Seasonal variations in *Anopheles* species (Diptera: Culicidae) and malaria incidence in Kodok locality, Upper Nile State, Republic of South Sudan

Venancio A. Okic[1]*, Nabil H. H. Bashir[2] and Yousif O. H. Assad[3]

[1] Department of Medical Entomology, Faculty of Public and Environmental Health, Upper Nile University, Malakal, South Sudan

[2] Department of Medical Entomology and Vector Control, Blue Nile National Institute for Communicable Diseases, University of Gezira, Wad Medani, Sudan

[3] Department of Pesticides and Toxicology, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan

Correspondence Author: Venancio A. Okic

Received 28 Aug 2023; Accepted 4 Oct 2023; Published 14 Oct 2023

Abstract

Malaria transmission in sub-Saharan Africa (SSA) is dominated by 3 widespread vectors: *Anopheles gambiae* s.s. Giles, *An. arabiensis* Patton, and *An. funestus* Giles (Diptera: Culicidae). Malaria is caused by the parasite *Plasmodium*, transmitted by the bite of an infected female *Anopheles*. A Cross- sectional study was conducted from April 2020 to March 2021 in Kodok locality (KL), Upper Nile State (UNS), Republic of South Sudan (RSS), to determine the larval and indoor resting density of adults, their seasonal changes, and relate them to the number of malaria cases in each KL area (hai), these are: Bilpam, Ochugi, Nevasha and Salam. These areas are surrounded with swamps and man-made ponds. The number of *Anopheles* and other mosquito adults in a given room was determined by using pyrethrum spray sheet (PSS) collection and the dipping method was used for larval collection from swamps and waste-water. The collected adults were identified as follows: *An. gambiae* s.s. (67 %), *An. arabiensis* (27.1%) and other mosquitoes (*An. funestus*, *Culex* and *Aedes*; 5.9%). The density/HH was determined, in the rainy-season (April-Oct.) and the dry- season (Nov. to March). *An. gambiae* larvae formed 85.4 % and other mosquitoes formed 14.6%. Malaria cases registered within 12 months in KL were 1,956, ranging from 96 to 271 case/month. The highest was during April and the lowest was that of August. It is concluded that 3 *Anopheles* species are present in the study area. *An. gambiae* is the dominant species during both seasons. All detected species densities and malaria cases were higher in the wet- season than the dry-season. This information must be seriously considered during the vector control programs.

Keywords: kodok locality, upper nile state, republic of south sudan, *An. gambiae s.s*, *An. arabiensis*, *Anopheles* density, seasonal change, malaria

1. Introduction

According to WHO, nearly half of the world's population up to 2020 was at risk of malaria. Most cases and deaths occur in SSA; *ca*.241 million cases and 627, 000 deaths. Children <5yr of age are the most vulnerable group; they accounted for about 80% of all malaria deaths in the WHO African Region [1]. The peak up period of transmission is during the rainy-season (April to October in Republic of South Sudan, RSS). *Plasmodium falciparum* is the dominant species of parasite and responsible for >90% of the cases in RSS [2]. *Anopheles* species (Diptera: Culicidae) are anthropophilic, bite in the morning or evening; they may bite indoors (endophagic) or outdoors (exophagic) behavior [3].

Population density (PD) of this *Anopheles spp.* varies seasonally in relationship to rainfall. While PD increases quickly with the first rains, the maximum PD is reached at the end of the rainy-season [4, 5]. Breeding sites are predominantly shaded swamp (S) and human-made wastewater (WW) throughout the year. *An. gambiae* is considered the main malaria vector in Kodok Locality (KL), since it is the most abundant species in the larval habitats during both the dry-and the rainy –seasons, followed by *An. arabiensis*, which is found to be involved in malaria transmission [6].

Malaria transmission rates can differ, depending on some local factors, *e.g*. rainfall patterns, availability of suitable larval habitats in the proximity to residential areas, types of households (HHs), the existing *Anopheles* species in the area, vegetation, *etc*. Some regions have a fairly constant number of cases throughout the year "malaria endemic". Other areas are characterized by malaria seasons, usually coinciding with the rainy- season [5]. KL of the Upper Nile State (UNS), RSS, is one of the areas that suffer from malaria throughout the year. Data is lacking about the vector's biology, ecology, habitats, behavior, *etc*.

