

REEVALUATION OF MALARIA PARASITES IN EL-FAYOUM GOVERNORATE, EGYPT USING RAPID DIAGNOSTIC TESTS (RDTs)

By

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Abstract

Malaria as a disease has been identified in Egypt since ancient times. Malaria was endemic in almost all parts of the country but prevalence showed a steady decrease by 1990, and regressed in most of the Governorates. Then by the end of 1998 till now Egypt become free from local transmission of malaria. All reported cases were imported mainly from Sudan. However, the outbreak of *falciparum* (1 case) and *vivax* (23 cases) that occurred (May 2014) in Aswan Governorate strongly indicated that malaria is reemerging in the country. El-Fayoum should be take special attention, rather than being the last residual focus. The efficient malaria vector *A. sergenti*, the proven vector *A. pharoensis* and the suspected vector *A. multicolor* were encountered. This work reevaluated malaria status by using RDTs in survey and Giemsa stained thick films to confirm positive cases and estimation of parasite rate, formula, densities and species, also to study the ecological and entomological efficacy factors.

The result showed that out of 2044 examined persons, 14 (0.68 %) were passive cases i.e. attending themselves to El-Fayoum Malaria Units after their return from Sudan. Microscopic examination of their stained thick films obtained from MOH&P shows that 9 (64.2%) out of passive cases were positive 3 of them are *P. falciparum* (33.3%) and the rest *P. vivax* 6(66.7%) The species formulas of *P. falciparum* and *P. vivax* were 33.3% and 66.7% respectively. Concerning the density class, only one *vivax* case was of low density class while the other cases were of high density class. All positive cases were males, imported from Sudan and most of them were merchants having trade activities in Sudan. All examined persons during active case detection ACD (1551) and neighborhood of detected cases NOD (479) were malaria negative by rapid diagnostic tests. The areas recording the highest number of imported cases were Abu Shanap, Aboxa (Ballona) and Kafr Aboud (Abshaway Center) but no *Anopheline spp* larvae were detected. While in Al Nazla *A. sergeni* and *A. multicolor* larvae were detected where there was no any imported case or even traveler to Sudan. If the situation is reversed i.e. an imported case inhabit Al Nazla, reemergence of local malaria may start. The situation of Kafr Fazara is greatly changed by using fine sand instead of clay in manufacturing red brick after prevention excavation of land. No imported cases or *Anopheline spp* larvae were recorded.

Key words: Egypt, El-Fayoum, Malaria, Reevaluation, Rapid diagnostic tests.

Introduction

Roll Back Malaria (RBM) strategy is implemented to maintain Egypt free of malaria and prevent reintroduction of gametocyte carriers. Epidemiologically, Egypt is classified within group 2 of the EMR countries where malaria was firmly under control and targeting the eradication (Halawani and Shawarby, 1957; WHO, 2004). The last focus of malaria in Egypt was in El-Fayoum which became free from transmission of malaria since 1998 and Egypt was preparing to be certificated as free of malaria. There were

few annual imported malaria cases since the year 1998. As regards malaria situation in Egypt; all detected cases were imported (WHO, 2012a). The outbreak of *P. falciparum* and *P. vivax* in southern part at Aswan Governorate at May 2014 strongly supports the idea that malaria is reemerging in the country (Kenawy, 2015). There are many factors which may contribute to the re-emergence of the disease in Egypt. Such factors include infection of local *Anopheline* mosquitoes by imported cases, continuous movement of populations between Aswan

Governorate and Sudan as well as the influx of large population from Africa and Asia to Egyptian Governorates for educational and religious purposes. Another risk factor was the environmental changes brought by water-sources development projects as Toshka (Shoukry and Morsy, 2011) and El Salam canal (Hassan *et al*, 2003). El Bahnasawy *et al*. (2011) recorded *An. multicolor*, *An. Sergentii* and *An. algeriensis* in Toshka District. They added that the endemicity of Chloroquine resistant *P. falciparum* on the Egyptian-Sudanese border pave the way for malignant malaria transmission particularly among travelers returning back from Sudan (El Bahnasawy *et al*, 2014). The imported malaria refers to infections acquired outside and brought into a national territory (WHO, 2012b). The origin of imported cases can be traced to a known malarious area outside the country to which the case has travelled (WHO, 2012c). Only *An. pharoensis* and *An. sergenti* are the proven vectors in Egypt. *An. pharoensis* is mainly responsible for *Plasmodium vivax* transmission while, *An. sergenti* is responsible for the *P. falciparum* transmission in El Fayoum (Kenawy, 1988; Wassim, 2014). Also, *An. multicolor* is suspected as a vector (Gad *et al*, 1964; Zahar, 1974; Kenawy *et al*, 1986). *An. sergenti* is the oasis vector or desert malaria vector due to its distribution across the Saharan belt in northern Africa into the Middle East, and its ability to cope with extreme climate condition (Sinka *et al*, 2010). *An. sergenti* is an important vector of malaria in the oases and in El Fayoum (Farid, 1956; El Said *et al*, 1986; Morsy *et al*, 1995a,b).

WHO (1996) recognized the urgent need for new, simple, easy, quick, accurate and cost-effective diagnostic tests for determining malaria parasites to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques were developed. This, in turn, led to an increase in the use of rapid diagnostic tests (RDTs) for malaria, which are fast and easy to perform, and do not require electricity or specific

equipment (Bell *et al*, 2006). Rapid diagnostic tests RDTs used to diagnose malaria are based on the use of immunochromatographic tests to detect the presence of plasmodium antigens. A rapid diagnostic test is a nitro-cellulose strip coated by a dye-labeled antibody for the antigen of the parasite. There are many types of these tests that differ according to the type of targeted antigen e.g. histidine-rich protein 2 (specific for *P. falciparum*), lactate dehydrogenase (parasite specific) and aldolase for *Plasmodium* species (Moody, 2002, FIND, 2013). Commercially, there are many manufacturers of malaria rapid diagnostic tests. WHO (2014b) evaluated the quality of many of these products and published the results.

