

Characteristics of *Anopheles arabiensis* larval habitats in selected urban areas in Kafta Humera district of Tigray Region, northwestern Ethiopia

Mekonnen Yohannes*

Department of Medical Parasitology and Entomology, School of Medicine, College of Health Sciences, Mekelle University; P.O.Box 1871, P.O.Box 1585, Ethiopia. Phone: 0914 708927

*Corresponding author: mekonnenyohannes@yahoo.co.uk

Abstract

Introduction: Mosquito Larval Source Management could be a valuable additional tool for malaria vector control especially in small urban hotspots where the breeding sites are limited in scope and where indoor residual spraying is not applied.

Objective: This study aimed to assess the abundance of potential mosquito breeding sites and identify the most important larval habitats of *Anopheles arabiensis* in urban settings of the agriculturally important district of Kafta Humera in western Tigray, northwestern Ethiopia.

Methods: Cross sectional larval surveys were conducted in October 2016 and 2017 in Setit-Humera town and nearby semi-urban communities of Adebay and Rawyan, in western Tigray. All water bodies encountered were sampled using standard dippers and their physical features recorded. A total of 132 potential mosquito breeding sites were visited and six aquatic habitats containing *Anopheles* larvae were identified: rain puddles, riverbed pools, vehicle ruts, leaking water pipes, dugouts, and artificial containers. Anopheline aquatic stages collected were reared and the emerged adults identified by morphological means. Sub-samples of *Anopheles gambiae* s.l were identified by PCR in a parallel insecticide susceptibility bioassay study. Logistic regression analysis was used to determine the key ecological factors explaining the different densities of mosquito larvae.

Results: Of the 132 habitats visited, 70% were positive for anopheline aquatic stages, of which 82% comprised of high larval density sites. Eighty eight percent of the larval positive habitats also had the pupal stages, indicating the high productivity of the habitats. Ninety nine percent of the 4,925 morphologically identified *Anopheles* species were *Anopheles gambiae* s.l, a subsample of which was identified as *Anopheles arabiensis* by PCR. *Anopheles arabiensis* larvae were more likely to be found in small and fully sunlit water bodies located within 10-20 meters of human habitations. Vehicle ruts were 12 times (OR = 12; $P < 0.001$) more likely to be colonized by anopheline larvae than puddles and 5 times (OR = ??? $P=0.01$) more likely than riverbed pools. Overall, human made water bodies were 5 times (OR = 5.19; $P = 0.001$) more likely to be colonized by anopheline larvae than natural water bodies. Besides, water bodies encountered in Raywan were nearly 6 times (OR = 5.64.; $p < 0.001$) and those in Adebay 5 times (OR=5.23; $P = 0.003$) more likely to host *Anopheles arabiensis* stages than water bodies in Humera town.

Conclusion: The findings suggest that targeting smaller human-made aquatic habitats could result in effective larval control of *Anopheles arabiensis* in the study area. Frequent surveillance of mosquito breeding sites, especially during the rainy season, is considered necessary for effective control planning.

Keywords: *Anopheles arabiensis*, urban, breeding site, larval habitat, Humera, Ethiopia

Introduction

Vector control, especially large-scale distribution of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), is among the key strategies that have contributed to the significant reduction in malaria morbidity and mortality in Ethiopia in the past 15 years. In order to maintain the current gains and progress further, attention should be given to the emerging threat of insecticide resistance (Messenger et al. 2017) and changes in behavior of *Anopheles arabiensis*, the main vector of malaria in Ethiopia. There is also a growing need to understand and plan control around special risk settings, including urban areas, water development projects and challenges posed by refugee populations and seasonal migrants (NMCT et al. 2014). As the population of sub-Saharan Africa rapidly urbanizes, there are worries that this

could lead to increased risk of malaria in cities and towns located in endemic and fringe areas of malaria transmission. Although urban areas are normally characterized by low malaria burden (Robert et al. 2003), unplanned urbanization can lead to the proliferation of larval habitats that can potentially contribute to malaria transmission (Dongus et al. 2009). On the other hand, as malaria transmission in urban settings is usually lower and more focal than in rural settings (Staedke et al. 2003; Hay et al. 2005), urban areas hold promise for vector control and integrated vector management (WHO 2013). Although nearly 84% of the Ethiopian population is still rural (CSA 2007), in view of the rapidly growing number of small- and medium-sized towns, there is a pressing need to give utmost attention and improve our understanding of the epidemiology of malaria in these