2. Materials and methods

2.1 Study area

KL (Fig. 1) is located in UNS (90 53' N and 32^0 07' E), RSS. The locality lies in western bank of the White Nile River, 306m above sea level; 49% R.H., and a population of *ca*. 7,709 (National Census 2012); the natural vegetation consisted of grasses (terrestrial and aquatic), and different tree species. The study sites: Salam, Nevasha, Bilpam and Ochugi (Fig.2).

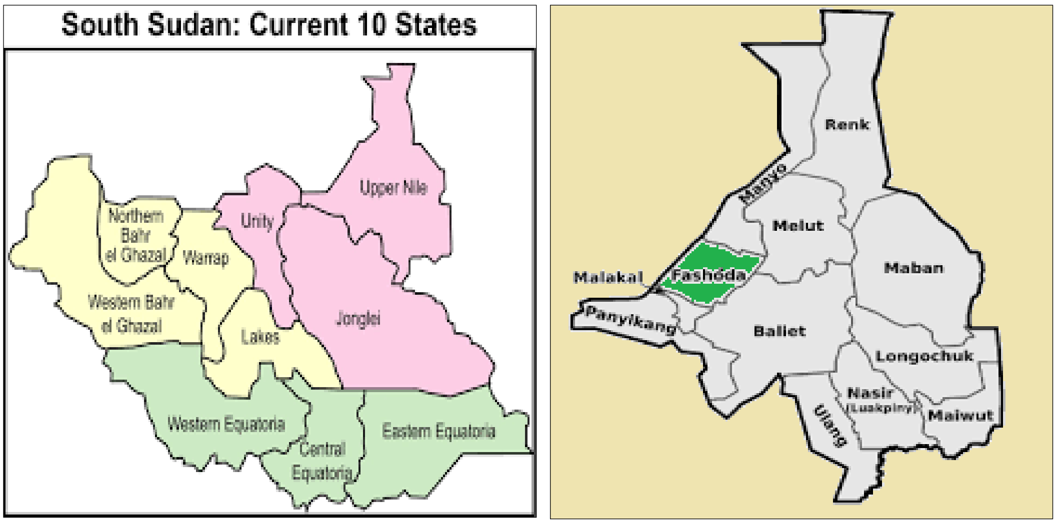

Fig 1: Upper Nile state map and its localities including Fashoda (Kodok)

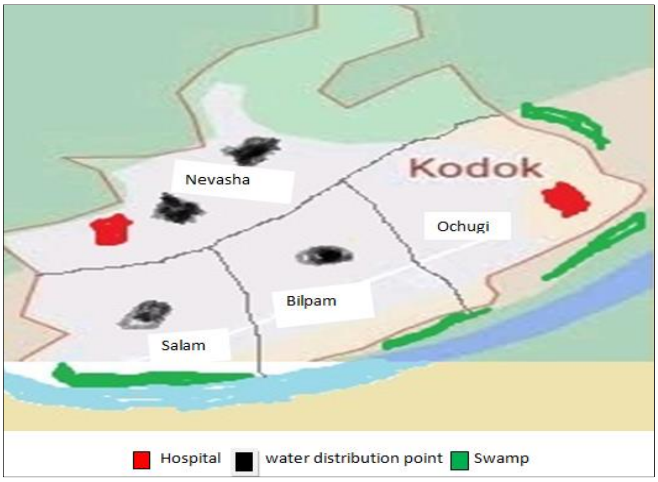

Fig 2: Map of Kodok town and the study area/Hai

2.2 Study design
This study was designed as a cross- sectional; counts were conducted bi-weekly [5].

2.3 Houses (HHs)
HHs (20) were examined /day/site (Hai); overall 80 HHs/KL. These HHs were randomly selected from a random list, choosing a 1st block, and within the block 3 HHs were included [5].

2.4 Breeding habitats
Larval survey was carried out in 8 breeding habitats (S and WW) in the 4 areas of the study. Larvae were collected from 2 breeding sites / area and 10 dips were taken / breeding site, with 2 visits / month, using the WHO recommended dipping technique. This was done for both permanent and seasonal habitats [5].

2.5 Entomological sampling method
Entomology studies were conducted from April 2020 to March 2021 in the 4 above-mentioned areas bi-weekly for collection of adults and larvae (1st and 3rd wk) from the HHs and the breeding sites (S and WW). Adults and larvae were collected as recommended [5]. Species were morphologically identified by using the proper key [7] under light microscope.

2.6 Data analysis
SPSS software Program, ANOVA (Ver.16) was used. The data tabulated to explain the possible relationship between the variables.