The present work aimed to reevaluate malaria parasites in El-Fayoum Governorate using RDTs in survey and Giemsa stained thick films to confirm positive cases and estimation the parasite rate, parasite formula, densities and species of parasites. Also studying ecological and entomological efficacy factors affecting such malaria status.

Subjects, Material and Methods

El-Faiyoum Governorate is more or less an agricultural oasis. It lies 90 km south west of Cairo in a depression averaging 20m, below the sea level. It is located between latitude 29° 45' and 30° 15' and longitude 30° 30' and 31°, it occupies an area of about 1778 km². The governorate is consisted of six centers; Fayoum, Sinnuris, Ebshway, Itsa, Tamiya and Yosif Alsedik. It is irrigated by Baher Youssef, a branch of the River Nile which breaks up into a number of streams before its water flow into Qaroun Lake. The lake lies in a depression averaging 45m, below the sea level. It is shallow lake of a depth about 6-7m, with a length of 45 km and a width of 9 km with averaging surface area of about 214 km². Preliminary studies include collection of data about passive cases attending to El-Fayoum malaria units from 2007 to 2014 were done. Data were analyzed showing the areas of highest number of imported positive malaria cases

to be the areas of interest in the present work. Three methods of malaria case detection were implemented as following: a) Passive case detection (PCD): Thick blood films were prepared for people attending from all districts of El Fayoum Governorates to Malaria Unites or El Fayoum Fever Hospitals by the Ministry of Health and Population (MOHP). Thick films were stained by Giemsa stain for microscopic examination. All slides of PCD obtained from MOH & P were examined to estimate the parasite densities and species formula of positive cases. b) Neighborhood of detected cases (NOD): Inhabitants of houses of the confirmed cases and their neighboring were visited and examined for malaria using rapid diagnostic tests RDTs. c) Active case detection (ACD): House-to-house visits for mass blood examination using RDTs were carried (from May to end of September) on selected areas in three districts; Abu Shanab (Ebshway Center) which contained the highest number of imported positive cases from 2007 until 2014, Al Nazlla and Qasr Elgeballi (Yosif Alsedik Center) where efficient malaria vectors were recorded, and Kafr Fazara and Abu Naoura area (Sinnuris Center) for comparing their epidemiological malaria situation with that assessed during 1995 & 1996 (Bassiouny, 1997;1999; 2001; Dahesh *et al*, 2009a).

The companies of rapid diagnostic tests RDTs used in present work were: 1- Abon Plus: used for detection and differentiation between of *Plasmodium falciparum* histidine rich protein II antigen and lactate dehydrogenase antigen of all *Plasmodium* species (P.F/PAN cassette for whole blood) REF IMA-T402 LOTMAL3060017. 2- Advanced Quality: used for detection and differentiation between *Plasmodium falciparum* histidine rich protein II antigen and specific *P. vivax* lactate dehydrogenase antigen (P.F/P.V cassette for whole blood) CODE NO: ITP114-TC40.

Thick blood films of all positive cases and 20% of negative cases were prepared from

the peripheral blood by finger prick using disposable lancets. The essential features of processing were use of Giemsa stain and examination under oil immersion (Gracia, 2001). Prior to this, suitable areas or fields were selected for examination by 10x lens. The criteria for selection were the presence of noticeable background and sufficient number of leukocytes 10-20 WBCs per one microscopic field (x700) according to standard method recommended (WHO, 1991). Parasite species and density class were detected for each positive slide.

Relevant information about the examined persons were collected include age, sex, job, history about travelling to endemic malarious areas or acquiring previous malaria infection, description of their houses, presence of animal shed and presence of breeding places near houses.

All the breeding places around examined people houses (10 meters apart) including uncovered irrigation canal, agriculture drains, swamps and surface water collections were investigated for *Anopheles* larval species by the recommended methods (WHO, 1975). Collected larvae were transferred in separate plastic-bags and transported to the laboratory. Last third and fourth larval instars were identified by adopting the standard keys (Krickpatrick, 1925; Ribeiro and Ramos, 1999; Azari-Hamidian and Harbach, 2009a,b).

The computer analysis data was carried out by PC using the Epi Info and SPSS 20 for windows software packages. The 0.05 cut-off value was used as a criterion for statistical significance and all statistical tests were interpreted in a two-tailed fashion.

Results

The results were shown in tables (1 to 14), figures (1 to 13), and photos (1 to 4).

Discussion

The statistical analysis of preliminary data during period (2007-2014) showed that Abu Shanab (Abshaway Center) was the village with the highest number of imported malaria cases inhabit so it was selected for active case detection survey in the present work. All cases had a histo-

ry of travelling to endemic African countries especially Sudan just before acquiring infection. The species of plasmodium found were *P. vivax* and *P. falciparum*. The difference between two species was not statistically significant.

In present work, out of 2044 examined persons 14 (0.68%) were passive cases attending themselves to El Fayoum Malaria Units after their return from Sudan. Microscopic examination of their stained thick films obtained from MOH & P showed that 9 (64.2%) out of passive cases were positive 3 of them are *P. falciparum* (33.3%) and the rest *P. vivax* 6 (66.7%). The species formula of *P. falciparum* and *P. vivax* were 33.3% and 66.7% respectively. The difference between two species was not statistically significant (table5). Concerning the density class of each species, table 6 indicates only one *vivax* case was of low density class (+) while the rest of cases were of high densities (+++/or ++++). The high parasitaemia may be due to duration between acquiring infection from Sudan until return and diagnosis in El-Fayoum Malaria Unit or may be due to the high number of sporozoites inoculated per bite of efficient *Anopheles* vector found in Sudan. When the hepatic schizonts rupture, they liberate approximately 10^5 - 10^6 microsites into circulation i.e. the product of 5-100 successful sporozoites. The rise of the parasite count is logarithmic initially, with a rising a sine wave pattern of parasitaemia in falciparum malaria but in most cases the parasite expansion terminate abruptly. Only *P. falciparum* and *P. knowlesi* have the capacity for untrammelled multiplication (Farrar *et al*, 2014).

