Impact of sugarcane irrigation on malaria vector *Anopheles* mosquito fauna, abundance and seasonality in Arjo-Didessa, Ethiopia

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Abstract

Background: Although irrigation activities are increasing in Ethiopia, limited studies evaluated their impact on malaria vector mosquito composition, abundance and seasonality. This study aimed at evaluating the impact of sugarcane irrigation on species composition, abundance and seasonality of malaria vectors.

Methods: Adult *Anopheles* mosquitoes were collected using CDC light traps from three irrigated and three non-irrigated clusters in and around Arjo-Didessa sugarcane irrigation scheme in southwestern Ethiopia. Mosquito collections were conducted in four seasons: two wet and two dry, in 2018 and 2019. Mosquito species composition, abundance and seasonality were compared between irrigated and non-irrigated clusters. *Anopheles* mosquitoes were identified to species using morphological keys and *An. gambiae* s.l to sibling species using PCR. Chi-square was used to analyze the association between *Anopheles* species occurrence and environmental and seasonal parameters.

Results: Overall, 2,108 female *Anopheles* mosquitoes comprising of six species were collected. Of these, 92.7% (n=1954) were from irrigated clusters and 7.3% (n=154) from the non-irrigated. *An. gambiae* s.l was the most abundant (67.3%) followed by *An. coustani* complex (25.3 %) and *An. pharoensis* (5.7%). PCR based identification revealed that 74.7% (n=168) of the *An. gambiae* s.l were *An. arabiensis* and 22.7% (n=51) *An. amharicus*. Density of *An. gambiae* s.l. (both indoor and outdoor) was higher in irrigated than non-irrigated clusters. The overall anopheline mosquito abundance during the wet seasons (87.2%; n=1837) was higher than the dry seasons (12.8%; n=271).

Conclusion: The ongoing sugarcane irrigation activities in Arjo-Didessa created conditions suitable for increased malaria transmitting *Anopheles* species diversity and abundance. This in turn could drive malaria transmission in Arjo-Didessa and its environs in both dry and wet seasons. Thus, currently practiced malaria vector interventions need to be strengthened and consider larval source management to reduce vector abundance in the irrigated areas.

Key Words: Malaria, Irrigation, *Anopheles* mosquitoes, vector density, *An. amharicus*, Ethiopia

Background

Irrigation based agriculture has been largely promoted to alleviate poverty and improve economic growth in Africa [1]. However, existing evidences show that irrigation might increase the risk of vector borne diseases such as malaria [2-5]. Manmade environmental modifications and expansion of unplanned water development schemes could enhance mosquito breeding and sustain malaria transmission [2-5]. In Ethiopia, where malaria is a major cause of morbidity and hospital admissions [6], irrigation activities may contribute to increased risk of the disease. Irrigations can enhance malaria transmission by increasing the number and diversity of mosquito breeding habitats (e.g. poorly managed irrigation canals and canal seepages) that can increase vector composition, density and longevity. This can ultimately increase risk of malaria and extend the duration of malaria transmission in irrigation areas in Ethiopia where the disease is seasonal and unstable [7-9].

Previous studies indicate higher malaria risk close to dam and irrigation schemes compared to communities living further away [9-11]. In northern Tanzania, a four-fold increase in the density of *An. arabiensis* and risk of malaria was documented in rice irrigation fields than in non-irrigated savannah villages [11]. In Ghana, higher larval and adult anopheline densities were observed in irrigated areas compared to non-irrigated areas in the rainy and dry seasons [12]. Similarly, in Ethiopia, villages practicing irrigated agriculture were shown to have increased malaria vector abundance [13], risk of malaria infection [14] and mosquito density [9, 10] compared to non-irrigated villages.

Although several studies reported an increase in mosquito density and malaria transmission associated with rice irrigation [2], little is known about the impact of sugarcane irrigation on malaria transmission in Africa [11]. Available data from cross-sectional studies failed to depict trends of temporal malaria vector dynamics at least in the two major seasons: dry and wet.

Thus, it deemed necessary to evaluate the current impact of sugarcane irrigation on vector distribution, abundance and seasonality pattern in a way to suggest vector control interventions and inform public health professionals [15]. Furthermore, knowledge on the dynamics and behavior of local *Anopheles* mosquitoes may help devise control tools to achieve malaria elimination goal [16]. Therefore, this study aimed to describe the impact of Arjo-Didessa sugarcane irrigation on species composition, seasonality and abundance of *Anopheles* mosquitoes.

