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Entomological study on species composition, behavior, longevity and probability of surviving sporogony of Anopheles mosquitoes in Lare District, Ethiopia

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In order to develop efficient malaria vector control strategies, this study was conducted to assess species composition, relative abundance and longevity of Anopheles mosquito malaria vectors in Lare district, south western Ethiopia. For this, Anopheles mosquitoes were collected using CDC light traps catch and pyrethrum spray collection for five months from May to September, 2016. Four kebeles (the smallest administrative unit in Ethiopia, three houses per each kebele) for CDC light trap catches collection and 20 households from each sampled kebeles for pyrethrum spray collection were selected randomly. A total of 2735 Anopheles mosquitoes belonging to four species were collected during the study period. *Anopheles gambiae* s.l. 1914 (69.9%) was the predominant malaria vector followed by *Anopheles pharoensis* 602(22%), *Anopheles nilli* 137(5%) and *Anopheles coustani* s.l. 82 (3.10%). Significant (p<0.05) variations existed in mean density of mosquitoes per month, but not in mean mosquito density per site (between sampled kebele). The highest mean longevity of *A. gambiae* s.l. was 9.4 days in July, while probability of surviving sporogony was 0.52 and 0.56 for *P. falciparum* and *P. vivax*, respectively. Thus, this study could contribute to the basic understanding of age, distribution and behaviour of anopheles mosquitoes in Lare district for evidence based malaria vector control program.

Key word: Anopheline species, longevity, sporogony, plasmodium, Ethiopia.

INTRODUCTION

Mosquitoes of the genus, *Anopheles* are important vectors of plasmodium parasites, causal agents of malaria. There are over 400 species of anopheles mosquitoes but only 30-40 of these acts as vectors of malaria. In the sub-Saharan Africa, approximately 20 out of 140 *Anopheles* species are known to transmit malaria to humans. Among these vectors, *Anopheles gambiae* Giles, *Anopheles cluzzii* Coetizee et al., *Anopheles. arabiensis* Patton and *Anopheles funestus* Giles are the most widely distributed and the most effective malaria

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vector species in tropical Africa (Coetzee et al., 2013; Gillies and Coetzee, 1987).

The non-random distribution of both anopheles larvae and adults includes fresh or salt-water, marshes, mangrove swamps, rice fields, grassy ditches, stream edges, rivers and small temporary rain pools. Many species prefer habitats with vegetations, others prefer habitats that have none. Some breed in open, sunlit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants (Nikookar et al., 2010).

The identification of mosquito species and associated distribution records are vital to build effective malaria control program and best indication for resistance of insecticide between species (Ramirez et al., 2009; Yehwalaw et al., 2010). In order to determine malaria transmission in a given area understanding vector behavior and diversity is crucial. To accomplish this goal, entomological study with Anopheles collection is important. The choice of method depends on the objectives of the study, even though human landing catch is golden standard; however, it has an ethical issue and less probability to catch different species of anopheles. On the other hand, CDC light traps has better access to catch more number with diversity of Anopheles mosquitoes (Ndiath et al., 2011). Coetzee (2004) reported that the distribution maps of mosquito vectors might not reflect the real species distribution in nature as it could be influenced by the collection efforts of entomologist. There is no doubt that entomological studies in Ethiopia are scarce and there is a need to focus on vector species biology and ecology. This is particularly important in the south western part of Ethiopia including Gambella region where malaria is endemic (Alemu et al., 2011; Krafsur, 1977).

The data from the district showed that malaria is one and the leading top ten diseases in the area with high incidence and morbidity (Lare District Report, 2016). This study aimed to assess the species composition, behavior, longevity and probability of surviving sporogony of Anopheles mosquito malaria vectors of Lare district, south western Ethiopia.

MATERIALS AND METHODS

Study area and period

The study was conducted in Lare district, Nuer zone, south west Ethiopia. Lare is bordered on the south and east by the Anuak Zone, on the west by the Baro river which separates it from Jikawo, and on the north by the Jikawo river which separates it from South Sudan. The study was conducted from April to September, 2016 G.C. (Figure 1).

