting characteristic amoeboid form as shown in figure 5.

Figure 6 showed applique forms of trophozoite and a schizont with the chromatin are already divided more than once. A Trophozoite that have started to divide precociously as shown in figure 7. Large monocyte and mononuclear leukocyte are presented in figure 8 & 9 respectively. Figure 10 showed an erythrocyte exhibiting basophilic stippling. A young gametocyte appeared as figure 11. Ookinete or zygote appeared as figure 12. Merozoites appeared as a daisy shape as figure 13. Sporogony phase appeared as figure 14. Sporozoites in gland of mosquito, and swarm of sporozoites appeared as figure 15.

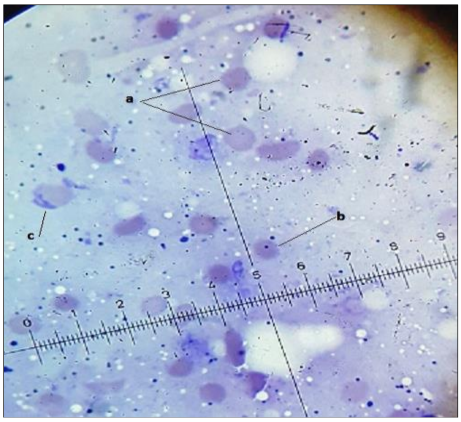

Fig 2: a. Normal RBCs. b. Trophozoite in fresh RBC. c. Very young trophozoite with a few Schüfuer's dots already in evidence.

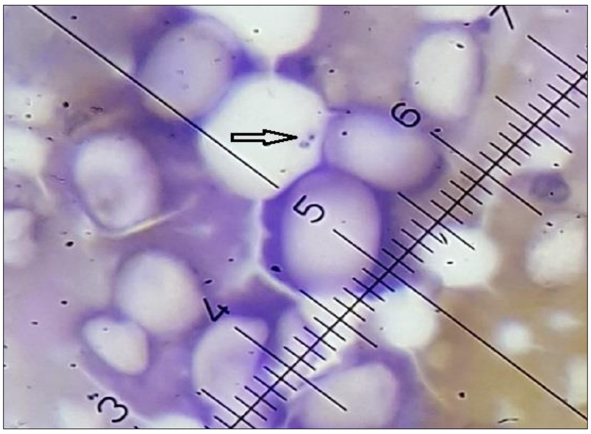

Fig 3: Ring forms of *Plasmodium* lying free outside the RBCs, 1000X.

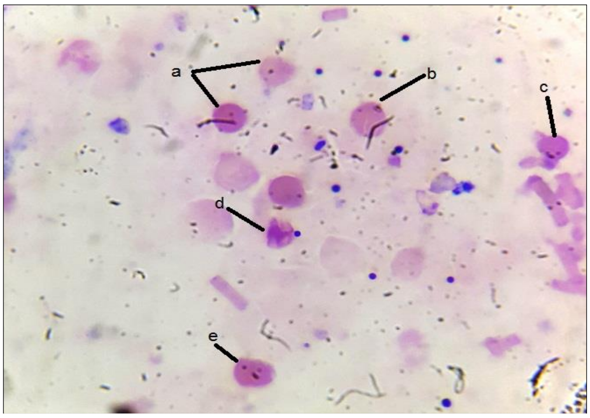

Fig 4: a. Trophozoite in fresh RBCs of mammals, b. ring stage marginal, c. trophozoite illustrating the typical band form, d. A microgametocyte with a typical large marginal chromatin mass, e. erythrocyte with three trophozoites, 2000X.

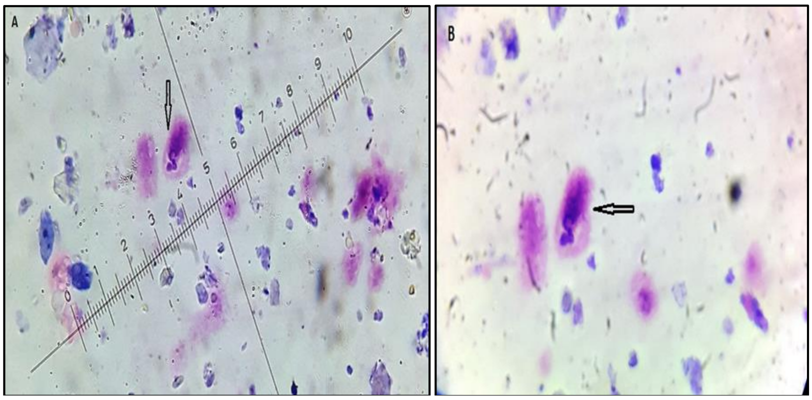

Fig 5: Trophozoite exhibiting characteristic amoeboid form, A.1000X. B. 2000X.