Most of positive cases inhabit Abu Shanap 3(33.3%) and Aboxa (Ballona) 3(33.3%) while one case (11.1%) from Kafr Aboud (Abshaway Center), Kohafa (Fayoum Center) and Kaabi (Sinnuris Center) (table 4). They were merchants (77.7%) and workers (22.3%) and the difference between two occupations was not significant.

The comparisons between negative and positive (or have a history of previous infection during last two years) groups according to their gender, occupation and history of travelling to endemic malarious area during 2015 were done. All positive group were males having a history of travelling to Sudan just before acquiring infection. The differences between two groups are highly significant. Concerning their occupation, the most of positive group were merchants (70%) and workers (30%) while most of nega-

tive group were unemployed (housewives, children or students) the difference was statistically significant. The nature of occupation explains the purpose of frequent travel to Sudan.

In the present work different localities were included for active case detection ACD and neighborhood of detected case NOD. Abu Shanap, Kafr Aboud and Aboxa-Ballona (Abshaway Center) were localities involved in survey of neighborhood of confirmed cases. The areas chosen for active case detection are Al Nazlla and Qasr Algeballi (Yosif Alsedik District), Abu Shanap (Abshaway Center) and area of Kafr Fazara and Abu Naoura (Sinnuris Center). The comparison between these localities according to occupations and history of travelling to malarious endemic area showed significant difference. Also, in the present study, the comparison revealed that Abu Shanap, Kafr Aboud and Aboxa- Ballona (Abshaway Center) recorded highest number of travelers to Sudan and highest number of positive malaria cases or with a history of infection during last two years. On other hand, Al Nazla and Qasr Algeballi areas (Yosif Al Sedik Center) and Kafr Fazara /Abo Naoura areas (Sinnuris Center) did not record any positive case or even had a history of previous infection. Also none traveled to endemic countries among the examined persons inhabiting these areas. The differences were significant.

Concerning occupations of the inhabitants of these localities, the highest percentages of merchants were concentrated in Abu Shanap and Aboxa (Ballona) in Abshaway Center, the localities which recorded the highest percentages of imported cases, with significant. The merchants inhabiting these localities have purchased small electric machines necessary for house, kitchen requirements and home décor accessories from Egypt then they have traveled frequently (more than twice yearly) to Sudan for sealing their goods so the high number of traveler merchants explains the presence of malaria imported cases in such localities. (Hassan *et al*, 2003) mentioned that the influx of large population from Africa and Asia to Egyptian Governorates for educational and religious purposes may leads to infection of local anopheline mosquitoes by imported cases and reemergence of malaria.

Concerning the breeding places in the present study, the near houses of examined individuals of the various localities, showed that Al Nazla area followed by Kafr fazara village recorded

highest number of individuals having breeding places near their houses 325 (76.8%) and 93 (22.0%) respectively while the rest of localities record very low numbers of breeding places, with significant difference.

All uncovered breeding places around houses (10 meter apart) in the localities were examined for larvae of *Anopheline* spp. In Al Nazla, out of 80 breeding sites, only 25 (31.2%) were positive for *Anopheles* spp. 15 (18.7%) sites contained *Anopheles multicolor* with density mean 4.0 ± 2.0 larvae per dip and 10 (12.5%) sites contained *Anopheles sergenti* with density mean 2.0 ± 1.0 larvae per dip. Also 50, 5, 3 and 3 sites of breeding places were investigated in Kafr Fazara, Abo Shanap, Aboxa (Ballona) and Kafr Aboud respectively. None showed *Anopheles* spp. larvae

Regarding status of Al Nazla, the area has a characteristic topography where mountains extend along more than 40 Km length. Battn Al Wadi valley lies in between these mountains. Along the slopes of mountains, subsoil water arises forming shallow clear semi running and semi shaded breeding places ideal for *Anopheles sergenti* larvae. *A. sergenti* is the important efficient vector for malaria (Farid, 1956; El Said *et al*, 1986). So, the presence of such efficient vector threatens the area. Although no malaria cases were recorded in that area in the present work, this area is considered the most dangerous or called of a malarious potentiality. i.e. the area is waiting the first imported case to start new outbreak. In 2004 and 2005 small outbreak occurred in Al Nazla area where two indigenous cases out of 42 examined in 2004 and 24 indigenous cases out of 16401 examined were recorded (kindly provided by Ministry of Health and Population. Such outbreak may be repeated if one of the imported cases inhabits Al Nazla area.

Regarding the present status of Kafr Fazara and Abo Naoura (Sinnuris Center), there were neither imported cases, nor travelers to endemic countries or *Anopheles* vec-

tors. Thus, its status is relatively good. In 1996 an intensive parasitological and entomological assessment of that area revealed that *A. sergenti* was the predominant species followed by *A. multicolor* and then *A. pharoensis* (Bassiouny 1997, 1999; 2001; Abdel Halim, 2008; Dahesh *et al*, 2009a,b; Mikhail *et al*, 2009). The breeding places of *Anopheles* spp larvae were scattered all over the village especially in an excavated land (about two faddens) in Abu Naoura area. Five red brick factories found in that area used clay of the excavated lands in manufacturing the red bricks. In turn, the excavation of land led to rising the subsoil water level and formation of large and wide breeding places (Bassiouny, 1997). In 1996, the parasitological studies revealed that out of 7236 examined individual, 32 were positive. Two of them were *P. vivax* and the rest *P. falciparum*. All the cases were indigenous and without history of travel to endemic areas (Dahesh *et al*, 2009).