settings. Setit -Humera is one of the populous towns in the country that could benefit from specialized municipal vector control strategies and surveillance (NMCT et al. 2014). Setit Humera is located in the high malaria endemic district of Kafta Humera in Tigray, northwestern Ethiopia. A number of other fast-growing towns in this area include Maikadra and Dansha.

The epidemiology of malaria in Kafta Humera is complex due to cross border trade, huge agricultural activity and seasonal migration of young workers. The towns in this area host tens of thousands of these non-immune migrant laborers during the weeding and harvesting season which overlaps with the main malaria transmission season. The migrant workers can not only increase the intensity of malaria transmission in the area, they can also serve as a source of infection back in their villages. To date, vector control in Kafta Humera area is limited to distribution of LLINs. Indoor residual spraying (IRS) operation has been discontinued in the area some 10 years back. Although larval control through environmental management and larviciding is an integral part of the malaria control initiatives in Ethiopia, in general, yet little has been done in the district due perhaps to limited expertise and the necessary basic resources. As a result, outbreaks are common, the most recent one being the one that has occurred in Humera in 2014. Mosquito Larval Source Management could thus be a valuable additional tool for malaria vector control in these areas. However, this requires knowledge of the distribution and characteristics of larval habitats for planning and implementing larval control strategies effectively. The purpose of the current study was to assess the abundance of potential mosquito breeding sites and identify particularly productive larval habitats of *An. arabiensis* in the town of Setit Humera and two semi-urban communities nearby.

Materials and Methods

Study area: The study was conducted in October 2016 and 2017 in the town of Setit-Humera (14°16'50"N, 36°37'03"E; at 580-603 meters above sea level-masl) and semi-urban communities of Adebay and Rawyan in Kafta Humera district of Tigray, northwestern Ethiopia. Setit-Humera town is located about 991 km northwest of Addis Ababa and 585 km west of Mekelle, the regional capital. Rawyan town (14°16'50"N, 36°37'03"E; at 603masl) is just 3kms southeast of Humera while Adebay town (14°16'50"N, 36°37'03"E; at 659masl) is 16kms northeast of the town. Kafta Humera district is bordered by Sudan to the west and Eritrea to the north. It is one of the most fertile agricultural zones in the region with large scale farming of cash crops such as sesame, sorghum, and

cotton. The area is rich in fertile black cotton soil and clay loam. Setit Humera is the administrative center of the district which covers an area of around 7000 Km². The area is a *kola* (lowland) zone with arid and semiarid climate and consisting of mainly wide-open plains. Temperatures rise to an average of 42°C between April and June and falls to between 25 and 35°C during the moderate months of June through February. The mean annual rainfall is 540.6 and varies from 357.8mm and 650mm minimum and maximum respectively. On average the main rainy season (June –September) contributes 85% to the annual rainfall totals (Niguse & Aleme 2015). This is the planting and weeding time and the harvesting season extends from October to December. During this period, over 600,000 young workers migrate into the Kafta Humera lowlands mainly from the whole of Tigray, northern Amhara, and Sudan.

As current population figures are not available (2017), the extrapolated population of Humera town from the 2007 census is about 36,074. According to the local administration, the population of Adebay is about 12,000 while that of Rawyan is around 8000. The most important water course in the study area is Tekeze River. Tekeze River is perennial and borders the town of Humera in the north and west. Four kilometers southeast of Humera town is found another seasonal river crossing the town of Rawyan east-west. The availability of water for both human and animal consumption is a serious problem in Kafta Humera district. Malaria and diarrhea are the major health risks in the zone. Malaria is associated with the onset and offset of rains and is transmitted by *Anopheles arabiensis* as in most parts of the country.