Mosquito sampling and identification

Lare district has 28 (twenty eight) kebeles (the smallest administrative unit in Ethiopia) among these, 4 (four) kebeles were randomly selected. A total of 12 households (three household per each kebele) were selected for mosquito sampling. Adult mosquitoes were collected once per month from the selected households (the households chosen one at the center and the others at the periphery of the kebele) (WHO, 1975) from May to September, 2016 using CDC light traps and pyrethrum spray collections. The collected mosquitoes were identified to species and complex level morphologically using standard keys by Gillies and Coetzee (1987); they were counted and then stored in Eppendorf tubes with desiccant (silica gel) for further laboratory processing at Sokoru Tropical Infectious Disease Research Center, Jimma University (TIDRC JU).

The CDC light trap collection

CDC light traps were set indoors (inside bed room) and outdoors (within 15-20 m radius outside the house), human dwelling (WHO, 1975; Mboera, 2005) and run from 18:00-06:00 h.

Pyrethrum spray collection

Pyrethrum spray collection (PSC) was used to collect indoor resting *Anopheles* mosquitoes from 6:00-7:30 h in 20 houses from each kebeles. The monitored houses were different from those used for light trap catches. Sampling was done once per month from May to September, 2016. Anopheles mosquito was sampled from each house once per month, from May to September, 2016. Prior to PSC, the inhabitant was asked to empty the house, any openings that could allow mosquito escaping were closed and the entire floor was covered with a white cloth from wall to wall in a single room. Then, a protected sprayer sprayed the room with Mobil flit (Bioygon SC. Jonhanson and Sun. Inc. USA) for about 5 min; the sprayed room was left closed for 15 min. Subsequently, the sheet was brought outside the room and knock-down mosquitoes were counted and identified.

Method of parity status determination

Half of the unfed female *Anopheles* mosquito collected from light trap catches were dissected for parity status. The abdominal and ovary dissection was conducted following the standards of WHO (1975). Ovaries with coiled tracheal skeins was considered as nulliparous while those with stretched out tracheoles was taken as parous as described by WHO (1975) and Fils et al. (2010).

Data analysis

The data was analyzed using SPSS statistical software version 16.0 (SPSS Inc, Chicago, IL, USA). Before analysis it was normalized by transforming into Log n +1 in SPSS. The significance test p-value less than 0.05 was considered significant during the analysis.

Daily survival rate (S) = $\sqrt[gc]{PR}$ Where gc = Estimated gonotrophic cycle of *A. gambiae* s.l. of the population. The gonotrophic cycle in area was estimated to be 3 days based on Krafsur (1977) study in west Ethiopia. Life expectancy (LE) = 1/ -LnS (Davidson, 1954). Degree of exophily (De) was calculated following: DE = 1 − (1/F-HGG) (Ameneshewa and Service, 1996).

Ethical considerations

The study was legalized by research review and ethical committee of Gambella University. Permission from the community was sought