In the present work, the recent status of Kafr Fazara is completely changed where new laws forbid the excavation of lands. So the brick factories use fine sand instead in manufacture of red brick blocks (photo3). Also the large excavated land of Abu Naoura is buried and most of it is constructed. Most irrigation canals near houses are covered (photo4). No imported cases were recorded among inhabitants and no one had a history of previous infection. No *Anopheles* larvae were detected.

The present reported fluctuation in the numbers of imported cases from 2007 until end of September 2015. The exacerbation each 3-4 years may reflect the instability of endemicity of the malarious status in Sudan where most imported cases were infected, with characteristic instability feature of hypoendemic and mesoendemic malarious areas (Bruce-Chwatt, 1985).

Recommendations

No doubt, the malaria is a difficult disease to control largely due to the highly adaptable nature of the vector and parasites involved.

Thus, people returning back from endemic areas as Sudan, Saudi Arabia and Yemen must be clinically and parasitological examined on arrival. People who have malaria have parasites available for malaria mosquitoes that bite them. If they are treated with appropriate drugs, the parasites disappear from their blood and are not available to the mosquitoes. This helps to prevent or reduce the introduced transmission of malaria.

Besides, at least there must be an annual screening of the different water sources to prevent the breeding of malaria mosquitoes. Also, the screening of dwellings to prevent malaria mosquitoes from entering and biting the people inside is a positive development measure. Where it's possible to put screens on windows and doors, it should be encouraged to use of a treated bed-net or residual treatment of walls because it reduces the number of malaria mosquitoes entering and leaving the building.

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References

Abdel Halim, AS, 2008: Efficacy of *Cinnamomum zeylanicum* on third stage larvae and adult fecundity of *Musca domestica* and *Anopheles pharoensis*. J. Egypt. Soc. Parasitol. 38, 2: 475-82

Azari-Hamidian, S, Harbach, RE, 2009: Keys to the adult females and fourth instar larvae of the mosquitoes of Iran (Diptera: Culicidae). Zootaxa (Magnolia Press 2078) 1-33.

Bassiouny, HK, 1997: Determination of epidemiological factor causing persistence of malaria, *P. falciparum* transmission in El-Faiyoum Governorate, Egypt: Final Report. WHO reference: Reg. File M5/72/1, EMRO.

Bassiouny, HK, 2001: Bioenvironmental and

meteorological factors related to the persistence of malaria in Fayoum Governorate retrospective study. East. Medit. Hlth. J. 7, 6:895-906.

Bassiouny, HK, Beljaev, AE, Awad, OM, Ahmed, MH, 1999: Parasitological profile of the surveyed population for malaria in an endemic area in Fayoum Governorate, Egypt. J. Egypt. Pub. Health Assoc. 74, 1/2:27-58.

Beier JC, Merdan AA, El Sawaf, BM, Abdel Kadder, M, 1987: Laboratory rearing techniques and adult life table parameters for *Anopheles sergenti* from Egypt. J. Amer. Mosq. Control Assoc. 3:266-70.

Bell, D, Wongsrichanalai, C, Barnwell, J, 2006: Ensuring quality and access for malaria diagnosis: how can it be achieved? In: Evaluating Diagnostics Review.

Bruce-Chwatt, LJ, 1985: Essential Malariology. 2nd edition, USA.

Dahesh, SM, Bassiouny, HK, El-Masry, SA, 2009a: Malariometric parasitological survey in El-Fayoum Governorate, Egypt. J. Egypt. Soc. Parasitol. 39, 1:213-25.

Dahesh, SM, Bassiouny, HK, El-Masry, SA, 2009b: Socioeconomic and environmental factors affecting malaria infection in Fayoum Governorate, Egypt. J. Egypt Soc. Parasitol. 39, 2:511-23.

El-Bahnasawy, MM, Saleh, NM, Khalil, MF, Morsy, TA, 2011: The impact of three anopheline mosquito species in Toshka, on the introduction of chloroquine resistant *P. falciparum* to Egypt. J. Egypt. Soc. Parasitol. 41, 3:573-92.

El-Bahnasawy, MM, Soliman, SA, Morsy, T A, 2014: Training nurses on dealing with arthropod-borne infectious diseases: Is it a mandatory nowadays in Sub-Saharan-Africa? Egyptian Military Medical Journal (EMMJ) 69, 1:32-50.

El-Said, S, Beier, JC, Kenawy, MA, Morsy, Z S, Merdan, AI, 1986: *Anopheles* population dynamics in two malaria endemic villages in Fayoum Governorate, Egypt. J. Amer. Mosq. Control Assoc. 2:158-63.

Farid, MA, 1956: The implications of *Anopheles sergenti* for malaria eradication programmes east of the Mediterranean Bull. WHO, 15:821-8

Farrar, J, Hotez, PJ, Junghanss, T, Kang, G, Lalloo, D, et al, 2014: Manson Tropical Diseases. Twenty third edition, England.

Foundation for Innovative New Diagnostics, (FIND) 2013: The Malaria Rapid Diagnostic Tests: An Implementation Guide, Geneva, Switzerland.