Methods and Materials

Study Setting

The study was conducted at Arjo-Didessa sugarcane irrigation scheme and its surroundings located at 395 km southwest of the capital, Addis Ababa, Ethiopia (Fig.1). Six study clusters were randomly selected out of 15 clusters from three districts: Jimma Arjo district (Abote Didessa), Bedele District (Command 5, Bildema Deru and Ambelta) and Dabo Hana district (Karka and Sefera Tabiya). They were selected on the basis of their proximity to the irrigation activities. The irrigation clusters (within and about 3 km from the irrigation area) were Command-5, Kerka and Abote-Didessa while the non-irrigated (4-10kms far from the irrigation area) were Ambelta, Bildema Deru and Sefera Tabiya. The shortest distance between the irrigated and the non-irrigated clusters was about 4-5kms and selection of the study clusters was by assuming an average *Anopheles* mosquito flight range of 3kms to control overlap/contamination of mosquitoes flying from the irrigated to the non-irrigated and vice versa. A cluster is defined as an area which has 150-200 households. Both clusters had similar eco-topography. Entomological surveys were conducted from January 2018 to August 2019 in four seasons: two wet seasons and two dry seasons in the six clusters.

The districts have a total population of 215,288 and the study clusters 50,000. The great majority of the population depends on subsistence farming. They raise cattle and cultivate mixed crops and cereals including sorghum, rice, corn/maize, peanut, vegetables and sugarcane. The altitude of the area ranges from 1300 to 2280 meters above sea level (masl) with mean annual rainfall of 1477mm. The irrigation area and its surroundings are known to be malarious [17]. It was formerly a wild life sanctuary (Didessa wildlife sanctuary), and since 2006, changed to a state owned sugarcane plantation development to supply the sugar factory. It is one of the biggest sugar development projects in Ethiopia, covering about 5000 hectares (ha) of land with future expansion plan of 80,000ha.

Mosquito Sampling and Processing

Adult *Anopheles* mosquitoes were collected using standard Centers for Disease Prevention and Control (CDC) light traps (Model: John W. Hock CDC Miniachure Light trap 512, USA) from eight randomly selected houses in each of the six clusters. A total of 384 houses (192 houses from irrigated clusters and 192 from non-irrigated clusters) were visited and 768 trapping nights (indoor and outdoor) spent through the collection time. During each sampling night, sixteen CDC light traps were set; one indoor in a sleeping room at about 1.5 m above the floor near the foot end of a person sleeping under long-lasting insecticide treated net and one outdoor at 5-8m near the same house used for indoor collection. The traps were kept running from 18:00 to 06:00 hours.

After 06:00 hour in the morning, the CDC light traps were labeled with identifier, collected and transported to the field laboratory for processing. Live and dead mosquitoes were retrieved by mechanical aspirator from collection bags and live mosquitoes were killed using Chloroform (99.8% Trichloromethane). Female *Anopheles* mosquitoes were sorted and identified morphologically under dissecting microscope to species using standard key [18]. Abdominal status of the mosquitoes was determined under dissecting microscope as unfed, freshly-fed, half-gravid or gravid. Culicine and male anopheline mosquitoes were also retrieved by aspirator from the bags, counted and recorded. Each female *Anopheles* mosquito was preserved individually in labeled Eppendorf tube over silica gel and stored for further processing. Sample processing was done at Arjo-Didessa International Center of Excellence for Malaria Research (ICEMR) Laboratory, Ethiopia.

Identification of *Anopheles gambiae* complex species

Among the total 1,418 *An. gambiae* s.l. collected during the survey, some 225 (~16%) were randomly selected and identified to species by using species-specific polymerase chain reaction (PCR) assay at the Molecular Biology Laboratory of Tropical and Infectious Diseases Research Center (TIDRC), Jimma University, Ethiopia. Briefly, genomic DNA was extracted using DNA extraction kit (Qiagen, DNeasy Blood and Tissue kit; Sigma Aldrich**,** Catalog#:69506) from legs and wings of each mosquito. PCR assay was carried out [19] using species specific primers with primers sequences of AG (5′-CTGGTTTGGTCGGCACGTTT-3; specific for *An. gambiae* s.s.), AG QD-B (5′-AGTGTCCAATGTCTGTGAAG-3′; specific for *An. quadriannulatus* species B, here after *An. amharicus*), AR (5′-AAGTGTCCTTCTCCATCCRA-3′; specific for *An*. *arabiensis*), and UN (5′-GTGTGCCCCTTCCTCGATGT-3′; common for all species) were mixed with PCR cocktail (Master mix, sample DNA and mH20). Amplification was done with 94°C/5 min x 1cycle, [94°C/ 30 sec; 54C/30 sec; 72°C/ 30 sec] x 35 cycles 72°C/10 min x 1 cycle; 4°C hold cycling conditions [19]. When amplification was complete, the amplicon was loaded on 1.5% agarose gel stained with ethidium bromide and run for gel electrophoresis. *An. arabiensis* from Sekoru insectary colony of Jimma University was used as a positive control.