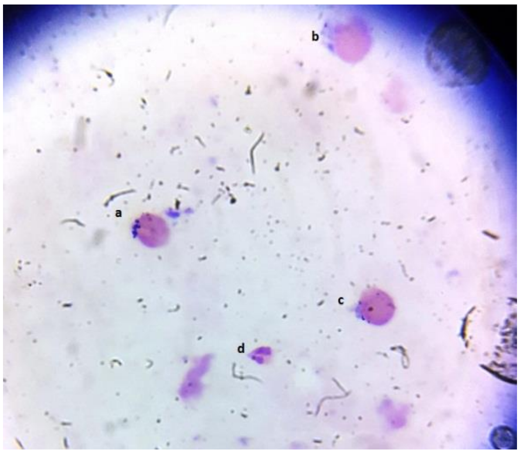

Fig 6: a, b, c. A trophozoite simulating the marginal or "applique" forms. d. A schizont with the chromatin already divided more than once. 2000X.

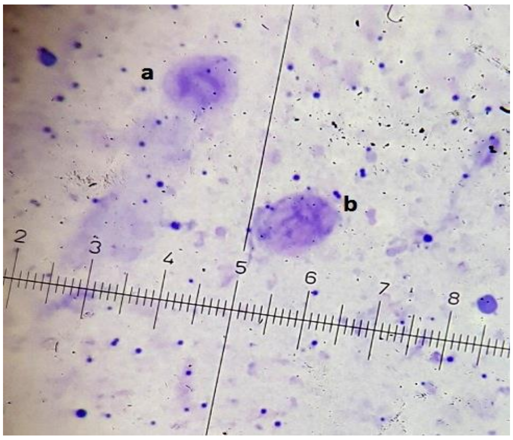

Fig 7: a. An older parasite still exhibiting the band form. b. Trophozoite that may have started to divide precociously, 1000X.

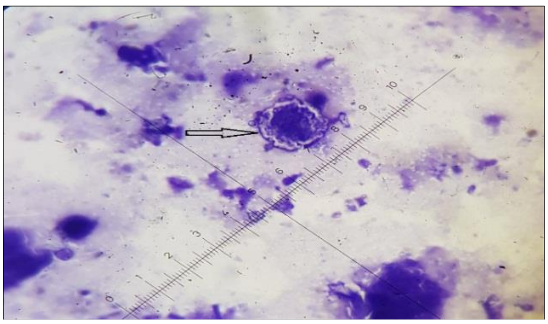

Fig 8: Large monocyte in the fresh bite of mosquito, 1000X.

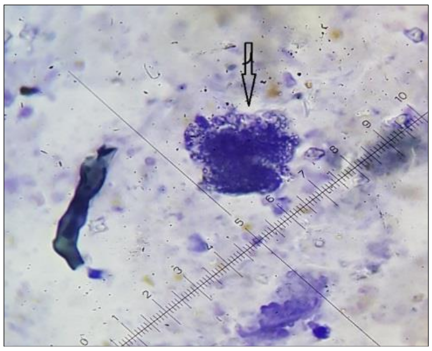

Fig 9: Mononuclear leukocyte in the fresh bite of mosquito, 1000X.

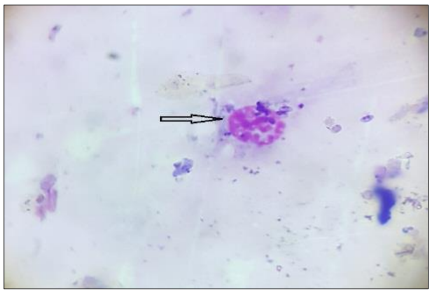

Fig 10: An erythrocyte exhibiting basophilic stippling, 2000X.