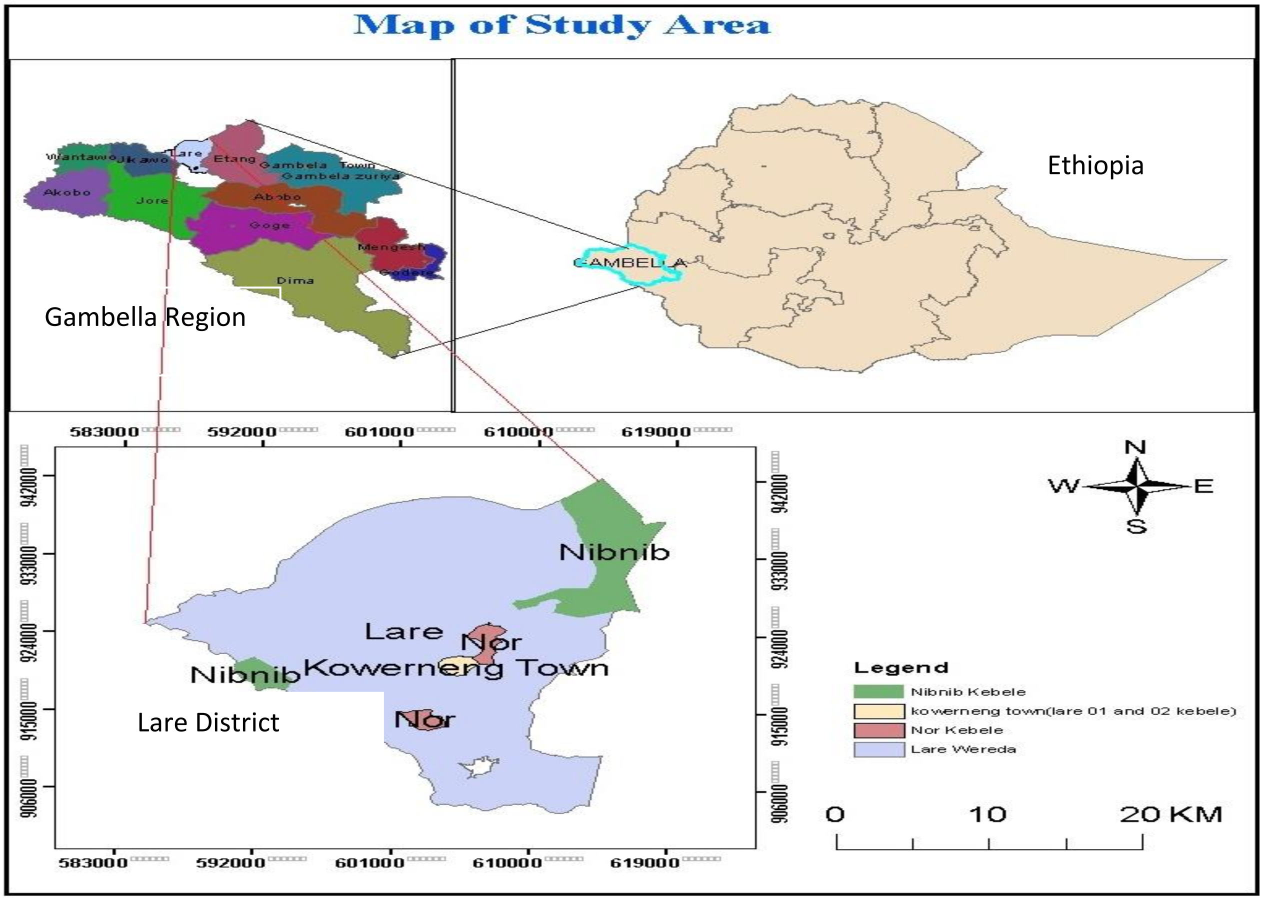

Figure 1. Map of south Western Ethiopia showing Lare district of the study area.

Table 1. Species composition and abundance of anopheles mosquitoes collected by CDC light trap and Pyrethrum spray catches in Lare district, southwest Ethiopia (May- September, 2016).

Anopheles **spp.**	**Total collected**	**Percentage**
A. gambiae s.l	1914	69.90%
A. pharoensis	602	22%
A. nilli	137	5%
A. coustani s.l.	82	3.10%
Total	2735	100%

before initiating the study by communicating the responsible zonal and district administrative offices through official letters from Gambella University. Similarly, household agreement and local oral consent were sought.

RESULTS

Anopheles mosquitoes species composition and relative abundance

A total of 2735 anopheles mosquitoes belonging to four species: *A. gambiae s.l.*, *A. pharoensis*, *A. nilli,* and *A. coustani* s.l. were collected from May to September, 2016 in the Lare district. Among the collected mosquitoes, the highest number of anopheles belonged to *A. gambiae* s.l (n= 1914; 69.9%) followed by *A. pharoensis* (n= 602; 22%) while the lowest density belonged to *A. coustani* s.l. (n= *82;* 3.1%) (Table 1).