3. Results
3.1 Mosquito species
3.1.1 Species identification and spatial distribution/area
Adults were identified as *An. gambiae*, *An. arabiensis* and others (*viz. An. funestus*, *Culex* and *Aedes*) [8]. Their distribution by area was as follows: Bilpam (*An. gambiae* s.s 65.8 %, *An. arabiensis* 27.6 %); Nevasha (*A. gambiae* s.s. 68.4 %, *An. arabiensis* 25.6 %); Ochugi (*An. gambiae* s.s 66.9 %, *An. arabiensis* 27.5 %) and Salam (*An. gambiae* s.s 67.1 %, *An. arabiensis* 27.4 %). The other mosquito species, following the same order of areas were: 6.6 %, 6.0 %, 5.6 %, and 5.5 % (Table 1).

Table 1: Spatial distribution of mosquito species population counts and percentages

Area	*An. gambiae*		*An. arabiensis*		*Others		Total
Bilpam	9,931	65.8 %	4,168	27.6 %	1,001	6.6 %	15,100
Nevasha	9,950	68.4 %	3,719	25.6 %	867	6.0 %	14,536
Ochugi	11,378	66.9 %	4,679	27.5 %	959	5.6 %	17,016
Salam	11,358	67.1 %	4,639	27.4 %	935	5.5 %	16,932
Total	42,617		17,205		3,762		63,584

*Others: *A. funestus*, *Culex* and *Aedes*.

3.1.2 Densities of adults (*An. gambiae* s.s. and *An. arabiensis)* resting indoors
The density of *An. gambiae* /HH (rooms) varied between different HHs. The density of *An. gambiae s.s* was 18/room and *An. arabiensis* was 7.6/room in the rainy- season (Table 2). But in the dry –season, *the* densities were 3.5 and 1.6, following the same order (Table 3).

Table 2: Rainy -season variation in *Anopheles* species and malaria reported cases

Species	Mean/density	SE ±	C. V. %	Malaria cases
An. gambiae	18.3	0.5	2.7	1,261 (64.5%)
An. arabiensis	7.5	0.2	2.6	
Others	1.8	0.1	5.5	

Table 3: Dry-season variation in *Anopheles* species and malaria reported cases

Species	Mean /densities	SE ±	C. V. %	Malaria cases
An. gambiae	3.5	0.2	5.7	695 (35.5%)
An. arabiensis	1.6	0.1	6.5	
Others	0.3	0.0	0	

3.1.3 Larvae (Number collected/area)

Total number of larvae collected was 6,538. Of this, *An. gambiae* constituted 85.4% (5,582) and other mosquitoes formed 14.6 % (956). *An. gambiae* was recorded in the different areas of the study as follows: Bilpam 84.9%, Nevasha 85.3 %, Ochugi 84.3% and Salam 86.8%. The result revealed that KL is heavily infested with *An. gambiae* s.s. (Table 4).

Table 4: Number of larvae collected in different areas

Area/Hai	*An. gambiae*		Others mosquitoes		Total
Bilpam	1,286	84.9 %	229	15.1 %	1,515
Nevasha	1,216	85.3 %	210	14.7 %	1,426
Ochugi	1,466	84.3 %	272	15.7 %	1,738
Salam	1,614	86.8 %	245	13.2 %	1,859
Total	5,582		956		6,538

3.1.4 Species numbers and percentages in breeding habitats

Swamps are available in 3 out of the 4 study sites, *viz*. Bilpam, Ochugi, and Salam. Bilpam and Salam have both S and WW. Hai Salam (Table 4) registered the highest number of larvae (1,869), out of these, 1,055 larvae were collected from the WW and the rest (804) from S. Ochugi (S only) ranked 2nd (1,738). Bilpam ranked 3rd (1,515) with its S (801) and WW (714), whereas Nevasha (WW only) ranked 4th (1,426 larva). The number of *An. gambiae* in S is not significantly lower than those in the WW, where both habitats are available (Table 5).