Survey of *Anopheles arabiensis* breeding sites:

Searches for potential *An. arabiensis* breeding sites were conducted in October 2016 and 2017. Standing waters in and around the three towns were checked for the presence of anopheline aquatic stages with a standard dipper (350 mls) (BioQuip Products, Inc. California, USA). From each habitat up to 10 dips were made depending on its size. During the survey, a habitat was first investigated for the presence of anopheline larvae visually. If anopheline aquatic stages could be seen in large numbers without dipping or nearly every dip contained anopheline larvae, the aquatic habitat was defined as having a high *Anopheles* density. Sites where only one or two dips out of 10 contained anopheline larvae, they were defined as having a low *Anopheles* density. The presence of pupae was also noted. For each larval habitat type, physical characteristics, including origin of habitat or habitat type (natural or human made), habitat size, light exposure, turbidity, presence of

emergent vegetation/ and proximity to human habitation were estimated and recorded visually.

Mosquito identification: All anopheline aquatic stages were collected in small wide-mouthed jerry cans and reared in white enamel plates in a field laboratory in Humera town. Upon pupation, they were transferred into glass beakers and placed in mosquito cages until emergence. Emerged adult mosquitoes were morphologically identified according to the key of Gillies and Coetzee (1987). Emerged female adults of *An. gambiae* s.l were used for insecticide resistance tests. Sub-samples of the test mosquitoes were processed for molecular identification using PCR and all identified as *An. arabiensis*. Hence, the anopheline mosquitoes morphologically identified as *An. gambiae* s.l in the present survey shall hereafter be referred to as *An. arabiensis*.

Results

Species composition: All the aquatic stages collected from the anopheline larval habitats sampled were reared. Of the emerged 4925 anopheline adults, 4922

(99.94%) were *An. gambiae* s.l (henceforth *An. arabiensis*) and the remaining 3 were identified to be *An. maculipalpis* (0.06%). Culicines were encountered in a couple of polluted waters but not quantified for the purpose of the present study.

Description of *An. arabiensis* larval habitat types: A total of 132 potential anopheline mosquito breeding sites were visited and six larval habitat types were identified in the area. These were grouped into 4 different types of habitats for data analysis: rain puddles (28), riverbed pools (54), vehicle ruts (40) and “Others” (10). The category “Others” included, pools from leaking water pipes (5), water tankers (2) and dug outs / ditches – in construction sites (3). Rain puddles and riverbed pools were considered as natural habitats while the rest were classified as temporary human made habitats. Of the 132 potential anopheline breeding sites, the most abundant type was riverbed pools (41%) followed by vehicle ruts (30%) and puddles (21%). Nearly 82% of the riverbed pools were recovered from Rawyan while 68% of the vehicle ruts were from Adebay town (Table 1).

Table 1: Frequency of potential anopheline mosquito breeding habitat types and presence of *An. arabiensis* aquatic stages in Adebay, Humera and Rawyan towns in western zone of Tigray, northwestern Ethiopia, Oct. 2016/17

Variables	Puddles	Riverbed pools	Vehicle ruts	Others	Total
Number of habitats visited n (%)	28 (21.2)	54 (40.9)	40 (30.3)	10 (7.6)	132
Proportion of <i>Anopheles</i> larvae	42.9 (12/28)	66.7 (36/54)	90 (36/40)	80 (8/10)	69.7 (92/132)
Proportion of <i>Anopheles</i> positive sites that had high larval density	75 (9/12)	44.4 (16/36)	83.3 (30/36)	87.5 (7/8)	81.5 (75/92)
Proportion of <i>Anopheles</i> pupae	100 (12/12)	69.4 (25/36)	100 (36/36)	87.5 (7/8)	87 (80/92)
Number of habitats visited n (%)					
Adebay	1 (3.1)	4 (12.5)	27 (84.4)	0	32
Humera	13 (40.6)	6 (18.8)	8 (25)	5 (15.6)	32
Rawyan	14 (20.6)	44 (64.7)	5 (7.4)	5 (7.4)	68
Proportion with <i>Anopheles</i> larvae					
Adebay	0 (0/1)	0 (0/4)	92.6 (25/27)	0	78.1 (25/32)
Humera	23.1 (3/13)	16.7 (1/6)	75 (6/8)	60 (3/5)	40.6 (13/32)
Rawyan	64.3 (9/14)	79.5 (35/44)	100 (5/5)	100 (5/5)	79.4 (54/68)