Indoor and outdoor density of Anopheles mosquitoes

CDC light traps placed outdoors revealed higher mean number of mosquitoes per traps per night than CDC light

Table 2. Mean indoor and outdoor number of Anopheles mosquitoes/trap- night houses in Lare district, southwest Ethiopia (May- September, 2016).

		Mean	±Std. error mean	95% CI difference		T	Df	p-value
				Lower	Upper			
A. gambiae s.l	Indoor	0.8678	±.03467	-0.32876	-0.19494	-7.831	59	0.00
	Outdoor	1.1296	±.04485					
A. pharoensis	Indoor	0.3498	±.04884	-0.31238	-0.17434	-7.056	59	0.00
	Outdoor	0.5932	±.06775					
A. nilli	Indoor	0.5932	±.06775	0.22468	0.41487	6.729	59	0.00
	Outdoor	0.2734	±.04499					
A. coustani s.l	Indoor	0.041	±.01510	-0.16188	-0.03178	-2.979	59	0.04
	Outdoor	0.1378	±.03321					

*p<0.05

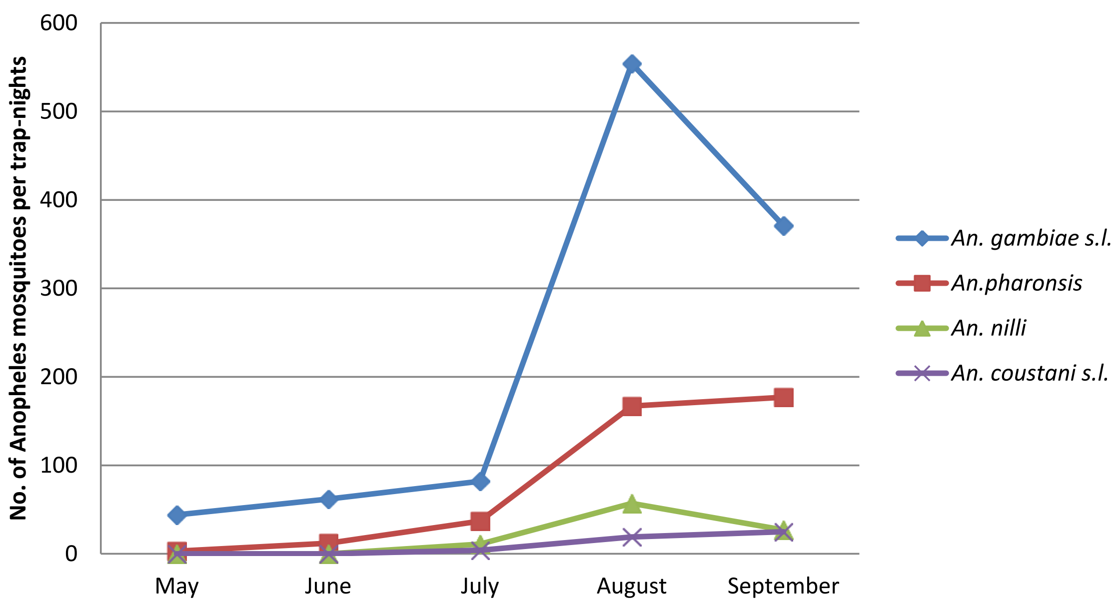

Figure 2. Monthly density of Anopheles *spp*. caught per CDC light trap per night in Lare district, south west Ethiopia (May- September, 2016).

traps placed indoor for each the four anopheles. Likewise, there were significant ($p< 0.05$) differences between indoor and outdoor mean mosquito number per trap per night for *A. gambiae s.l.* ($t= -7.831$, $p < 0.00$), *A. pharoensis* ($t= -7.056$, $p = 0.00$), *A. nilli* ($t= 6.729$, $p= 0.00$) and *A. coustani s.l*. ($t= -7.056$, $p= 0.00$), respectively (Table 2).