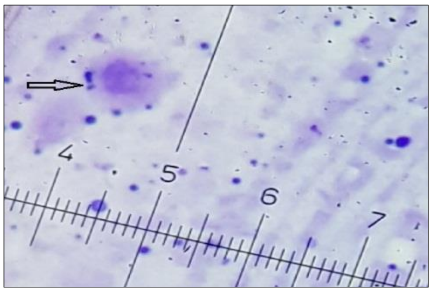

Fig 11: Trophozoite, (probably a young gametocyte).

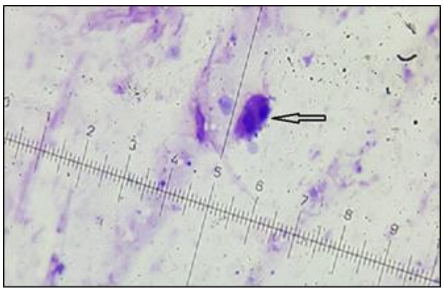

Fig 12: Ookinete or zygote, 1000X.

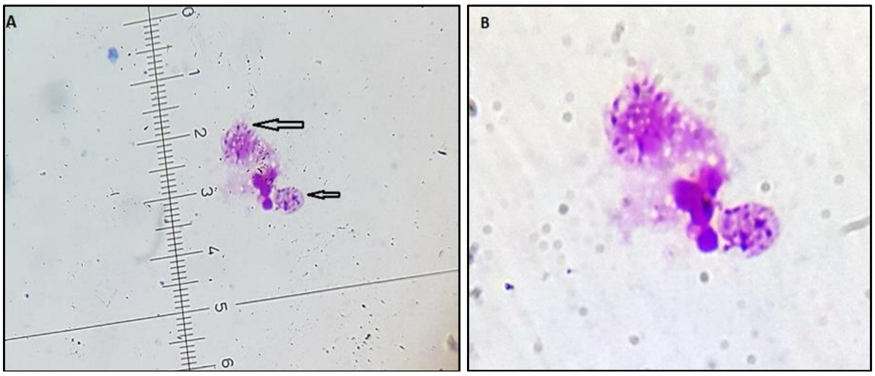

Fig 13: Daisy shape merozoites, A. 1000X. B. 2000X.

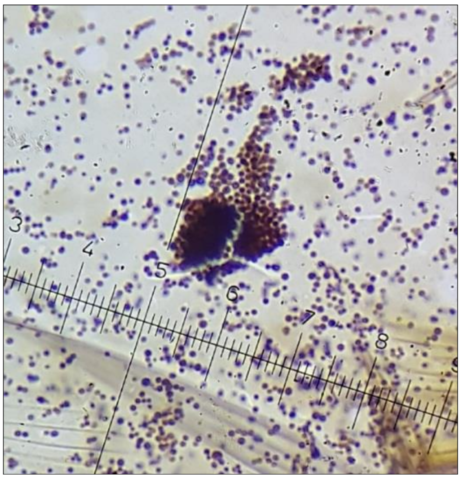

Fig 14: Sporogony occur with mass of pigment, 1000X.

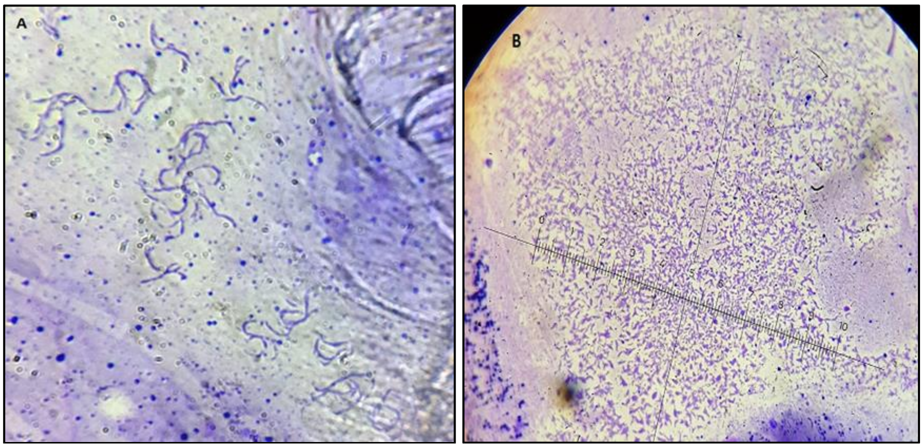

Fig 15: A. Sporozoites in gland of mosquito, 2000X. B. Swarm of sporozoites, 1000X.