Table 5: Larvae species collected at breeding habitat/area

Areas	Breeding sites	*An. gambiae*		Others		Total
Bilpam	S	674	84.1 %	127	15.9 %	801
	WW	612	85.7 %	102	14.3 %	714
Nevasha	WW	1216	85.3 %	210	14.7 %	1,426
Ochugi	S	1466	84.3 %	272	15.7 %	1,738
Salam	S	695	86.4 %	109	13.6 %	804
	WW	919	87.1 %	136	12.9 %	1,055
Total						6,538

S = swamp, WW = wastewater

3.2 Malaria cases

3.2.1 Dry- *vs.* wet–season

During the rainy-season (April to October) the cases were 64.5% out of 1,956 cases (KL population is <8,000, *i.e. ca*. 25% of KL population), during the year of the study in the locality (Table 6). The rest of the cases were reported during the dry-season (November to March). During this year, the numbers of *An. gambiae* were almost 5x higher than *An. arabiensis.*

Table 6: Seasonal variation of *Anopheles* and malaria cases

Season	Larvae (No. & %)		*An. gambiae* (No. & %)		*An. arabiensis* (No. & %)		Malaria cases (No. & %)	
Rainy (April-Oct.)	4,193	75.7%	35,009	84.3%	7,015	86.8%	1,261	64.5%
Dry (Nov.- March)	1,364	24.3%	6,454	15.7%	2,007	13.2%	695	35.5%
Total	5,557	100%	41,463	100%	9,022	100%	1,956	100%

3.2.2 Per month

The highest number and percentage of malaria cases in the study area was registered during April 2020 (13.9%), and the lowest was during August 2020 (5%). May and October ranked 2nd and 3rd. The rest of the months of the study period registered cases percentages ranging from 5.8 to 8.8%. (Table 7).

Table 7: Total number of *Anopheles* and malaria cases /month

Months	Larvae (No & % of the total)		Adult				Malaria cases	
			An. gambiae (No & % of the total)		*An. arabiensis* (No & % of the total)		No.	%
2020-Apr	874	15.7%	7,679	18.5%	3,191	35.4%	271	13.9
May	707	12.7%	6,692	16.1%	638	7.1%	246	12.6
Jun	675	12.2%	5,859	14.1%	558	6.2%	159	8.1
Jul	585	10.5%	5,005	12.1%	508	5.6%	136	7.0
Aug	515	9.3%	4,198	10.1%	1,642	18.2%	96	5.0
Sep	446	8.0%	3,250	8.0%	299	3.3%	145	7.4
Oct	391	7.0 %	2,326	5.6%	179	1.9%	208	10.6
Nov	343	6.2%	1,783	4.3%	572	6.3%	169	8.6
Dec	297	5.3%	1,498	3.6%	438	4.9%	172	8.8
2021-Jan	274	5.0%	1,346	3.2%	391	4.3%	114	5.8
Feb	250	4.5%	1,090	2.6%	339	3.8%	124	6.3
Mar	200	3.6%	737	2.0%	267	3.0%	116	5.9
Total	5,557	100%	41,463	100%	9,022	100%	1,956	100%

4. Discussion

The official data about malaria in KL/month showed that about 1,965 cases were reported during the study period. However, as expected, this number is for those who reported to the hospitals and/or health centers only. Many cases do not visit these facilities for one reason or another. The study population of KL live along the banks of the White Nile River, which experiences seasonal flooding that usually provides favorable temporary and permanent breeding sites for mosquitoes in general [8], *viz. An. gambiae* and *An. arabiensis.*

Both species are efficient malaria vectors [1]. These 2 species are detected in very high numbers and densities in KL habitats

[5]. The number of *An. gambiae* is by far (*ca*. 5x) higher than the other mosquito's species (*An. arabiensis*, *An. funestus*, *Culex and Aedes*) in terms of indoor resting PD and larvae. Hai Ochugi took the lead regarding the PD. *An. arabiensis* ranked 2nd. The PD of *An. gambiae* in larvae in the swamps in the 3 areas was lower than in the WW during the study period. The number of larvae in WW breeding sites, for sure, strongly contributes to the adult population.