Determination of positive and negative mosquito breeding sites: Among the 132 potential mosquito breeding sites inspected in the three study sites, 70% were found to be positive for *An. arabiensis* larval stages (Table 1). Of these, nearly 82% had high larval density and 87% contained the pupal stages. Nearly 67% of riverbed pools and over 80% of vehicle ruts and others were positive for *An. arabiensis* aquatic stages. On the other hand, over 50% of the puddles did not harbor *An. arabiensis* larval stages during the survey period. Among the sites positive for *An. arabiensis* larval stages, 69% of riverbed pools and over 87% of the puddles, vehicle ruts and other habitats harbored the pupal stages (Table 1).

Spatial distribution of anopheline larval habitats: The distribution of anopheline larval habitats was heterogeneous across the three study sites. The most

common potential anopheline larval habitat in Adebay town was vehicle ruts (84%), of which 93% harbored *An. arabiensis* aquatic stages. The seasonal stream about 0.5km south of Adebay was already dry during the survey period except one big pool and 3 smaller ones nearby. In Humera, rain puddles (41%) and vehicle ruts (25%) accounted for 66% of the potential *An. arabiensis* breeding habitats. Three fourth of the vehicle ruts and nearly one fourth of the puddles were positive for *An. arabiensis* aquatic stages. The contribution of the residual pools left by the receding Tekeze River was minimal during the survey period. The same was true with the big puddles located at the southern periphery of the town. On the other hand, riverbed pools (65%) and rain puddles (21%) were the most widespread habitats in Rawyan town. Nearly 80% of the riverbed pools and 64% of the puddles were positive for *An. arabiensis* aquatic stages.

Overall, in Rawyan 79% of the 68 habitats contained *An. arabiensis* aquatic stages whereas 78% of the 32 habitats in Adebay and 41% of the 32 habitats in Humera town were positive for *An. arabiensis* aquatic stages. All the positive breeding sites in Adebay were

created by vehicle ruts. On the other hand, puddles and riverbed pools in Rawyan town constituted over 81% (44/54) of the *An. arabiensis* positive breeding sites (Figure 1).

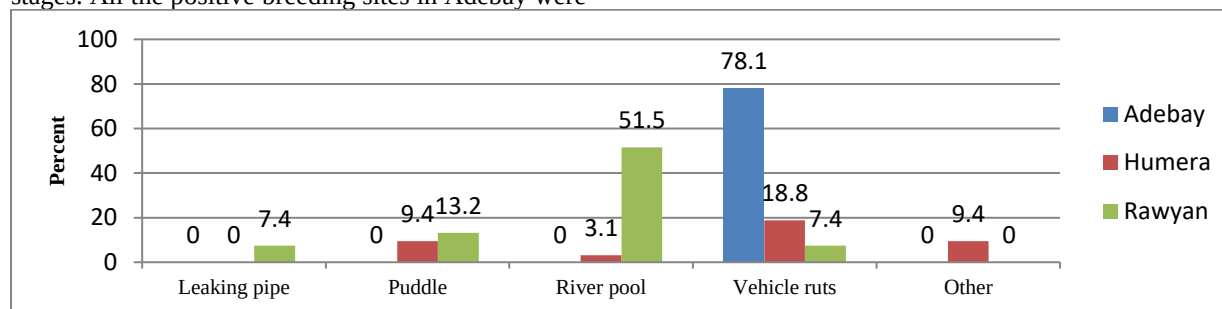


Figure 1: The percentage of habitat types positive for *An. arabiensis* aquatic stages in Adebay, Humera and Rawyan towns in Kafta Humera district of Tigray, northwestern Ethiopia, Oct. 2016/17

Characterization of *An. arabiensis* larval habitats:

The key parameters of the potential anopheline breeding sites are summarized in table 2 below. Accordingly, logistic regression analysis showed that vehicle ruts were 12 times (OR = 12; $P < 0.001$) and riverbed pools nearly 3 times (OR = 2.67; $P = 0.04$) more likely to be colonized by *An. arabiensis* larvae than puddles. Overall, human made water bodies

(vehicle ruts, leaking water pipes, dugouts and artificial containers) were 5 times (OR = 5.19; $P = 0.001$) more likely to be colonized by *An. arabiensis* aquatic stages than natural water bodies (riverbed pools and puddles). Overall, among the larval habitat types, vehicle ruts were found to be the most productive habitats in terms of the proportion that had harbored the larval and pupal stages of *An. arabiensis* as well as the level of larval density.