- Gad, AM, Kamel, OM, Abdel Hafez, M, Taha, AM, 1964:** A survey of malaria in Sinai, The J. Egypt. Pub. Hlth. Assoc. 39:163-74
- Garcia LS, 2001:** Diagnostic Medical Parasitology. 4th edition; ASM Press; Washington, DC.
- Halawani, A, Shawarby, AA, 1957:** Malaria in Egypt: History, epidemiology, control and treatment. J. Egypt. Med. Assoc., 40, 1:753-92.
- Hassan, MA, Kenawy, H, Abdelsattar, A, So-wiwm, M, 2003:** GIS-based prediction of malaria risk in Egypt. East. Mediterr. Hlth. J. 9, 4:549.
- Kenawy MA, 1988:** *Anopheles* mosquitoes (Diptera: Culicidae) as malaria carriers in A.R. Egypt: History and present status. J. Egypt. Pub. Hlth. Assoc. 63:67-85
- Kenawy MA, 1991:** Development and survival of *Anopheles pharoensis* and *An. multicolor* from Faiyum, Egypt. J. Amer. Mosq. Control Assoc. 7:551-5
- Kenawy MA, Beier JC, Asiago, CM, El-Said S, Roberts CF, 1990:** Interpretation of low level *Plasmodium* infection rates determined by ELISA for anophelines (Diptera: Culicidae) from Egyptian oases. J. Med. Entomol. 27:681-5.
- Kenawy MA, Beier JC, El-Said, S, 1986:** First record of malaria and associated *Anopheles* in El Gara Oasis, Egypt. J. Amer. Mosq. Control Assoc. 2:101-3
- Kenawy, AM, 2015:** Review of *Anopheles* mosquitoes and malaria in ancient and modern Egypt. Egypt. Acad. J. Biol. Sci. 8, 1:15-32.
- Kirkpatrick, TW, 1925:** Mosquitoes of Egypt. Cairo Government Press.
- Mikhail, MW, Al-Bursheed, KM, Abd El-Halim, AS, Morsy, TA, 2009:** Studies on mosquito borne diseases in Egypt and Qatar. J. Egypt. Soc. Parasitol. 39, 3:745-56.
- Moody, A, 2002:** Rapid diagnostic tests for malaria parasites. Clin. Microbiol. Rev. 15, 1:66-78.
- Morsy, TA, El Kadry, AA, Salama, MMI, Sabry, AA, El Sharkawy, IMA, 1995a:** Studies on bionomics and vector competence of adult Anopheline mosquitoes in El Faiyum Governorate, Egypt. J. Egypt. Soc. Parasitol., 25, 1:213-44.
- Morsy, TA, El Kadry, AA, Salama, MMI, Sabry, AA, El Sharkawy, IMA, 1995b:** Studies on Anopheline larvae in El Faiyum Governorate, Egypt. J. Egypt. Soc. Parasitol. 25, 2:329-54.
- Ribeiro, H, Ramos, HC, 1999:** Identification keys of the mosquitoes (Diptera: Culicidae) of continental Portugal, Acores and Madeira. Euro. Mosq. Bull. 3:1-32.
- Shoukry, NM, Morsy, TA, 2011:** Arthropod borne diseases at Toshka, Upper Egypt. World J. Zool. 6, 2:126-33.
- Sinka, ME, Bangs, MJ, Manguin, S, Coetzee, M, Mbogo, CM, et al, 2010:** The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis, Parasites & Vectors, 3:117, pp.34 <http://dx.doi.org/10.1186/1756-3305-3-117>
- Wassim, NM, 2014:** Secondary structure and sequence of ITS2-rDNA of the Egyptian malaria vector *Anopheles pharoensis* (Theobald). J. Egypt. Soc. Parasitol. 44, 1:197-204.
- WHO, 1975:** Manual on Practical Entomology in Malaria: WHO division of malaria and other parasitic disease. Part II- Methods and technique, Geneva, Switzerland.
- WHO, 1982:** Biological control of vector disease: WHO Expert Committee on Vector Biology & Control. WHO, Tech. Rep. 6, Ser. No. 679, Geneva,
- WHO, 1991:** Basic malaria microscopy. Part 1- Geneva: WHO. Learner's Guide 17- 24.
- WHO, 1996:** WHO information consultation on recent advances in diagnostic techniques and vaccines for malaria: a rapid dipstick antigen capture assay for the diagnosis of *falciparum* malaria. Bull WHO 74:47-9.
- WHO, 2004:** Report in the Responsiveness of the RBM Programme to country needs in the WHO/EMRO.
- WHO, 2012a:** Malaria Report 2011, Geneva.
- WHO, 2012b:** WHO Global Malaria Programme, Geneva, Switzerland.
- WHO, 2012c:** Disease surveillance for malaria elimination: An operational manual, Geneva.
- WHO, 2014a:** World Malaria Report 2014. Geneva, Switzerland: WHO Press.
- WHO, 2014b:** Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 5 (2013). Geneva, Switzerland: WHO Press.
- Zahar AR, 1974:** Review of the ecology of malaria vectors in the WHO/EMR