Data analysis

Data entry and analysis was made using Microsoft Excel (Version 2016, Microsoft Corporation, Washington, USA) and IBM SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) statistical software packages and had been summarized with frequencies (n) and percentages (%) by species, season and irrigation levels. Chi-square ($\chi2$) test was used to compare mosquito variation by irrigation level and season and the test was assumed significant at a p-value of less than 0.05. Indoor and outdoor mosquito density for each species per household was calculated as:

“D = n/trap-night”

where ‘D’ is density for individual mosquito species and ‘n’ is the number of mosquitoes for every species, ‘trap-night’ represents the trapping night spent in each house of all clusters. Note that the frequency of collection, the number of traps used and the number of nights spent in each season and in each cluster was similar.

Ethical Considerations

Ethical clearance was obtained from the Institutional Review Board (IRB) of Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia (Ref.: ***No. ALIPB/IRB/012/2017/18***) and National Ethics Review Committee (NERC), Ethiopia. Permission was also obtained from East Wollega and Buno Bedele Zonal Health Offices, Oromia Regional State, Ethiopia. Verbal consent was obtained from household owners to set CDC light traps.

Results

Species composition of *Anopheles gambiae* complex

Among the 225 *An. gambiae* s.l. diagnosed using PCR, 74.7% (n=168) were *An. arabiensis*, 22.7% (n=51) *An. amharicus* (formerly known as *An. quadranulatus* B) and the remaining 2.6% (n=6) samples were not amplified (Fig. 2).

Anopheles species composition and abundance

Overall 2,108 (38.8%) anopheline and 3,326 (61.2%) culicine mosquitoes were collected from the six clusters during the study period. Among the 2,108 anopheline mosquitoes, 92.7% (n=1954) were from the irrigated clusters and 7.3% (n=154) from the non-irrigated (control) clusters (Table 1). The *Anopheles* species from the irrigated clusters were *Anopheles arabiensis, An. amharicus*, *An. coustani* complex, *An. pharoensis, An. funestus* group and *An. squamosus*. The species from the non-irrigated clusters were *An. arabiensis*, *An. coustani* complex, *An. pharoensis* and *An. squamosus*. *An. amharicus* and *An. funestus* group were collected from the irrigated clusters only. PCR based analyses of sub-samples of *An. gambiae* s.l from non-irrigated clusters revealed only *An. arabiensis*.

Overall, *An. gambiae* s.l. (n=1418; 67.27%) was the most abundant species followed by *An. coustani* complex (n= 534; 25.33%), *An. pharoensis* (n=120; 5.69%), *An. squamosus* (n=25; 1.19%) and *An. funestus* group (n=11; 0.52%). The *Anopheles* mosquito abundance in the irrigated clusters was significantly ($\chi2$=61.404, df = 4, P<0.001) greater than in the non-irrigated clusters. *An. gambiae* s.l was more abundant in the irrigated clusters (n= 1,347; 95%) than in the non-irrigated (n=71; 5%). Similarly, *An. coustani* complex, *An. pharoensis* and *An. squamosus* showed higher abundance in the irrigated clusters relative to the non-irrigated clusters.

Mosquito density and seasonality

Majority of mosquitoes were collected from outdoor (n=1247, 59.2%) than indoor (n=861, 40.8%) and the difference was statistically significant ($\chi2$=188.07, df = 4, P< 0.001). Density of *An. gambiae* s.l was higher in the irrigated than in the non-irrigated clusters. Individual indoor and outdoor average mosquito densities revealed that 3.46 mosquitoes per night per house indoor and 3.55 outdoor for *An. gambiae* s.l. in the irrigated clusters whereas for the non-irrigated clusters it was 0.21 mosquito per night indoor and 0.16 outdoor in the wet season (Table 2). The indoor and outdoor densities of *An. coustani* complex was 0.36 mosquito per night indoor and 2.15 outdoor in the irrigated clusters but it was 0.1 mosquito per night indoor and 0.18 mosquito per night outdoor in the non-irrigated clusters.

About 86% (n=1813) of the total *Anopheles* mosquitoes were collected during the wet seasons and the remaining 14% (n=295) during the dry seasons (Table 3) and the difference was statistically significant ($\chi2=70.423$, df = 4, $P<0.001$). In the wet seasons, indoor and outdoor density of *An. gambiae* s.l. was highest followed by *An. coustani* complex and *An. pharoensis* from the irrigated villages (Fig. 3).