Monthly dynamics of Anopheles species

Over all, the highest, 841 (39.4%) and lowest, 115 (5.4%) anopheline mosquitoes densities were observed in August and May, respectively (Figure 2). The abundance of *A. gambiae* s.l and *A. nilli* peaked during August, while *A. pharoensis* and *A. coustani* s.l. peaked during September. One way ANOVA revealed that there were significant ($p < 0.05$) mean density differences between the monitored months for *A. gambiae* s.l ($F= 27.247$, $p= 0.000$), *A. pharoensis* ($F= 65.648$, $p = 0.000$), *A. nilli* ($F=5.296$, $p= 0.000$) and *A. coustani s.l.* ($F= 13.874$, $p = 0.000$), respectively.

Regarding mosquito abundance per sampled kebeles, total mean densities of *A. gambiae* s.l. ($F= 0.184$, $p = 0.907$), *A. pharoensis* ($F= 0.038$, $p = 0.990$), *A. nilli* ($F= 2.038$, $p = 0.119$) and *A. coustani* s.l. ($F= 0.038$, $p = 0.485$) were shown not to be significantly different between kebeles (Appendix Tables 1 and 2).

Table 3. Monthly parity rates, daily survival rates and life expectancy of *A. gambiae s.l.* in Lare district south west Ethiopia (May-September, 2016).

	Total dissected	No. of parous	Parity rate in (%)	Daily survival rate	Life expectancy
May	15	8	53%	0.81	4.7
June	24	16	67%	0.88	7.8
July	46	34	74%	0.9	9.4
August	107	70	65%	0.87	7.2
September	57	33	58%	0.83	5.4
Mean	49.8	32.2	63%	0.86	6.9

Table 4. Monthly parity rates, daily survival rates and life expectancy of *A. pharoensis* in Lare district south west Ethiopia (May-September, 2016).

	No. of dissected	No. of parous(P)	Parity rate (PR)	Daily survival rate(S)	Life expectancy in days (LE)
May	5	2	40%	0.74	3.3
June	11	5	45%	0.77	3.8
July	13	7	54%	0.81	4.7
August	14	9	64%	0.86	6.6
September	27	19	70%	0.89	8.6
Mean	14	8.4	55%	0.81	5.4

Parity rate and longevity of Anopheles mosquitoes

The highest parity rate (0.74, 0.70), probability of daily survival rate (0.9, 0.89) and life expectancy (9.4, 8.6) of *A. gambiae s.l.* and *A. pharoensis* were observed in July and September, respectively (Tables 3 and 4).

The highest mean probability of surviving sporogony (PSS) of *A. gambiae* s.l. and *A. pharoensis* for *Plasmodium falciparum* (0.52, 0.49) and *Plasmodium vivax* (0.56, 0.53) were observed in July and September, respectively, with corresponding highest expected infective life in days for *P. falciparum* (3.3, 2.4) and *Plasmodium vivax* (4.1 and 3.2) of the two *Anopheles* spp., respectively (Tables 5 and 6).

Indoor resting behavior and degree of exophily

Out of 602 collected anopheles mosquitos by pyrethrum spray catches, the highest (459) were *A. gambiae* s.l followed by *A. pharoensis* (93) (Table 7*).* Furthermore, fed to gravid ratios was 2.04:1, 3.04:1, 2.8:1 and 8:1 for *A. gambiae* s.l., *A. pharoensis*, *A. nilli* and *A. coustani* s.l., respectively (Table 3). The degree of exophily (DE) for predominant malaria vector was 0.51 (Table 7).

DISCUSSION

This study assessed the density of anopheles mosquitoes in selected site in Lare district and their potential role as malaria vectors. The distribution of anopheles mosquitoes in the four kebele revealed that *A. gambiae s.l., A. pharoensis, A. nilli* and *A. coustani* s.l. were found in sympathry. No significant differences in mosquito densities between kebeles were found. This could be because the study areas have similar environmental conditions, homogeneous people in socio cultural activities and the presence of several similar mosquito breeding sites. *A. gambiae* s.l. was the most abundant malaria vectors in the study area which is consistent with other parts of Ethiopia. Hence, *A. gambiae s.l.* is the principal vector of malaria in sub-Saharan Africa in general, East Africa and Ethiopia in particular (Taye et al., 2016; Coetzee, 2004; Tesfaye et al., 2011) it may be responsible for the presence of heavy burden of malaria incidence and morbidity in the area.