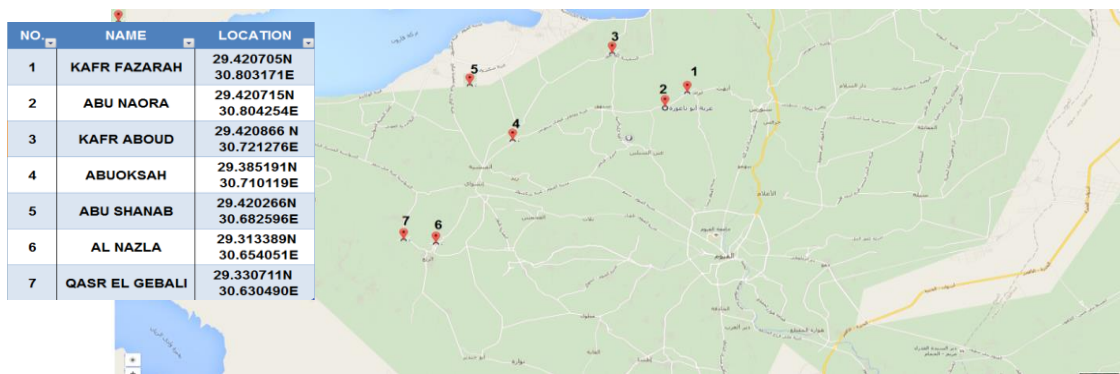


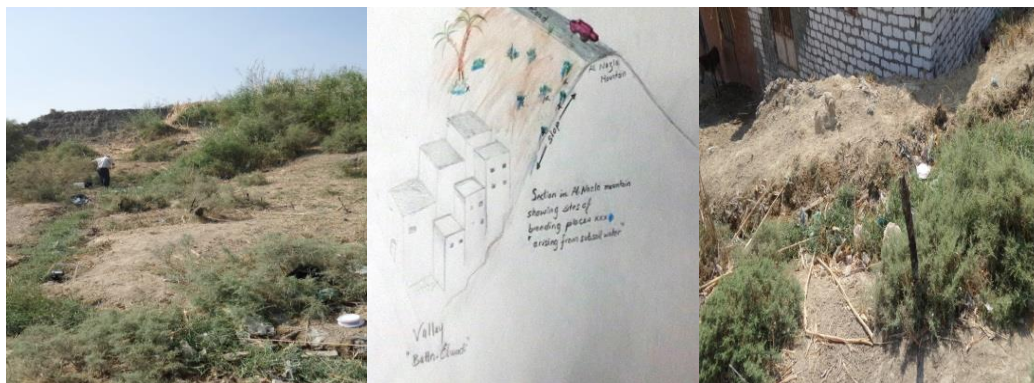
Figure 1: Fayoum Governorate showing locations and GPS of areas of present work.



Fig. 2: Kafr Fazara and Abu Naoura (Sinnouris District) showing location of the red brick factories



Fig. 3: El-Nazla (Yosif Al-sidek District) showing characteristic topography of Gabal El-Nazla and Batn El-Wadi

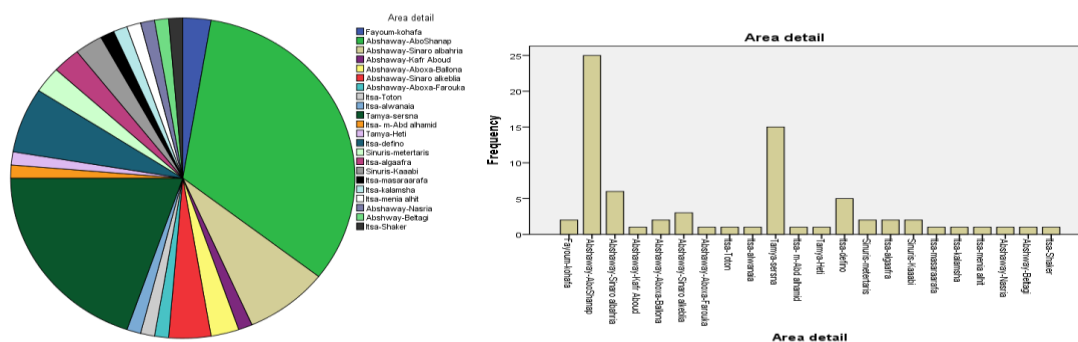


Photos 1, 2 and fig. 4: Subsoil water arising along slop between Al Nazlla mountain and valley (Battn Al Wadi) forming ideal breeding places for *A. sergenti*.



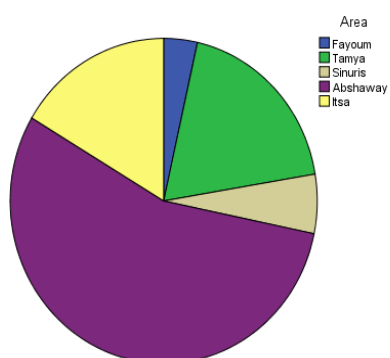
Photo 3left: Red brick factory in Kafr Fazara village using fine sand instead of clay in manufacturing of red brick
Photo 4right: Covered irrigation canal near houses in Kafr Fazara village

Fig. 5,6: Imported malaria cases during (2007-2014) in varies Fayoum Governorate Districts



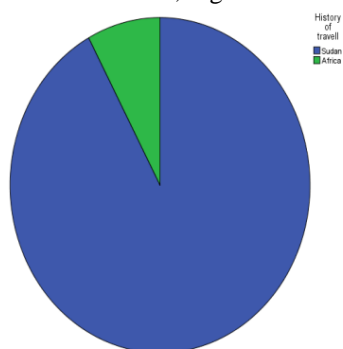
X=204.8 P<0.001

Table 1, Fig. 7: Imported malaria cases during (2007-2014) in Fayoum. Governorate Districts ($X^2 = 60.31$, $P < 0.001$)



District	No of cases	Percent %
Fayoum	2	2.6 %
Tamya	16	21.1 %
Sinuris	4	5.3 %
Abshaway	40	52.6 %
Itsa	14	18.4 %
Total	76	100.0 %

Table2, Fig. 8: Countries imported cases came from during (2007-2014) $X^2=53.8$ $P < 0.001$



Country	No. of cases	Percent%
Sudan	70	92.1 %
Africa (other than Sudan)	6	7.9 %
Total	76	100.0 %

Table 3 and Fig. 9: Percentage of infection with different *Plasmodium spp* among imported cases (2007-2014) $X^2= 1.31$, $P< 0.05$

<i>Plasmodium spp</i>	No. of cases	Percent
Positive <i>P.f</i>	43	56.6%
Positive <i>P.v</i>	33	43.4%
Total	76	100.0%

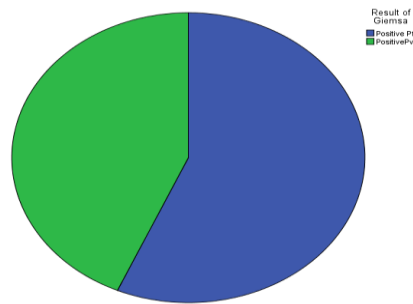


Fig. 10: Diagram illustrating methods of case detection, No. of persons in various localities included.