Discussion

Sugarcane irrigation activities, in Arjo-Dedissa area of southwestern Ethiopia, were associated with increased *Anopheles* species diversity, abundance and density during dry and wet seasons. Interestingly, *Anopheles amharicus* was recorded from sugarcane irrigated areas in the present study. Indeed, irrigation provided suitable breeding grounds for malaria vector mosquitoes in the area and increased mosquito species composition and abundance. This could emanate from the availability of several and suitable *Anopheles* species breeding microhabitats as a result of the uninterrupted sugarcane irrigation activity. *Anopheles arabiensis*, *An. amharicus*, *An. coustani* complex, *An. pharoensis, An. funestus* group and *An. squamosus* were collected from the irrigated sugarcane plantation areas while *An. arabiensis*, *An. coustani* complex, *An. pharoensis* and *An. squamosus* were collected from non-irrigated areas. This could explain the relevance of sugarcane irrigation schemes in supporting breading of diverse *Anoheles* species. Similar studies from Ethiopia [13] and elsewhere in Africa [20-22] suggest that irrigation agricultural practices influence *Anopheles* mosquito species diversity. The presence of such diversified malaria transmitting *Anopheles* species could contribute to increased risk of malaria transmission and complicate disease prevention and control process in the irrigation scheme.

Occurrence and distribution of *An. amharicus* (formerly known as *An. quadranulatus* species B) in irrigation schemes was recorded for the first time in this study. *Anopheles amharicus* was reported for the first time in Ethiopia by Hunt *et al.,* [23] about 18 km east of the present study area [24]. Although much is unknown about the geographic distribution of this species in Ethiopia [24], its co-existence with *An. arabiensis* in the present study indicate that these two species might have similar breeding habitat and ecologic preferences. Changes in microclimate and increased water ponding resulted from diversified habitat types such as irrigation canals, hippo trench and manmade pools might favor breeding and distribution of *An. amharicus* in the irrigated clusters.

The two secondary malaria vectors in Ethiopia, *An. pharoensis* and *An. funestus* group [25, 26], were also recorded in the study area. Similarly these two vector species were linked with irrigation practices in Central Ethiopia [9]. A study in northern Tanzania indicate that *An. funestus* group was increased following introduction of irrigation schemes [11]. The study showed that semi-permanent ponds formed due to poorly maintained water systems were the main breeding habitats of *An. funestus* around irrigation schemes. The occurrence of *An. funestus* group both in the dry and wet seasons in the present study suggests that irrigation areas created conducive breeding grounds for this species throughout most of the year. This species has become a common mosquito species in areas with water resources development in Ethiopia [9, 10]

This study clearly shows that *Anopheles* species in the irrigated clusters were far greater populated and diverse than those in the non-irrigated clusters in dry and wet seasons. Higher abundance of *An. gambiae* s.l. primarily comprising *An. arabiensis*, the major malaria vector, in the irrigated villages shows the role of sugarcane irrigation in enhancing abundance of the primary vector in the country. Poorly managed irrigation creates fresh and small sunlit breeding habitats that favor fast *An. arabiensis* breeding [27]. Similarly, *An. arabiensis* predominated irrigated fields in Ethiopia [9, 13, 28, 29], Northern Tanzania [11] and Ghana [12]. A previous study in Ethiopia also showed that an increase in canal water release was associated with an increase in larval density of *An. arabiensis* [30]. Another study associated that *An. arabiensis* gravid females to be more attracted to sugarcane pollen-associated volatile sweet attractants [31] which might be the reason for the greater abundance of this species in the sugarcane irrigated fields in the present study. Overall, as vector abundance is one of the direct predictors for malaria transmission; our study suggested a high risk of malaria transmission around the irrigated fields unless proper vector intervention strategies are implemented.

In the present study, *Anopheles* mosquito density was higher outdoor than indoor, which could compromise the effectiveness of indoor-based vector interventions (LLINs &IRS). In agreement to our finding, similar outdoor biting activity of anophelines was documented in southwestern

Ethiopia [32]. *Anopheles arabiensis* had the highest density outdoors in the irrigated clusters during the wet season. Obliviously, the wet seasons created significantly higher number of additional breeding habitats than the dry seasons which ultimately can increase the vector density. Larval source management in the irrigated fields could help reduce vector density/abundance [33] and irrigation schemes should therefore consider additional larval source management strategies for malaria vector control in such settings.