Table 2: Factors associated with the presence of *An. arabiensis* larvae in the three study towns in Kafta Humera district, western Tigray, northwestern Ethiopia, October 2016/2017

Characteristic	Variables	Number of Water bodies n (%)	Anopheline larval habitats n (%)	Univariate analysis ^a OR (^b 95% CI)	p-value
Towns	Adebay	32 (24.2)	25 (78.1)	5.23 (1.75 - 15.61)	0.003*
	Rawyan	68 (51.5)	54 (79.4)	5.64 (2.25 - 14.12)	<0.001*
	Humera	32 (24.2)	13 (40.6)	1	
Water bodies types	Puddles	28	12 (42.9)	1	
	Riverbed pools	54	36 (66.7)	2.67 (1.04 - 6.82)	0.04*
	Vehicle ruts	40	36 (90)	12 (3.35 - 42.97)	<0.001*
	Other**	10	8 (80)	5.33 (0.95 - 29.81)	0.057
				6.15 (2.42 - 15.65)	<0.001*
Size (m ²)	≤ 1	107	83 (77.6)	1	
	> 1	25	9 (36)	1	
Stability and size	Temporary <1m2	52	46 (88.5)	3.73 (1.34 - 10.35)	0.01*
	Permanent <1m2	55	37 (67.3)	1	
Proximity (meters)	<20m	69	56 (81.2)	3.63 (1.48 - 8.90)	0.005*
	20 - 100m	28	17 (60.7)	1.30 (0.48 - 3.57)	0.609
	>100m	35	19 (54.3)	1	
Water turbidity	Turbid	79	48 (60.8)	1	
	Clear	53	44 (83)	3.16 (1.35 - 7.37)	0.008
Source of breeding site	Human made	50	44 (88)	5.19 (1.99 - 13.56)	0.001*
	Natural	82	48 (58.5)	1	

^aOR = Unadjusted Odds Ratio; ^b95% OR Confidence Interval

The analysis also showed that proximity to human dwellings, size and turbidity of water bodies appeared to influence the presence of *An. arabiensis* aquatic stages. Water bodies within 20 meters of houses were positively associated with the presence of *An. arabiensis* larvae (OR = 3.63, $P = 0.005$). Over 50% (69/132) of the sampled water bodies were within 20m of human dwellings and of these 81% (56/69) were positive for *An. arabiensis* larvae. Water bodies <1m²

in size appeared to harbor *An. arabiensis* aquatic stages 6 times (OR = 6.15, $P < 0.001$) more frequently than water bodies of a relatively bigger size. The size of the majority (81%; 107/132) of the water bodies visited was less than 1m². Nearly 78% (83/107) of these water bodies harbored *An. arabiensis* aquatic stages. On the other hand, there was a negative association between turbidity of water bodies and presence of anopheline larvae. Clear water bodies

were 3 times (OR = 3.16, $P=0.008$) more likely to harbor *An. arabiensis* aquatic stages than turbid/semi-turbid water bodies. Overall, water bodies encountered in Rawyan and Adebay were on the average 5.4 times more likely to host *An. arabiensis* aquatic stages than water bodies in Humera town.