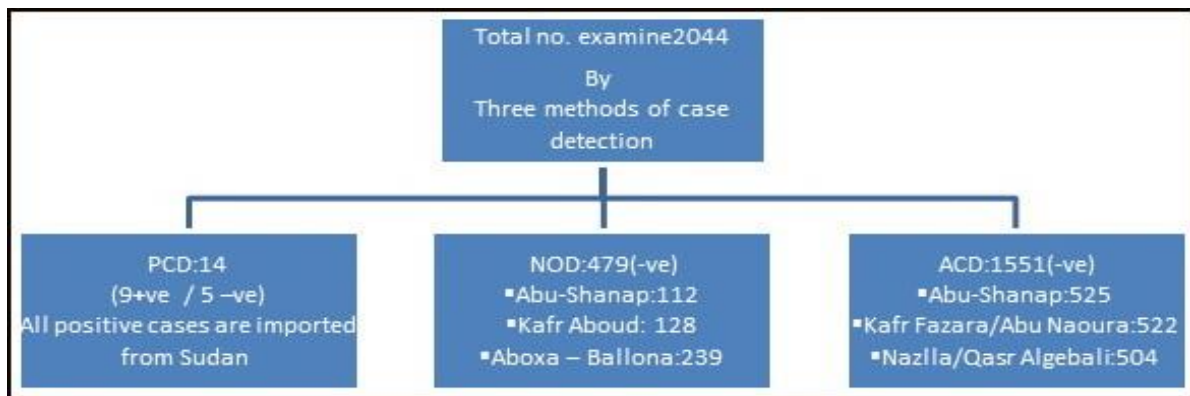


Table 4, Fig.11: Localities of the imported malaria cases during 2015.

Locality	No. of cases	Percent%
Fayoum-kohafa	1	11.1
Abshaway-AboShanap	3	33.3
Abshaway-Kafr Aboud	1	11.1
Abshaway-Aboxa-Ballona	3	33.3
Sinuris-Kaaabi	1	11.1
Total	9	100.0

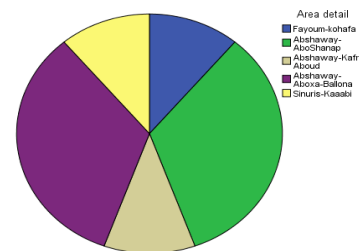


Table 5: Percentage of different *Plasmodium spp* infection among imported cases during 2015($X^2=2.44$, $P> 0.05$)

	No. of cases	Percent
Positive <i>P.f</i>	3	33.3
Positive <i>P.v</i>	6	66.7
Total	9	100.0

Table 6, Fig. 12: Percentage of parasite density classes with different *Plasmodium spp* among imported cases during 2015. $X^2=2.66$, $P> 0.05$ FL: Low density of *P. falciparum* (+/++). FH: High density of *P. falciparum* (+++/++++). VL: Low density of *P. vivax* (+/++). VH: High density of *P. vivax* (+++/++++).

Parasite density class	No. of cases	Percent%
FL	0	0.0%
FH	3	33.3%
VL	1	11.1%
VH	5	55.6%
Total	9	100.0%

Table7: Occupation of positive imported cases during 2015.

Occupation	No. of cases	Percent%
Merchant	7	77.7%
Worker	2	22.3%
Total	9	100%

$X^2=2.77$, $P> 0.05$

Table 8: A comparison between negative and positive (or have a history of previous infection during last two years) groups according to gender during 2015.

Sex	Negative		Positive or a history of infection during last 2 years		Total	
	No.	%	No.	%	No.	%
Male	1033	51.0	20	100.0	1053	51.5
Female	991	49.0	0	0.0	991	48.5
Total	2024	100.0	20	100.0	2044	100

$$X^2=19.01, P<0.001$$

Table 9: A comparison between negative and positive (or a history of infection during last two years) groups according to travelling history to endemic malarious area during 2015.

History of travelling	Negative		Positive or a history of infection		Total	
Did not travel	1998	98.7%	0.0	0.0%	1998	97.7%
Travel to Sudan	26	1.3%	20	100%	46	2.3%
Total	2024	100.0%	20	100.0%	2044	100%

$$X^2=877.2, P<0.001$$

Table10: A comparison between negative and positive (or a history of infection during last 2 years) groups according to their occupation during 2015.

Occupation	Negative		Positive or a history of infection		Total	
	No.	%	No.	%	No.	%
Student, children or housewife	1793	88.6	0	0	1793	87.7
Farmer	117	5.8	0	0	117	5.7
Employer	12	0.6	0	0	12	0.6
Merchant	13	0.6	14	70	27	1.3
Worker	89	4.4	6	30	95	4.6
Total	2024	100.0	20	100.0	2044	100

$$X^2=768.0, P<0.001$$

Table11: A comparison between localities included in active case detection ACD and neighborhood of detected cases according to travelling history to endemic malarious area during 2015.