Significant variation existed in the density of *Anopheles* spp. during the season, with the highest densities being in August-September. The study demonstrated that all the four Anopheles mosquitoes collected during the study period exhibited significant outdoor biting activity. It is similar to Woyessa et al. (2004) studies in which one of *A. gambiae* s.l, *A. arabiensis* showed exophagic feeding behavior. The high outdoor biting density was observed by *A. gambiae s.l*, this contrasted with prior report by Fornadel et al. (2010a), in which outdoor biting was not observed. This implies a behavioral shift favoring outdoor host-seeking, possibly as the result of intense selection pressure imposed by the indoor application of insecticides. This observation was consistent with other studies both in Africa and elsewhere reported by

Table 5. Probability of surviving sporogony of *Plasmodium* species in *A. gambiae s.l.* by month in Lare district south west Ethiopia (May- September, 2016).

	Atom. Temperature (°C)	EIP pf in days	EIP pv in days	PSS of pf	PSS of pv	Expected of infective life days for pf	Expected of infective life in days for pv
May	35.25	5.8	5.1	0.3	0.34	-1	-0.3
June	34.33	6.1	5.3	0.46	0.51	1.8	2.5
July	34	6.2	5.4	0.52	0.56	3.3	4.1
August	33.67	6.3	5.5	0.41	0.46	0.9	1.7
September	34.11	6.1	5.3	0.32	0.37	-0.8	0.01
Mean	34.3	6.1	5.32	0.4	0.5	0.84	1.6

*EIP= Extrincic incubation period, pf = *Plasmodium falciparum, pv= Plasmodium vivax,* PSS = probability of surviving sporogony.

Table 6. Probability of surviving sporogony of *Plasmodium* species in *A. pharoensis* by month in Lare district south west Ethiopia (May- September, 2016).

Month	Temperature (°C)	EIP *Plasmodium falciparum* in days	EIP *Plasmodium vivax* in days	PSS *Plasmodium falciparum*	PSS *plasmodium vivax*	Expected of infective life days for *Plasmodium falciparum*	Expected of infective life days for *plasmodium vivax*
May	35.25	5.8	5.1	0.18	0.21	-2.4	-1.7
June	34.33	6.1	5.3	0.2	0.25	-2.2	-1.4
July	34	6.2	5.4	0.27	0.32	-1.4	-0.6
August	33.67	6.3	5.5	0.38	0.44	0.34	1.1
September	34.11	6.1	5.3	0.49	0.53	2.4	3.2
Mean	34.3	6.1	5.32	0.3	0.35	-3.26	-0.6

Table 7. Total collected anopheles mosquitoes by pyrethrum spray catches with their abdominal status in Lare district south west Ethiopia (May- September, 2016).

Abdominal status	*A. gambiae s.l*	*A. pharoensis*	*A. nilli*	*A. coustani s.l*	Total
Fed (F)	308	70	17	24	418
Have Gravid (HG)	117	16	4	2	140
Gravid (G)	34	7	2	1	44
Total	459	93	23	27	602
F: HG and G	2.04 : 1	3.04 :1	2.8 : 1	8 : 1	
Degree of Exophily	0.51	0.67	0.64	0.87	

Fornadel et al. (2010b) on an increased proportion of outdoor host-seeking in response to indoor residual spray or long lasting insecticide nets. It is now widely accepted that outdoor transmission will need to be addressed in order to achieve the goal of eliminating malaria from many endemic areas (Meyers et al., 2016). The finding showed relatively high degree of exophily observed for *A. gambiae s.l.,* this high degree of exophily indicates

A. gambiae s.l. revealed tendency to outdoor resting. However, the observed degree of exophily by *A. coustani s.l., A. nilli* and *A. pharoensis* was anticipated since the three Anopheles species are known exophilic species as reported elsewhere (Korgaonkar et al., 2012; Fils et al., 2010; Kibret et al., 2009; Walker, 2002).