The seasonal abundance and PD results of the present work of *Anopheles* species improved the understanding on these vectors and their roles in malaria transmission. The result confirmed that PD of *An. gambiae* s.s. is influenced by rains. The PD increased at the beginning of the rainy- season (April and October), and the peak PD was registered towards the end of the rainy- season. As mentioned earlier, KL human population is around 8,000. The reported cases were *ca.* 1,956 during one yr. That is about 25% of the population. Approx. 64.5% of the cases occurred during April to October. The rest was reported during the dry -season. Three of these areas have both S and WW. The WW habitat proved to be invested with significant numbers of *Anopheles* larvae. Therefore, the concept of larval source management (LSM) must be adopted in this locality. This concept proved to be highly effective in the first half of the 20th century. But was largely disbanded in favor of IRS. Today, LSM continues to be used in large-scale mosquito abatement programs in many countries. The hand-application of larvicides can reduce transmission by 70-90% in settings where mosquito larval habitats are defined, e.g. small ponds and WW, but is largely ineffectual where habitats are so extensive, like swamps. The latter habitat cannot be covered on foot. Swamps experience substantial flooding. LSM can be an effective method of malaria control, especially when combined with LLINs [9]. Vector control (VC), in many countries, depends on use of insecticides both indoors and outdoors. Other developments for VC use ovitraps, space-spray, biological control agents, *etc.* Safer VC agents, *e.g*. insect growth regulators (IGRs) and natural plant products (botanical extracts), proved effective. Bacterial insecticides (e.g. *Bacillus thuringiensis israeliensis*) proved to be effective in many countries. But, in areas like KL swamps seems to be difficult to be adopted for logistic and technical reasons. Environmental management methods seem to be the logical solution for KL and the similar localities near the White Nile River and the other rivers, accompanied by awareness-raising and capacity-building of the community. Inter-sectoral coordination is required, even though it is very work-intensive and costly [10-12].

Dealing with WW ponds by several practical measures of management, manipulation and modifications is possible *via* community, NGOs and/or governmental efforts. More intensive and innovative measure are required for dealing with the permanent swamps. The river is just 1 km away from the residential areas. The larval percentages, as expected, were higher in the rainy -season than in the dry- season. *An. gambiae* s.s. also has wide variations with the highest peaks in April up to October.

Further effects could have been provided by other permanent water sources located outside the study areas, but no sampling was carried out. A good example is found in River Nile located <1 km away from the farthest breeding site in the 3 areas; well within the flight range of gravid *An. gambiae* females [13, 14].

References

1. WHO. World malaria report day, Geneva, WHO, 2020.
2. Ministry of Health, Government of Southern Sudan. Prevention and treatment guidelines for primary health centers and hospitals, 2006, 89-90.
3. Republic of South Sudan. Malaria Indicator Survey (MIS). In: World Health Organization. World Malaria Report 2020, 2017. *https://malariasurveys.org/documents]-*
4. Gilles MT, Coetzee MA. Supplement to Anophelinae of Africa south of the Sahara. South Africa Inst. Med. Res, Johannesburg, 1987, No. 55.
5. Okic VA, Bashir NHH, Assad YOH. *Anopheles* species (Diptera: Culicidae) in kodok locality, upper Nile state, Republic of South Sudan. International Journal of Mosquito Research. 2023;10(5):14-18. DOI: https://doi.org/10.22271/23487941.2023.v10.i5a.691
6. Atieli H, Menya D, Githeko A, Scott T. House design modifications reduce indoor resting malaria vector densities in rice irrigation scheme area in western Kenya. Malaria Journal. 2009;8:108. doi: 10.1186/1475-2875-8-108.
7. Precious A, Dzorgbe M, Godfred F, Leonard KA, Memuna MM, Dziedzorm KDS, *et al*. Diversity in breeding sites and distribution of *Anopheles* mosquitoes in selected urban areas of southern Ghana. Parasit Vectors. 2017;10(1):25. doi: 10.1186/s13071-016-1941-3.
8. Gilles M, De Meillon B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). South African Inst. Med. Res., Johannesburg, 1968, No 54.
9. Fillinger U, Lindsay SW. Larval source management for malaria control in Africa: myths and reality. Malaria Journal. 2011;10:353-362.
10. Raghavendra K, Barik TK, Reddy NBP, Sharma P, Dash AP. Malaria vector control: from past to future. Parasitol. Res. 2011;108:757-779.
11. Naseem S, Malik MF, Munir T. Mosquito management: A review. Journal of Entomology and Zoology Studies. 2016;4(5):73-79.
12. Adam EIY, Assad YOH, Eltohami MME, Bashir NHH, Abdelrahman SH, Nour BYM. Natural Anopheline mosquito repellents and phytochemical analysis of ethanol and hexane leaf extracts from four plant species. Highlights in BioScience. 2021;4:1-10.
13. Thomson MC, Connor SJ, Quinones ML. Movement of *Anopheles gambiae* s.l. malaria vectors between villages in The Gambia. Medical and Veterinary Entomology. 1995;9:413-419.
14. Kaufmann C, Briegel H. Flight performance of the malaria vectors *Anopheles gambiae* and *Anopheles atroparvus*. J Vector Ecol. 2004;29:140-153.