History of travel		Localities included in ACD & NOD					
		(Abshaway)			Al-nazlla /Qasr algebali	kafr fazara /Abu naoura	Total
		Abu-Shanap	Kafr Aboud	Aboxa/ Ballona			
Persons didn't travel	No.	617	128	242	504	522	1998
	%	30.9%	6.4%	15.0%	25.2%	26.1%	100.0%
Person travel to Sudan	No.	26	1	1	0	0	43
	%	60.5%	2.3%	0.2%	0.0%	0.0%	100.0%
Total No. of person	No.	643	129	243	504	522	2041**
	%	31.5%	6.3%	11.9%	24.7%	25.6%	100.0%

X²=843.0, P<0.001 ** Two positive cases (one from Fayoum & one from Kaabi, Sinnuris) and one negative case from El Fayoum excluded

Table12: A comparison between localities included in active case detection ACD and neighborhood of detected cases according to No. of positive cases during 2015 or with history of infection during last two years.

Malarious case*	Localities included in ACD &NOD					
	(Abshaway)			(Yosif Al-sedik) Al-nazlla /Qasr algebali	(Sinnuris) kafr fazara /Abu-naoura	Total
	Abu-Shanap	Kafr Aboud	Aboxa/ Ballona			
Negative	636	128	233	504	522	2023
%	31.4%	6.3%	11.5%	24.9%	25.8%	100.0%
Malarious*	7	1	10	0	0	18
%	38.9%	5.6%	55.6%	0.0%	0.0%	100.0%
Total No.	643	129	243	504	522	2041**
%	31.5%	6.3%	11.9%	24.7%	25.6%	100.0%

*Malarious case referred to one of +ve imported passive cases during 2015 or had a history of previous infection during last two years.

X² =38.5, P<0.001** Two positive cases (one from Fayoum & one from Kaabi. Sinnuris) and one negative case from El Fayoum excluded

Fig. 13: Fluctuation of positive imported cases during period from 2007 to September 2015. ($X^2 = 33.6$, $P < 0.001$)

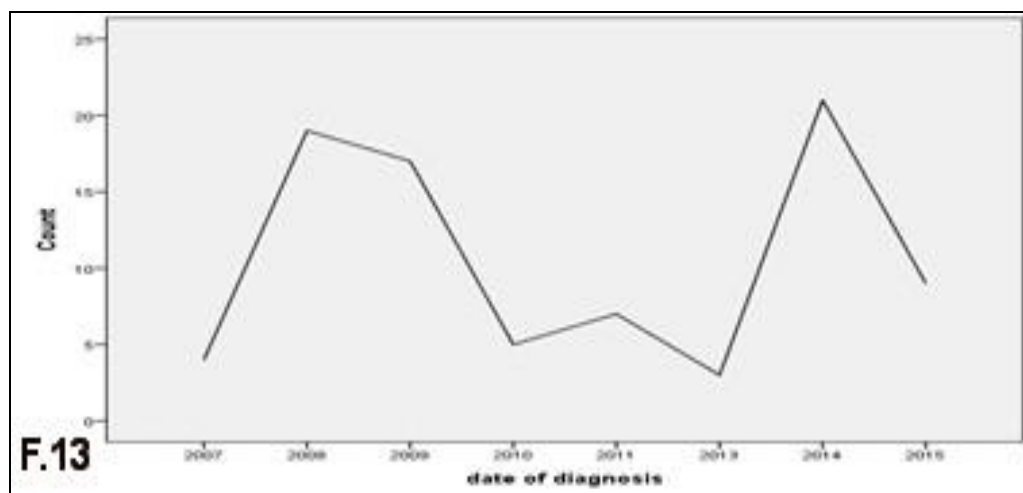


Table13: A comparison between localities included in active case detection ACD and neighborhood of detected cases according to occupation of persons during 2015.

Occupation		Localities included in ACD &NOD					
		(Abshaway)			(Al-nazilla /Qasr algeballi	kafr fazara /Abu naoura	Total
		Abu-Shanap	Kafr Aboud	Aboxa/ Ballona			
Student, children or housewife	No.	580	125	208	434	446	1793
	%	32.3%	7.0%	11.6%	24.2%	24.9%	100.0%
Farmer	No.	20	2	9	34	52	117
	%	17.1%	1.7%	7.7%	29.1%	44.4%	100.0%
Employer	No.	7	0	0	5	0	12
	%	58.3%	0.0%	0.0%	41.7%	0.0%	100.0%
Merchant or free work	No.	14	1	6	0	3	24
	%	58.3%	4.2%	25.0%	0.0%	12.5%	100.0%
Worker	No.	22	1	20	31	21	95
	%	23.2%	1.1%	21.1%	32.6%	22.1%	100.0%
Total	No.	643	129	243	504	522	2041**
	%	31.5%	6.3%	11.9%	24.7%	25.6%	100.0%

$X^2 = 74.4$, $P < 0.001$ ** Two positive cases (one from Fayoum and one from Kaabi, Sinnuris) and one negative case from El Fayoum excluded

Table14: A comparison between localities included in active case detection ACD and neighborhood of detected cases NOD according to presence of breeding places near houses (10 meters around house) of persons during 2015.

Presence or absence of breeding places near house (10 meters around) of examined person.		Localities included in ACD & NOD					
		(Abshaway)			(Yosif Al-sedik) Al-nazlla /Qasr algeballi	(Sinnuris) kafr fazara /Abu naoura	Total
		Abu-Shanap	Kafr Aboud	Aboxa/ Ballona			
No breeding places near houses	No.	640	128	242	179	429	1618
	%	39.6%	7.9%	15.0%	11.1%	26.5%	100.0%
Breeding places near houses	No.	3	1	1	325	93	423
	%	0.7%	0.2%	0.2%	76.8%	22.0%	100.0%
Total	No.	643	129	243	504	522	2041**
	%	31.5%	6.3%	11.9%	24.7%	25.6%	100.0%

$X^2 = 842.0$, $P < 0.001$, ** Two positive cases (one from Fayoum and one from Kaabi, Sinnuris) and one negative case from El Fayoum excluded