This study had several caveats. The first one was lack of monthly stratified data for mosquito abundance. Entomological indicators such as human blood index, sprorozoite rate and entomological inoculation rates were not determined. The study is a part of a large NIH funded ongoing study and we plan to assess these indicators in the future works. The role of *An. amharicus* in malaria transmission in the study setting requires further investigation. Research is also required to evaluate the effectiveness of larval source management around irrigated schemes for mosquito control.

In conclusion, environmental modifications due to sugarcane irrigation schemes create conditions suitable for mosquito diversity and propagation. The increased number of malaria vector mosquitoes in the irrigated areas may drive malaria transmission both during the dry and wet seasons. Understanding the role of *An. amharicus* on malaria transmission in the irrigated area is important to devise tailor-made vector interventions. Current malaria vector control interventions need to incorporate larval source management to reduce vector abundance in irrigated areas.

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Authors' contributions

AD, DY and GY conceived and designed the study; AD and DH involved in data collection, field supervision and data analysis. AD performed the laboratory analysis and drafted the manuscript. AD, DH and AT conducted the field work and collected the data. MCL developed map of the study area. DY, SK, AA, MCL and GY critically reviewed the manuscript. All authors read and approved the final manuscript.

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1. Composition and abundance of *Anopheles* species in sugarcane irrigated and non-irrigated areas of Arjo-Didessa irrigation scheme, Southwest Ethiopia,2018-2019

	Cluster	*An. coustani* n (%)	*An. funestus* n (%)	*An. gambiae s.l* n (%)	*An. pharoensis* n (%)	*An. squamosus* n (%)	Total n (%)
Irrigated Clusters	Command 5	267 (22.2)	4 (0.3)	849 (70.6)	82 (6.8)	0 (0.0)	1202 (100)
	Abote Didessa	6 (15.0)	3 (7.5)	31 (77.5)	0 (0.0)	0 (0.0)	40 (100)
	Karka	208 (29.2)	4 (0.6)	467 (65.6)	16 (2.2)	17 (2.4)	712 (100)
Non-Irrigated Clusters	Ambelta	28 (28.0)	0 (0.0)	49 (49.0)	22 (22)	1 (1.0)	100 (100)
	Bildema Deru	24 (64.9)	0 (0.0)	6 (16.2)	0 (0.0)	7 (18.9)	37 (100)
	Sefera Tabiya	1 (5.9)	0 (0.0)	16 (94.1)	0 (0.0)	0 (0.0)	17 (100)
	Total, n (%)	534 (25.33)	11 (0.52)	1418 (67.27)	120 (5.69)	25 (1.19)	2108 (100)

Table 2. Indoor and outdoor Anopheline mosquito density in irrigated and non-irrigated clusters of Arjo-Didessa irrigation scheme, Southwestern Ethiopia, 2018 & 2019

	Irrigated Clusters		**Non-Irrigated Clusters**	
***Anopheles* Species**	Indoor	Outdoor	Indoor	Outdoor
An. coustani complex	0.36	2.15	0.10	0.18
An. gambiae s.l	3.46	3.55	0.21	0.16
An. pharoensis	0.24	0.27	0.06	0.05
An. squamosus	0.01	0.08	0.01	0.03
An. funestus group	0.02	0.04	0.00	0.00

Table 3: Seasonal abundance of *Anopheles* species at Arjo-Didessa Irrigation Scheme, Southwest Ethiopia, 2018 & 2019

Season	*An. coustani* n (%)	*An. funestus* n (%)	*An. gambiae s.l* n (%)	*An. pharoensis* n (%)	*An. squamosus* n (%)	Total n (%)
Irrigated	481 (24.61)	11 (0.56)	1347 (68.94)	98 (5.01)	17 (0.87)	1954 (100.0)
Dry season	106 (45.49)	5 (2.24)	97 (43.50)	13 (5.83)	2 (0.90)	223 (100.0)
Wet season	375 (21.66)	6 (0.35)	1250 (72.21)	85 (4.91)	15 (0.87)	1731 (100.0)
Non-irrigated	53 (34.42)	0 (0.00)	71 (46.10)	22 (14.29)	8 (5.19)	154 (100.0)
Dry season	18 (25.00)	0 (0.00)	44 (61.11)	5 (6.94)	5 (6.94)	72 (100.0)
Wet season	35 (42.68)	0 (0.00)	27 (32.93)	17 (20.73)	3 (3.66)	82 (100.0)
Total (n (%))	534 (25.33)	11 (0.52)	1418 (67.27)	120 (5.69)	25 (1.19)	2108 (100.0)