A new host is infected when a mosquito carrying sporozoites (figure 15) injects them into the body of her victim, along with the salivary (anticoagulant) secretion. The minute parasites are quickly carried to remote parts of the body, for mosquitoes generally insert their proboscis directly into the capillaries when they bite, and thus the malaria organisms are injected into the blood stream as effectively as if placed there by a hypodermic needle. The sporozoites entered cells of the lymphoid-macrophage (reticuloendothelial) system within 30 minutes after being deposited in the body [22]. Female anopheles bites human again and ingests gametocytes (female gamete and male gamete), the current study distinguished Trophozoite probably a young gametocyte figure 11. After a sexual phase in mosquitoes the gametes, ookinete or zygote will be released figure 12. Oocyst in gut of mosquito appeared as a daizy shape figure 13. The sporogony occurs figure 14, and then oocyst burst and release the sporozoites which migrate to salivary gland of mosquito figure 15.

Schematic life cycle of *Plasmodium* sp. by Cox [24] in five phases: 1. Sporozoites injected by mosquito, 2. Exoerythrocytic schizogony in liver, 3. Erythrocytic schizogony in red blood cell, 4. Gametocyte taken up by mosquito, 5. Sporogony and sporozoites production in mosquito. In our study, nearly most of the phases of life cycle were appeared in gut and salivary of mosquitoes as well as Sporozoites, Gametocyte and Sporogony; these findings revealed to the parasites which dormant in mosquitoes. That present results are in agreement with Markus [25] who revealed that hypnozoites do not occur in such infections, they may be take place in mosquito- transmitted (i.e. sporozoites-initiated).

This phenomena was called hypnozoites which was recorded by Krotoski [26] who discovered of dormant exoerythrocytic stages, hypnozoites when infected with *P. vivix*. Recently, Markus [27] concluded unlikely that hypnozoites are the only hidden parasites obstacle to malaria eradication.

3.3 Morphology of *Plasmodium* species abundance

There are four species of *Plasmodium* were responsible for spread of malaria in humans in all of the world, *P. falciparum*, *P. vivax*, *P. ovale, P. malariae;* Recently, the fifth species *P. knowlesi* was recorded by Jiram *et al.* [1] is also risky for human.

The current study diagnosed *Plasmodium falciparum* from three characters that related with: First character is the marginal or "applique" forms, figure 6. Second character is young parasites lying free in blood figure 3; these characters were descripted by Manwell, [22]. Third character is segmented schizoint stage precociously, figure 7; that descripted by Radfar [23].

Whereas, *P. vivax* appeared also in three diagnostic characters in this study: First character is large amoeboid trophozoite, figure 5. Second character is group of merozoites with mass of pigment, figure 14. Third character is Swarm of sporozoites figure 15, [22]. That similar to Al-Mukhtar [11] who revealed to the *P. vivax* has been transmitted by all the species of anopheline in Iraq.

In addition of one characteristic related to *P. fallax* that is a daisy shaped in exoerythrocytic segmenter. Figure 13, [22].

In Iraq, malaria transmission was regarded as unstable in the whole country [28]; so, the current finding revealed to predictions of retransmission of malaria in Baghdad is probability at any time. This data can update the information system surveillance and strategies into the national program of malaria elimination.

4. Conclusion

Xenomonitoring of the vector mosquitoes (Diptera: Culicidae) to diagnose blood protozoa is perfect method. The current study revealed the presence of *Plasmodium* sp. in Anopheline female mosquitoes which were collected indoor from different areas in Baghdad city, this result proved that malaria is still in Baghdad in two area: Al Ameria (in the west) and Al Geraiat (in the north). According to the morphology study, three species of *Plasmodium* were appeared: *P. falciparum, P. vivax* and *P. fallax.*

This study concluded nearly most of the phases of life cycle were appeared in gut and salivary of mosquitoes as well as Sporozoites, Gametocyte and Sporogony; these findings revealed to the parasites which dormant in mosquitoes (hypnozoites).

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