Discussion

The purpose of the current study was to assess the abundance of potential mosquito breeding sites and identify the particularly productive larval habitats of *An. arabiensis* in the towns for informed larval intervention. Accordingly, the findings of this study revealed that the main potential breeding sites of *An. arabiensis* in these urban settings are riverbed pools, rain puddles, vehicle ruts, leaking water pipes, dugouts and artificial container habitats which are characteristic of many urban areas in Africa (De Silva and Marshal 2012; Mahgoub et al. 2017). Of the 132 standing water bodies visited 70% were positive for *An. arabiensis* aquatic stages, 82% of which had high larval density. Nearly 88% of the larval positive sites also harbored the pupal stages reflecting the high productivity of the habitats. The findings also indicated that human made habitats, such as vehicle ruts, leaking water pipes, dug outs and artificial container habitats, were more likely to be colonized by *An. arabiensis* aquatic stages than the natural habitats. Eighty eight percent of the human-made habitats had *An. arabiensis* aquatic stages compared to 59% of the natural habitats. It should be noted that although riverbed pools were categorized under natural habitats, many of the larval positive side pools in Rawyan were associated with anthropogenic activities, such as sand excavation from the riverbed. Overall, vehicle ruts were found to be the most productive / preferred in terms of occurrence, abundance (density) and maturity (presence of late instars and pupae) of the aquatic stages of *An. arabiensis*.

In this study, human made surface waters in close vicinity to human habitation were more likely to be colonized by *An. arabiensis* larval stages than those far away from human dwellings. Proximity of households to standing water is proved to be a significant risk factor for increased malaria infection in Nazareth / Adama town in Ethiopia (Yohannes & Petros 1996; Peterson et al. 2009) as well as other urban areas in sub-Saharan Africa (Diédhiou et al. 2016; Staedke et al. 2003). Geographical localization and mapping of breeding sites would thus be needed to spatially rank malaria risk in urban settings and focus control activities on a small scale.

The distribution of anopheline larval habitats was heterogeneous across the three study sites. In Rawyan, riverbed pools were the highest contributors during the

survey period, accounting for approximately 65% of the larval positive sites. During the rainy season (June to September) the contribution of Rawyan River appears to be minimal as it remains full until mid October, depending on the amount of rainfall and temporal distribution. Its influence is expected to increase after October as the river recedes. On the other hand, the majority of the productive breeding sites in Humera and Adebay towns were temporary rain - dependent habitats and their contribution would be limited to the rainy season. In Adebay, vehicle ruts, along the streets of the town, were the only productive breeding sites of *An. arabiensis*, whereas in Humera vehicle ruts and rain puddles, in that order, accounted nearly 70% of the positive *An. arabiensis* breeding habitats. In Humera, *An. arabiensis* was also found breeding in water tankers and dug outs in construction sites which accounted for 23% of the vector positive sites. This indicates that filling puddles, vehicle ruts and dugouts and dealing with container habitats can significantly reduce the population of *An. arabiensis* mosquitoes in these towns. In Humera, as searches for breeding sites were confined to the streets and other open areas, additional larval habitats are likely to exist inside private and public compounds.

As elsewhere in sub-Saharan Africa (Gillies and Coetzee 1987; Mereta et al. 2013), *An. arabiensis* breeding sites in the study area were characteristically small, sunlit and without emergent vegetation. Smaller fully sunlit water bodies are generally characterized by high water temperature that promotes microbial growth and rapid larval development time (Paaijmans et al. 2010; Machault et al. 2009). In this study, *An. arabiensis* showed a preference for breeding in clear water and this is consistent with many studies (Diédhiou et al. 2016; Robert et al. 1998; Shililu et al. 2003). However, nearly 61% of the semi-turbid/turbid pools were also inhabited by *An. arabiensis* aquatic stages. This is consistent with the report of Ye-Ebiyo et al. (2003), where *An. arabiensis* was found in turbid water. It should be noted that turbidity here was not due to water pollution. Besides, it would be difficult to compare outcomes from different studies if the degree of turbidity is not measured.

Overall, the findings of this study suggest that targeting rain puddles and smaller human-made aquatic habitats could result in effective larval control of *An. arabiensis* in the study area. In view of the absence of indoor residual spraying in the district as a whole, measures targeted against the larval stage of *An. arabiensis* should be of utmost priority as a complement to treated nets and prompt access to treatment. Paving the side streets with selected soil material and/or cobble stones would dramatically reduce the problem in Adebay and Humera towns.

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