The mean monthly parous rates were high, 50% and suggested that a large proportion of the *A. gambiae* s.l. and *A. pharoensis* of the study site had already practiced haematophagy. These findings are in agreement with those of Taye et al. (2016) and Olayemi and Ande (2008). In contrast to this, relatively low parity rate was reported by Ndoen et al. (2012). Mosquito lifespan is one component of the lifetime transmission potential of an individual mosquito. Hence, the longevity of an adult Anopheles may affect its power of transmitting malaria. The probability of daily survival of the mosquitoes remained very high throughout the five months, suggesting that *A. gambiae s.l. and A. pharoensis* are well adapted to the environmental conditions of the study area. *A. gambiae* s.l. and *A. pharoensis* are the principal and secondary malaria vectors in sub-Saharan Africa and Ethiopia, respectively. Therefore, they are well adapted to the prevailing tropical conditions in the region. Relatively higher mean life expectancy was observed in July for *A. gambiae s.l.* as compared to Taye et al. (2016). A long-lived adult female mosquito allows for increased opportunities to encounter an infected human host, the malaria parasites to multiply and reach the salivary glands after an infective blood meal (Susanna and Eryando, 2012).

Relatively high probability of surviving sporogony was envisaged in this study as compared to study conducted in Ethiopia (Taye et al., 2016) and Indonesia (Ndoen et al., 2012). This may be due to high mean annual temperature of the study period because temperature has an impact on the extrinsic incubation period of the *Plasmodium* species, as ambient temperature of the area increase, the extrinsic incubation period of *Plasmodium* species decrease (Susanna and Eryando, 2012; Olayemi and Ande, 2008).

In conclusion, this study showed that *A. gambiae* s.l. was the predominant malaria vector in the Lare district. This species is a major vector of malaria in East Africa, in particular, Ethiopia. The highest abundance parity rate of this major malaria vector was observed in August and July. Furthermore, the highest longevity and probability of surviving sporogony were in July. Consequently, national vector control programs for indoor residual spray intervention in the Lare district should be conducted in mid to late July.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Appendix 1. Mean density/ trap nights of Anopheline mosquitoes by months (May- September / 2016) Lare District south west Ethiopia.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Mean density An.gambiae s.l.	Between Groups	2301.775	4	575.444	27.427	.000
	Within Groups	1153.958	55	20.981		
	Total	3455.733	59			
Total Mean density An.pharoensi	Between Groups	1076.725	4	269.181	65.648	.000
	Within Groups	225.521	55	4.100		
	Total	1302.246	59			
Total Mean density An.coustani s.l.	Between Groups	15.333	4	3.833	5.296	.001
	Within Groups	39.812	55	.724		
	Total	55.146	59			
Total Mean density An.nilli	Between Groups	66.975	4	16.744	13.874	.000
	Within Groups	66.375	55	1.207		
	Total	133.350	59			

Appendix 2. Mean density/trap-nights of Anopheline mosquitoes vs kebeles of the study site.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Mean density An.gambiae s.l.	Between Groups	33.787	3	11.262	.184	.907
	Within Groups	3421.946	56	61.106		
	Total	3455.733	59			
Total Mean density An.pharoensi	Between Groups	2.613	3	.871	.038	.990
	Within Groups	1299.633	56	23.208		
	Total	1302.246	59			
Total Mean density An.coustani s.l.	Between Groups	5.428	3	1.809	2.038	.119
	Within Groups	49.718	56	.888		
	Total	55.146	59			
Total Mean density An.nilli	Between Groups	5.651	3	1.884	.826	.485
	Within Groups	127.699	56	2.280		
	Total	133.350	59			